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HORMONE RESEARCH

From Developmental Endocrinology to Clinical Research

LWPES/ESPE 8TH JOINT MEETING

ABSTRACTS

LWPES/ESPE 8th Joint Meeting Global Care in Pediatric Endocrinology

in collaboration with APEG, APPES, JSPE and SLEP

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Plenary Lectures

PL1-01 Plenary I

Early and lifelong remodelling of our epigenomes by nutrition

Claudine Junien[1]

[1]Genetics and Epigenetics of Metabolic Diseases, Neurosensorial Diseases and Development, Hospital Necker - Enfants Malades, Paris, France

The phenotype of an individual is the result of complex interactions between genome, epigenome and current, past and ancestral environment leading to a lifelong remodelling of our epigenomes. The genetic information expression contained in the genome is controlled by labile chromatin-associated epigenetic marks. Epigenetic misprogramming during development is widely thought to have a persistent effect on the health of the offspring and may even be transmitted to the next generation. The epigenome serves as an interface between the environment and the genome. Dietary factors - including folate involved in the one-carbon metabolism - and other social and lifestyle exposures have a profound effect on many aspects of health including ageing and do so, at least partly, through interactions with the genome which result in altered gene expression with consequences for cell function and health throughout the life course. Depending on the nature and intensity of the environmental insult, the critical spatiotemporal windows and developmental or lifelong processes involved, epigenetic alterations can lead to permanent changes in tissue and organ structure and function, or, to phenotypic changes that can (or cannot) be reversed using appropriate epigenetic tools. Moreover, the flexibility of epigenetic marks may make it possible for environmental, nutritional and hormonal factors, or endocrine disruptors to alter — during a particular spatiotemporal window in a sex-specific manner — the sex-specific methylation or demethylation of specific CpGs and/or histone/chromatin modifications underlying sex-specific expression of a substantial proportion of genes. Moreover, genetic factors, the environment and stochastic events change the epigenetic landscape during the lifetime of an individual. Epigenetic alterations leading to gene expression dysregulation accumulate during ageing are important in tumorigenesis and age-related diseases. Given several encouraging trials, prevention and therapy of age- and lifestyle- related diseases by individualized tailoring to optimal epigenetic diets or drugs are conceivable. However these interventions will require intense efforts to unravel the complexity of these epigenetic, genetic and environment interactions and to evaluate their potential reversibility with minimal side effects.

PL1-02 Plenary I

Aquaporin channel disorders and clinical implications

Daniel G Bichet[1]

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Nephrogenic diabetes insipidus (NDI), which can be inherited or acquired, is characterized by an inability to concentrate urine despite normal or elevated plasma concentrations of the antidiuretic hormone, arginine vasopressin (AVP). Polyuria, with hyposthenuria, and polydipsia are the cardinal clinical manifestations. About 90% of patients with congenital NDI are males with X-linked recessive NDI (OMIM 304800) and have mutations in the arginine-vasopressin receptor 2 (AVPR2) gene that codes for the vasopressin V2 receptor; the gene is located in chromosome region Xq28. In about 10% of the families studied, congenital NDI has an autosomal recessive or autosomal dominant mode of inheritance (OMIM 222000 and 125800). Mutations have been identified in the aquaporin-2 gene (AQP2, OMIM 107777) which is located in chromosome region 12q13.

NDI is clinically distinguishable from neurohypophyseal diabetes insipidus (OMIM 125700) by a lack of response to exogenous AVP and by plasma levels of AVP that rise normally with increase in plasma osmolality. Hereditary neurohypophyseal diabetes insipidus is secondary to mutations in the gene encoding AVP (OMIM 192340). Other inherited disorders with complex polyuro-polydipsic syndrome with loss of water, sodium, chloride, calcium, magnesium, and potassium include Bartter syndrome (OMIM 601678) and cystinosis (OMIM 219800), while long-term lithium administration is the main cause of acquired NDI.

The 2003 Nobel Prize in chemistry was awarded to Peter Agre and Roderick MacKinnon, who solved two complementary problems: How does a cell let one type of ion through the lipid membrane to the exclusion of other ions? And how does it permeate water without ions? This contributed to a momentum and renewed interest in basic discoveries related to the transport of water and indirectly to diabetes insipidus. The AQP2 mutants encoded in recessive NDI are mostly misfolded and retarded in the endoplasmic reticulum (ER), while AQP2 mutants in dominant NDI are able to pass the ER and oligomerize with wild type (wt) AQP2. The proper apical membrane targeting of sufficient amounts of wt-AQP2 is impaired explaining the dominant NDI.

We propose that all families with hereditary diabetes insipidus should have their molecular defect identified because early diagnosis and treatment of affected infants can avert the physical and mental retardation that results from repeated episodes of dehydration.

PL2-01 Henning Andersen Award Lectures

Suppressor of cytokine signaling-2 inhibition of STAT signaling in the growth plate

Chloe Pass[1,2]*; Vicky E MacRae*[1]*; Syed F Ahmed*[2]*; Colin Farquharson*[1]

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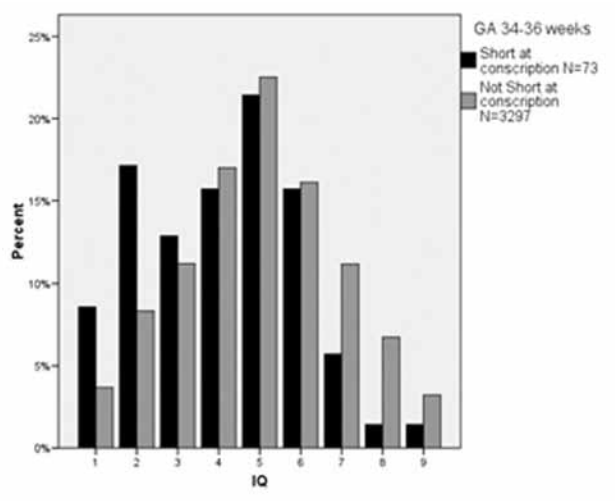
Introduction Postnatal growth is tightly controlled by Suppressor of Cytokine Signaling-2 (SOCS2), possibly through regulation of GH and IGF-1 signaling. SOCS2 null mice are characterized by enhanced body size and bone length. SOCS2 protein may also mediate the effects of IL-1β and TNFα in chronic inflammatory disorders. The precise mechanisms by which SOCS2 inhibits the GH/IGF-1 axis and pro-inflammatory cytokine signaling at the growth plate are unclear and require further study.

Methods The temporal expression of SOCS2 in wild-type (wt) costochondral chondrocytes in response to GH and IGF-1 was investigated by Western Blotting (WB). STAT signaling by chondrocytes from wt and SOCS2 knockout (ko) mice was studied in response to GH (500ng/ml), IGF-1 (50ng/ml), and pro-inflammatory cytokines IL-1β and TNFα (10ng/ml). Phosphorylation of STATs1, 3 and 5 was determined by WB.

Results SOCS2 expression increased in response to GH, an effect that increased with time to peak after 48hrs. SOCS2 expression was unaffected by IGF-1. Initial studies showed that STATs 1 and 5 phosphorylation increased significantly ($P<0.01$) in response to GH in wt cells. This analysis was extended to a comparison of SOCS2 ko and wt chondrocytes. Phosphorylation of STATs1, 3 and 5 was increased in both wt and ko cells in response to GH exposure for 15 minutes. In comparison to wt chondrocytes, ko cells showed increased STAT3 phosphorylation in response to exposure with GH (15mins), IL-1β (24hrs) and TNFα (24hrs). STATs1 and 5 showed similar levels of activation in both wt and ko cells. The noted lack of STAT phosphorylation in response to IGF-1 was not unexpected as the IGF-1 receptor lacks specific tyrosine-based motifs recognised by STATs. GH stimulation of wt chondrocytes for between 15 and 60 minutes resulted in the phosphorylation of STATs1, 3 and 5 that peaked at approximately 30 minutes and declined thereafter. In contrast, STATs1, 3 and 5 activation in ko cells was prolonged showing no decline by the end of the study period.
Conclusion We have confirmed that in growth plate chondrocytes, SOCS2 inhibits GH, IL-1β and TNFα signaling via STAT proteins. It is therefore likely that the increased length of long bones of SOCS2 knockout mice (MacRae et al. 2009) is due to increased and prolonged chondrocyte STAT signaling. It is also possible that SOCS2 may contribute to the growth retardation observed in children with chronic inflammation and may act as a potential therapeutic target.



Figures Males born before 32 weeks [fig.1] and between 34 and 36 weeks [fig.2]. Stanine scores of those who were short at conscription compared with those not short at conscription. All males born SGA are excluded.

PL2-02 Henning Andersen Award Lectures

Postnatal growth and intellectual performance among males born preterm

Maria E Lundgren[1]*; Torsten Tuvemo*[1]*; Jan Gustafsson*[1]
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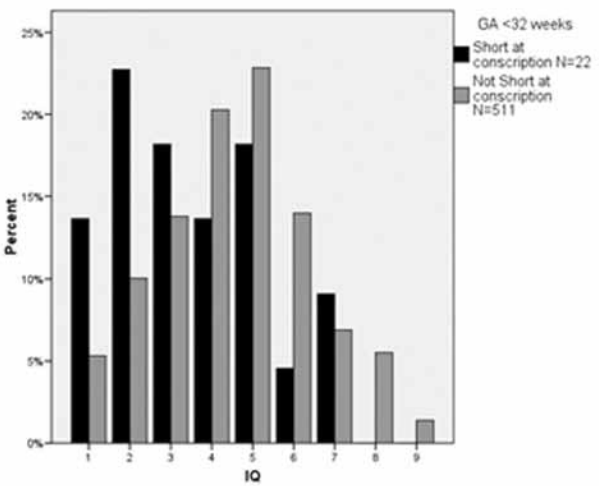
There is a lack of long term follow-up studies on growth and development of infants born preterm. In the present work, our objective was to study the association between preterm birth and intellectual performance, with special emphasis on postnatal growth.
Methods The cohort included 272 046 males, born from 1973 to 1978, without malformations and alive at 18 years, of whom 248 446 were conscripted. Birth characteristics were obtained from the Swedish Medical Birth Register and information on intellectual performance (given as Stanine scores), final height and BMI was taken from the Swedish Conscript Register. Multiple logistic regression analysis was performed.
Results Preterm birth was associated with subnormal intellectual performance compared with birth at term. Short adult stature among the males born preterm enhanced the risk of low intellectual performance. Moreover, a high adult BMI in the males born preterm was associated with a 50% increased risk of a subnormal performance as compared to those with BMI below 25 kg/m^2.
Conclusions In male conscripts, being born preterm is associated with lower results in the test of intellectual performance as compared with males born at term. Moreover, short adult stature and a high adult BMI further enhance the risk of subnormal intellectual performance in this particular group of males.



PL3-01 Plenary III

The GH receptor: Update on mechanism and actions

Michael J Waters[1]
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Until recently, the accepted model for GH-dependent activation of the receptor was hormone induced dimerization, which brought two JAK2 tyrosine kinase molecules bound to the receptor cytoplasmic domain together, so that they could cross-activate as for tyrosine kinase receptors. However, a wealth of data now supports the existence of a constitutive receptor dimer, which mandates a conformational change/receptor realignment as the mechanism of activation. We determined the crystal structure of the extracellular domain in the absence of hormone and compared it to the published structure of the bound form in order to identify the conformational change. The only salient difference was a change in the disposition of a loop in the lower beta-sandwich domain (see below), but mutation of this loop did not influence signalling through JAK2. It appeared that activation must involve subunit realignment, and we were able to show that relative rotation of subunits can activate the receptor, but not fully. To identify additional conformational changes we have used three approaches (1) cysteine crosslinking down the transmembrane domain of the full length receptor (2) replacing the extracellular domain with a jun zipper, which holds the receptor extracellular domain in manageably fixed orientations and (3) FRET with fluorophores placed just below the transmembrane domain. These approaches led us to conclude the activation mechanism involves a rotation coupled with a drawing together of the extracellular domain, resulting in (surprisingly) movement apart of the receptor subunits just below the membrane, which we suggest displaces the inhibitory pseudokinase domain of one JAK2 from the catalytic domain of the other JAK2 and vice versa.
Mutation of the loop referred to above abrogated ERK signalling, but not JAK2/STAT5 activation. This led us to identify a second tyrosine kinase signalling system directly activated by the receptor, involving a Src family kinase and ERK, acting via PLCg and increased cytosolic calcium. We could show this pathway in cells lacking JAK2 and in mice with a mutation in the box 1 sequence which abrogates their ability to activate JAK2. Using microarray analysis we have identified transcripts specific to this pathway in mice, and have identified liver regeneration as a process critically dependent on this pathway. Studies are in progress to identify the target of GH-dependent ERK signalling responsible. Supported by NHMRC(Australia).

PL3-02 Plenary III

Human ALS deficiency: clinical, endocrine and metabolic consequences

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The majority of IGF-I (and IGF-II) circulates in the serum as a complex with IGFBP-3 or IGFBP-5, and an acid labile subunit (ALS). The well-established function of ALS is to prolong the half-life of the IGFs-IGFBP-3/IGFBP-5 binary complexes.
From the description of the first case of ALS deficiency, the number of mutations identified in the *IGFALS* gene has rapidly increased, suggesting that ALS deficiency may be prevalent in a subset of patients with extraordinarily low serum levels of IGF-I and IGFBP-3 that remain abnormally low upon GH stimulation. Fourteen different mutations of the human *IGFALS* gene have been identified in the seventeen patients identified to date. Eleven patients were found to be homozygous and six were compound heterozygous. The mutations showed an autosomal recessive pattern of inheritance.
Postnatal growth was clearly affected. Commonly, height SDS before puberty was between -2 and -3, and were approximately 1.4 SD shorter than the midparental height SDS. Adult height SDS was higher than prepubertal height, but still 1.0 SD lower than the midparental height SDS. Pubertal delay was found in 50% of the male patients, while in the remaining, puberty started relatively late.
Human ALS deficiency results in a peculiar IGF-I deficiency. Whereas circulating levels of IGF-I decrease dramatically, local production appears to be preserved. In addition to IGF-I other members of the circulating IGF system are also affected. Circulating IGF-II**,** IGFBP-1, -2, and -3 levels were all reduced, with the greatest reduction observed for IGFBP-3. Insulin resistance, characterized by normal glucose levels, hyperinsulinemia and low levels of IGFBP-1, was a common finding. In addition, some patients presented low bone mineral density (BMD). The pathophysiological mechanisms explaining these findings are still only partially understood.
In summary, human ALS deficiency, the first monogenic defect involving an insulin-like growth factor binding protein, represents a unique condition in which the lack of ALS protein results in the disruption of the entire IGF circulating system. Despite a profound circulating IGF-I deficiency, there is only a mild impact on postnatal growth. Perhaps, the preserved expression of locally produced IGF-I under the stimulation of normal or even increased GH levels, might be responsible for the preservation of linear growth near or within normal limits.

PL4-01 Plenary IV

Biology and function of nuclear steroid hormone receptors

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Nuclear steroid hormone receptors (NRs) form a large gene superfamily, acting as hormone-inducible transcription factors and require a number of co-regulators and complexes for their hormone-dependent functions. Such transcriptional controls by nuclear receptors have been recently revealed to require chromatin reorganization and histone modifications as epigenomic rearrangement. More recently, a number of epigenetic regulators supporting nuclear receptor function have emerged, and histone modifying enzymes as well as chromatin remodelors have been identified as transcriptional co-regulators as multi-subunit complexes. The roles of the complexes will be discussed with the newly identified complexes by us (Kitagawa et al., Cell, 113, 905, 2003; Fujiki et al., Nature, 459, 455, 2009).
Physiological impacts of nuclear receptors could be verified by gene disruption of nuclear receptors in mice. We had ablated the Vitamin D receptor (VDR) in mice, and observed rachitic disorders in the mutant mice only after weaning, representing a mouse model for human type II rickets patients (Yoshizawa et al., Nat Genet., 16, 391, 1997). Likewise, the significance of androgen receptor (AR) in androgen actions was confirmed in mice (Kawano et al., PNAS, 100, 9416, 2003). Together with recent our findings of the estrogen receptor α (ERα) function in osteoprotective estrogen actions (Nakamura et al., Cell, 130, 811, 2007), the physiology of AR/ERs/VDR will be overviewed.

PL4-02 Plenary IV

Inherited endocrine diseases involving G proteins and G protein-coupled receptors

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Naturally occurring mutations in the G protein Gs-a subunit, and in a number of G protein-coupled receptors (GPCRs) have been identified in human diseases. Loss of function mutations in GPCRs for various hormones lead to hormone resistance manifest as hypofunction of the gland expressing the affected GPCR. Conversely, GPCR gain of function mutations lead to hormone-independent activation and hyperfunction of the involved gland. Our lab has focused on the extracellular calcium-sensing GPCR (CaR) expressed primarily, but not exclusively, in parathyroid glands and kidney. Loss of function CaR mutations lead to a form of hyperparathyroidism termed familial hypocalciuric hypercalcemia, an apparent exception to the general pattern described above, but in fact reflecting resistance to the normal inhibition of parathyroid hormone (PTH) secretion by the "hormone" agonist, extracellular Ca^{++}. CaR gain of function mutations cause autosomal dominant hypocalcemia due to activation of the receptor at subphysiologic concentrations of serum Ca^{++}, leading to "inappropriate" inhibition of PTH secretion. I will describe recent work that helps inform design of novel therapeutics targeting this important GPCR.

PL5-01 Plenary V

IGFs for life

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The IGF system comprises two structurally similar ligands (IGF-I and IGF-II), six IGFBP and two receptors (IGF-R). For both IGF-I and IGF-II, signal transduction is mediated by IGF-1R. By contrast, IGF-2R is responsible for ensuring IGF-II clearance. The IGF system has been widely implicated in intermediate metabolism, proliferation, cell differentiation and survival and development. Due to its ubiquitous expression and action, defects in this system lead to many fetal, postnatal and tumoral growth disorders. The parameters of the IGF system are controlled in a complex manner dependent on hormonal stimuli, the IGF-producing tissue and developmental stage. The physiology of the somatotropic axis (GH, IGFs) has been defined more accurately by genetic modification experiments in mice. IGF-II (encoded by an imprinted gene) and IGF-I play key roles in the control of growth, in terms of both weight and height, during fetal development, and IGF-I is also involved in postnatal growth. IGF-1R mediates the action of both these factors and *IGF-1R* knockout results in death immediately after birth. Partial or conditional gene invalidations have therefore been used in studies focusing on the physiology of IGF-I and its receptor in the postnatal period and during adulthood. IGF-1R has been implicated in the control of longevity. In addition, invalidation or overexpression of the *IGF-1* gene in the liver, the organ controlling IGF-I concentration (endocrine) in the bloodstream, have demonstrated the respective effects on growth and tissues of endocrine or paracrine-autocrine IGF 1. In humans, abnormalities in the *IGF I and IGF-1R* genes has been described in some fetal and postnatal growth retardations, whereas epigenetic defect of 11p15 leading to the abolition of *IGF II* expression and associated with the biallelic expression of *H19* has been observed in the majority of the fetal growth retarded Silver-Russell syndromes. Conversely, *IGF II* is often overexpressed in tumors and in fetal overgrowth syndromes (e.g. Beckwith Wiedemann syndrome), in which other epigenetic (methylation) or genetic defects in the 11p15 region housing the *IGF II* gene have been identified, some of which have been implicated in the risk of tumor formation. Thus the ubiquitous production and action of IGFs affect the physiology of all tissues and body functions, from the fetal stage, through postnatal development and into adulthood, and may go as far as controlling the longevity of the organism.

PL5-02 Plenary V

Lessons from three dimensional protein homology modelling for steroidogenic diseases

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Adrenal steroid hormones are vitally important. Deficient adrenal steroid biosynthesis causing a lack of glucocorticoid and mineralocorticoid hormones leads to hypoglycaemia, hypotension, salt loss and death. Because of the importance of adrenal steroids, defects in steroidogenesis and steroid action are rare and have serious consequences. By studying the molecular genetics of these rare defects the cause of the diseases can be described and further insights in steroid physiology can be obtained. In recent years delineating of structure-function relationships has proven a very valuable tool to understand underlying molecular mechanisms. Our group was able to identify numerous sequence variations in *CYP21A2, CYP11B1, CYP17A1, HSD3B2* or *NR3C2* which were further investigated using novel protein homology models. While limited structural information is available in the absence of crystal structures for cytochrome P450 enzymes and short chain dehydrogenases involved in steroidogenesis, methods predicting the three-dimensional structure of these enzymes have been used. Homology modelling is a technique used to predict the three dimensional structure of proteins based on the observation that proteins with similar amino acid sequences have a tendency to adopt similar three-dimensional structures. Homology modelling predicts the three-dimensional structure of a protein based only on its amino acid sequence and its alignment with the solved crystal structures of proteins with similar sequences. Although such structures obtained by modelling are less accurate than those derived experimentally, they are invaluable since they provide a testable hypothesis in the absence of experimental data. Until recently, homology models of mammalian cytochrome P450 enzymes have been based solely on the structurally determined bacterial cytochrome P450s. The recent availability of the crystal structures of more closely related mammalian cytochrome P450s have contributed towards the reliability of homology models. For short-chain dehydrogenases we still have to rely on bacterial templates. Several steroid hormone receptors like the mammalian mineralocorticoid receptor have already been crystallized. By using protein homology modelling we were able to gain fundamental insights in the individual mechanism of mutations in steroidogenic enzymes and steroid receptors. Essential structures for the function of these proteins have been delineated and are the basis for our future studies.

PL5-03 Plenary V

Exploring the utility of a European register of disorders of sex development

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Research & audit are vital for the management of disorders of sex development (DSD) and associated genital anomalies. However, the information that is required to investigate issues such as aetiology, management and long-term outcome require good clinical ascertainment with the least amount of selection bias. Furthermore, a number of questions that need to be addressed can only be answered by multicentre studies. The 2006 Consensus Workshop jointly hosted by ESPE and LWPES on the diagnosis and management of DSD stressed the need for the creation and maintenance of a register in centres of expertise. Such registers do exist in many regional centres, but they lack international uniformity and ability to cross-talk. The ESPE Research Unit grant has allowed the creation of a web-based register which uses a core dataset based on the revised DSD nomenclature. This web-based register has now become an integral part of EuroDSD, a collaborative project of EU funded research, launched in spring 2008. The register will include a portfolio that supports many aspects of DSD research including capabilities to link clinical data resources on a case-by-case basis as well as providing services that support scientific research into DSD. The cornerstone of this proposed virtual research environment (VRE) model is based upon site autonomy. In this, each clinical site is solely responsible for deciding what data sets it can share, with which partner sites and in what context. Given this, the design of the VRE is driven by security, incorporating both the needs of the clinical community and ethical oversight required on information governance. It is hoped that in the long-term, tools such as these would allow the development of international research strategies for the diagnosis of previously unsolved cases of DSD as well as assessment of long-term management.

PL5-04 Plenary V

Update on the diagnosis and treatment of isolated *SHOX* haploinsufficiency

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Abnormal growth and consequent short stature is the commonest reason of referral to a pediatric endocrine unit. Among the recognized causes of short stature, isolated *SHOX* (short stature homeobox-containing gene) defects are the most frequent monogenic cause of short stature. *SHOX* is located in pseudoautosomal region of the X and Y chromosomes and it encodes a cell-specific homeodomain protein that act as a transcription activator in osteogenic cells. Two active copies of *SHOX* gene are required for its normal function. It has been well established that *SHOX* haploinsufficiency causes a wide spectrum of short stature phenotypes. Heterozygous *SHOX* deletions or point mutations are observed in 56 to 100% of patients with Leri-Weill dyschondrosteosis (LWD) and in 1 to 14% of children with short stature without any apparent skeletal dysplasia, usually classified as idiopathic short stature (ISS). LWD is a dominant inherited skeletal dysplasia characterized by disproportionate short stature, mesomelic limb shortening and the Madelung deformity of the forearm (bowing of the radius and distal dislocation of the ulna).
It is noteworthy that patients that carried *SHOX* defects exhibited a broad phenotypic variation regarding the severity of short stature and/or the presence of Madelung deformity. However, the most frequent clinical finding in patients with *SHOX* mutations was the presence of height disproportion. So, it is logical to select ISS children with disproportional short stature and/or a family history of LWD to undergo the molecular analysis of *SHOX*. Our data confirm the usefulness of this approach, and we also showed that the simpler sitting height : height ratio for age and sex (SH/H SDS) effectively selected a group of children that presented a higher frequency of *SHOX* mutations (3.2% in non-selected ISS children vs. 22% in children selected by SH/H).
The growth deficit due to *SHOX* haploinsufficiency is estimated in -2 SD of height. Short stature observed in patients with Turner Syndrome is partially explained by the haploinsufficiency of *SHOX*. Because rhGH therapy in TS patients improves growth velocity and consequently, final height, the treatment of short stature due to *SHOX* defects is proposed. Thus, the molecular diagnosis of *SHOX* defect has therapeutic implications. Studies have reported optimistic responses to treatment with recombinant human growth hormone (rhGH) alone or with the concomitant use of gonadotropin-releasing hormone analog (GnRHa).

PL6-01 Plenary VI

Genome-wide genetic profiling of type 1 diabetes: But what next?

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We now have a genome-wide profile for the genetic basis of the familial clustering of the multifactorial disease, type 1 (insulin-dependent) diabetes. The expected, L-shaped, allelic spectrum of few large effects and many smaller effects is dominated by genes that regulate immune responsiveness to pancreatic islet antigens: from HLA antigen recognition to dephosphorylation of signalling cascades and interleukin-2 (IL-2) cytokine regulation.
We can now study the effects of these common alleles on the common characteristics of the immune system to identify inherited biomarkers that may be precursors of autoimmunity and that could be targeted in future clinical trials to prevent the disease.
For example, we have shown that the type 1 diabetes predisposing alleles of

the susceptibility gene, the interleukin-2 receptor, IL2RA, are associated with altered levels of the receptor on memory and naive T cells, highlighting these subpopulations in human blood as candidates for components of the balance between healthy immune homeostasis and pathogenesis.
The development and education of the immune system is affected by environmental exposures, infection and diet, linked to the likelihood that a susceptibility genotype of many predisposing alleles across the genome will lead to type 1 diabetes. The challenge now is to put together these observations with the molecular disease pathways in an effort to identify which environmental exposures can be modified to modulate autoimmune disease precursors towards avoiding type 1 diabetes early in life.

PL6-02 Plenary VI

Adiponectin

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Adiponectin is an adipocyte-derived hormone that circulates in plasma. Numerous studies suggest that adiponectin increases insulin sensitivity, but the underlying mechanism is not fully understood. Adiponectin mediates protective effects against lipotoxic insults to maintain fatty acid homeostasis, thus complementing leptin in anti-steatotic and anti-apoptotic functions. Adiponectin is an endogenous insulin sensitizer with the capacity to normalize dyslipidemia and exert anti-atherogenic actions. Such insulin sensitizing effects have been attributed to a sequence of events that induce AMPK activation, inactivate acetyl coenzyme-A carboxylase (ACC) and decrease malonyl coenzyme-A (CoA) levels, a process that may stimulate FA oxidation in skeletal-muscle. However, it is more likely that endogenous adiponectin exerts its effects in a skeletal muscle-independent way, mainly through action in organs such as the liver. The liporegulatory properties of adiponectin have further been highlighted by its' ability to cause TG depletion in liver to ultimately minimize ectopic lipid deposition in non-adipose tissues.
Recent evidence suggests that adiponectin may also promote survival of β-cells in pancreatic islets. Adiponectin has been observed to moderately suppress cytokine- and FA-induced β-cell apoptosis in order to prevent autoimmune and lipotoxicity-induced dysfunction. Furthermore, adiponectin has been reported to stimulate AMPK in cultured MIN6 β-cells and purified rat islets, thus demonstrating its' potential to enhance FA oxidation in β-cells through ACC regulation; this is consistent with the phosphorylation of ACC observed in cultured human islets. These data therefore underline the promising anti-apoptotic functions of adiponectin, which may reduce the formation of toxic lipid metabolites to counteract lipotoxicity-induced β-cell destruction; furthermore, adiponectin may play a pivotal role in compensatory β-cell growth.

Symposia

S1-01 Symposia I

Closed loop devices

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The requirements for an artificial pancreas are: an insulin pump, continuous glucose sensor, and a control algorithm. All are available today. The problems are: time delays in the onset of SQ insulin and SQ sensor lag times, the accuracy of continuous glucose sensors, biologic variation in insulin action and meal absorption, and rapid changes in insulin sensitivity associated with exercise. Possible solutions to the delays in onset of insulin action are a novel more rapid acting insulin (such as ViaJect), microneedle injection, local warming, hyaluronidase, and intraperitoneal or intravascular insulin deliver. Another issue is whether to give bi-hormonal replacement with glucagon as well as insulin. Most control algorithms currently utilize either a proportional-integral-derivative algorithm, or a model predictive control algorithm.
Initial closed loop studies may be aimed at preventing nocturnal hypoglycemia by suspending insulin delivery when hypoglycemia is predicted or detected, and using algorithms which treat-to-range, preventing extremes of hyper and hypoglycemia. Closed loop control may also be very practical in an intensive care unit setting where there is one-to-one nursing and intravenous insulin delivery allowing for rapid onset of insulin action. Another area for utilization of closed-loop therapy is at the onset of diabetes. Studies are currently underway to determine if early stabilization of glucose levels at the onset of diabetes using closed loop control can prolonged the remission phase.

S1-02 Symposia I

Islet transplantation

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The primary goals of islet transplantation (ITX) is the achievement of glycemic control in the absence of severe hypoglycemic episodes, improvement of quality of life and prevention or reversal of the chronic, progressive complications associated with diabetes. Insulin independence, although desirable, is not considered a primary objective of ITX. C-peptide production following islet transplantation may contribute to some of the improvement of diabetic complications observed post-transplant. However, in the context of ITX, a decline of renal function has also been reported in some studies, while more recent reports have shown stable renal function and lack of worsening diabetic nephropathy at long-term follow-up, or an initial decline of renal function that stabilizes without further worsening in the long-term. Strict selection of islet transplant candidates without previous renal dysfunction (*i.e.*, micro-albuminuria and low estimated glomerular filtration rates) and timely implementation of nephro-protective and anti-hypertensive therapies (i.e., Angiotensin-Converting Enzyme inhibitors and/or Angiotensin-Receptor Blockers) may have accounted for the different clinical outcomes observed across different ITX studies. Immunosuppressive protocols void of nephrotoxicity are highly desirable, and indeed, ongoing clinical trials are showing promising results in patients undergoing conversion of either CNI or mTOR-inhibitors to MPA maintenance, with preservation of both renal and islet function. While a steady progress in the 1-year and 5-year ITX survival has been observed, the benefits of ITX need to be carefully weighed against the risks associated with the need for chronic immunosuppression and its side effects. The clinical applicability of ITX beyond the most severe cases of Type 1 DM will require the development of successful immunosuppressive regimens that minimize or eliminate nephrotoxic drugs, and the introduction of novel strategies to replace systemic immunosuppression with local immunomodulatory strategies, nanoencapsulation and tolerance induction, which are currently being explored in pre-clinical and clinical trials.

S1-03 Symposia I

In vivo generation of glucose-responsive insulin-secreting cells from human embryonic stem cell-derived pancreatic endoderm

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Development of a cell therapy for diabetes would be greatly aided by a renewable supply of human beta-cells. We have previously described the in vitro generation of pancreatic endoderm cells from human embryonic stem (hES) cells. We now show that the hES cell-derived pancreatic endoderm efficiently generates glucose-responsive endocrine cells after implantation into mice. Upon glucose stimulation of the implanted mice, human insulin and C-peptide are detected in sera at levels similar to those of mice transplanted with ~3000 human islets. Moreover, the insulin-expressing cells generated after engraftment exhibit many properties of functional beta-cells, including expression of critical beta-cell transcription factors such as PDX1, NKX6-1, and MAFA, expression of the pro-hormone processing enzymes PCSK1 and PCSK2, appropriate processing of proinsulin, and the presence of mature endocrine secretory granules. Finally, in a test of therapeutic potential, we demonstrate that implantation of hES cell-derived pancreatic endoderm protects against streptozotocin-induced hyperglycemia. Together, these data provide definitive evidence that hES cells are competent to generate glucose-responsive, insulin-secreting cells.

S2-04 Symposia II

Global prevalence and health implications of malnutrition

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Maternal and child undernutrition is highly prevalent in low-income and middle-income countries, resulting in substantial increases in morbidity, mortality and overall disease burden. More than 3.5 million children under five die unnecessarily each year due to the underlying cause of undernutrition, and millions more are permanently disabled by the physical and mental effects of a poor dietary intake in the earliest months of life. Undernutrition includes a wide array of effects including intrauterine growth restriction (IUGR); underweight; stunting; wasting; and less visible micronutrient deficiencies. Using recent data and growth standards, today it is estimated that 13 million children are born annually with IUGR, 112 are underweight and 178 (or 32%) million children under 5 years suffer from stunting. The vast majority of these children

live in south-central Asia and sub-Saharan Africa. Poor fetal growth or stunting in the first 2 years of life leads to irreversible damage, including shorter adult height, lower attained schooling, reduced adult income, and decreased off spring birth weight. Although fewer children are severely wasted compared to those affected by stunting, these children (19 million) are at very high risk of death and their treatment should be taken as a priority. As the WHO Child Growth Standards demonstrate, children up to the age of 5 years across large populations - regardless of their racial or ethnic background - grow to remarkably similar heights when given the best possible growth conditions in early life. To continue to allow underprivileged environments to affect children's development not only perpetuates the vicious cycle of poverty but also leads to an enormous waste of human potential. Because undernutrition is an intergenerational problem, the prevention of maternal and child undernutrition is a long-term investment that will benefit the present generation and their children.

S2-05 Symposia II

Experimental evidence for early nutrition programming of later health

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A number of prospective epidemiological-based studies and other recent meta-analyses have lent validity to the hypothesis that the developmental environment is capable of adjusting an individual's risk of adult (non-communicable) disease. In order to explore the multitudinous mechanistic pathways toward adult disease that the 'developmental environment' may confer, one must turn to an appropriate animal model. Many have been developed that recapitulate one aspect of developmentally programmed non-communicable disease risk e.g. hypertension for cardiovascular disease, insulin resistance or obesity for Type 2 Diabetes. In these animal models, exposure to a particular pattern of nutrient deficit or excess during a susceptible window of development alters the trajectory of cellular, tissue or organ growth to ultimately constrain later functional capacity of that cell, tissue or organ. Ageing, without exception, exacerbates any programmed sequalae. A number of endocrine axes, in particular, the hypothalamic-pituitary-adrenal or gonadal axes have proved susceptible to developmental programming, with clear sex-specificity for both. The long-term quality-of-life effects of these subtle alterations to endocrine function have been little characterised and assumptions are invariably made between developmental programmed changes to endocrine function and susceptibility to a given disease. For the reproductive axis, as an example, we find that early life insults, such as undernutrition, may subtly impact upon the gonadal progenitor cell complement and later hypothalamic-pituitary-gonadal axis function with little effect however, on lifetime reproductive capacity and fecundity. Thus to conclude, there is substantial evidence for early nutritional programming of later physiological and endocrine function, but the extent to which programming effects may be attributed toward actual clinical outcome requires further work.

S3-06 Symposia III

The molecular basis of adrenarche

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Adrenarche has fascinated endocrinologists for the past several decades, largely because this process occurs only in human beings and a very few higher primates. Despite many years of study, the importance of adrenarche in normal human physiology remains obscure. In addition, several misconceptions have clouded our understanding of adrenarche. First, the zona reticularis of the postnatal adrenal gland is not equivalent biochemically to the fetal adrenal cortex. Second, adrenarche does not begin abruptly at about age 6-8 but occurs gradually from infancy. Nevertheless, we do know that the zona reticularis of the human adrenal cortex contains a good combination of enzymatic machinery to synthesize dehydroepiandrosterone sulfate (DHEAS) efficiently and to the exclusion of cortisol or aldosterone production. These characteristics include high expression of cytochromes P450c17 (CYP17A1) and b5 (CYB5) with low expression of 3β-hydroxysteroid dehydrogenase type 2, as well as the cholesterol side chain cleavage enzyme system (CYP11A1) and steroid sulfotransferase 2A1 (SULT2A1). Some recent concepts about adrenarche and the zona reticularis that will be discussed include the development of better cell models and attention to the efflux of DHEAS from these cells.

S3-07 Symposia III

Clinical implications of premature adrenarche

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The term "adrenarche" was coined in 1947 by Fuller Albright. He defined it in a strictly biochemical sense: " … the age of entrance of adrenal cortical 'N'-hormone on the scene is termed 'adrenarche'; …". The age of entrance of this 'N'-hormone was dated between the 6th and 8th year of life. We now know that 'N'-hormone corresponds to dehydroepiandrosterone-sulfate (DHEA-S), the dominating C_{19}-steroid of the adrenal cortex. However, recent research has revealed that adrenarche is actually a gradual process starting much earlier than hitherto believed because production of DHEA-S and its metabolites is already present in preschool children (Remer 2005).
The increase in adrenal C_{19}-steroids leads to clinical signs of androgenicity, e.g. pubic hair. Precocious appearance of clinical signs of androgenicity warrants medical clarification. In particular, steroid producing tumors, various disorders of steroid metabolism and precocious central puberty have to be excluded. Premature appearance of clinical signs of androgenicity is called idiopathic in case these entities have been ruled out and it is attributed to a premature augmentation of adrenal androgen secretion.
Clinical effects of this idiopathic premature augmentation of adrenal androgen secretion on growth, development, body composition as well as hormonal and metabolic status in affected individuals will be discussed.
Remer et al., J Clin Endocrinol Metab 2005;90:2015.

S3-08 Symposia III

Family relationships and adrenarche

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Life history theorists have proposed that humans have evolved to be sensitive to specific features of early childhood environments and that exposure to different environments biases children toward development of different reproductive strategies, including differences in timing of adrenarche and puberty. The current research provides a longitudinal test of this theory. Assessments of family environments, based on interviews with mothers and fathers, were conducted in preschool, and children were then followed prospectively through middle childhood. Adrenal hormones were assayed in a selected subsample of 120 children (73 girls) at age 7, and parent and child reports of secondary sexual characteristics were collected in the full female sample of 180 girls at age 11. Higher quality parental investment (from both mothers and fathers) and less father-reported Marital Conflict/Depression forecast later adrenarche. Consistent with a life history perspective, quality of parental investment emerged as a central feature of the proximal family environment in relation to timing of adrenarche.

S4-09 Symposia IV

Hypoglycemia, functional brain failure and death

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Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes. The vast majority of episodes of iatrogenic hypoglycemia are corrected after the glucose level is raised. Nonetheless, an estimated 6% to 10% of deaths in type 1 diabetes, and an unknown proportion in type 2 diabetes, are due to hypoglycemia. While profound, prolonged hypoglycemia can cause brain death, most fatal episodes are the result of other mechanisms, presumably ventricular arrhythmias (*J Clin Invest* 117:868, 2007). Hypoglycemia triggers

an increase in sympathoadrenal activity that causes abnormal cardiac repolarization, a condition known to be associated with fatal ventricular arrhythmias. Patients with structural diabetic autonomic neuropathy are particularly vulnerable. Recent antecedent iatrogenic hypoglycemia causes functional autonomic failure that impairs sympathoadrenal defenses against subsequent hypoglycemia, the concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes (*Diabetes* 57:3169, 2008). Recent data indicate that HAAF includes reduced baroreceptor sensitivity, a plausible mechanism of sudden death (*Diabetes* 58:360, 2009). Clearly, although long-term glycemic control is in the best interest of people with diabetes, that should be at the lowest level that can be accomplished safely.

S4-10 Symposia IV

Prevention of hypoglycemia in children

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Hypoglycemia is indeed the limiting step in the ability to achieve and maintain optimal glycemic control in children and teens with type 1 diabetes. What is clear is that the risk of hypoglyccemia is not uniform among individuals with type 1 diabetes nor between centres caring for these children and teens. These data suggest that there are modifiable risk factors for hypoglycemia in this population. This presentation will focus on the following factors: (i) more phsyiological insulin delivery; (ii) frequency of monitoring of blood glucose concentrations; (iii) vigilance during nobasal conditions such as intercurrent illness and exercise; and (iv) resetting of glycemic targets in the most vulnerable. While severe hypoglycemia can be significantly decreased in these children and teens, it cannot be entirely prevented, thus mandating knowledge of treatment of these severe episodes.
Please note that this is the abstract for the workshop entitled;
Hypoglycemia, the limiting factor to achieve optimal glycemic control on Sept 11th at 10:45 a.m.

S4-11 Symposia IV

Hypoglycemia a conundrum

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Both hyperglycemia and hypoglycemia are associated with an increased mortality rate. This U-shaped relationship between extremes of dysglycemia and mortality may be biological markers of poor outcomes. The fear of hypoglycemia-induced mortality resurfaced recently with the use of more aggressive insulin treatments, though direct causality has not been established. In fact, despite increased rate of hypoglycemic events in the intensively treated arms of many landmark studies, improved clinical outcomes without mortality changes is the case. In recent trials designed to achieve near-normal glycemic targets mortality rate was unchanged. Two studies are the exception; the ACCORD trial where the intensive glycemic treatment arm had to be prematurely terminated due to increased unexplained mortality, and the NICE-SUGAR STUDY, where intensive insulin therapy in intensive care units was associated with a higher mortality. The association between hypoglycemia and increased mortality rate is apparent without evidence for causality. Hypoglycemia can produce a variety of symptoms but the principal problems arise from an inadequate supply of glucose as fuel to the barin. During careful short and long term neurological evaluation in the largest trial for individuals with type 1 diabetes (DCCT), the intensively treated arm with a higher rate of hypoglycemia had no neurological deficit. While it is "easier to kill the brain", it is more difficult to "kill the heart". There is a paucity of evidence that low blood sugars causes myocardial ischemia or generate cardiac arrhythmias, particularly in patients with longstanding diabetes that tend not to have reperfusion injury and not to counteregulate. Hypoglycemia is common during hospitalization in patients with and without diabetes, and is more frequent in the elderly and or those with organ failure and or polypharmacy. The use of insulin or oral antidiabetic agents frequently causes hypoglycemia, but rarely death. Hypoglycemia in hospitalized patients appears to be a strong biomarker of poor prognosis and is associated to organ failure. Hypoglycemia predicts mortality, but in several studies, multivariate analysis has shown that it is only a marker. The use of intensive insulin regimens needs to be carefully evaluated, particularly in elderly patients with other comorbidities. While hypoglycemia is an undesirable event, there is no proof that it is a direct cause of increased mortality.

S5-12 Symposia V

Assessment of pubertal timing (PT) and determinants

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The physiological variations in PT tend to increase and may exceed 5 years, raising questions about mechanisms and significance. 1) Several hypothalamic neuromodulators could account for physiological variations in PT. Among them, oxytocin is a facilitator in rodents and deserves studies in humans. 2) Environmental factors are thought to be minor determinants of PT that is mainly dependent on genetic factors. However, endocrine changes related to obesity epidemics and endocrine disruptors can account for increasing impact of environment on PT. The hypothalamic mechanism is complex as shown for the insecticide DDT whose action involves estrogen receptors, glutamate receptors and orphan dioxin receptors. 3)Self-evaluation of PT can help in studying PT significance in the global developmental process of adolescence. In comparison with physician assessment, self-evaluation of PT in boys and girls versus peers accounts respectively for 61/69% sensitivity and 77/70% specificity in differentiating early from normal puberty. Overall, there is a distorted estimation towards earlier timing. While age at first intercourse is not affected by PT, 59, 41 and 24% among adolescents with early, normal and late timing of puberty self-report sexual activity by 16 yrs of age, respectively. Except drug abuse, the scores of delinquency self-reported by early and late adolescent boys are higher than those with average PT. Multivariate analysis of the determinants of delinquency, however, shows that PT is not a significant predictor as opposed to factors such as social aspects including peer characteristics. Among 47 adolescent sexual offenders, 36% self estimated PT to be normal whereas 30% and 34% felt to be early or late and victimized peers or younger children, respectively. The interrelation between pubertal timing and adolescence warrants further studies.
Supported by Belgian FNRS and BSGPE.

S5-13 Symposia V

New mutations in abnormal puberty

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Puberty represents a complex biological process of sexual development that can be influenced by genetic, nutritional, environmental and socioeconomic factors. The activation of pulsatile hypothalamic GnRH secretion is a key event in the onset of puberty. A network of hypothalamic neurons is critical for GnRH release and, consequently, pituitary gonadotropin secretion and gonadal steroid production during pubertal maturation. Precocious puberty is defined as the development of secondary sexual characteristics before the age of eight years in girls and nine years in boys. There are several causes of precocious puberty, and it is of utmost importance to distinguish between central precocious puberty (gonadotropin-dependent), which results from premature activation of the hypothalamic-pituitary-gonadal-axis, and gonadotropin-independent precocious puberty. Central precocious puberty has striking female gender predominance and most of these cases are considered idiopathic. However, it is known that genetic factors play a fundamental role in the timing of pubertal onset, as illustrated by a similar age of menarche among members of ethnic groups and in mother-daughter, monozygotic twin and sibling pairs. The GPR54-kisspeptin system is as one of the most important neuroendocrine regulator of puberty initiation. A number of loss-of-function mutations in the GPR54 gene have been associated with a normosmic isolated hypogonadotropic hypogonadism phenotype in humans. Consistent with this, mice lacking GPR54 (gpr54-/-) exhibit a phenotype similar to that of humans with normosmic hypogonadotropic hypogonadism. Conversely, one activating mutation (Arg386Pro) was also described in this receptor in a Brazilian adopted girl with idiopathic central precocious puberty. In vitro studies have shown that this missense mutation leads to prolonged activation of intracellular signaling pathways in response to kisspeptin. More recently, two novel KiSS1 mutations (P74S and H90D) were associated with idiopathic central precocious puberty in an apparently complex

mode of inheritance. Therefore, activating mutations of the GPR54 and KiSS1 genes seem to be implicated in the pathogenesis of human central precocious puberty in humans.

S5-14 Symposia V

Biosocial aspects of puberty: Natural selection and age of first reproduction in pygmy populations

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Studies aiming at understanding variation in timing of human pubertal growth and development have focused mostly in western populations. These studies have uncovered genes involved in regulating timing of pubertal onset and body growth in large European samples. In parallel, anthropological studies have reported the secular trends and the strong influence of environmental factors such as nutrition, on age of puberty onset in Western populations. Variation in timing of puberty in hunter-gatherer and other small scale societies are much less understood and averages can vary considerably: Average age of menarche for instance, can vary from 13 years old in some South American Indians to 17 years old in some East African pastoralists. While the vast heterogeneity of environments is recognized as one important factor leading to this variability, genetic diversity is also likely to play a key role in shaping growth and timing of pubertal onset in these populations. Most interestingly, genetic variability in these traits is not produced by chance, but it is the product of the particular adaptive strategies and selective pressures of each population. Studying pygmy populations in the Philippines and Africa, (who are exposed to very high mortality environments) we have argued that small body size in pygmies can be explained as a life history trade-off between selection for extended growth (where larger size is associated with increased fertility) on the one hand, and selection for early reproduction (where earlier reproduction is associated with increased fertility) on the other, with the balance pending towards the latter in these populations due to their exceptionally high mortality rates (Migliano et al., 2007). While selection for early reproduction and consequent early growth cessation has been identified in pygmy populations, little is known on the genetic factors underlying this phenotype. Large scale association studies are not possible in these hunter-gatherer groups given the small population sizes; in spite of that, the recent findings of genes involved in the regulation of both pubertal onset and adult height in European populations, bring the opportunity to explore the relationship between natural selection acting on the phenotype, and the underlying genetic mechanisms leading to early puberty in human pygmies.

SS01 New Molecular Tools for Pediatric Endocrinology

Molecular Biology for Clinicians

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Fundamental concepts in the area of molecular biology will be presented in this lecture. These include biology's and biotechnology's central dogma; the 'human genome project'; the discordance between human gene number and corresponding protein number; and gene cloning techniques. Procedures used to determine gene number and location (Southern blotting) and levels of gene expression at the RNA (Northern blotting, Reverse Transcription/Polymerase Chain Reaction, micro-array/gene chip) and protein (Western blotting, proteomics) level will be presented. Additionally, three examples describing the cloning of genes/cDNAs and production of the respective recombinant therapeutic proteins will be offered. Finally, functional genomic concepts and protocols will be discussed including production of transgenic and gene-disrupted (knocked out) animals as well as methods to down regulate gene expression using antisense, ribozyme, or small inhibitory RNAs. The lecture will stress the 'basics' of the various protocols with clinical examples cited.

S6-15 Symposia VI

Screening for thyroid disorders in premature infants

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Permanent primary congenital hypothyroidism (PPCH) can occur in premature neonates but is *not* more common than in term newborns: in fact, neonates with PPCH tend to be born *post*-term and macrosomic. After 24h of life, mean TSH values are similar in premature and term neonates, so the same cut-off for recall can be used. Sampling tends to occur late in premature neonates so that neonatal nurseries have to be reminded of the importance of timely screening for PPCH. Transient hypothyroxinemia of prematurity (THOP) is defined as a state of low plasma free thyroxine with a normal plasma TSH. In contrast to the benefits of pre-symptomatic treatment of PPCH thanks to biochemical screening, controlled trials have shown that thyroxine treatment of premature neonates with THOP does not result in an overall improvement of IQ. *Post-hoc* analysis of one such trial has suggested an improved psychomotor development in neonates born at < 27 weeks, while a deleterious effect was observed in those born > 29 weeks. Given the absence of benefit from treatment, population-based screening of neonates for THOP is not recommended. Likewise, population-based biochemical screening for congenital hyperthyroidism is not recommended, because the condition is very rare and can be recognized clinically.

S6-16 Symposia VI

Effectiveness of a national program to optimize growth and development of preterm infants using nutrient enhanced formulas, additional effects on insulin sensitivity and body composition

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Advances in medicine, specifically neonatal care, have allowed an increased proportion of premature infants of low and extremely low birth weight to survive These infants represent a challenge for the neonatal intensive care units and paediatricians in order to allow their survival and lower their incidences of postnatal chronic morbidities. Among these morbidities concern has been raised during the last two decades with regard to the association between low birth weight and increased incidence of cardiovascular disease and alterations in glucose homeostasis in later life. The effect of low birth weight and future adult disease has been mostly explored in newborns at term. The effect in VLBW preemies has not been clearly demonstrated. Moreover some discordant findings in young adult premies compared to SGA term young adults have been reported. The hypothesis proposed to explain the development of these long term alterations is called the Developmental Origin of adult diseases which include the fetal adaptation to in utero adverse environment and the additional contributions of the patterns of growth in infancy and childhood. Some short term effects of nutrient impact in postnatal growth in preterms have been reported. The National Health Service (NHS) in Chile modified existing national program that provided free milk formula to all preterm infant with birth weight <1500 g and/or 32 wks followed in NHS clinics during the first 12 months corrected age (CA). Before, all infants received Term Formula (TF 20kcal/oz) until 6 mo corrected age (CA) followed by Fortified Cow's Milk (added Fe, Zn, Cu and ascorbic acid) until 12 mo CA. We evaluated the impact of this program on growth, development, insulin sensitivity and body composition from birth to 2 years in a subset of children discharged before the program was started and compared with the data obtained in the group born after the program was initiated. This is to our knowledge the first study evaluating the impact of a nutritional intervention with the prolonged use of an enriched formula. The cost-effectiveness of this type of intervention and the potential unintended consequences need to be assessed in order to select best use of limited resources.

S6-17 Symposia VI

Endocrine pathology of the premature infant – adrenal function

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About 80 % of the fetal adrenal gland volume is comprised of the fetal zone producing dehydroepiandrosterone sulfate (DHEAS). The fetal zone cannot make cortisol from cholesterol due to the low expression of the 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme. Some premature newborns present with hypocortisolism which has been associated with vasopressor resistant hypotension, bronchopulmonary dysplasia, and poor outcome. On the other hand, high cortisol concentrations have been associated with severe intraventricular hemorrhage and death. Low cortisol concentrations could be explained by the immaturity of adrenal steroidogenesis due to low 3β-HSD activity. However, our studies revealed that low cord and first day cortisol levels associated with low DHEAS concentrations suggesting that adrenal 3β-HSD deficiency does not explain early hypocortisolism; it may be of central origin on the basis of reported low ACTH levels. Hydrocortisone treatment of sick premature newborns with low serum cortisol levels could be beneficial, but there is an increased risk for intestinal hemorrhages especially during the simultaneous use of indomethacin or ibuprofen for the closure of patent ductus arteriosus. Additional trials on early hydrocortisone supplementation targeted at premature newborns with low cortisol and/or DHEAS concentrations might reveal the clinical significance of low serum cortisol concentration after very premature birth.

S7-18 Symposia VII

New concepts in prolactin biology

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Prolactin is currently viewed as a hormone of pituitary origin, whose production (i.e. serum levels) is controlled by dopamine agonists, whose biological actions relate exclusively to reproductive functions, and whose unique associated pathology is hyperprolactinemia. Studies performed during the past few years have considerably widened our perception of prolactin biology: i) in addition to the endocrine hormone, locally-produced prolactin has been documented in various human tissues (e.g. the mammary gland and the prostate), where it acts via autocrine/paracrine mechanisms, ii) there is increasing evidence supporting the role of prolactin in human breast and prostate tumorogenesis, especially when it is locally-produced, iii) we recently reported the first gain-of-function variant of the prolactin receptor, that was identified in patients presenting with breast tumors (benign or cancer), iv) we have engineered human prolactin variants acting as pure competitive antagonists of the prolactin receptor, and we have demonstrated their ability to down-regulate the actions triggered by local prolactin or by the constitutively active receptor variant in experimental models; these compounds represent a novel class of molecules of therapeutic interest as a potential alternative to dopamine agonists. These novel aspects of prolactin biology will be presented.

S7-19 Symposia VII

Hyperprolactinemia in children and adolescents

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The prevalence of functional and organic hyperprolactinemia increases from childhood to adulthood. Clinical manifestations vary depending on gender and time of onset. Difficulties on diagnostic aspects are partly due to the fact that it is only recently that adequate reference ranges have been found for both genders throughout puberty. These difficulties become even more evident when different prolactin isoforms with varied biological activity are evaluated. According to our experience, gylcosilated prolactin is present, although variable, during puberty. Additionally, we observed high-molecular weight isoforms present in this period. In patients with asymptomatic hyperprolactinemia, the presence of altered proportions of prolactin isoforms should be evaluated.

The evolution of prolactinomas in children and adolescents is still controversial. Girls have more prevalence of microprolactinomas with signs and symptoms related to hyperprolactinemia and the resulting hypogonadotrophic hypogonadism. In males, the greater incidence of macroadenomas would not be related to a later diagnosis and results in the presence of neuro-ophthalmologic signs. Diagnosis requires both documented presence of sustained hyperprolactinemia and radiographic evidence of pituitary adenoma. Dopaminergic agonists are the initial therapy of choice in pediatric age. Finally, molecular biology and genetic studies have brought new insights into the pathogenesis, clinical behavior and different therapeutic responses.

S7-20 Symposia VII

Epidemiology of prolactinomas

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Pituitary adenomas are benign but clinically relevant intracranial neoplasms because of hormonal hyperproduction or tumor mass effects in most of the cases. A recent study drew specialists' attention on their prevalence, showing that pituitary adenomas are several times more frequent than previously estimated – 1:1064 people.

Among all types of adenomas prolactinomas are the predominant entity, estimated on about 45% to even 66% in some of the series. According to their size they could be divided to micro- (<10 mm) and macroadenomas (>10 mm), which are unevenly distributed. Microprolactinomas, being prevalent mostly in women in reproductive age, whereas macroadenomas show inclination to male sex. It is still unclear whether macroprolactinomas in men are due to a delay in diagnosis or to an initially more aggressive potential. In general, preponderance in female favour is observed (10:1), however losing its power with aging. Prolactinomas are rare in childhood, increasing in number in adolescence, presented mostly by macroadenomas, and repeating the adult pattern in regard to sex distribution.

Although the most part of prolactinomas appear in a sporadic setting, there is a certain number that arise in familial settings such as multiple endocrine neoplasia type 1 (MEN1) and familial isolated pituitary adenomas (FIPA). This fact is of great clinical importance as these adenomas show a more aggressive behaviour compared to their sporadic counterparts. Even more, bearers of AIP mutation (found in about 15% of FIPA kindreds and rarely in a sporadic setting) are younger at diagnosis and more difficult to manage.

In conclusion, epidemiological studies on prolactinoma prevalence play an important role, giving insight on the pathophysiological causes and the burden of the disease in a certain sub-population of patients.

1. Daly AF et al. High prevalence of pituitary adenomas: a crosssectional study in the province of Liege, Belgium. Journal of Clinical Endocrinology and Metabolism 2006; 91:4769–4775.
2. Verge's B et al. Pituitary disease in MEN type 1 (MEN1): data from the France–Belgium MEN1 multicenter study. Journal of Clinical Endocrinology and Metabolism 2002 87 457–465.
3.Daly AF et al. Aryl hydrocarbon receptorinteracting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. Journal of Clinical Endocrinology and Metabolism 2007; 92:1891–1896

S8-21 Symposia VIII

State-of-the-art imaging in the evaulation of congenital hyperinsulinism

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Congenital Hyperinsulinism is increasingly recognized as a frequent cause of persistent hypoglycemia in neonates. Pancreatic uptake of 18F-fluoro-L-dihydroxyphenylalanine (18F-DOPA), has led to a major breakthrough in the diagnosis and treatment of these patients. Research studies have shown that 18F-DOPA Positron Emission Tomography (PET) imaging along with clinical and genetic testing can help guide therapy in these patients. PET imaging has led to the identification of two distinct types of congenital hyperinsulinism, focal and diffuse. In focal hyperinsulinism, the 18F-DOPA PET scan can identify a focal lesion within the pancreas that can be resected and may result in a cure.

In the diffuse type of hyperinsulinism activity is seen throughout the pancreas. Patients with diffuse disease refractory to medical therapy may undergo a palliative subtotal pancreatectomy. This does not lead to a cure. Experience at the Children's Hospital of Philadelphia and several centers in Europe indicate that 18F-DOPA PET imaging has a high sensitivity for the diagnosis of focal lesions and high accuracy for anatomic localization. This lecture will review the current data, and describe techniques, protocol recommendations, patterns of uptake and pitfalls in diagnosis. Imaging of patients with congenital hyperinsulinism using 18F-DOPA should be strongly considered prior to surgery.

S9-22 Symposia IX

Children and adolescents with type 2 diabetes: Characteristics of the TODAY study participants at baseline

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Systematic data on optimal methods to treat type 2 diabetes (T2DM) in adolescents are lacking. In response, the TODAY study group, comprised of 15 clinical centers in the US funded by NIDDK, designed a prospective, randomized trial to evaluate treatment regimens and the clinical course of T2DM in youth. The trial compares the efficacy of 3 treatment arms in maintaining glycemic control among 700 participants over 2-6 years. The 3 arms consist of standard diabetes education (SDE) plus: (i) metformin plus placebo, (ii) metformin plus rosiglitazone, and (iii) metformin plus intensive lifestyle program incorporating nutrition, physical activity, and behavior modification. The primary outcome is time to treatment failure, defined as HbA1c 8% for 6 months. Secondary outcomes include measures of beta cell function and insulin resistance, body composition, nutrition, physical activity and aerobic fitness, cardiovascular risk factors, microvascular complications, quality of life, psychological outcomes, as well as the influence of individual and family behaviors on treatment response and the relative cost effectiveness of the 3 treatment arms. Enrollment was completed on February 28, 2009. The demographics, family and medical history, disease characteristics, and prevalence of comorbidities in this well-characterized cohort at the time of entry into TODAY will be described.

S9-23 Symposia IX

The HEALTHY trial

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In response to the increase in type 2 diabetes in youth, the HEALTHY trial was designed to determine if risk factors for type 2 diabetes could be modified with a middle school-based multi-component intervention. The study was conducted in 42 middle schools, 6 at each of 7 locations; 21 schools were randomized to receive the intervention and 21 acted as controls. The modifiable risk factors measured were indicators of adiposity and glycemic dysregulation: body mass index 85th percentile, fasting glucose 100 mg/dL, and fasting insulin 30 μU/mL. In the 6th grade, 6358 students were enrolled and those remaining in the schools were followed to end of 8th grade. Health screening data were collected in the 6th and 8th grades. The intervention consisted of four integrated components: (1) changes in the quantity and nutritional quality of food and beverage offerings; (2) physical education class lesson plans and accompanying equipment to increase both participation and number of minutes spent in moderate-to-vigorous physical activity; (3) brief classroom activities and family outreach vehicles to increase knowledge and enhance decision-making skills; and (4) communications and social marketing strategies to enhance and promote change through messages, images, events, and activities.

S9-24 Symposia IX

Update to Finnish trials aimed at prevention of type 1 diabetes

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The incidence of type 1 diabetes (T1D) has increased markedly over the past decades among children and adolescents in developed countries. Accordingly there is a definite need for effective preventive measures. Such measures can be categorized into primary strategies aimed at preventing the initiation of the disease process, secondary prevention aimed at stopping the progression of the disease process to clinical diabetes, and tertiary prevention aimed at restoring the failing beta-cell function or preventing diabetic complications. Several prevention trials have been performed in Finland over the last 15 years. The TRIGR (Trial to Reduce IDDM in the Genetically at Risk) pilot set out to test the feasibility of weaning high-risk infants to a hydrolyzed formula. The study comprised 230 infants with a family member affected by T1D and HLA-conferred disease susceptibility. The participants were randomized to receive either a highly hydrolyzed formula or a regular cow's milk based formula whenever breast milk was not available over the first 6-8 months of life. The 10-year follow-up data show that the early nutritional intervention reduced the cumulative incidence of diabetes-associated autoantibodies by 40-60% except for GAD antibodies. The first results of another pilot trial testing the hypothesis that bovine insulin is an exogenous determinant of T1D will become available within the next few months. In this pilot infants with HLA-defined diabetes susceptibility recruited from the general population were randomized to be weaned to either an insulin-free formula or a regular cow's milk based formula containing bovine insulin. A secondary prevention trial has been performed in young autoantibody-positive children with HLA-conferred T1D susceptibility derived from the background population within the DIPP (Diabetes Prediction and Prevention) study. This trial assessed whether daily administration of intranasal insulin decreases the progression rate to clinical T1D. The inclusion criteria were HLA-defined disease susceptibility and positivity for two or more diabetes-associated autoantibodies in at least two sequential samples. The progression rate turned out to be identical in those randomized to intranasal insulin and in those randomized to placebo. These experiences indicate that prevention of diabetes after the disease process has been initiated is a true challenge. Accordingly more efforts should be put into modalities aimed at primary prevention.

S10-25 Symposia X

The GH-IGF system in human longevity

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To examine the relationship of the GH-IGF axis to human longevity, a unique cohort of individuals with exceptional longevity, their offspring, and -matched controls without a family history of unusual longevity, was studied in collaboration with Professor Nir Barzilai at Einstein (The LonGenity Cohort). Published observations from this cohort have demonstrated a favorable lipoprotein profile and favorable genotypes for polymorphisms in the cholesteryl ester transfer protein (CETP) gene, an apolipoprotein C-3 (APOC-3) promoter variant, and a deletion at the 3' UTR of the adiponectin gene. We observed that female offspring of centenarians have higher serum IGF-I but significantly reduced reported maximal heights compared to age-matched controls, suggesting a state of IGF-resistance (male offspring had identical heights and serum IGF-1 levels as controls). We screened the IGF1R gene of centenarians and controls and discovered a common longevity-associated SNP and 5 novel mutations amongst female centenarians. Lymphocytes from affected individuals demonstrated a decrease in IGF-1 receptor number and signaling. These data demonstrate for the first time in humans, that the IGF1R is a genetic determinant of longevity in a gender specific manner (similarly to what is observed in the IGF1R heterozygous KO mice). We also studied the frequency of the common exon-3 deletion polymorphism of the GH receptor (D3GHR) in this cohort. D3GHR homozygosity rose with age and was 4-fold more common in centenarians versus controls. This observation was confirmed in a second cohort. Male centenarians were shorter than their cohort, and had lower serum IGF-I. Lymphocytes from d3/d3-GHR carriers displayed significantly slower

growth rates and lower activation of ERK at baseline, but higher growth and ERK-activation in response to GH treatment, as compared to fl/fl-GHR carriers indicating an altered GH-dose-response in d3-GHR-carriers. On the other hand, among centenarians, IGF-I levels were correlated positively with mental performance, and in 70-years old males, higher IGF-I levels were associated with lower rates of cardiovascular disease. Thus, exceptional longevity is associated with enrichment in several genotypes, including some within the GH-IGF axis and these in turn, are associated with significant measurable phenotypes. Our findings provide intriguing possibilities regarding genotype-specific pharmacologic interventions to enhance healthy aging.

S10-26 Symposia X

Longevity versus healthspan

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Longevity is defined as the period of time from birth to death. This contrasts with healthspan, the period of life which is characterized by freedom from disability and disease, with the ability to enjoy an independent life without functional limitations. GH/IGF-I deficiency in worm, fly and transgenic and knockout animal models and calorie restriction are associated in the laboratory setting with increased longevity. Life-long calorie restriction or growth hormone deficiency are not viable options for humans. In addition, life expectancy of humans has doubled from 1900 to the present. Americans over the age of 65 years numbered 3.1 M in 1900, 25.7M in 1980, 34.9M in 2000 and are projected to exceed 65.6M in 2030. The aging of the baby boomers now presents major societal, health, and economic challenges for the next quarter century and beyond. It is commonplace to reach 80 years of age or more. The progressive reduction of muscle mass that starts at mid puberty and continues through life is one of the most important age-related changes in body composition. This reduction in muscle mass is one of several factors that contribute to frailty of aging; this is a major factor in shortening healthspan. Therapeutic strategies to slow or reverse this process will be discussed.

S11-27 Symposia XI

A journey up and down the GH/IGF1 axis

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Ross Clark was a long time colleague and friend and it is a pleasure to have this opportunity to discuss his contributions to GH and IGF biology. He was an integrative physiologist, viewing the GH-IGF1 axis as part of a neuroendocrine cascade controlling growth and metabolism. Ross developed sampling and infusion techniques in rodents for detailed investigations of the role of pulsatility in GH signalling both on IGF1 and other target genes, and on the mechanisms of GH control. The GH/IGF1 system has proved a good target for imaging and genetic manipulation under physiological and pathophysiological conditions at all scales, from molecular and subcellular events in single cells, to multicellular organization and function at the whole-organism level. Ross's work with IGF1 was crucial in making this pleiotropic growth factor widely available for clinical use, but he was also interested in the role of binding proteins for both GH and IGFs, and in the GH secretagogue forerunners of ghrelin, and his contributions remain relevant to the key questions in these fields. Integrative physiology has often been characterised as seeing how "black boxes" interact in the context of the whole organism: Ross played an important part in starting to open the boxes.

S12-28 Symposia XII

What is different in type 2 diabetes in South Asians

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South Asians have experienced rising prevalence of obesity, type 2 diabetes (T2DM: from 3% to 12%), and coronary heart disease (from 2% to 10%) in the last 25 years. They develop insulin resistance (IR), T2DM, and consequent morbidity and mortality a decade earlier and at lower body mass index (BMI), due to their phenotype of relatively lower muscle mass, higher fat, and more visceral fat ("thin-fat"). Even healthy adolescents with normal BMI may have high BP, high triglycerides and low HDL cholesterol. Thus lower BMI cutoffs are needed, and are in use for adult Indians; similar reduction may be needed for adolescents. Asia has a high incidence of maternal malnutrition and micronutrient deficiencies and therefore low birth weight (LBW). This adverse fetal programming not only affects individuals, but also future generations, as it might be inherited. LBW predisposes to later IR, especially with rapid postnatal growth. These factors, combined with a rapid demographic, nutritional and socioeconomic transition, have resulted in the epidemic of T2DM and its complications. Food is becoming cheaper, more available, and more diabetogenic: high carbohydrate/ high saturated fat/ reducing fiber content. Levels of physical activity are dropping with mechanization, rising urbanization and over-crowding. The epidemic and its complications are increasingly affecting the young and the underprivileged, impacting the economically productive phase of life, and imposing a heavy economic burden. The low ratio of public to private health expenditure, and high out-of-pocket health spending worsens matters. Three-fourths of India lives on under 2 dollars a day: any chronic disease further drives families into poverty. To tackle the problem, better and cheaper medical care, and prevention through lifestyle education are needed. Also needed are public health interventions aimed at improving intrauterine and childhood environments (better maternal and infant nutrition, opportunities for physical activity) which may prove more effective.

S12-29 Symposia XII

Diabetes in American Indian populations

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Diabetes is a common and serious disease among American Indian children and adults. Among children, it has been best characterized among the Pima Indians of the Southwestern U.S. and in some First Nations communities in Canada. Recently, American Indian children have been included in SEARCH, a survey of diagnosed diabetes in several U.S. locations and different ethnicities.

Although classification of the type of diabetes is often difficult, the vast majority of American Indian children with diabetes have clinical features of type 2 diabetes. Recent attempts to classify their diabetes using measures of diabetes-associated antibodies and c-peptide secretion, which are not routinely measured in clinical practice, have indicated that classification is not straightforward, as many American Indian children who might be classified as having type 2 diabetes clinically also have diabetes-associated antibodies or low c-peptide secretion, characteristics of typical type 1 diabetes.

The Pima Indian longitudinal population study provided data on diabetes incidence and prevalence over four decades. The incidence of diabetes, thought to be almost exclusively type 2, is much higher in children in this population than it is elsewhere. Unfortunately, the risk of microvascular complications, especially diabetic nephropathy, has a similar dependence on duration of diabetes as it does in those who develop type 2 diabetes in adulthood. Therefore, Pima Indian children who develop diabetes have a high incidence of complications, including end-stage renal disease, by middle adulthood. Because of the dramatic increase in the age-specific incidence rates of diabetes in youth, the problem of serious diabetes complications in young or middle adulthood is likely to worsen. Diabetes prevention efforts in adults, including American Indians, have had some success. Applying similar interventions in youth is an important challenge in pediatrics.

S12-30 Symposia XII

Type 2 diabetes in indigenous Australian children – high rates and a management challenge

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The influence of Western civilisation in the last 250 years has seen a large increase in metabolic disease in indigenous Australians. The life expectancy of indigenous Australians is17 years less than that of the total Australian population, and the most common cause of death is cardiovascular disease. Rates of diabetes in adults are at least 3x higher than other Australians, however data in

indigenous children are limited. A recent study of T2DM in indigenous children in Western Australia found incidence rates of 38.5 per 100 000 per year, the highest internationally reported incidence of T2DM in children aged 0-16. A number of factors contribute to these high rates –lifestyle, poor maternal nutrition, low birth weight, poor compliance with prescribed treatment, poor attendance rates and gestational diabetes. Relevant data supporting the role of these factors will be presented. Similar to reports in other indigenous groups, rates of early renal disease and hypertension are high in indigenous Australian children.
The large size of Australia and small population make delivery of health care to indigenous people, many of whom live in rural and remote areas challenging. For example, Western Australia is the largest state in Australia, it covers 2,525,500 square kilometres (4 x the area of Texas). Despite the huge size, the population is only 2 million. The entire state is serviced by one Paediatric Diabetes Unit. Some of the approaches to the issues which compound the care of indigenous children in Australia will be presented. The role of HbA1c as a useful diagnostic tool in remote communities will be discussed. Preventative programs coordinated by indigenous groups have had some success in remote communities with lifestyle changes and these will also be discussed.

S13-31 Symposia XIII

Genomewide association studies (GWAS) for the pediatric endocrinologist

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Genome-wide association (GWA) studies have been used to find common genetic variants that are associated with polygenic traits and diseases, including several relevant to pediatric endocrinology. However, the modest effects of these common variants has meant that mapping and validating associated loci requires large numbers of samples and collaborations. Within the Genetic Investigation of ANthropometric Traits (GIANT) consortium, we have taken a collaborative approach and are using GWA studies representing over 100,000 individuals to identify common variants associated with anthropometric traits, including height and measures of obesity. Earlier iterations of these efforts, involving studies of up to 30,000 samples, successfully identified 10 loci for body mass index and over 40 loci for height. These results have implicated both expected and novel biological pathways, and have highlighted the role of central nervous system in regulating body weight as well as several distinct pathways in the regulation of height.
Thus far, the associated variants we have identified explain only a small fraction of the expected contribution of genetic factors to population variation. Clearly, many of the causal loci and even causal variants at known loci remain to be discovered. Thus, further efforts and analyses will likely uncover additional genetic determinants, although the relative contributions to trait variation of common and rare variants, and discovered vs undiscovered loci, remains to be seen. These efforts will include expansion of GWA studies both in sample size and in the number and types of variants that can be assayed, studies of individuals at phenotypic extremes, comprehensive and deep sequencing of known loci, more complex analyses, and, eventually, comprehensive sequencing of the genome in large numbers of individuals. Clinically useful predictive information will likely only emerge for a subset of diseases and traits, and will depend both on the predictive power of genetic variants and on the available clinical interventions. A main goal remains uncovering causal pathways that could shed light on underlying biology and potentially guide the development of new interventions or therapies.

S13-32 Symposia XIII

Use of genome-wide approaches to investigate variation in pubertal timing

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Recent, remarkable progress has been made in identifying genes (*e.g. GPR54, FGFR1, PROK2, PROKR2, FGF8, TAC3, TACR3*) that, when mutated, cause disorders of pubertal development such as hypogonadotropic hypogonadism and Kallmann syndrome. Although the timing of puberty is highly heritable (50-80% of the variation in pubertal timing is determined by genetic factors), much less is known about the genes that regulate pubertal timing in the general population. Our understanding of why, for example, one young woman begins puberty at age 9 years and another at 12 years remains incomplete. This presentation will (i) provide an update on the genes implicated in disorders of puberty, (ii) review the role that common genetic variants in these and other genes play in regulating pubertal timing, and (iii) discuss alternative avenues of investigation that may help identify genetic regulators of pubertal timing focusing on data emerging from the initial genome-wide association studies (GWAS) designed to investigate pubertal timing in the general population.

S13-33 Symposia XIII

Genome-wide association for obesity-related traits – what have we learned?

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The genetic contribution to inter-individual variation in common obesity has been estimated at 40% to 70%. Despite a relatively high heritability, the search for obesity susceptibility genes has not been trivial. In the past 15 years, candidate gene and genome-wide linkage studies have been the two main genetic epidemiological approaches to identify obesity susceptibility loci, but their success has been limited as only a handful of variants were found to be consistently associated with obesity-related traits.
However, in recent years, genome-wide association has dramatically changed the pace of gene discoveries for common disease, including obesity. Three waves of large-scale high-density genome-wide association studies have already discovered at least 15 previously unanticipated genetic loci incontrovertibly associated with BMI and obesity risk. Most loci, but not all, were found to also associate with childhood BMI and risk of early-onset obesity, some of which with even larger effects than those found in adults. The physiological role of most of these loci in relation to obesity is still poorly understood. However, many of them locate near genes that are highly expressed in the brain, including the hypothalamus, supporting a role for the nervous system in body weight control.
The combined contribution of the recently discovered loci to inter-individual variation in obesity risk is sobering small and their predictive value is typically low, suggesting that many more obesity susceptibility loci remain to be uncovered. One approach is to further increase the sample size of the discovery stage, which will provide more power to identify new loci with smaller effects. Therefore, the GIANT (Genomic Investigation of Anthropometric Traits) consortium, an international collaborative initiative that focuses on anthropometric traits, has initiated a fourth wave of genome-wide association meta-analyses for BMI including data on more than 120,000 individuals. Another approach is to focus on refined traits that are more accurate proxies for adiposity, such as body fat percentage and waist. Furthermore, genome-wide association studies in pediatric cohorts might identify loci that have not been found in studies of adults. The latest findings of various new approaches will be reviewed.
These newly discovered loci are set to shed new light on the complex physiology of obesity and might lead to the development of more effective preventive and therapeutic interventions.

S13-34 Symposia XIII

Identification of genetic variants conferring increased risk to autoimmune thyroid disease

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In keeping with other common diseases, the identification of novel genetic variants conferring susceptibility to autoimmune thyroid disease via candidate gene studies and linkage analysis has proved problematic. Recent genome-wide association studies are however now, beginning to reveal a number of novel genetic variants in many diseases. The Wellcome Trust Case Control Consortium (WTCCC) in the UK has recently completed two large association studies in eleven common diseases. In the smaller of the two studies 5,500 individuals

were investigated, which included 900 subjects with Graves' disease, using a genome-wide set of 14,500 nonsynonymous coding single nucleotide polymorphisms (nsSNPs). Whilst the strongest association signal was identified in the human leukocyte antigen (HLA) region (P value < 10^{-20}), association was also confirmed with the previously reported, Graves' disease specific locus, the thyroid stimulating hormone receptor gene (*TSHR*) and Fc receptor-like 3 gene (*FCRL3*), with a further nine novel regions showing evidence for a trend of association with P value $\leq 10^{-3}$(4). In conjunction with previously replicated loci, including the *CTLA4, PTPN22 and CD25* genes, significant progress is now being made in unravelling the complex genetic background to thyroid autoimmunity. This in turn is also enabling us to better understand the mechanisms leading to the autoimmune disease process in general and in specific diseases. Ultimately it is likely that this will not only lead to the development of new therapies but also bespoke targeting of treatments.

S14-35 Symposia XIV

Weird animal genomes and the evolution of human sex chromosomes

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Whether a baby develops as a boy or girl depends on a single gene on the Y chromosome. In humans and other mammals, females have two X chromosomes, but males have a single X, and a Y that bears a gene (SRY) that induces testis differentiation and switches on hormones that masculinize the embryo. The human X is a middle-sized chromosome, rich in "brains-and-balls" genes involved in reproduction and intelligence (often both), and thought to have had a major role in human evolution. The tiny Y is a genetic wasteland – full of genetic junk and bearing only 45 genes, most of which are active only in testis. How did human sex chromosomes get to be so weird?
Our strategy is to compare the chromosomes, genes and DNA in distantly related mammals and even birds and reptiles (which have completely different sex determining systems). The genomes of Australia's unique kangaroos and platypus, now completely sequenced, are a goldmine of new information. Kangaroo sex chromosomes reveal that only part of human X and Y chromosomes were original and part added recently. The bizarre platypus sex chromosomes (more related to those of birds) tell us that our sex chromosomes are relatively young.
The human X and Y evolved from an ordinary chromosome pair as the Y degraded progressively. The Y chromosome is a relic of the original chromosome pair, and the genes on it (including the sex determining gene SRY) are relics of genes on this ancestral chromosome. The Y chromosome is degrading rapidly due to its isolation from recombination with the X. At the rate it is degrading, I predict that the Y will disappear in just 5 million years. If humans don't become extinct, new sex determining genes and chromosomes will evolve, maybe leading to the evolution of new hominid species.

S14-36 Symposia XIV

The pseudoautosomal regions, *SHOX* and disease

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The pseudoautosomal regions represent blocks of sequence identity between the mammalian sex chromosomes. In humans, they reside at the ends of the X and Y chromosomes and encompass roughly 2.7 Mb (PAR1) and 0.33 Mb (PAR2). While PAR2 resembles the overall sequence composition of the X chromosome and exhibits only slightly elevated recombination rates, PAR1 behaves like an autosome and displays distinct structural and functional features. As part of its important function in mediating male meiosis, PAR1 is subjected to one mandatory crossover during this process and it exhibits one of the highest recombination frequencies throughout the entire human genome and, probably as a consequence of its structural features, displays a significantly faster rate of evolution. It therefore represents an exceptional model to explore the correlation between meiotic recombination and evolutionary forces such as gene mutation and conversion. At least thirty genes lie within the human pseudoautosomal regions, and these genes exhibit 'autosomal' rather than sex-specific inheritance. All genes within PAR1 escape X inactivation and are therefore candidates for the etiology of haploinsufficiency disorders including Turner syndrome (45, X). However, the only known disease gene within the pseudoautosomal regions is the ***S***hort stature ***HO****meboX* gene *SHOX*. Its functional loss is causally related to various short stature conditions and disturbed bone development.

S15-37 Symposia XV

Updating the rationale and outcomes of weight loss surgery in youth

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Extreme obesity is recognized as major health problem, threatening the health of more than 2 million U.S. children and adolescents. Diet and lifestyle interventions for pediatric patients have not been successful in achieving and maintaining meaningful improvements in weight and health. Bariatric surgery achieves meaningful and sustained weight loss in adults and adolescents. Published data demonstrate that gastric bypass and adjustable gastric banding are commonly used for adolescents. Outcomes data have demonstrated significant improvements in type 2 diabetes and insulin resistance, dyslipidemia, sleep efficiency, obstructive sleep apnea, hypertension and cardiac hypertrophy, proteinuria, depressive symptoms, quality of life, and body composition in extremely obese adolescents. It is important to begin to consider the impact of timing of surgery on weight loss outcome and health outcome of these young patients. The medical community and public have justifiably been concerned about the safety of offering surgery for obesity to youth. However, on the other hand, recently analyzed outcome data lead us to the competing concern that if effective treatment is not offered soon enough, the effectiveness of the treatment may be compromised. Analysis of the outcome of 60 adolescents who underwent laparoscopic gastric bypass in Cincinnati was recently performed. BMI in the entire cohort at baseline was 60.2 kg/m^2. One year following surgery, BMI decreased 37%, irrespective of starting BMI; thus only 17% of patients achieved a non-obese BMI (<30 kg/m^2). Most of those with baseline BMI in the superobese range (eg., >55kg/m^2) remained morbidly obese postoperatively despite aggressive surgical therapy. In summary, the window of opportunity to effectively reverse adolescent morbid obesity may close during adolescent years for those who are gaining weight rapidly. It is important that clinicians note that pre-operative BMI serves as a predictor of nadir BMI at 1 year postoperatively, and this finding must be factored into decision-making regarding timing of surgical referral for adolescent bariatric surgery. This treatment paradigm may be even more applicable to patients with biologically-driven (hypothalamic) obesity. Certainly much more research will be needed before the precise role of weight loss surgery will be known in these more rare groups of patients.

S15-38 Symposia XV

Pediatric bariatric surgery – experimental therapy and "ultima ratio" in selected patients only – no general application neither today nor in the future

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Bariatric surgery is an effective weight loss treatment for adults with a BMI above 40 kg/m² and there are sufficient data to calculate complication risks for adults. Data for adolescents are insufficient and there are no data for children. No surgical treatment studies were suitable to be included in the latest Cochrane review on interventions for treating obese children (Cochrane Database Syst Rev. 2009:CD001872).
Very limited data from case series suggest bariatric surgical interventions can lead to moderate to substantial weight loss and improvement of metabolic disturbances in adolescents in the short term and to some immediate health benefits through resolution of comorbidities, such as sleep apnea or asthma. Harms vary by procedure.
Bariatric surgery is no causal therapy for obesity or the metabolic syndrome. In addition, there is a long list of unsolved problems and risks. Bariatric surgery should be strictly limited to highly selected patients with ife-threatening comorbidities under well defined conditions (see Endocrine Society Clini-

cal Pratice Guideline, JCEM 93, 4576-4599, 2008). We should not discuss on whether or not obese children should be subjected to bariatric surgery. We should rather ask: *Which are the right patients to operate and what is the benefit? Which patients do have the highest benefit?*
During decision making a high value has to be placed on avoiding anatomical and functional changes in developing children, on avoiding unforeseen complications associated with lifelong exposure to these changes, and on avoiding the costs and perioperative complications of these procedures.
Pediatric societies argue against bariatric surgery for preadolescent children and for any patient who has not mastered the principles of healthy dietary and activity habits as well as for any patient with an unresolved eating disorder, untreated psychiatric disorder, or Prader-Willi-Syndrome.
Bariatric surgery will also be in the future only an 'ultimate ratio' in a few highly selected children and adolescents. It is suggested that this kind of experimental therapy will be of interest only for the next 10 – 15 years. Then the endocrine and metabolic mechanisms leading to the great benefits of bariatric surgery (body weight reduction and metabolic improvements) will be better understood and new pharmacological treatment options as well as preventive measures will be available and replace surgical treatment options.

S15-39 Symposia XV

Insulin sensitizers for the treatment of pediatric obesity

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Obese children are at high risk for adult obesity and co-morbidities; thus inadequate or delayed intervention may promote or accelerate the development of serious complications. Complication rates are shaped by race, ethnicity, and family history; certain groups are at higher risk, and intervention must be individualized. People referred to specialists for treatment of obesity have not responded to standard lifestyle counseling. Intensive lifestyle counseling can sometimes be effective, but it is time-consuming, expensive, and often disappointing. Insulin sensitizers (metformin) may reduce body fat and fasting glucose and insulin levels and attenuate the risks of type 2 diabetes. The safety profile and costs of metformin are acceptable. A balanced, tailored approach makes sense.

S15-40 Symposia XV

Insulin sensitizers in pediatrics – con

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Obesity is strongly associated with insulin resistance, a risk factor for type 2 diabetes and other obesity-related comorbid conditions. Individuals who develop type 2 diabetes as children are likely to experience the microvascular and macrovascular complications of this disease at younger ages than individuals who develop diabetes as adults. In addition to lifestyle interventions, insulin sensitizers have been utilized to promote weight loss and improve the metabolic profile of overweight, non-diabetic children, with the ultimate goal of diabetes prevention. The use of insulin sensitizers has been associated with very modest weight loss in blinded, randomized, controlled clinical trials. Whether or not insulin sensitizers prevent the development of type 2 diabetes in children with insulin resistance, impaired fasting glucose, or impaired glucose tolerance is not known. While insulin sensitizers (metformin) have been shown to effectively reduce the rate of progression from pre-diabetes to type 2 diabetes in adults (albeit, not as well as lifestyle modification) they have not been shown to be efficacious in the treatment of obesity. More research is indicated to establish the range of potential uses of insulin sensitizers in children.

S16-41 Symposia XVI

Vitamin D biology revealed by genetic mouse models

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We employed mice with targeted deletion of genes encoding the 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]-synthesizing enzyme, 25 hydroxyvitamin D-1alpha-hydroxylase [1α(OH)ase$^{-/-}$], and of the nuclear receptor for 1,25$(OH)_2$D, the Vitamin D receptor (VDR), and additional mouse mutants to examine both skeletal and extra-skeletal effects of vitamin D. In terms of skeletal and calcium homeostasis, we found that optimal dietary calcium absorption required both 1,25$(OH)_2$D and the VDR, however serum calcium and phosphorus alone were sufficient to mineralize skeletal tissue independent of 1,25$(OH)_2$D. Parathyroid hormone (PTH) secretion was also modulated primarily by ambient serum calcium but the enlarged parathyroid glands which the mutants exhibited and abnormal epiphyseal growth plate development could only be normalized by the combination of calcium and 1,25$(OH)_2$D. These studies therefore defined specific roles for 1,25$(OH)_2$D, and calcium in modulating skeletal and calcium homeostasis. Endogenous 1,25$(OH)_2$D and the VDR were essential for baseline trabecular bone formation and exogenous 1,25$(OH)_2$D could also be shown to increase bone volume. Finally severe abnormalities in the regulation of the renin/ angiotensin sytem leading to hypertension and cardiac abnormalities, were observed in 1α(OH)ase$^{-/-}$ mice, which were correctible by 1,25$(OH)_2$D. These studies therefore define specific functions of vitamin D both in calcium homeostasis and skeletal health, and in important extra-skeletal functions consistent with its broad actions as a nuclear receptor activator.

S16-42 Symposia XVI

Mother child vitamin D deficiency

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Vitamin D deficiency is common worldwide, due to limited sun exposure. Pregnant women and newborns are particularly vulnerable. Maternal vitamin D deficiency puts the fetus and newborn at risk for neonatal hypovitaminosis D and hypocalcemia, rickets, respiratory infections, intrauterine growth retardation and future low bone mass. It is also implicated in enhanced risk of types 1 and 2 diabetes mellitus, autoimmunity and cancers. Since neither the amount of sun exposure in tropical countries, nor the diet fortification in temperate countries is effective in preventing vitamin D deficiency, pharmacological supplementation is unavoidable, at least in vulnerable groups. Supplementation regimens for pregnancy and lactation are under investigation. Doses of > 2000 to 4000 IU of vitamin D per day appear necessary to raise maternal and neonatal serum 25OHD to levels > 20-30 ng/ml.
In Lucknow and surrounding districts in north India (latitude 26.8 °N), the population prevalence of vitamin D deficiency among rural adolescent girls was 89%. Sun exposure correlated with serum 25 OHD. Skin pigment and modest tradition of clothing contributed to the existence of vitamin D deficiency despite many hours of outdoor activity. Girls were worse off than their brothers of similar age, and gender was a significant predictor of serum 25 OHD in a multivariate model. Among pregnant women, 84% of urban and 74% of rural subjects had vitamin D deficiency (serum 25OHD < 20 ng/ml), as did 96% of newborns. Oral vitamin D_3, 120,000 units each in the 5th and 7th month antenatal visits, resulted in median maternal 25OHD of 21 ng/ml at delivery. Infant anthropometry at birth, 3, 6 and 9 months were preserved in comparison to untreated controls.
To conclude, vitamin D deficiency is rampant in urban and rural Indian girls, pregnant women and their newborns. Preliminary studies of antenatal supplementation doses in second and third trimesters show encouraging results, but need further elucidation in future studies.

S16-43 Symposia XVI

Clinical features of FGF23, vitamin D, and bone

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The novel fibroblast growth factor, FGF23, plays an indispensable role in the development of the skeleton and the maintenance of skeletal health. This part of the session will: 1) review the role of FGF23 as a necessary mediator of mineral homeostasis; 2) demonstrate how changes in the availability or action of FGF23 results in disease; and 3) offer promise as to how manipulation of this system may hold potential for future therapeutic use. FGF23 is thought to be most abundantly expressed in osteocytes, and its major action is on regulating renal phosphate conservation and vitamin D metabolism. This novel system serves as a means by which the skeleton can communicate with kidney, thereby informing the organ directly mediating mineral homeostasis of the body's demand for skeletal mineral. Inappropriate activation of the bone-kidney FGF23 axis results in renal tubular phosphate losses and characteristic rachitic/osteomalacic bone diseases. Conversely, inhibition of the system or elimination of FGF23 results in dangerously excessive phosphate retention and a risk for inappropriate precipitation of mineral in soft-tissues. Clinical disorders illustrating this axis will be discussed. Finally, attention to vitamin D as a mediator of pediatric bone disease, and the basis for current supplementation requirements will be discussed.

S17-44 Symposia XVII

Transcriptional regulation of adrenal development

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In humans, the adrenal gland develops from the intermediate mesoderm at around 4 weeks gestation and undergoes a series of distinct morphological and functional changes throughout pre- and post-natal life. Two key transcriptional regulators of adrenal development are the nuclear receptors DAX-1 (*NR0B1*) and steroidogenic factor-1 (SF-1, *NR5A1*, Ad4BP). Mutations or deletions of DAX-1 result in X-linked adrenal hypoplasia congenita (AHC). Boys with this condition typically present with salt-losing adrenal failure in early infancy or throughout childhood and show evidence of hypogonadotropic hypogonadism (HH) and impaired spermatogenesis in adolescence. DAX-1 mutations are a relatively frequent cause of adrenal hypoplasia in males, especially with a family history of X-linked adrenal failure or when HH is present. Alternative presentations of X-linked AHC include isolated mineralocorticoid insufficiency, premature sexual maturation, adrenal hypoplasia in girls with skewed X-inactivation and adult-onset adrenal failure/HH. Despite this clinical insight, the molecular etiology of X-linked AHC remains poorly understood as DAX-1 paradoxically represses transcription in several assay systems. However, recent data have also shown transcriptional activation by DAX-1, and dysregulated adrenal stem cell development may also be mechanistically important in the etiology of this condition. The related nuclear receptor SF-1 regulates transcription of many key target genes involved in adrenal development and function. Sf-1 knockout mice have adrenal and gonadal agenesis and a small number of patients with adrenal failure due to SF-1 mutations have been described. More recent studies have shown that partial loss of SF-1 function more frequently results in 46,XY DSD or primary ovarian insufficiency. Adrenal reserve has been shown to be normal in these patients, although long-term follow up studies will be required. Other transcription factors implicated in adrenal development from studies in mice include Wt1, FoxD2, Gli3, Pbx1 and Cited2. Although mice with targeted deletions of *Pbx1 or Cited2* have adrenal agenesis, we have been unable to identify significant changes in these factors in humans with adrenal hypoplasia. The search continues, therefore, for new transcriptional regulators and networks that could account for adrenal hypoplasia where the underlying cause is currently unknown.

S17-45 Symposia XVII

P450 oxidoreductase deficiency

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P450 oxidoreductase (POR) is a 680 amino acid protein that transfers electrons from NADPH to all microsomal (type 2) cytochrome P450 enzymes. The human genome encodes 50 distinct type 2 P450s, including most of the hepatic enzymes that metabolize drugs and xenobiotics, and three steroidogenic enzymes: P450c17 (17α-hydrodxylase/17,20 lyase), P450c21 (21-hydroxylase), and P450aro (aromatase). Disruption of the POR gene in mice is lethal early in embryonic development, hence the discovery of autosomal recessive human POR mutations was surprising (Nat Genet 36:228, 2004). Severely affected infants of both sexes have ambiguous genitalia due to their steroidogenic disorder: the males are under-virilized because of defective 17,20 lyase activity of P450c17, and the females are partially virilized because of defective aromatase activity and because of the diversion of 17OHP to dihydrotestosterone via the "backdoor pathway" to androgens that bypasses DHEA, androstenedione and testosterone. Severely affected infants also have the Antley-Bixler skeletal malformation syndrome (ABS), characterized by craniosynostosis and radio-ulnar or radio-humeral synostosis, plus other, lesser skeletal defects. ABS may also be caused by autosomal dominant mutations in fibroblast growth factor receptor 2 (FGFR2), but these patients have normal steroidogenesis and normal genitalia (Am J Hum Genet 76:729, 2005). Mild forms of POR deficiency have been described resembling isolated 17,20 lyase deficiency and in otherwise asymptomatic adults with infertility. The effect of a specific POR mutation will vary with the P450 to which it is donating electrons: the loss of one enzymatic activity may not predict the loss of a different POR-dependent activity (Pharmacogenet Genomics 18:569, 2008). The incidence of disease-causing POR mutations is not known, but the sequence variant A503V is found on 28% of all human alleles, varying from 19% among African-Americans to 37% among Chinese-Americans (PNAS 105:1733, 2008). The activity of this common allele is reduced with P450c17 and with hepatic CYP3A4 (which metabolizes about 40% of clinically-used drugs), but has normal activity with P450c21 (JCEM 93:2913, 2008) and with hepatic CYP1A2 and CYP2C19. Thus the common A503V variant of POR may contribute to human variations in fertility and drug metabolism.

S17-46 Symposia XVII

Clinical and molecular-genetic spectrum of lipoid CAH and noncalssical lipoid CAH

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Lipoid congenital adrenal hyperplasia (CAH) is the most severe form of CAH. Lipoid CAH is caused by the defect in either the steroidogenic acute regulatory (StAR) protein or the P450scc. Because fetal testis is affected, 46, XY genetic male are born with female or severe undervirilized genitalia. Recently, 46, XY males with normal genitalia and late onset adrenal insufficiency have been reported. These forms are defined as nonclassic lipoid CAH.
We present new patients with nonclassic lipoid CAH, caused by mutations of the StAR gene. The first Japanese patient with normal male genitalia showed adrenal failure at 9 months of age. He is now 15 years of age, and have developed spontaneous puberty (his testicular volume, 18 ml and genital stage Tanner IV). This patient had R188C and Q258X of the StAR gene (Katsumata and Nakai et al. manuscript in preparation). The other two Japanese patients were siblings (30 years-old male and 36 years-old male).These two patients had Q258X and R272C mutations of the StAR gene. Semen analysis of the elder brother showed normal sperm formation (Shibata et al. manuscript in preparation). This is the first report of normal pubertal development and sperm formation in nonclassic lipoid CAH.

S17-47 Symposia XVII

Molecular mechanism of adrenal tumorigenesis

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Cushing syndrome (CS) is caused by adrenocortical tumors (ACTs). Until recently, most textbooks, as well as reviews on the subject would venture little beyond the common adrenocortical adenoma (ADA) and the rare carcinoma (ACA). At most, they would mention primary pigmented nodular adrenocortical disease (PPNAD) and corticotropin (ACTH)-independent macronodular adrenocortical hyperplasia (AIMAH) or massive macronodular adrenocortical disease (MMAD), as two rare forms of CS. We have identified no less than 6 types of bilateral adrenocortical hyperplasias (BAH). They are divided into two groups, macro- and micro-nodular hyperplasias on the basis of the size of the associated nodules. In macronodular disorders, the greatest diameter of each nodule exceeds 1 cm; in the micronodular group nodules are less than 1 cm. Although nodules less than 1cm can occur in macronodular disease (especially the form associated with McCune-Albright syndrome), and single large tumors may be encountered in PPNAD (especially in older patients), the size criterion has biologic relevance, as we rarely see a continuum in the same subject: most patients are either macro- or micro-nodular. There are two additional basic characteristics that we use in this classification of BAHs: 1) the status, hyperplasia or atrophy, of the surrounding cortex and 2) the presence of pigment within the lesion or the surrounding cortex. Hyperplasia or atrophy of the "normal" cortex, the internodular cortex around the lesions in bilateral diseases, can assist in subgrouping both macronodular and micronodular forms of BAH. Pigment in adrenocortical lesions is most often lipofuscin. The molecular defects causing most BAHs are linked to the cAMP-signaling pathway. This is an exciting time in the study of ADTs: Not only a number of new disorders have been described, but we also now know that most of the benign adrenocortical lesions associated with CS are linked in one way or another to cAMP-signaling dysregulation. We also know that cancer forms in the context of p53 mutations and/or IGF-II upregulation. This new knowledge has yet to lead to new therapies: it is our hope that pharmacological therapy of CS by molecular targeting will replace adrenalectomy for benign lesions and lead to better outcomes in cancer. Finally, large genome-wide association and prospective studies will clarify the role of certain genetic variants in the formation of "incidentalomas" in the general population.

S18-48 Symposia XVIII

Genetic causes of hypospadias

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Hypospadias can be caused by single gene disorders, epigenetic disorders, and multifactorial disorders. Here, I will primarily focus on *MAMLD1* that has been shown to be a causative gene for hypospadias.
We identified three different nonsense mutations and a splice acceptor site mutation of *MAMLD1* in patients with hypospadias. These patients had apparently normal testicular function until early childhood, but showed compromised testosterone production and testicular microlithiasis from ~7 years of age.
The murine homolog was specifically expressed in fetal Sertoli and Leydig cells around the critical period for sex development. We also showed that MAMLD1 was controlled by SF1 and transactivated the non-canonical Notch target gene Hes3 in nuclear bodies. Furthermore, transient knockdown experiments using siRNA showed significantly reduced testosterone production and CYP17A1 activity in murine Leydig tumor cells.
We also constructed knockout mice for Mamld1. The male knockout mice had normal reproductive function, although testosterone production was reduced. However, they showed postnatal obesity and metabolic syndrome with exaggerated food intake. Mamld1 was expressed in the hypothalamus.
These findings suggest that *MAMLD1/Mamld1* is involved in reproduction and energy metabolism, and exert a major effect on reproduction in humans and on energy metabolism in mice.

S18-49 Symposia XVIII

Hormones and genes in sexual development

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Disorders of sex development (DSD) encompass a very large spectrum of phenotypes, from minor malformations of the genitalia (hypospadias, cryptorchidism, hypertrophy of the clitoris) to sexual ambiguity. Taken altogether, these anomalies have an estimated frequency of 0.5% to 1%. Moreover, DSD have a major impact on quality of life. Recently, the debate about the management of DSD patients has intensified over issues of gender assignment and the indication for early genital surgery. Yet the scientific data on patient outcome have remained poor. The main obstacles to the optimal management of these patients have been a combination of lack of controlled outcome data and the lack of understanding of their pathophysiology, which prevents precise diagnostic categorization of patients. Despite much progress in the past 15 years, the molecular mechanisms underlying mammalian sex determination, are still far from understood, and the molecular basis of sex reversal in the majority of XY patients—about 75%—cannot yet be explained.
We will review the hormones and genes known to be involved in sexual development, as well as strategies to identify novel genes involved in sex development, from animal models to human genetic screens. We will also illuminate how this knowledge is translated to the bedside by providing diagnostic strategies for patients affected with DSD.

S18-50 Symposia XVIII

Long term follow-up of patients with DSDs

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In times when molecular medicine now drives most academic minds, informations regarding the adult life of paediatric patients are scarce. This is particularly true when it comes to sexual matters, due to patients' modesty. From 1999 to 2009, we investigated prospectively a cohort of patients who were treated for various DSDs. Our approach was based on contacting the whole set of patients recalled from 1964-1995 registries through mail or phone and asking them for participation to our evaluation, to evaluate the consequences of doctors' or parents' decisions regarding medical or surgical procedures during infancy. The cohort included 83 patients with 21-hydroxylase deficiency, 15 with PAIS, 6 with CAIS, 2 with 5alpha-reductase deficiency, 48 French ambiguous 45,X/46,XY DSDs, 5 patients with 17-ketoreductase deficiency. 111/159 patients agreed to participate and we were not able to analyze the reasons for the others' refusal. Patients had anatomical examination of their genitalia, and filled specific questionnaire evaluating their sexual behavior and reproduction. We reported the collected data in two separate publications in 2006 and 2007. Our main findings were that we grossly underestimated the difficulties that most patients have to face after reaching adulthood, among which this abstract will only touch a few examples. Most of them have not seen any specialist since their pediatric age. As an example, among 14 women born with Prader IV or V, 6 only had an active sexual life. 8/35 with Prader 1-5 CAH had children. Among 15 patients with 46,XY PAIS with AR mutations, mean penile length was 40 mm. None reported any penile activity with a hetero or homosexual partner. Among 48 patients with 45,X/46,XY mixed gonadal dysgenesis, 19 were reared as males, and 29 as girls. Puberty was initially spontaneous in all boys, but a testosterone treatment had to be added in 11. Mean final height was 156 cm in boys and 154 cm in girls. It became clear to us that many of our initial medical or surgical decisions were and may still be taken regardless of a precise knowledge of their sexual activity, reproduction performances, and self sexual esteem in the long term. Despite continuous improvements in surgical techniques, many questions regarding sexual function in both sexes remained to be answered. We identified potential areas for improvement, notably during late childhood, puberty and early adulthood when medical and psychological support may be needed the most.

S19-51 Symposia XIX

Skeletal development in children: Beyond getting longer, denser, heavier

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The growing bone faces the difficult task of remaining stable while at the same time being challenged by increasing length and increasing loads. Bone can only withstand these loads if its axis is aligned with the direction of the largest forces to which it is exposed. To this end, a feedback mechanism must exist in the growth plates which ensures that bone growth proceeds in the direction of the predominant mechanical forces. Although the actions of this mechanism are obvious in everyday clinical practice, mechanistic insights are scarce. Apart from controlling growth plate activity, the growing bone adapts to increasing loads by enlarging its circumference. This occurs through periosteal apposition, which is the responsibility of periosteal osteoblasts. According to mechanostat theory, periosteal apposition is regulated by mechanical requirements. An alternative model, called sizostat hypothesis, maintains that a master gene or set of genes regulate bone growth in width to reach a preprogrammed size, independent of mechanical requirements. The virtues of these two hypotheses have been the subject of much discussion, but experimental data are few and far between. Future research will have to address the question how periosteal bone cells manage to integrate mechanical, hormonal and other input to shape bones that are as strong as they need to be.

S19-52 Symposia XIX

Manipulation of anabolic and catabolic mechanisms in bone repair (translational)

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The emergence of effective pharmaceutical treatments for osteoporosis and metabolic bone disease have stimulated active efforts to investigate the effects of similar compounds in bone repair. As well as questions relating to the safety of osteoporosis treatments in patients sustaining osteoporotic fracture, optimisation and combinations of these therapies are being explored as an adjunct to fracture healing. Many of these measures may be useful to Children undergoing reconstructive procedures such as limb lengthening.
Riggs and Parfitt have suggested classifying osteoporosis drugs according to their principle action, ie anabolic or anti-catabolic. It is similarly useful to expand on this concept and think of bone repair as a system of coordinated anabolic and catabolic responses. The anabolic response can be further divided into non-specific anabolism (wound repair) and specific (bone) anabolism. One must also remember that agents can affect both anabolic and catabolic processes, and the relative effects are situation and time dependent.
In this manner pharmaceutical agents can be classified according to their effects on the various parts of the process. For example, PTH is a very specific anabolic, ie it acts on committed and mature bone cells. PTH has no known effect on non-specific anabolism (ie wound repair phenomena of cell recruitment and angiogenesis). PTH also indirectly increases catabolism. Bisphosphonates are anti-catabolic but eventually have indirect effects which reduce bone formation via remodelling. At high doses at least experimentally bisphosphonates can modulate effects on non-specific anabolism such as angiogenesis. This classification system can be applied to novel therapies which are emerging such as RANKL or SOST inhibition such that the likely effective scenarios for their application in bone repair can be deduced and then tested.
Clinical data are emerging on the effects of pharmaceutical agents on bone repair, but these data are currently quite limited.

S20-53 Symposia XX

The consensus conference on insulin resistance in children: definition, measurement, risk assessment, treatment and prevention

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Insulin resistance (IR) in adults has been recognized for decades as a cardinal feature in the development of type 2 diabetes mellitus and as a strongly associated factor in the pathogenesis of cardiovascular risk. It has also become clear, based on substantial evidence from pediatric studies that IR is significantly related to obesity and levels of cardiovascular and metabolic risk factors in children and adolescents. In addition, there are some unique features for IR in childhood, with relation to puberty, children born small for gestational age, prematurity, some developmental syndromes and treatment with glucocorticoids or growth hormone. Screening for insulin resistance requires information about prevalence, potential adverse effects on child health and treatment; and sensitivity, specificity and cost of the tests used. It is generally agreed that it is not practical to use the hyperinsulinemic euglycemic clamp – the gold standard -, FSIVGTT or OGTT to screen large groups of children. Fasting insulin measurement is simple, safe and relatively inexpensive, but there is significant variability among assays and among ethnic groups, genders and pubertal stages, and a lack of sensitivity and specificity in diagnosing IR. The prevalence of IR in children is unknown, but there are high-risk populations, e.g. polycystic ovary disease and obesity. Because of the strong tracking effect of obesity and the cardiovascular risk factors from childhood into adulthood, it is now acknowledged that caregivers for children can play an important role in the prevention of type 2 diabetes and cardiovascular diseases later in life. However, there is a lack of clarity as to what IR means in childhood, and there are no data on treatment of isolated IR in children. Caregivers would benefit from a better understanding of how it is best assessed, in what clinical disorders it occurs, what are its consequences and whether it can be treated or prevented. To explore these issues, the European Society for Pediatric Endocrinology (ESPE), the Lawson Wilkins Pediatric Endocrine Society (LWPES), the International Society for Pediatric and Adolescent Diabetes (ISPAD), the Asia Pacific Paediatric Endocrine Society (APPES), the Australasia Paediatric Endocrine Group (APEG), the Sociedad Latino-Americana de Endocrinologia Pediatrica (SLEP), and the Japanese Society for Pediatric Endocrinology (JSPE) convened a panel of expert physicians for a consensus conference on IR in children.

Club Sessions

CL1-01 Bone/Growth Plate/Turner Club

Bone development, growth and structure

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Turner syndrome has effects on both bone length and bone strength. The underlying mechanisms include both direct effects of monosomy X on the skeleton and indirect effects, mediated particularly by estrogen deficiency. To understand these effects, it is necessary first to understand how the chondrocyte, osteoblast, and osteoclast cause bone growth and remodeling, how these processes change with age, and how they are modulated by estrogen. Bone elongation requires growth plate chondrocyte proliferation and hypertrophy. The resulting new cartilage is then remodeled by osteoclasts and osteoblasts into trabecular bone. The trabeculae formed beneath the central growth plate are subsequently resorbed, allowing expansion of the medullary cavity, whereas the trabeculae formed beneath the peripheral growth plate coalesce to elongate the cortex. The cortex also enlarges radially due to periosteal bone formation, at times accompanied by endosteal bone resorption. Bone density depends on the balance between osteoblastic bone formation and osteoclastic bone resorption during the processes of skeletal growth and remodeling. Perturbations in bone mineral acquisition during childhood often have only transient effects because bone mineral density is governed by a dynamic homeostatic system that returns the density to a set point determined by genetic and current environmental conditions.

During childhood, major developmental changes occur in the skeletal system. The growth plate undergoes programmed senescence, causing growth to slow. This developmental program appears to result from gradual exhaustion of the proliferative capacity of the growth plate chondrocytes. Epiphyseal fusion is triggered when growth plate chondrocytes have exhausted their proliferative capacity, and thus fusion is a consequence, not a cause, of growth cessation. Estrogen accelerates growth plate senescence and thus secondarily hastens epiphyseal fusion. Estrogen affects not only bone length but also bone density such that estrogen deficiency causes a high turnover state with increased bone resorption.

CL1-02 Bone/Growth Plate/Turner Club

Wnt/β-catenin, FoxOs, and oxidative stress in the pathogenesis of metabolic disorders

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In contrast to the conventional thinking that loss of estrogens at menopause is the seminal mechanism of osteoporosis, bone loss begins as early as the early part of the third decade in both women and men; substantial trabecular bone loss occurs in sex steroid sufficient young adult women and men; and after the first few years of accelerated bone loss in postmenopausal women, bone mass and strength decline in both sexes at the same rate. Consistent with these clinical observations, mechanistic studies in mice show that aging, and specifically increased oxidative stress, rather than age-associated failure of other organs, is a fundamental pathogenetic mechanism of age-related bone loss and strength, leading to, among other changes, a decrease in osteoblast lifespan and bone formation. Loss of estrogens or androgens accelerates the effects of aging on bone by decreasing defense against oxidative stress. Oxygen radical-induced activation of the FoxO family of transcription factors defends against such an increase by up regulating free radical scavenging and DNA repair enzymes, thereby representing an indispensable homeostatic mechanism for skeletal health. Consistent with this, loss or gain of function of FoxOs decreases and increases bone mass respectively. Albeit, excessive or protracted FoxO activation diverts ß-catenin away from Wnt signaling, leading to a decreased osteoblastogenesis. Excessive FoxO activation may also lead to a decrease in bone strength, independently of bone mass, by compromising the bone vasculature and the hydration of the aging skeleton. Fascinatingly, attenuation of Wnt-mediated transcription, resulting from an autosomal dominant missense mutation in LRP6 or LRP5 —co-receptors for the Wnt-signaling pathway has been linked recently genetically not only to premature osteoporosis, but also to coronary artery disease as well as several features of the metabolic syndrome including hyperlipidemia, hypertension, and diabetes, but not obesity. Hence, antagonism of Wnt-signaling by oxidative stress—induced activation of FoxOs with increasing age may be a common molecular mechanism contributing to the development not only of involutional osteoporosis, but several pathologies like atherosclerosis, insulin resistance, and hyperlipidemia—all of which become more prevalent with advancing age (Manolagas and Almeida, Mol Endocrinol. 2007 21:2605-14).

CL1-03 Bone/Growth Plate/Turner Club

SHOX gene. The impact on cartilage and bone growth

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The short stature homeobox-containing gene on the sex chromosomes, *SHOX*, encodes a homeodomain transcription factor responsible for a significant proportion of long-bone growth. Patients with mutations or deletions of SHOX (or its enhancers), including those with Turner syndrome who are haploinsufficient for *SHOX*, have variable degrees of growth impairment, with or without a spectrum of skeletal anomalies consistent with dyschondrosteosis. Also, up to 10% of patients diagnosed as idiopathic short stature have a SHOX defect.

To shed light on the mechanism underlying SHOX-related disorders of bone formation, overexpression of *SHOX* in primary oral fibroblasts and primary chondrocytes was carried out. A comparative functional analysis of mutant and wildtype SHOX indicated that SHOX expression leads to cell cycle arrest and apoptosis suggesting a role of SHOX in hypertrophic differentiation. These events are associated with alteration in expression of several genes including pRB, p53 and the cyclin kinase inhibitors p21 and p27.

SHOX is a transcription factor and one of its first direct target genes represents NPPB encoding the natriuretic peptide BNP. Binding of SHOX to the NppB promoter was demonstrated *in vivo* by chromatin fixation and immunoprecipitation and lack of promoter activation was shown in two SHOX mutants from patients with Leri-Weill syndrome. Thus, SHOX and BNP act on the same pathway in bone development. Further target genes of SHOX are currently under investigation. Its closely related paralogous gene, *SHOX2,* also functions as

a key regulator of endochondral ossification and regulates *Runx2,* an important regulator of chondrogenesis.

CL1-04 Bone/Growth Plate/Turner Club

Bone status in Turner syndrome. Evaluation. Effect of GH and oestrogens

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Turner Syndrome (TS) is often listed as a cause of secondary osteoporosis in children and adolescents. However the International Society for Clinical Densitometry (ISCD) published a position statement in 2007 which indicated that the definition of osteoporosis in this age group requires the presence of a clinically significant fracture history and a bone mineral content or density Z score that is less than or equal to -2.0, adjusted for age, gender and body size, as appropriate.
There have been numerous studies that have assessed bone density in children and adolescents with TS using a variety of modalities. Many of the early studies that utilised dual-energy X-ray absorptiometry (DXA) of the lumbar spine did not correct for short stature leading to an overdiagnosis of osteoporosis. Subsequent studies that have adjusted for body size have not identified a significant problem in the paediatric age group. However some studies that have examined the peripheral skeleton using modalities such as pQCT have suggested a selective deficit in cortical bone density and thickness. The impact of growth hormone (GH) treatment on bone status has been examined with some conflicting results although the majority do not suggest an additional benefit from GH treatment. Oestrogen treatment has been shown to be important in optimising bone mineral accretion with the timing of its introduction being felt to be important.
Although there is evidence to indicate an increased fracture risk in adult women with TS which may relate to the timing of oestrogen replacement there is little current evidence for this in children and adolescents with TS. In view of the lack of evidence for an increased fracture risk and low bone mineral density in paediatric subjects with TS it is doubtful that it should continue to be listed as a cause of osteoporosis in children. Routine assessment of bone density during childhood and adolescence for girls with TS is not indicated although assessment at the time of transition to adult care is recommended.

CL2-05 DSD Working Group

The European DSD registry

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Research & audit are vital for the management of disorders of sex development (DSD) and associated genital anomalies. However, the information that is required to investigate issues such as aetiology, management and long-term outcome require good clinical ascertainment with the least amount of selection bias. Furthermore, a number of questions that need to be addressed can only be answered by multicentre studies. The Consensus Workshop jointly hosted by ESPE and LWPES on the diagnosis and management of DSD stressed the need for the creation and maintenance of a database in centres of expertise. Such databases do exist in many regional centres, but they lack international uniformity and an ability to cross-talk. With the help of an ESPE Research Unit grant, Drs Ahmed (Glasgow, UK), Bertelloni (Pisa, Italy), Drop (Rotterdam, Netherlands), Hiort (Luebeck, Germany) & Hughes (Cambridge, UK) collaborated with each other and with Professor Richard Sinnott at the National E-Science Centre in Glasgow to develop a web-based register which uses a core dataset based on the new DSD nomenclature. This web-based register has now become an integral part of a Virtual Research Environment (VRE) of EuroDSD, a programme of EU funded research, launched in spring 2008. The utility of the VRE to include an extensive portfolio of targeted services/ tools to support many aspects of DSD research including capabilities to link clinical data resources on a case-by-case basis as well as providing a variety of DSD-targeted bioinformatics applications and services to support scientific research into DSD is currently being explored. The cornerstone of the proposed VRE model is based upon site autonomy. In this, each clinical site is solely responsible for deciding what data sets it can share, with which partner sites and in what context. Given this, the design of the VRE is driven by security, incorporating both the needs of the clinical community and ethical oversight required on information governance. It is hoped that in the long-term, tools such as these would allow the development of international research strategies for the diagnosis of previously unsolved cases of DSD as well as assessment of long-term management.

CL2-06 DSD Working Group

Application of an ESPE DSD e.learning webportal

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The aim of the web portal is to provide entrance to an interactive learning environment for an up-to-date program on DSD including normal development, pathophysiological mechanisms, diagnostic and therapeutic interventions, psychological counseling, outcome and whenever applicable, implementation of study results and provision of guidelines. Target groups are medical students, residents, fellows, specialists, consultants, teachers around the world The portal is developed in the English language and two levels of learning are foreseen: *a. basal* (medical student): focus is the understanding of the normal development and its patho-physiology with its clinical and social implications; *b. advanced* (postdoc etc): the fellow is additionally invited to analyze and diagnose disorders, to solve problems related to these disorders, to appraise scientific evidence and to communicate with professionals and patients (parents). A forum functionality enables the users to post comments and remarks and discuss certain topics. The forum will be used for specific discussions on a case or study results or knowledge sharing.
Following an intensive phase of construction the webportal has gone live in October 2008. Currently authors have been invited to contribute content on DSD to the portal. The main emphasis is on data entry, i.e text for chapters and case descriptions. The development of the functionality for scoring of questions and the development of formative assessment of competencies of students and portal users is a separate entity requiring specific expertise. The educational background and objectives have been summarized (1).
Editorial working groups have been formed, consisting of a group of medical editorial contributors.(see below). Further extension of these groups with experts from several pediatric endocrinological societies is foreseen. As author and/or editor they will be responsible for the content of the program. A technical staff is involved in the construction, lay out and function of the web portal.
(1) Development of an ESPE e-learning portal: educational considerations K. Grijpink-van den Biggelaar, S.L.S. Drop, L. Schuwirth. Horm Research (2009), in press.

CL2-07 DSD Working Group

Technological advances in the genetic analysis of DSD

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It is now 20 years since the identification of SRY as a primary testis-determining gene and the molecular characterization of many of the key enzymes and receptors involved in androgen synthesis and action. Although significant progress has been made since then in identifying other components involved in sex development, we are still unable to find an underlying genetic cause in many individuals with these conditions (e.g. gonadal dysgenesis, "PAIS"). Efforts to identify specific genetic causes of DSD are impeded by several factors such as phenotypic variability, the lack of specific biochemical markers and the high frequency of "unusual" genetic mechanisms such as sex-limited dominant inheritance or de-novo events. Indeed, as DSD is usually associated with infertility, classic large pedigrees amenable to linkage or mapping studies are relatively rare. Traditionally, a "candidate gene" approach has been used to identify many cases of DSD, which can be time consuming and costly. However, the explosion in new nano-technologies that we are witnessing means that we could potentially identify the genetic causes of DSD on a high-throughput scale, and can address some "newer" genetic mechanisms that might be important in influencing disease phenotype (eg digenic or oligogenic inheritance, gene dosage, epigenetic regulation). For example, the development of a DSD

"resequencing chip" could allow the rapid analysis of many of the known or candidate genes for DSD simultaneously in one individual, whereas "next generation sequencing" technologies allow sequencing of several thousand PCR products or large areas of the genome in a matter of hours. CGH and SNP-based technologies are allowing detection of copy number variants (CNV) in the genome with an increasing unprecedented degree of resolution so that small deletions or duplications can be identified, which are likely to be critical during sex development. Finally, tissue-based studies of DNA methylation, exon array analysis and proteomics could uncover epigenetic influences, altered splicing and post-translational modification events, respectively, which might influence sex development or act as "fingerprints" for specific conditions. The challenges will be cost, scale, organization/collaboration, bioinformatics, bioethics, predicting in vivo functional effects, and integrating this wealth of information into an appropriate systems model relevant to DSD. Nevertheless, potentially exciting advances lie ahead.

CL2-08 DSD Working Group

The EuroDSD project and consortium

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EuroDSD is an acronym for a collaborative project of clinical and basic scientists working in the field of disorders of sex development (DSD). EuroDSD is currently supported by the European Commission under the 7th European Framework Programme (FP7) and receives funding from May 2008 until April 2011 within the topic of the natural course and pathophysiology of rare diseases. EuroDSD has 13 partners from 6 European countries; combining 6 clinical centres with 6 research institutions plus a private administrative partner. Research in DSD is desperately needed to provide a basis for diagnostic pathways, medical decision making, and distinct treatment advice.
EuroDSD aims at linking a European patient-based data collection and analysis tool with research on development of novel diagnostic strategies to identify currently unknown causes of DSD. It will design and validate a DSD GeneChip to offer the opportunity for rapid genetic analysis in DSD. A selected group of patients will be characterized by genome analysis with CGH array technology. EuroDSD will study steroid analysis by GC/MS and LC/MS/MS in urine and plasma to identify unique steroid metabolomes that will be a "fingerprint" to diagnosis. A special focus is on elucidation of androgen action as a major impact of genital differentiation. Selected mutations of the androgen receptor are investigated to correlate in-vitro function with long-term post-pubertal outcome. Also, the molecular functions of the androgen receptor during embryology are elucidated due to identification of relevant co-regulators of androgen action. Sex-specific methylation target genes will be identified in patients with androgen insensitivity syndrome. The EuroDSD project is complemented by the elaboration of a DSD e-learning webportal, which is targeted within the ESPE e-learning activities.
The EuroDSD project offers a unique networking opportunity for European-based research on rare disorders of sex development, and we hope that this consortium can lead to a sustainable association of clinicians and basic scientists propagating research in this field.

CL2-09 DSD Working Group

The rights of the affected infant

Garry L Warne

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An infant who has DSD, like all infants, is vulnerable, dependent and in need of protection.This paper will consider specific ways in which DSD imposes additional vulnerability, on whom the infant is dependent, and from what he or she needs to be protected.
Infants who have DSD share all of the rights of children enshrined in the U.N. Declaration on the Rights of Children (1959) and the Convention of the Rights of the Child (1989). An infant is more helpless than an older child and the infant's fate is inextricably entwined with the situation in which the parents find themselves. For parents of children with DSD, this can be very distressing. Infants have a right to a secure attachment to a caregiver, but this can be threatened if the mental health of the caregiver is unstable. The infant's temperament, which can be affected by prenatal stressors and genetic factors, as well as by maternal mental health factors, may also affect bonding.
An infant with DSD has a right to be treated with respect and dignity - as a baby, not a case; its anatomical differences shielded from public gaze; given a temporary name if the parents want to use one; followed up by support staff who have been well informed about the special needs of mother and baby.
The infant has the right to parents who have been well informed about the condition, the possible outcomes, the support that is available and the treatment options. Every infant with a DSD has the right to be given a name and a sex.
The infant has a right to protection: from ill-conceived advice to the parents that adds to their distress and indirectly affects their ability to provide care; from decisions about surgery that for many reasons might not be in the best interests of the child; from infanticide (known to occur in some countries); from the effects on development of the underlying genetic or hormonal condition, if prenatal treatment is available; from the negative effects of maternal mental illness; from being devalued and treated as different.
The infant with DSD has the right to be thought of as a person who will grow up to be a child, a teenager and an adult and to have access to education, the opportunity to be fully integrated in the community and to medical care that will promote the best possible physical, emotional and spiritual happiness and wellbeing, across the lifespan.

CL2-10 DSD Working Group

Long-term outcome and quality of life in children and young adults

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Children and adolescents with disorders of sex development (DSD) are exposed to specific stressors like surgery, lack of virilization in boys and overvirilization in girls. Entering puberty, the adolescents become aware of the developmental discrepancies in comparison with their peers. There is a lack of empirical data for children and adolescents despite supposed age-specific demands. Within the clinical evaluation study of the German network DSD/Intersexuality we investigated the impact of the DSD on the psychosocial well-being. Methods: We assessed the psychosocial aspects of children and adolescents between four and 16 years between 2005 and 2007 in Germany, Austria and Switzerland. Health-related quality of life (HrQoL) and mental health were measured with generic instruments. Additionally we used disease-specific questionnaires. Results: 166 children (4-12 years) and 66 adolescents (12-16 years) with DSD and their parents participated, 170 raised as girls, 62 raised as boys. For statistical group comparisons we used four diagnostic subgroups: girls with 46,XX & overvirilization/CAH (N=95), girls with 46,XY & partial androgen effects (N= 55), girls with 46,XY without androgen effects (N= 20), boys with lack of virilization (N= 62). Results from the HrQoL and the mental health did not reveal differences between the four diagnostic groups. Children's HrQoL is impaired concerning emotional well-being in comparison with norm data. Adolescent's HrQoL is not impaired in comparison with the norm group. We did not identify high risks for mental health problems. However, outcomes related to adolescent developmental tasks like sexual activities demonstrated impaired participation, especially girls with DSD report fewer activities. Adolescents with visible differences deal less open with their condition and report more shame and stigmatization. Discussion: The impairments concerning HrQoL in children show quite plainly the impact of DSD on the emotional well-being. The fact that mental health is not affected in pre-pubertal children can be explained with social support and personal resilience. But the daily routine of children seems to be influenced by the DSD. While we did not find gross impairments for adolescents, but being different is a source of fear and insecurity. In future, interdisciplinary health care teams have to focus this pressure of conformance and to openly discuss it with the children & adolescent in context of treatment decisions.

CL2-11 DSD Working Group

Sexual function in adults with a past history of DSD

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Newborns with gender-atypical external genitalia may face long-term adverse social, psychological, and functional consequences, including uncertainty regarding the proper gender assignment; gender-related stigma within the family, in the peer group, and with romantic erotic partners; marked self-consciousness and stigma anticipation leading to impairment of self-esteem and to avoidance of romantic/erotic relationships; and difficulties in performing peno-vaginal intercourse and achieving insemination or conception if the gonads are fertile. These consequences are mostly documented in case reports or described as clinical impressions.

Follow-up studies of corrective surgery show great variability in outcomes and patient satisfaction. Apart from purely medical outcome criteria (e.g., acute surgical complications, intra- or early-postsurgical nerve conductance, and later anatomic changes such as vaginal stenosis, clitoral atrophy, compromised urination, or irregular tissue growth during puberty), also diverse aspects of cosmesis, gender-confirming appearance, sexual functioning, and reproductive potential need to be evaluated.

Long-term psychosocial outcomes are characterized by small samples of often heterogeneous DSD conditions, few outcome variables, and a multitude of surgical techniques employed. Nevertheless, available data, most frequently on 46,XX CAH, permit a number of tentative conclusions. (1) Physicians rate aesthetic outcomes more positively than patients. (2) The gender-confirming goal of genital surgery is more easily met in female-assigned patients, but is not sufficient to prevent problems with gender identity, when marked behavioral masculinization is present. (3) Male-raised patients with marked behavioral masculinization tend to maintain a male gender identity in spite of small size and/or atypical appearance of their genitalia. (4) DSD patients as a group tend to be delayed in attaining psychosexual milestones, partly related to sexual avoidance. (5) Functional variability after surgery is high. (6) Assessment tools for routine clinical evaluation are needed.

The timing of genital surgery remains controversial. Recent consensus conferences recommend not to perform genital surgery between 12 months and adolescence (except for compelling medical indications). The majority of DSD patients surveyed favor early surgery. Vaginal dilatation should not be prescribed before adolescence.

CL3-12 Obesity Club

Genomewide association studies for adult obesity: Implications for children?

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Recent technological advances and the massive increases in scale and statistical rigour of genomewide association (GWA) studies has allowed the robust demonstration of common genetic variants for adult BMI and obesity risk that are consistently replicable in other populations. The first such common genetic variation shown to be associated with adult BMI was in the *FTO* gene region, published in 2007 by Frayling et al. This was closely followed by variation downstream of *MC4R* in 2008 by Loos et al. Several further loci, in or near *TMEM18, GNPDA2*, *KCTD15, NEGR1, BDNF, ETV5, MTCH2 and SH2B1*, were reported in 2009 to be adult obesity risk variants by studies from the GIANT international consortium and the deCODE Genetics group.

To date all the published GWA-BMI studies primarily focussed on the association with adult BMI. The initial reports of common variants in/near to *FTO and MC4R* also included demonstration of their relevance to childhood BMI and childhood obesity, and these have since been confirmed in other childhood studies. A further four of the six new variants for adult BMI identified by the GIANT consortium also showed association with childhood BMI and/or childhood obesity (*TMEM18, GNPDA2*, *KCTD15 and NEGR1*). However, the joint publication by deCODE Genetics did not include any childhood populations. Although reported to be associated with severe obesity, BMI and body fat in childhood, the effects for most of these obesity risk variants on growth and weight gain during infancy and childhood have not yet been established. We have recently analysed data from the population-based ALSPAC birth cohort in order to identify the effects of these adult obesity susceptibility loci on weight gain and growth during specific periods of early life. Our findings show that use of adult obesity genetic susceptibility variants discovered through large-scale GWA studies may help to us better characterise the early childhood phenotypic pathways to later obesity.

CL3-13 Obesity Club

How does allelic variation in *FTO* convey effects on body weight

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There are 2 recognized genes in the DNA interval implicated by the various association studies reported for this region of the genome: *FTO* was cloned from a mouse deletion (that does not include obesity as a resultant phenotype) and named "fused toe" because as associated phenotype. *FTO* (renamed "Fat mass- and obesity-associated locus") has been implicated in nucleic acid demethylation, is highly expressed in the arcuate nucleus of the hypothalamus, where it is reduced in mice by fasting; *FTM* (KIAA1005 or *RPGRIP1L* in human) whose transcription start site is located only 200 base pairs from the 5' end of *FTO* and about 61 kb from the 47-kb interval containing the BMI associations. and which is transcribed in the opposite direction from *FTO. FTM* is a structural component of the ciliary body, present on all cells, that plays a role in a wide range of cellular and organ functions. Elements of the cilium are mutant in Bardet-Biedl syndrome. In mice, fasting and environmental cooling, both of which lead to increased hunger, are associated with decreases in expression of both *FTO and FTM* in the hypothalamus. One SNP (rs8050136) in intron 1 of *FTO*, which is located near rs9939609 at the peak of the statistical association for adiposity, is also a binding site for a transcription factor, CUTL1. Interestingly, binding of CUTL1 to this site in human fibroblasts, reduces the expression of both *FTO and FTM*, consistent with their being under joint regulation *in vivo*. Both genes are plausible candidates to influence energy homeostasis, *FTO* possibly by virtue of its demethylase activity through which it might influence the expression of other genes; *FTM* by virtue of its role in the complex cell signaling pathways or structural development in which ciliary body participates. It is possible, of course, that both genes are involved in conveying the strong statistical association of this genetic interval with obesity. In fact, a GWAS is inherently biased towards the discovery of intervals containing several contributing genes.

CL3-14 Obesity Club

Imprinting and childhood obesity: The Prader-Willi syndrome paradigm

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Prader-Willi syndrome (PWS) is the most frequently diagnosed genetic cause of obesity and it was the first recognized human disorder related to genomic imprinting. It results from failure of expression of genes that are only expressed from the paternally inherited chromosome 15 by one of three main mechanisms: paternal deletion of the 15q11.2 region; maternal uniparental disomy of chromosome 15 or a defect in the imprinting process in 15q11.2.

The obesity in PWS typically begins between 2-4 years of age if the diet is not appropriately managed. Remarkably, as neonates there is an almost complete absence of an appetite drive. The appetite gradually increases in infancy and early childhood such that by about 8 years of age the individual with PWS has an insatiable appetite. Through careful longitudinal studies we have been able to discern 6 distinct nutritional phases in PWS. This makes PWS an ideal model system to dissect the various metabolic and hormonal components in appetite regulation and the development of obesity.

Free Communication

FC1-001 Growth

Imprinted genes that affect body size promote growth of embryonal malignancies

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Proliferation rates decline postnatally in many normal tissues causing somatic growth to slow. Some imprinted genes that affect body size, including *Mest, Plagl1*, *Peg3, and Igf2,* are expressed at high levels in multiple tissues of the mouse and rat embryo but are then downregulated in postnatal life, suggesting that these genes contribute to normal somatic growth deceleration. Embryonal cancers comprise cells that maintain embryonic characteristics and rapid rates of proliferation. We hypothesized that in embryonal cancer cells, abnormal persistent rapid proliferation occurs in part because of abnormal persistent high expression of growth-promoting imprinted genes.
Consistent with this hypothesis, analysis of existing microarray data showed elevated expression of *MEST, PLAGL1*, *PEG3, and IGF2* (~2-16 fold) in rhabdomyosarcoma and Wilms' tumor samples, xenografts, and cell lines compared to non-embryonal tumors of childhood. To confirm, we assessed mRNA expression by real-time PCR in 6 rhabdomyosarcoma cell lines and found increased expression of *MEST , PEG3, and IGF2*, compared to 10 non-embryonal cancer cell lines (P<0.05). We next suppressed expression of of *MEST, PLAGL1*, *PEG3, and IGF2* in the Rh30 rhabdomyosarcoma cell line using siRNA interference. Proliferation, assessed by tritiated thymidine incorporation, was inhibited by siRNA directed at *MEST, PEG3, PLAGL1, and IGF2*, compared to negative control siRNA (P<0.002). Knockdown of the target gene was confirmed with real-time PCR, and growth inhibition was confirmed with a second siRNA for each RNA target. We conclude that some imprinted genes that affect body size, including *MEST, PEG3, PLAGL1, and IGF2*, show high levels of expression in some embryonal tumors and contribute to growth of rhabdomyosarcoma cells. Because physiological downregulation of these genes contributes to physiological growth deceleration, persistent expression in these tumors may contribute to their persistent pathological proliferation, suggesting a link between the mechanisms that regulate normal childhood growth and growth of childhood cancers.

FC1-002 Growth

A genetic program limiting body size in mammals

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Cell proliferation is rapid in early life but decelerates with age, causing somatic growth to slow and eventually cease, thus setting a fundamental limit on adult body size. We hypothesized that growth deceleration results from a growth-limiting genetic program that occurs simultaneously in multiple tissues. Consistent with this hypothesis, we recently identified a postnatal genetic program that occurs coordinately in multiple organs in mice. In the current study, we found that this program is highly conserved in rats (by microarray) and that proliferation is the major function of the age-downregulated genes (by gene ontology analyses). We then selected 8 age-downregulated genes for further study (Mest, Plagl1, Peg3, Ezh2, Mdk, Gpc3, Mycn, Meis1), confirming temporal changes by real-time PCR (all $P < 0.01$). Knocking down expression of 7 of these 8 genes by siRNA transfection inhibited proliferation (all $P < 0.05$) in murine fetal hepatocytes, supporting the hypothesis that these genes are growth-promoting and therefore that declining expression in vivo contributes to growth deceleration. Using chromatin immunoprecipitation, we assessed histone modifications (Acetyl-H3, trimethyl-H3K4, and trimethyl-H3K27) in 3 genes (Peg3, Plagl1 and Mdk). We found significant declines in trimethyl-H3K4 with age for all 3 genes in multiple organs. Trimethyl-H3K4 is associated with transcriptional activation, suggesting that the observed declines in methylation coordinate the downregulation of multiple genes. To investigate whether this genetic program is driven by age (a biological clock) or by proliferation (a cell-cycle counter), we inhibited growth in newborn rats by inducing tryptophan deficiency for 4 wk. Microarray analysis revealed that the genetic program was slowed by growth inhibition ($P < 0.0001$). These findings, confirmed by real-time PCR for the 8 selected genes, suggest that the changes in gene expression are driven by a cell-cycle counter. Finally, microarray data comparison showed that the program of gene downregulation progresses more slowly in rats than mice ($P < 0.001$), suggesting that the program time course is evolutionarily modulated to adjust the duration of growth and hence adult body size. Taken together, our findings suggest that proliferation in early life causes epigenetic changes, which downregulate proliferation-stimulating genes, causing somatic growth to slow and eventually cease, thus imposing a fundamental limit on adult body size.

FC1-003 Growth

Heterozygous *OTX2* mutations are associated with variable pituitary hormone deficiency

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Objective: Recent studies have suggested that *OTX2* is involved in pituitary function and development, as well as in eye and brain development. To date, however, pituitary dysfunction has been reported only in four *OTX2* mutation positive patients. Thus, we performed mutation and functional analyses of *OTX2*, and genotype-phenotype correlation in *OTX2* mutation positive patients.

Subjects: We studied 89 Japanese patients consisting of 28 patients with ocular malformation (group 1) and 61 patients with various combined pituitary hormone deficiency (CPHD) but without eye abnormality (group 2).
Mutation analysis: We identified three *de novo* heterozygous frameshift mutations (p.S135fsX136 in case 1, p.K74fsX103 in case 2, and p.A72fsX86 in case 3), a heterozygous nonsense mutation (p.G188X in two unrelated cases 4 and 5), and a heterozygous 2.86 Mb deletion involving OTX2 (case 6) in group 1. No mutation was identified in group 2.
Clinical features: Cases 1–6 had anophthalmia or microphthalmia and variable degrees of developmental retardation. Cases 1, 2, 4, and 6 exhibited short stature. Pituitary function studies revealed isolated GH deficiency in cases 1, 2, and 6, and CPHD (severe GH, TSH, and PRL deficiencies and possible LH and FSH deficiencies) in case 4. Cases 3 and 5 had apparently normal pituitary function. Magnetic resonance imaging delineated an ectopic posterior lobe in cases 2 and 4 and pituitary gland hypoplasia in cases 4 and 6.
Functional studies: The wild-type, the p.S135fsX136 and the p.G188X OTX2 proteins localized to the nucleus and bound to the target sequences within the promoters of *IRBP* involved in eye development and *HESX1, POU1F1, and GNRH* relevant to pituitary development and function, whereas the p.K74fsX103 and p.A72fsX86 proteins lacking a part of homeodomain could not localize to the nucleus. Furthermore, while the wild-type OTX2 protein markedly transactivated the above promoters, such transactivation functions were significantly reduced for the mutant OTX2 proteins without a dominant negative effect.
Conclusions: The results indicate that heterozygous *OTX2* loss-of-function mutations result in variable pituitary hormone deficiencies and pituitary structure abnormalities with no correlation between genotype and phenotype. In addition, OTX2 mutations appear to be rare in patients with pituitary hormone deficiencies alone.

FC1-004 Growth

Humanin is a developmentally-regulated circulating factor whose levels are determined by IGFBP-3: establishment of normative ranges and initial pharmacokinetics

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Humanin (HN) is a mitochondrially-encoded, 24-aa peptide, neuronal survival factor. Unpublished data from our laboratory revealed potent insulin-sensitizing effects of humanin and its analogs. We previously showed that HN is a high affinity and specific IGFBP-3-binding partner. OBJECTIVE: To establish normative ranges and to determine the effects of circulating IGFBP-3 on the pharmacokinetics of HN in mice. HYPOTHESIS: we postulated that injection of HN into IGFBP-3 KO mice would result in lower amplitude and faster clearance when compared with wild-type (WT) littermates. METHODS: IGFBP3KO mice were generated on a C57B mixed background. We developed a novel ELISA for human and mouse HN that utilizes antibodies purified with protein A/G column chromatography and further purified with an HN-ligand affinity column with a sensitivity of 0.1 ng/ml. Plasma was collected from C57/BL6 mice of both genders at 4, 8, 16 and 32 weeks of age. Additionally, 11-week old male mice (WT and IGFBP3KO) were given intra-peritoneal injections of 2 mg/kg HNG (a potent analog of HN with an S14-G substitution and preserved affinity for IGFBP-3) and EDTA plasma was collected at multiple time points (0-180 minutes). RESULTS: Normative ranges for humanin in male and female mice were established and showed a rapid age-dependent decline. Baseline levels of HN were 3-fold lower in IGFBP3KO as compared with WT littermates (2-10 ng/ml); $P<0.05$. HN levels (ng/ml, mean+/-SD) in WT mice given intraperitoneal HNG show a peak HN level at 60 minutes post injection of 58+/-5, while IGFBP-3 KO mice show a peak HN level at 15 minutes post injection of 15+/-6 ($p<0.01$). The peak HN level in WT mice represented a 30-fold increase from baseline levels, while the peak level in IGFBP-3KO mice was significantly lower at a 5 fold difference from baseline, $P<0.005$. CONCLUSIONS: Humanin is developmentally regulated in a gender-specific manner. Circulating IGFBP-3 enhances endogenous HN levels as well as the duration, peak level, and rate of clearance of injected HNG. These findings may prove important in the development of humanin-related therapies of neurological and metabolic diseases by allowing the development of specifically targeted, more potent and longer lasting humanin-based therapeutics.

FC1-005 Growth

The primordial growth disorder 3-M syndrome connects ubiquitination to the cytoskeletal adaptor OBSL1

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3-M syndrome (MIM 273750) is an autosomal recessive primordial growth disorder characterized by pre-natal growth restriction in the absence of recognizable maternal or placental pathology and by the failure of post-natal catch-up growth resulting in significant proportionate short stature. The syndrome is associated with distinct facial features, radiological abnormalities and normal intelligence. Final adult height is in the range of 115 to 150 cm. Mutations in the *CUL7* gene cause 3-M syndrome, the protein product, CUL7, is a member of the Cullin family which forms multi-subunit E3 ligases within the ubiquitin-proteasome pathway.
We have identified 3-M patients that do not carry *CUL7* mutations, and performed high-density SNP mapping in these families to identify a second locus at 2q35-q36.1. Subsequently we discovered 7 distinct null mutations from 10 families within the gene *OBSL1,* their phenotype being indistinguishable from that seen in those with *CUL7* mutations. OBSL1 exists as 3 groups of isoforms (A-C) and is a putative cytoskeletal adaptor protein which localizes to the nuclear envelope and is homologous to obscurin, a key component of the sarcomere. All mutations thus far have been identified within the first 6 exons of the gene and affect all known OBSL1 isoforms. It is predicted that the mutations induce a nonsense mediated decay response leading to loss of OBSL1. Thus 3-M syndrome is the null phenotype of human OBSL1. OBSL1 is postulated to have a role in protein-protein interaction and that disruption of OBSL1 would result in either skeletal or cardiac myopathy. However 3-M patients with *OBSL1* mutations do not exhibit any muscular/cardiac symptoms and it is possible that overlapping functions between OBSL1 and obscurin may explain this. Using co-immunoprecipitation and immunoblotting, we are able to demonstrate that OBSL1 interacts with CUL7. Additionally we have shown that knockdown of OBSL1 by siRNA leads to down regulation of CUL7 indicating a role for OBSL1 in the maintenance of CUL7 protein levels.
Our findings suggest that both CUL7 and OBSL1 are involved within the same molecular pathway and the identification of the ubiquitinated target of the CUL7 SCF complex will further enhance our understanding of how the CUL7/OBSL1 pathway regulates human growth. To our knowledge this is the first description of mutations in a cytoskeletal adaptor protein causing significant growth impairment and implicating OBSL1 as a key regulator of growth.

FC1-006 Growth

Identification of the chondrogenesis related SOX5 and SOX6 transcription factors as SHOX interacting proteins

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SHOX (short stature homeobox-containing gene), located in the pseudoautosomal region (PAR1) of the X and Y chromosomes, encodes a homeodomain transcription factor involved in human skeletal growth. Mutations/deletions of *SHOX* and/or a downstream region containing enhancer elements of *SHOX* transcription, represent the molecular cause of approximately 70% of the cases of Léri-Weill dyschondrosteosis (LWD), a skeletal dysplasia associated with disproportionate short stature. No molecular defect has been detected in the remaining 30% of LWD cases, suggesting that other genes could be implicated as the molecular cause of this pathology.

In order to identify potential gene candidates, we set out to: 1) Identify proteins which interact with SHOX with the scope to determine the signalling pathways in which SHOX participates, and; 2) Identify the molecular defect in the LWD patients with no known molecular defect by screening the genes encoding the proteins previously identified.

We approached the identification of SHOX interacting proteins by a yeast two hybrid screen using a 9.5-10.5 dpc mouse cDNA library and SHOXa as the bait. Among the large number of SHOX interacting proteins, we identified two transcription factors involved in chondrogenesis: SOX5 and SOX6. Coimmunoprecipitation assays in human cells confirmed the existence of these interactions. Moreover, we demonstrated the coexpression of SHOX, SOX5 and SOX6 in the same zones of human embryonic growth plate sections. Subsequently, we carried out mutation screening of *SOX5 and SOX6* in 25 LWD or possible LWD individuals without a known molecular defect. We screened the coding regions and intron/exon boundaries of the two genes by dHPLC and sequencing. No mutation was identified in our cohort, suggesting that mutations in these genes are not the molecular cause of LWD.

Thus, we have identified the chondrogenesis related SOX5 and SOX6 transcription factors as the first two SHOX interacting proteins. Although the functions of these interactions remain to be investigated, these findings suggest that SHOX may participate in various developmental stages of chondrogenesis via its association with these transcription factors.

FC2-007 Bone

Postnatal deletion of the G-protein subunit alpha ($G_s\alpha$) in epiphyseal chondrocytes inhibits longitudinal bone growth

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Patients with Albright hereditary osteodystrophy (AHO) often display brachydactyly and premature closure of the epiphyseal growth plate. AHO results from loss-of-function mutations in one copy of the *GNAS* gene, which encodes the α-subunit of the stimulatory G-protein (Gsa). Gsa is thought to be a primary downstream target of the PTH/PTHrP receptor (PPR). Recently we have shown that ablation of PPR from epiphyseal chondrocytes of postnatal mice induces abrupt fusion of the growth plate. In the current work we explore the role of Gsa in the growth plate of postnatal mice.

For this purpose we crossed mice with collagen-2 (Col2) driven tamoxifen-inducible Cre (Col2-CreERt) with mice harboring floxed exon-1 of the Gsa gene. Ablation of Gsa exon-1 was induced by injection of tamoxifen (tam) at 3 and 7 days of age. All mice employed in the study were harboring floxed exon-1 of the Gsa gene and injected with tam. Col2-CreERt negative mice were used as a control.

We found that ablation of Gsa in Col2-expressing chondrocytes at 3-4 days of age dramatically delayed growth of long bones. Histological analysis of long bones revealed permanent disruption of bone epiphyses and altered morphology of the growth plate. Fourteen days after tam administration epiphyseal cartilage was narrowed in Col2-CreERt positive mice with decreased numbers of cells and extent of both the proliferative and hypertrophic layers. Only remnants of epiphyseal cartilage were observed thirty days after tam injection. Nine days after Gsa ablation we observed decreased chondrocyte proliferation and increased differentiation. Interestingly, we observed an increase of TUNEL-positive cells at the chondro-osseous junction while the number of TRAP-positive cells (osteo/chondroclasts) was not changed. Metatarsal bones dissected 2 days after tam administration and cultured *ex vivo* displayed decreased longitudinal growth, which was prevented by administration of forskolin, an activator of adenylyl cyclase.

In conclusion, we have shown that Gsa is essential for normal linear growth of young postnatal mice. Differences in growth plate phenotype between our Gsa -inactivated mice and mice in which PPR was inactivated in a similar manner suggest that other mediators of PPR signaling may play essential roles in the biology of the epiphyseal growth plate.

FC2-008 Bone

microRNAs are involved in nutritional induced catch up growth in the epiphyseal growth plate

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MicroRNAs (miRNAs) are small endogenous RNAs that regulate target mRNAs by binding to their 3'-UTRs. They have been reported to be involved in a variety of functions, including skeletal development and longitudinal growth. Catch-up (CU) growth is a period of accelerated growth that occurs when growth inhibitory conditions resolve. The local mechanisms by which nutrition affects growth at the epiphyseal growth plate (EGP) are still not elucidated.

To study the mechanisms regulating nutritional induced CU growth, pre-pubertal rats were subjected to 10 days of 40% food restriction, followed by a renewal of the regular food supply. Humeri were used for morphometric analysis. Total RNA was extracted from tibial EGPs and loaded on miRNA microarrays. Results were confirmed by real-time PCR, and changes in the predicted targets of these miRNAs were followed using Western immunoblot. Findings were compared with EGPs from animals fed ad libitum.

Humerus and EGP lengths were significantly smaller in the food-restricted group. When food restriction was removed, the rats showed an instantaneous increase in weight and EGP length, later accompanied by an increase in humerus length. Our previous studies found that nutritional manipulation induced dramatic changes in the expression of several genes[1]; however, no significant change was detected in growth-related genes. Further studies suggested the involvement of additional regulatory mechanisms, namely, miRNAs. Using miRNA microarrays, we found that numerous miRNAs were expressed in the postnatal EGP. Furthermore, nutritional manipulation led to significant changes in the expression of several miRNAs, including the cartilage-specific miR140. We also noted a dramatic change in several potential targets of these miRNAs, including STAT-3, GDF-10, TGFβ1 and IGFBP7, all of which are expressed in the EGP and may have an anti-proliferative effect on the EGP or bone.

These results may have important implications for understanding the mechanism of the EGP growth. The present study is the first, to our knowledge, to show the involvement of miRNA in growth regulation in the post-natal EGP and the first to show the effect of nutrition on miRNA expression in vivo. Involvement of miRNA in the regulation of growth may open a new era of

research and may enable the development of new treatment for children with growth abnormalities.

1. Even-Zohar N et al. Bone 42 Pp: 505-515, 2008

FC2-009 Bone

Aromatase inhibition causes osteopenia in prepubertal male and female rats and results in polycystic ovaries in female rats

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Pharmaceutically induced estrogen deficiency by aromatase inhibitors is proposed as a novel treatment modality for growth enhancement in children with short stature. Since estrogen is also essential for bone metabolism and gonadal development, adverse effects on those tissues may be expected during aromatase inhibitor treatment. We assessed the effects of aromatase inhibition on growth and on bone quality and gonadal morphology in prepubertal rats. Female prepubertal rats (n=36, 26 days old) were ovariectomized, placebo-treated or treated with the aromatase inhibitor exemestane at a dose of 10, 30 or 100 mg/kg/week for 3 weeks. Male prepubertal rats (n=36, 26 days old) received placebo or exemestane treatment at a dose of 10, 30 or 100 mg/kg/week for 6 weeks. Parameters of linear growth were recorded. High resolution X-ray microtomography was performed for quantitative analysis of bone parameters (trabecular number and thickness, bone volume, calcium density) in the epiphyseal and metaphyseal segments of the right femur. Morphology of the uterus, ovaries, testes and seminal vesicle was assessed by histological analysis.

In females, exemestane at the highest dose marginally increased growth, but also resulted in a lower ovarian weight, the presence of multiple ovarian cysts and absence of corpora lutea. In male rats, exemestane treatment resulted in a pattern of decreased growth, without apparent effects on gonadal morphology. In both genders, exemestane treatment resulted in a reduction of cancellous bone due to thinning and loss of trabeculae in the epiphyseal and metaphyseal segments.

In conclusion, aromatase inhibition by exemestane has a sexually dimorphic effect on growth, but causes osteopenia in both genders. In addition, a polycystic ovary syndrome phenotype is induced in females. Although the rat model may not be representative for studying human growth, the results of the present study warrant serious consideration of the potential side effects of aromatase inhibition on bone and gonadal development.

FC2-010 Bone

Hypophosphatasia: treatment of life-threatening disease using bone-targeted human recombinant tissue non-specific alkaline phosphatase

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Hypophosphatasia (HPP) features low serum alkaline phosphatase (ALP) due to deactivating mutation(s) within the gene that encodes the "tissue nonspecific" isoenzyme of ALP (TNSALP). Consequently, inorganic pyrophosphate, a natural substrate for this ectoenzyme, accumulates extracellularly and blocks skeletal mineralization. There is no established medical treatment.

ENB-0040 is a bone-targeted, human recombinant, TNSALP fusion protein prevented HPP in a TNSALP knockout mouse model of the infantile form (JBMR 23:777, '08). In a phase 1, month-long, multi-center, open-label protocol, 6 adults received 1 IV infusion of 3 mg/kg ENB-0040 followed by weekly SC injections of 1 or 2 mg/kg.

Here, we report findings from our 6-mo, open-label protocol involving 5 patients with life-threatening HPP (ages 6-36 mo at baseline), with up to 6 mo of ENB-0040 treatment. Previously, each had shown worsening skeletal disease and respiratory symptoms predicting a lethal outcome.

At age 7 mo, patient 1 with infantile HPP received a single IV infusion of 2 mg/kg of ENB-0040 followed by 1 and then 3 mg/kg SC 3X/wk. During therapy, there was substantial remineralization of the skeleton, weaning from ventilatory support, and improved growth and motor development. After receiving only 3 wk of treatment, Patient 2, also with infantile HPP, showed skeletal remineralization (figure 1), and then improved ventilation.

Patient 2 shows substantial metaphyseal remineralization and apparent growth plate narrowing after 21 days of subcutaneous injections of ENB-0040, with further improvement after 78 days of treatment.

Both patients 1 & 2 have been released from hospital to continue ENB-0040. The remaining 3 patients, including one with perinatal HPP, have varying degrees of skeletal disease and have each been treated for at least 1 mo. The infant with perinatal disease thus far shows correction of characteristic hypercalcemia after 8 wk of SC treatment.

There have been no drug-related serious adverse events, or development of anti-ENB-0040 antibodies.

Substantial skeletal remineralization and improved clinical status has been demonstrated in association with ENB-0040 bone-targeted enzyme replacement therapy in 2 severely affected infants with HPP in this short term study.

FC2-011 Bone

A novel inactivation mutation of the ENPP1 gene is causing a new form of autosomal recessive hypophosphatemic rickets

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Human disorders of phosphate (Pi) handling and hypophosphatemic rickets without hypercalciuria can result from mutations in PHEX, FGF23 and DMP1 genes, presenting as X-linked, autosomal dominant and autosomal recessive patterns, respectively.
We have characterized an enlarged consanguineous Bedouin family displaying autosomal recessive Hypophosphatemic rickets. Two patients presented with short stature and bowing legs. A third patient has normal stature and was apparently healthy except for persistent non-union of post traumatic fracture of his right tibia. Laboratory findings included hypophosphatemia, hyperphosphaturia, and elevated plasma alkaline phosphatase. Serum calcium, PTH, vitamin D metabolites and urinary calcium to creatinine ratios were within normal ranges. Plasma FGF23 levels were low.
The structure and size of the family were suitable for positional cloning of the causing gene through linkage study. After excluding linkage to the three above mentioned known genes, we carried out a genome wide search for the chromosomal region containing the mutated gene. We identified the mutation, located in the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene. ENPP1 generates inorganic pyrophosphate that is an essential physiologic inhibitor of calcification. The mutation is a single nucleotide change that causes substitution of a strictly conserved amino acid. The population of 236 healthy Bedouins was tested and polymorphism was excluded. The function of the mutation was tested by transfection of a pSVT7 expression vector harboring the mutated full-length *ENPP1* coding sequence into cells and measuring the NPP activity in comparison to the activity in cells transfected with the normal sequence vector. We found that the mutation completely abolished NPP activity.
Previously, inactivating mutations in *ENPP1* reported to cause generalized arterial calcification in infancy and additional aberrant calcification conditions. Our surprising results clearly implicate the ENPP1 gene to hypophosphatemic rickets but cannot be explained in terms of current known functions of this enzyme.

FC2-012 Bone

Treatment of hypoparathyroid adults and adolescents with parathyroid hormone (PTH) 1-34 induces marked changes in bone turnover and structure: a biochemical and histomorphometric study

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The effects of PTH on bone are complex; chronic elevated levels of PTH are catabolic in hyperparathyroidism, yet intermittent administration is anabolic in osteoporosis. PTH deficiency, (hypoparathyroidism) is associated with low bone turnover and high bone mass. Hypoparathyroid patients treated for 3 years with "replacement dose" PTH (maintaining serum calcium at or near the lower limit of normal), showed a marked increase in bone turnover markers without changes in bone density (BMD) by DXA. To better understand the effects of PTH on bone, we treated 5 hypoparathyroid patients (2 adults, 3 adolescents) with replacement dose human PTH 1-34 twice or thrice daily for 1 year. Bone turnover markers and DXA (hip/spine) were assessed every 6 months; double-labeled iliac-crest biopsies were performed before and after 1 year of PTH. Data are presented as mean±SD, baseline vs 1y.
PTH increased bone turnover markers to supranormal levels: osteocalcin (53±61 vs 307±44 ng/mL), bone-specific alkaline phosphatase (15±14 vs 38±9 µg/L), N-telopeptide (76±104 vs 457±147 nmol BCE/mmol Cr), pyridinoline (75±62 vs 165±74 nmol/mmol/Cr), deoxypyridinoline (22±23 vs 63±28 nmol/mmol/cr), p<0.05 for all comparisons. Histomorphometry revealed that PTH further increased already supranormal cancellous bone volume/total volume (BV/TV, 27.6±4.9 vs 43.5±6.3%, p<0.01) and trabecular number (Tb.No, 2.8±0.3 vs 4.3±0.9 #/mm, p< 0.05) and further decreased trabecular separation (Tb.Sp, 265±36 vs 139±50 µm, p<0.05). Trabecular width did not change, suggesting that the increase in trabecular number was due to intratrabecular tunneling. Cortical porosity/cortical area also increased (Ct.Po.No/Ct.Ar, 4.7±2.0 vs 6.9±2.6 #/mm2, p<0.05). Cancellous bone remodeling was decreased at baseline but increased above normal after PTH: mineralized perimeter (Md. Pm, 4±4 vs 26±4%, p<0.01); bone formation rate (BFR, 0.046±0.061 vs 0.273±0.058 µm2/µm/d, p<0.01). Similar changes were seen in endocortical and intracortical remodeling. Despite these significant increases in bone turnover and volume, BMD z-score did not change. Without affecting BMD, 1 year of PTH therapy stimulated bone turnover and was anabolic at the iliac crest, evidenced by marked increases in bone volume and changes in bone structure beyond the age-specific norms. The apparent discrepancy between histomorphometry and densitometry warrants further exploration. Larger, long-term studies are ongoing to determine if these effects persist.

FC3-013 Reproduction

Prostaglandin-D2-synthase mutation: a new genetic cause of isolated cryptorchidism in boys

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The process of testicular descent is not fully understood, but several factors, such as Hoxa-10, epidermal growth factor (EGF) and calcitonin gene-related peptide (CGRP), together with androgens and insulin-like hormone 3 (Insl3), have been suggested to regulate it. Lipocalin-type prostaglandin D2 synthase (L-PGDS) and hematopoietic prostaglandin D2 synthase (H-PGDS) are two enzymes expressed in the testes and involved in the nuclear translocation and expression of SOX9, a central factor in testis determination during foetal life. Knockout of L- and H-PGDS in male mice induces delayed testicular organisation and a uni- or bilateral testis migration defect.
The aim of this work was to find out whether L-PGDS and H-PGDS abnormality could be associated with cryptorchidism in boys. We studied 60 boys with isolated cryptorchidism (N=25), cryptorchidism associated with hypospadias (N=5) or anorchia (N=30) and 30 controls.
No L-PGDS abnormality was identified among the studied patients. Conversely, an H-PGDS gene abnormality was identified in one boy referred to our pediatric endocrinology clinic at 15 months with bilateral cryptorchidism and severe growth retardation (-4DS). Basal plasma FSH and LH were very low (<0.5 and 1 UI/l, respectively). HCG stimulation testing showed a subnormal testosterone response (0.2 to 2 ng/ml) along with right testis migration. The gene abnormality consisted of a triple mutation: ([c.271 A>G (p.Ile91Val)], [c.383 T>C (p.Met128Thr)], [c.559G>A (p.Val187Ile)]) transmitted by the mother. Functional analysis of the triple mutant and the p.Met128Thr single mutant showed about a 40% decrease in glutathione transferase activity (indispensable for PGDS activity) and total prostaglandin D2 synthase abolition. Taken together, these data clearly demonstrate, for the first time, that H-PGDS defect can be responsible for cryptorchidism in human.

FC3-014 Reproduction

FSH regulates anti-Müllerian hormone gene expression through PKA-mediated SOX9 activation and SF1 nuclear translocation

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FSH increases anti-Müllerian hormone (AMH) gene expression in prepubertal Sertoli cells through the classical cyclic AMP (cAMP) pathway. No classical cAMP response element is present on the AMH promoter. SOX9, SF1, GATA4 and AP1 are activated by cAMP in diverse cellular types and have response elements on the AMH promoter. Our aim was to test whether AP1, GATA4, SF1 and/or SOX9 could be involved in cAMP-mediated upregulation of AMH expression in prepubertal Sertoli cells. A previously validated prepubertal Sertoli cell line (SMAT1) was incubated for 24 h with or without 1 mM dbcAMP in the presence or absence of inhibitors of PKA (H89), PI3-K (LY294002), p38-K (SB203580) or MAPK (PD98059). AMH promoter activity was assessed after

transfection with luciferase vectors containing 3 kb of the AMH promoter, either wild-type or with mutated sites for GATA (-74, -168 and -408), AP1 (-203), SOX9 (-141) or SF1 (-92 and -218). Mutations of SOX9 and SF1 sites, but not those of GATA4 and AP1 sites, abolished AMH promoter response to dbcAMP. The PKA inhibitor H89, but not the other kinase inhibitors, impaired wild-type promoter response to cAMP. We next assessed whether an increased availability of SOX9 and SF1 in Sertoli cell nuclei was observed after cAMP incubation. In real time RT-PCR and Western blot analyses, SOX9 expression was upregulated in SMAT1 cells after cAMP incubation. H89 precluded SOX9 upregulation. SF1 expression was not modified by cAMP. By immunocytochemistry, we observed that SOX9 was always nuclear, whereas SF1 was translocated from the cytoplasm to the nucleus after cAMP incubation. In conclusion, cAMP activation of PKA results in an increased bioavailability of SF1 and SOX9 in prepubertal Sertoli cells, resulting from SF1 nuclear translocation and SOX9 upregulated expression. cAMP-activated SF1 and SOX9 are responsible for the increase in AMH promoter activity, after binding to specific response elements present in the proximal AMH promoter.

FC3-015 Reproduction

Anogenital distance (AGD) as a marker of androgen exposure is replicated in humans

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Background: AGD is sexually dimorphic in rodents and is routinely used as a reliable indicator of the effects of prenatal androgen and anti-androgen exposure in reproductive toxicological studies. Evidence suggests that AGD may also be a sensitive biomarker of human fetal androgen exposure. However, data on AGD in humans are sparse with no longitudinal data collected during infancy.

Objective: To determine AGD from birth to two years in boys and girls and relate this to other anthropometric measures

Materials and methods: Infants were recruited from a prospective single centre UK birth cohort study. AGD was measured from the centre of the anus to the base of the scrotum in boys and to the posterior fourchette in girls. Measurements were performed longitudinally at birth and at 3, 12, 18 and 24 months of age.

Results: 463 male and 426 female full term infants provided 2168 longitudinal AGD measurements (median = 2 measurements per infant). Mean AGD (SD) at birth was 19.8 (6.1) mm in boys and 9.1 (2.8) mm in girls ($p < 0.0001$). AGD increased rapidly up to 12 months in both sexes and plateaued thereafter. AGD at birth in boys was approximately 2 fold greater than in girls and a similar relationship was maintained at all time points. In boys, AGD was correlated with weight only at 3 months ($r = 0.14$, $p = 0.03$). AGD was positively correlated with penile length at birth ($r = 0.18$, $p = 0.003$). Penile growth from birth to 3 months was correlated with an increase in AGD ($r = 0.20$, $p = 0.001$) and was the sole predictor of increase in AGD during this period ($\beta = 0.28$, 95% CI = 0.09 - 0.47). Centile curves for AGD was produced from the data and preliminary results suggest it is reduced in cryptorchidism.

Conclusion: We report novel, longitudinal data for AGD during infancy. Association of AGD with penile length at birth and penile growth at 3 months suggests the effect of prenatal and post-natal androgens respectively. The study reports the first indirect evidence of change in AGD in response to neonatal surge in androgens. Association of AGD with weight in boys was weak and inconsistent in infancy indicating that correcting AGD for weight may render this measurement less reliable. The availability of normative data provides a means of utilising this biological marker of androgen action in population studies of the effects of environmental chemicals on human genital development.

FC3-016 Reproduction

Perfluorooctanoic acid (PFOA) and pubertal maturation in young girls

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Background: Polyfluoroalkyl compounds (PFCs) and their salts, such as perfluorooctanoic acid (PFOA), have been reported to change mammary gland structure and function in laboratory animals. We explored the relationship between serum PFOA concentration and pubertal maturation in young girls.

Methods: Within the NIH Breast Cancer and the Environment Research Centers (BCERC), we conducted a study of multiple environmental biomarkers, including PFOA and other PFCs in serum of young girls (age 6-7 years at entry) from two sites (N=689 girls). Pubertal staging (breast (B) and pubic hair (PH)) has been conducted by clinicians or trained research staff, every year or more frequently, for as long as four years. After calculating adjusted geometric means for all PFCs, we examined the relationship between PFOA serum concentration at the beginning of the study, with body mass index (BMI) and pubertal Stage 2 at baseline and one year follow-up.

Results: Detectable serum levels of five PFCs, including PFOA, were found in >95% of the girls. The PFOA median was 6.4 ng/ml (range <LOD 0.1 to 55.9 ng/ml), with 24.9% having values above the 95th percentile for children 12-19 years (NHANES 2003-2004 population (8.6 ng/ml)). At the follow-up visit, 28.3% of girls had reached Tanner stage B2+, 19.2% were PH2+ and 30.3% had a BMI percentile for age >85. In analyses where serum PFOA was modeled as a continuous variable, we found a direct relationship with pubertal breast status and an inverse relationship with BMI percentile at the follow-up visit, with adjustment for age, race, site and caregiver education.

Conclusions: It appears that PFOA acts as an endocrine disruptor although perhaps not by the usual mechanism. Although the relationship with BMI was inverse, there was a direct relationship with breast maturation. We continue to explore these complex relationships in models including other covariates.

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The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.

FC3-017 Reproduction

New loci for congenital hypopituitarism: overlap with Kallmann syndrome

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Background: To date several loci (*KAL1, FGF8, FGFR1, PROK2, and PROKR2*) have been implicated in Kallmann syndrome (KS). These genes are expressed in the olfactory placode where GnRH neurons originate. Notably, adenohypophyseal and olfactory placodes arise from a common preplacode. Thus, we hypothesize that genes involved in early GnRH ontogeny also underlie some cases of congenital hypopituitarism.
Methods: Fifty-seven patients with congenital hypopituitarism were analyzed for mutations in the 5 KS loci. *FGFR1 and PROKR2* mutants were evaluated in vitro with AP-1- and OCFRE-Luc reporter assays in L6 myoblasts and Egr1-Luc reporter and Aequorin reporter Ca mobilization assays in HEK293 cells, respectively. For the *FGF8* mutant, mRNA expression studies were performed using a *FGF8* minigene construct in HEK 293 cells.
Results: We identified 6 heterozygous mutations in 7 of 57 probands (*KAL1* c.1627G>A (p.V543I); *FGFR1* c.1595T>C (p.M532T) and c.1447C>T (p.P483S); *FGF8* c.216G>A (p.T72T), *PROKR2* c.253C>G (p.R85G) and c.254G>A (p.R85H) in 2 probands). Changes were not seen in > 200 healthy adult controls. The Fgfr1 and Prokr2 mutants exhibit altered function in vitro: Fgfr1 p.P483S, Prokr2 p.R85G and p.R85H cause loss-of-function, while Fgfr1 p.M532T causes gain-of-function. The synonymous p.T72T change in Fgf8 significantly decreases *FGF8* mRNA expression levels. Subjects harboring mutations had a combination of pituitary hormone deficiencies; one subject had an ectopic pituitary gland on MRI, and 2 had septo-optic dysplasia. No mutations were found in *HESX1, LHX3, LHX4, PITX2*, *OTX2, SIX6, PROP1 and POU1F1*. Of interest, the Kal1 and one Prokr2 mutations (p.V543I, p.R85H) were previously identified in KS patients. The *PROKR2* mutations in this cohort prompted us to study Prokr2 expression in the pituitary using a Prokr2-EGFP transgenic mouse line. EGFP was expressed in the posterior pituitary as well as in some anterior pituitary cells requiring further identification.
Conclusions: 1) This report expands the number of loci underlying congenital hypopituitarism; 2) This is the first report demonstrating an overlap of genes involved in both KS and congenital hypopituitarism; 3) The expression of Prokr2 in the murine pituitary is consistent with these human data suggesting a role for Prokr2 in pituitary development/function.

FC3-018 Reproduction

Final reproductive outcome of high-dose sex steroid treated tall boys and girls

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Introduction: Sex steroid treatment to reduce final height of tall boys and girls has been available since the late 1950s. It has been suggested that this treatment may interfere with normal fertility. We evaluated fertility and gonadal function in tall men and women who did or did not receive high-dose sex steroid treatment in adolescence.
Methods: Reproductive and gonadal function was assessed by standardized interview, endocrine parameters and ultrasound imaging of the gonads in 116 and 219 tall Dutch men and women aged 25-45 years. Of these, 60 men and 149 women had been treated (mean follow-up 25 years).
Results: *Men:* 66 men had attempted to conceive. The probability of conceiving their first pregnancy within one year was similar in treated and untreated men (26 out of 36 (72%) vs. 24 out of 30 (79%), log rank P=0.6). Testis volume, sperm quality and concentration and serum levels of LH, FSH, and inhibin B were comparable between treated and untreated men. However, treated men had significantly reduced age-adjusted serum T levels (mean (SD) 14.1 (3.8) vs. 16.0 (5.8) nmol/l, P = 0.03) as well as serum AMH levels (13.4 (6.7) vs. 16.3 (9.7) µg/l, P = 0.04). *Women:* 124 women had attempted to conceive. The probability of conceiving was significantly reduced in the treated group (n=44 out of 85 (51%) vs. n=27 out of 39 (69%), log rank P = 0.03). In addition, treated women were more frequently treated for subfertility (n=27 out of 85 (32%) vs. n=3 out of 39 (8%), OR=5.7 95%CI 1.6-20.3). Antral follicle counts and serum hormone levels were comparable between treated and untreated women. However, the number of women (n=36 out of 171 (21%)) with a hypergonadotropic profile, suggestive of early ovarian failure, was markedly increased, irrespective of treatment (median FSH (IQR) 7.6 (6.1-10.7) IU/l (ref. 2-8 IU/l)). Moreover, tall women had high serum AMH levels (median (IQR) 4.4 (1.7-8.1) µg/l (ref. 0.5-7 µg/l)).
Conclusion: At a mean follow-up of 25 years after high-dose sex steroid treatment we conclude that in tall treated men fertility is not affected, albeit that testosterone levels are reduced. However, tall treated women are at risk for subfertility in later life. Their time to pregnancy is significantly increased. Moreover, in a considerable number of tall women a hypergonadotropic profile was found, regardless of treatment. However, AMH levels were increased, suggesting deviant follicular dynamics.

FC4-019 Systems Biology & New Technologies

Use of continuous glucose monitoring with a proportional-integral-derivative algorithm to achieve tight glucose control in the pediatric intensive care unit

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Background: Recently, tight glucose control in the intensive care unit (ICU) setting has been a subject of much debate. Adult and pediatric studies show mixed results with regard to overall improved morbidity and mortality in intensively ill patients. Many of the studies with poorer outcomes have had significant hypoglycemia associated with tight glucose control. To date, the use of an insulin algorithm for tight glucose control along with a continuous glucose monitor (CGM) to prevent hypoglycemia has not been studied. We aimed to use an insulin algorithm with a CGM to safely achieve tight glucose control in the ICU, while preventing hypoglycemia.
Methods: PICU patients ages 6m to 18yrs with hyperglycemia defined as a blood glucose (BG) level > 150 mg/dl twice or one BG >200mg/dl were enrolled in a single-center feasibility study. A CGM was placed and a bedside computer with a proportional-integral-derivative (PID) algorithm received the sensor glucose (SG) via RF signal. Subjects were on IV insulin administered via a standard ICU infusion pump. A subject weight and BG entered into the algorithm started the study with an initial insulin infusion recommendation. All insulin infusion recommendations were verified prior to implementation by study personnel. SG values were received every 5 min by the algorithm. The algorithm alarmed for BG checks every 2 hrs or earlier if the algorithm deemed it necessary. The algorithm determined insulin dosing based both on glucose level and rate of change of the discrete BG values. The target BG for the algorithm was 120mg/dl.
Results: Data was analyzed for sensor accuracy, time to target BG(120±30mg/dl), % BG in target range, and episodes of hypoglycemia (BG < 60mg/dl). 9 subjects (mean age of 12.4±4.5 yrs.) are currently enrolled for an ave of 39 hrs each (range 18-108 hrs). The median absolute difference between BG and SG was 10 mg/dL, and the median relative absolute difference was 8.4 %. There were no episodes of hypoglycemia.

Data Summary						
Mean Starting BG (mg/dl)	Mean Time to Target BG (hrs)	Mean % BG 61-70mg/dl	Mean % BG 71-90mg/dl	Mean % BG 91-150mg/dl	Mean % BG 151-180mg/dl	Mean % BG >181mg/dl
151±71	4.8±5.1	0.1±0.3	4.5±6.3	70.1±23.5	10.9±11.3	14.3±23

Conclusion: Tight glucose control without significant hypoglycemia is achievable in the ICU using an insulin algorithm with CGM. Larger randomized controlled trials are needed to look at overall benefit of this therapy.

FC4-020 Systems Biology & New Technologies

Overnight closed-loop (CL) glucose control in children and adolescents with type 1 diabetes (T1D): towards home testing

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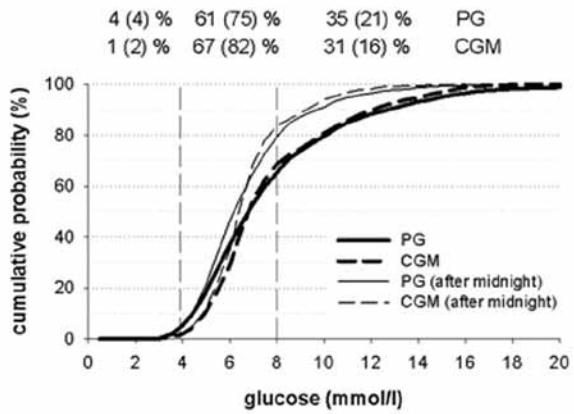
We performed 3 CL inpatient studies (APCam01, 02 & 03) in young people with T1D. In total, 17 subjects were studied (M 8; age 13.5±3.6 years, BMI 21.0±4.0 kg/m^2, duration diabetes 6.4±4.1 years, A1C 8.5±1.8%, mean ± SD) over 33 nights with CL and 21 nights with CSII. APCam01 compared CL against CSII (N = 12; self-selected meal and prandial insulin at 18:00; CL from 20:00 to 08:00). APCam02 evaluated CL after large slowly and rapidly absorbed meals (N = 6; 129±34 g CHO dinner with prandial insulin at 18:00; CL from 18:30 to 08:00). APCam03 compared CL and CSII following exercise (N = 9; 55% VO_2max exercise from 18:00 until 18:45 with 5min rest; CL from 20:00 to 08:00). Real-time subcutaneous (sc) continuous glucose monitoring (CGM) data were fed into a model predictive controller every 15 minutes, which calculated sc insulin infusion for manually adjusted insulin pump. Reference plasma glucose was measured every 15min. During CL, no rescue carbohydrates were given. CL increased time in target and reduced risk of hypoglycaemia, see Table. Figure shows cumulative plasma glucose and CGM distributions during APCam01, 02 & 03 from the start of CL (values in brackets are from midnight). We conclude that inpatient overnight CL in young people with T1D is safe, efficacious, and improves significantly glucose control compared to CSII. Outpatient testing is the next goal.

Overnight closed loop vs CSII during APCam01 & 03 (N=21)

	Closed loop	CSII	p
Mean Plasma glucose (mM)	∗7.0 (†6.3)	8.1 (7.0)	NS (NS)
Plasma glucose ≤ 3.9 mM (% of time)	6.8 (6.3)	20 (28)	0.01 (0.002)
Plasma glucose in target 3.9 - 8.0 mM (% of time)	66 (77)	38 (40)	0.01 (0.007)
Plasma glucose > 8 mM (% of time)	28 (17)	43 (32)	NS (NS)
Insulin infusion (U/h)	1.1 (1.0)	1.1 (1.0)	NS (NS)

∗ from 20:00 to 08:00; † from 00:00 to 08:00

Figure. Plasma glucose and CGM during closed loop in APCam01, 02 & 03 (N = 33) from start of control and after midnight. Time spent below, at, and above target (3.9 – 8.0mM) is displayed at top.



FC4-021 Systems Biology & New Technologies

Production of normaly functioning beta cell line from 8 to 10 weeks human embryos

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Regenerative medicine is an important avenue in the research of new treatments for diabetes. Using an original approach, we have been able to generate a beta cell line which could possibly be used for diabetes cell therapy.
Method
We have shown that, a 8 to 10 weeks human fetal pancreas, when grafted in a SCID mouse, develops and, after several months in vivo contains insulin producing cells. We have then developed an experimental strategy based on lentiviral mediated gene transfer to generate immortalized insulin secreting human pancreatic beta-cell lines. We have used human 8 to 10 Wks embryos obtained after legal abortion according to French law and with the agreement of the French Health authorities. After dissection, the fetal pancreatic anlage was transduced with recombinant lentiviruses expressing SV40T and human telomerase reverse transcriptase (hTERT) with both genes flanked by loxP sites for later Cre recombinase-based excision. Expression of both genes were under the control of the insulin promoter. Such transduced human pancreas were grafted in SCID mice. After 4 to 6 months an insulinoma developed. Cells from this tumoral mass were dispersed and after several passages we have been able to derived functional beta cell lines. Such cells are stable after more than 60 passages.
Results
These cells contain insulin and all the markers of a normal beta cell that were looked for were present in those cells. When cultured in a proper medium glucose stimulated insulin secretion was observed. At variance with a normal beta cell they display a high degree of proliferation as shown by BrDU incorporation. We have then deleted the immortalizing transgene by transduction with Cre expressing lentiviral vector. The efficacy of this excision procedure is about 90%. Under such condition, beta-cell proliferation sharply decreases and by contrast insulin content and glucose-stimulated insulin secretion increases. Both cell lines, when grafted into diabetic SCID mice can control for several months the blood glucose level to normal values.
Conclusion
With our technology we have been able to generate for the first time normal functional human beta cells. These cells have the capacity to produce insulin when grafted into living animals. They will be an interesting material for cell replacement therapy. We are now working on the excision procedure to be able to securely delete the transgene from all immortilized cells.

FC4-022 Systems Biology & New Technologies

Novel targets of steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) in the adrenal

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Background: Steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) is a nuclear receptor that plays a central role in adrenal and gonadal development, steroidogenesis, lipid metabolism and tumorigenesis. To date, more than 40 SF-1 regulated genes have been identified. Most of these genes play a critical role in endocrine function, and have been shown to contain promoters or enhancers that can be up-regulated by SF-1.
Aim: Using a "reverse discovery" approach, we aimed to manipulate SF-1 expression levels or binding in adrenal cells to identify a novel subset of SF-1 dependent target genes which themselves could represent novel regulators of adrenal development, steroid metabolism or tumorigenesis.
Methods: 1) Using human adrenal cell lines and Amaxa nucleofection we used pIRES vectors to either a) overexpress human SF-1 or b) knock-down SF-1 (shRNA) together with GFP expression. FACS analysis allowed transfected cells to be harvested. RNA was extracted from these cells and subjected to

microarray analysis (Affymetrix ST Gene Array 1.0). Western blots confirmed SF-1 protein changes and qRTPCR was used to confirm key changes seen. Gene clustering and differences in expression was analysed using Partek and Bioconductor R. 2) ChIP-on-Chip was used to detect SF-1 binding targets in adrenal cells using an anti-human SF-1 antibody, promoter arrays (Affymetrix Human Promoter 1.0R), and MAT/CisGenome software. 3) The resulting datasets were compared for novel targets and networks, which were analysed using GeneGo MetaCore systems biology software.
Results: Using this combinatorial approach we were able to identify steroidogenic acute regulatory protein (STAR) and CYP11A1 as major SF-1 targets in these systems. Novel SF-1 targets included regulators of cholesterol metabolism, membrane transporters (e.g. ABCA5), a previously uncharacterized calcium-dependent domain factor, and regulators of vasculogenesis/cell adhesion (e.g. ITGA9).
Conclusions: We have identified several known and novel SF-1 targets in adrenal cells. Defects in these systems could result in forms of adrenal hypo- or hyper-function in children and adults where the molecular cause is currently unknown and could in the future represent tissue-specific targets for pharmacotherapy in Cushing syndrome or adrenal cell carcinoma.

FC4-023 Systems Biology & New Technologies

Evoked inflammation of human adipose in vivo allows identification of novel human adipocyte and macrophage-secreted inflammatory proteins

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Introduction: Adipose inflammation is a crucial step in obesity-related metabolic consequences; hormones and cytokines secreted by adipocytes and inflammatory macrophages, many unknown, are important in the pathophysiology. We have used microarray profiling of human adipose tissue before and after 3 ng/kg endotoxin to determine genes modulated by inflammation, and identified several novel and unexplored factors predicted to encode secreted proteins. We have chosen a subset of these for validation and exploration of cellular origin.
Methods: A group of 86 genes of interest, including positive controls, was chosen based on degree of modulation and biologic relevance. These genes were analyzed by RT-PCR in subcutaneous human adipose samples obtained from healthy volunteers(n=7) before and at 4, 12, and 24 hours after low-dose(0.6 ng/kg) endotoxin. Those validated as present and modulated by endotoxin in adipose were studied in 1)differentiated primary human adipocytes 0-48 hours after 100 ng/ml endotoxin vs control and 2)primary human macrophages at resting state and after polarization to classically-activated M1 (overnight treatment with 100 ng/mL endotoxin and 20 ng/mL IFNγ) and alternatively-activated M2 (overnight treatment with 20 ng/mL IL4) phenotypes.
Results: The majority of genes chosen, 64 (75%) were confirmed to be present and upregulated at any time point in human adipose after low-dose endotoxin. Of these (49, 77%) were upregulated by inflammation in primary adipocytes ($p<0.05$). Compared to resting macropages, 55 genes (86%) were upregulated in M1 macrophages ($p<0.05$). Only 16 genes (25%) were upregulated in M2 macrophages, and all to a significantly lesser degree than in the M1 phenotype. Summary of cell source of genes determines that 11% are expressed and modulated by inflammation in adipocytes, 20% from M1 macrophages, 65% from both cell types, and 4% from neither (stromal cells). Many of these genes have not previously been identified or explored in these cell types.
Conclusions: The majority of human adipose genes identified to be modulated by inflammation by microarray analysis were validated by RT-PCR in independent human adipose samples. Almost all of these genes were expressed and modulated in inflammatory adipocytes and/or M1 macrophages, suggesting these cell types as the predominant mediators of adipose tissue inflammation. These genes represent novel candidates for study and treatment of obesity-related adipose inflammation.

FC4-024 Systems Biology & New Technologies

Single nucleotide polymorphism (SNP) array analysis of 47 short children born SGA results in the detection of 5 copy number variants and 2 cases of uniparental disomy

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SNP array analysis is a powerful tool to detect copy number variants (CNVs). A major advantage of SNP array analysis in comparison to other array systems is the extra SNP genotyping information, which enables the detection of copy-number neutral chromosomal aberrations such as uniparental disomies (UPD) and loss of heterozygosity (LOH). UPD can result in rare recessive disorders, or developmental problems due to the effects of imprinting.
Aims: Genome wide screening for CNVs to identify genetic causes of growth disorders.
Patients and methods: We performed an Affymetrix GeneChip® Mapping 250K NspI SNP array analysis in 47 short but otherwise healthy children who were classified as small for gestational age (SGA), and belong to the Tuebingen fraction of the NESTEGG cohort.
An advantage of the Affymetrix array in comparison to competitors is that it can also detect uniparental disomies (UPDs) which are known to be associated with abnormalities of growth.
Results: In 5 children pathogenic CNVs were detected: a 22q11 deletion, a terminal deletion of 15q (resulting in IGF1R haploinsufficiency), a duplication upstream of SHOX (Xp), a duplication in Xq, and a deletion in 2q. In addition, a case with UPD of chromosome 7 (UPD 7) and one with UPD of chromosome 14 (UPD 14) were detected. The child with UPD 7 had a birth weight (BW) of -2.5 SDS, showed a prominent forehead and bone deformities (clubfeet, late dentition). Growth hormone (GH) treatment was started at a height of -2.9 SDS resulting in good catch-up. The patient with UPD 14 was small at birth (weight -4.0 SDS, length -2.5 SDS, and head circumference -2.9 SDS), had typical bone deformities (kyphosis, wedge-shaped vertebra Th 2), precocious puberty and good catch-up on GH.
Conclusion: By means of the Affymetrix SNP array analysis we detected CNVs in 5 and a UPD in 2 out of 47 short children born SGA. This high frequency (14.9%) illustrates that this methodology is suited to investigate cohorts of children with short stature of unknown origin. This diagnostic approach is expected to provide information on novel gene defects associated with short stature.

FC5-025 Autoimmunity & Perinatal

Pulmonary autoimmunity is a feature of autoimmune polyendocrine syndrome type 1 and is associated with reactivity to the bronchial autoantigen KCNRG

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Patients with autoimmune polyendocrine syndrome type 1 (APS-1) suffer from multiple organ-specific autoimmunity with autoantibodies against target tissue-specific autoantigens. Endocrine and nonendocrine organs such as skin, hair follicles, and liver are targeted by the immune system. Despite sporadic observations of pulmonary symptoms among APS-1 patients, an autoimmune mechanism for pulmonary involvement has not been elucidated.
We have recently reported (Proc Natl Acad Sci U S A. 2009;106:4396-401)

a subset of APS-1 patients with respiratory symptoms. Eight patients with pulmonary involvement were identified. Severe airway obstruction was found in 4 patients, leading to death in 2. Immunoscreening of a cDNA library using serum samples from a patient with APS-1 and obstructive respiratory symptoms identified a putative potassium channel regulator (KCNRG) as a pulmonary autoantigen. Reactivity to recombinant KCNRG was assessed in 110 APS-1 patients by using immunoprecipitation. Autoantibodies to KCNRG were present in 7 of the 8 patients with respiratory symptoms, but in only 1 of 102 APS-1 patients without respiratory symptoms. Expression of KCNRG messenger RNA and protein was found to be predominantly restricted to the epithelial cells of terminal bronchioles. One of the patients with severe steroid dependent chronic obstructive respiratory symptoms was switched to the immunosuppressive drug mycophenolate mofetil (1500 mg/day) in 2005. On this regimen, his prednisolone dose could be decreased to 5 mg/day and he is now free of respiratory manifestation with 4 years of follow-up.
We conclude that autoantibodies to KCNRG, a protein mainly expressed in bronchial epithelium, are strongly associated with pulmonary involvement in APS-1. These findings will facilitate the diagnosis, understanding and management of the pulmonary manifestations of APS-1.

FC5-026 Autoimmunity & Perinatal

Neonatal exendin-4 administration normalizes epigenetic modifications at the proximal promoter of proliferator-activated receptor γ coactivator-1α (PGC1-α) in the adult IUGR liver

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Intrauterine growth retardation (IUGR) has been linked to the development of type 2 diabetes in adults. The abnormal metabolic intrauterine environment affects the development of the fetus by permanently modifying gene expression and function of susceptible cells. Uteroplacental insufficiency impairs hepatic mitochondrial function in prediabetic IUGR animals. PGC1-α is a transcriptional coactivator and metabolic regulator that promotes mitochondrial biogenesis and is critical for normal mitochondrial function. However it is not known if PGC1-α expression is also altered in adult IUGR liver. Exendin-4 (Ex4), a long-acting agonist of the glucose dependent insulinotropic hormone (GLP-1), improves mitochondria function, normalizes hepatic insulin resistance, and prevents diabetes in the IUGR rat. The aim of this study was to determine if IUGR alters hepatic PGC1-α expression, if Ex4 normalizes PGC1-α expression, and whether changes in expression are linked to epigenetic modifications at the PGC1-α promoter.
Hypotheses: 1) Hepatic PGC1-α expression is decreased in adult IUGR liver. 2) Neonatal Ex4 treatment of IUGR animals will restore hepatic PGC1-α expression to control levels. 3) Decreased PGC1-α expression is due to silencing histone modifications at the proximal promoter of PGC1-α. 4) Neonatal Ex4 treatment of IUGR animals will reverse aberrant histone modifications at the PGC1-α promoter, thus allowing normal PGC1-α expression.
Methods: IUGR newborn pups were treated with a 6-day course (day 1-6) of Ex4 and liver was harvested for analyses at 8 weeks of age. Gene expression was measured by quantitative (q)-PCR. Histone modifications were evaluated by chromatin immunoprecipitation assays and q-PCR.
Results: PGC1-α gene expression was decreased in IUGR animals compared to control by 80% (p=0.017) and Ex4 treatment normalized PGC1-α expression to levels equal to control samples (p=0.36). In adult IUGR liver, H3 acetylation was 0.16±0.09 of control, H3K4 trimethylation was 0.19±0.05 of control and p300 binding was 0.12±.04 of control at the PGC1-α promoter. Neonatal Ex-4 treatment of the IUGR pups increased acetylation of H3 by 80% (p=0.029), increased trimethylation of H3K4 by 80% (p=0.045) and p300 binding by 95% (p=0.038) at the PGC1-α promoter.
Summary: We speculate that Ex4 normalizes hepatic mitochondrial function by increasing HAT activity, which in turn mediates epigenetic modifications at PGC1-α.

FC5-027 Autoimmunity & Perinatal

From genetics to the switch from insulin injections to oral treatment in neonatal diabetes mellitus/monogenic diabetes of early infancy (NDM/MDI): the experience of the French NDM study group

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NDM/MDI is a rare (around 1/300 000 newborns) but potentially devastating condition. Two main groups have been recognized on clinical grounds, transient NDM (TNDM) and permanent NDM (PNDM), which differ in the duration of insulin dependence early in the disease.
The aim of our study is to unravel the causes of such rare forms of diabetes and to translate this knowledge into a pharmacogenomic approach to improve as much as possible the quality of life of our young patients.
Our study group has begun as a retrospective study and has evolved into a prospective multi-center effort. Patients were recruited on a voluntary basis through a network of physicians involved in the treatment of diabetes mellitus. Between 1995 and 2009, 236 patients with NDM were referred to the French network for the study of NDM. In this cohort, our genetic studies on 184 cases so far have established a genetic diagnosis in 114 patients: 49 with PNDM were explained by a mutation in either *KCNJ11* encoding the Kir6.2 subunit of the b-cell K_{ATP} channel (35%), or the preproinsulin *(INS)* gene (8%), or *ABCC8* encoding the high affinity sulfonylurea receptor SUR1 (5%), or the glucokinase (*GCK*) gene (1%); 65 with TNDM were explained by a chromosome 6 anomaly (48%), or a *ABCC8 (24%) or KCNJ11* (3%) mutation.
Neuromotor and neuropsychological abnormalities were observed in association with the K_{ATP} channel mutations, but not in the groups with chromosome 6 or *INS* anomalies. The most striking clinical implication was the radical change in the treatment of 29 patients with a K_{ATP} channel mutation we were able to switch from insulin therapy to an oral sulfonylurea drug (glibenclamide mostly and glipizide) at our center, in a context approved by the Health Authorities, as the sulfonylureas are contra-indicated in children in France. Insulin injections stopped in 27, with an excellent metabolic control (mean HbA1C from 8.2 (5.9-10.1) to 6.5 (5.1-9) % after the switch), decrease in the insulin dose in one, but was unsuccessful in one patient.
These recent advances in the field of NDM illuminate how the molecular understanding of some monogenic forms of diabetes may lead to an unexpected improvement of the treatment in children affected by this condition.
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FC5-028 Autoimmunity & Perinatal

Permanent neonatal diabetes caused by compound heterozygous SUR1 mutations with opposite functional effects

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Introduction: Neonatal diabetes is a rare disorder defined as diabetes mellitus occurring in the first 6 months of life. Heterozygous activating mutations in the KCNJ11 gene encoding the Kir6.2 subunit of the pancreatic beta cell KATP channel are the most common cause of permanent neonatal diabetes (PNDM). In addition, heterozygous activating mutations in the ABCC8 gene encoding the SUR1 regulatory subunit of this channel as well as mutations in the insulin (INS) gene have been recently reported.

Subjects and Methods: We describe a patient born after sperm donation to a healthy mother after an uneventful pregnancy. At the age of 3 months he presented with fever, convulsions and persistent hyperglycemia. HbA1C was 9.9%, anti-GAD antibodies were negative. Insulin treatment was started. Genetic analysis: Genomic DNA was extracted from peripheral lymphocytes, and direct sequence of the KCNJ11, ABCC8 and INS genes has been undertaken.
Results: Genetic analyses of the Kir6.2 and INS were normal. A compound heterozygote mutation for a novel missense mutation, S459R in exon 9 and a splicing mutation, c.3992-9G>A in intron 32, of the ABCC8 gene were found. The S459R in exon 9 is predicted to be an activating mutation. The G>A mutation at nucleotide c.3992-9 (c.3992-9G>A) is an inactivating mutation and has previously been reported in patients with **congenital hyperinsulinism**. This is one of the 2 mutations which accounts for more than 90% of cases of congenital hyperinsulinism in the Ashkenazi Jewish population. The S459R mutation is conserved across species and is on opposite parental chromosomes (in trans). The mother was found to be a carrier of the S459R mutation.
Conclusions: This is the first disease phenotype reported to be a result of compound heterozygosity for both gain- and loss-of-function mutations i.e a mutation causing neonatal diabetes and a mutation causing congenital hyperinsulinism, respectively. The phenotype is of neonatal diabetes. Treatment with sulphonylurea was initiated and resulted in improved glycaemic control (HbA1C 6.5% compared with 9.4% during treatment with an insulin pump). We predict that the sperm donor is heterozygous for the c.3992-9G>A mutation and is therefore a carrier of congenital hyperinsulinism/neonatal diabetes. Appropriate genetic counseling was given.

FC5-029 Autoimmunity & Perinatal

Hyperaldosteronism, hyponatremia and hyperkalemia are associated with a low renal mineralocorticoid receptor expression in healthy newborns: evidence for physiological aldosterone resistance

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The human kidney is characterized, in the neonatal period, by a functional immaturity, responsible for impaired sodium reabsorption, resembling partial aldosterone resistance. To demonstrate this neonatal hormonal resistance, we conducted a prospective study in healthy newborns and their mothers.
A highly significant difference was observed between aldosterone and renin levels in newborns compared to their mothers (817±73 vs 575±55 pg/ml and 79±10 vs 15±2 pg/ml, respectively, P<0.001). These results contrasted with signs of functional hypoaldosteronism in newborns: hyponatremia (132.6±0.7 mmol/l), hyperkalemia (5.7±0.3 mmol/l) and urinary sodium loss (urinary Na+/K+= 2.0±0.4; Nau/creatu = 19.6±8.3), consistent with a partial aldosterone resistance at birth. Reference values for urinary aldosterone concentrations in neonates were also determined for the first time (106±10 pg/µg creat), and appeared as the best index for accurate evaluation of mineralocorticoid sensitivity at birth, at variance with plasma aldosterone.
Since aldosterone renal effects are mainly mediated through its binding to the Mineralocorticoid Receptor (MR), we next hypothesized that this hormonal resistance could be related to a weak MR level in the distal nephron. We analyzed its mRNA and protein expression, using two complementary methods (qPCR and immunohistochemistry), during renal development. We demonstrated that, both in mouse and human, MR mRNA and protein expression follows a biphasic temporal profile with a transient peak during gestation (between 15 and 24 gestational weeks in human and at E18 in mice), a low expression at birth and a progressive increase during the postnatal period. This cyclic renal expression was specific to the mineralocorticoid signaling pathway, as MR expression was tightly correlated with the evolution of the 11 beta-hydroxysteroid dehydrogenase type 2, which confers specificity to the aldosterone-MR signaling pathway and the alpha subunit of the Epithelial sodium Channel, one major MR target gene, at variance with the expression of other markers of the distal nephron (Glucocorticoid Receptor, Aquaporin 2 and Arginine-vasopressine Receptor).
Herein, we demonstrate, for the first time, that a low MR expression level at birth could account for the partial aldosterone unresponsiveness in neonates. These results could ultimately lead to new therapeutic strategies for the management of sodium loss in preterms and neonates.

FC5-030 Autoimmunity & Perinatal

Novel *GLIS3* mutations in two patients with resistant hypothyroidism, neonatal diabetes, hepatitis, renal cystic dysplasia, osteopenia and pancreatic exocrine dysfunction

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Mutations in the *GLIS3* gene (encoding the transcription factor GLI similar 3) are a rare cause of neonatal diabetes and congenital hypothyroidism with 6 affected cases from 3 families reported to date. Additional features include congenital glaucoma, hepatic fibrosis, polycystic kidneys and facial dysmorphology. We report two new cases:
Patient 1 was born to first cousins at 35 weeks gestation weighing 1.17kg. She developed diabetes on day 3 and was commenced on an insulin pump. Initial TSH was >150 mU/l and FT4 4.3pmol/l. Despite using supraphysiological doses of daily thyroxine followed by IV liothyronine her TSH remained elevated. No thyroid tissue was identified on neck ultrasonography although thyroglobulin measured 170 micrograms/L. Thyroid recovery followed initiation of thyroxine 30mcg/kg/day divided into four doses at 114 days. She also had renal cystic dysplasia, corneal clouding, neonatal hepatitis, osteopenia with a severe thoracolumbar scoliosis and pancreatic exocrine dysfunction.
Patient 2 was born to non-consanguineous parents at 35 weeks gestation with a birth weight of 1.44kg. He developed diabetes on day 3, treated with continuous IV insulin and then converted to an insulin pump. His initial TSH was 898mU/l and FT4 2.7pmol/l and thyroglobulin measured >500 micrograms/l. Suppression of TSH proved difficult despite normal FT4 measurements on 14mcg/kg/day thyroxine. He also had polycystic kidneys and hepatitis but no eye or pancreatic exocrine disease.
Homozygous partial *GLIS3* deletions were suggested by the failure of PCR amplification in the probands. Gene dosage analysis showed that the parents were carriers of a deletion encompassing exons 1-2 (case 1) and exons 1-4 (case 2) of the 11 exon gene. Genome wide SNP analysis did not reveal a common ancestral *GLIS3* haplotype in patient 2.
Our results confirm partial gene deletions as the most common type of *GLIS3* mutations, accounting for 4 of 5 families identified to date. Both patients have neonatal diabetes, severe resistant hypothyroidism in the presence of elevated thyroglobulin, hepatic dysfunction, renal dysplasia and facial dysmorphism. Osteopenia and pancreatic exocrine dysfunction have not previously been associated with *GLIS3* mutations. We report the first case of a recessive *GLIS3* mutation causing neonatal diabetes and congenital hypothyroidism in a child from a non-consanguineous pedigree, highlighting the importance of molecular genetic testing in any patient with this phenotype.

FC6-031 SGA & Turner

Independent effects of weight gain and fetal programming on the metabolic complications in smal for gestational age (SGA) adults

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Introduction: The relationship between a low birth weight and the development of insulin resistance (IR) and the Metabolic Syndrome (MS) has been well

documented at different periods of life. But little is known on the progression of MS in young adults born SGA.
Aim: We have previously reported an increased gain of fat mass with ageing in young adults born SGA beyond the simple effect of time. We hypothesized that being born SGA would promote a marked progression of IR and MS not only reflecting this gain of fat mass but reflecting also fetal programming.
Subjects and methods: 1273 subjects selected from a community-based cohort, all born full-term, either SGA (BW< 10th p) or appropriate for gestational age (AGA) (25th <BW<75th p) were prospectively followed over 7.5 yr. Metabolic syndrome was assessed using the WHO definition.
Results: All comparisons are adjusted on gender, age, BMI, socio-economical status and family history of metabolic disorders. * Same model with delta BMI instead of BMI

	AGA (n=697)	SGA (n=576)	p value
Baseline			
Age (years)	22.1 ± 3.8	22.0 ± 3.8	0.78
Sex ratio %	47/53	45/55	0.68
BMI (kg/m2)	22.6 ± 3.7	22.4 ± 4.2	0.39
MS n (%)	1 (0.14)	15 (2.60)	0.008
HOMA-IR	1.02 ± 0.5	1.18 ± 0.9	0.001
Follow-up			
Follow-up duration	7.5 ± 2.6	7.4 ± 2.5	0.49
BMI (kg/m2)	24.1 ± 4.3	24.2 ± 5.3	0.65
Delat BMI (kg/m2)	+ 1.5 ± 2.4	+ 1.8 ± 2.7	0.02
Fat mass (%)	22.1 ± 8.2	23.1 ± 8.9	0.04
HOMA-IR	1.27 ± 0.6	1.41 ± 0.8	0.004
Delta HOMA-IR	0.25 ± 0.6	0.24 ± 0.9	0.61/0.28*
MS n (%)	34 (4.9)	51 (8.9)	0.05/0.08*

All comparisons are adjusted on gender, age, BMI, socio-economical status and family history of metabolic disorders. * Same model with delta BMI instead of BMI

Conclusion: At both visits, subjects born SGA are more insulin resistant and have a significantly higher prevalence of MS in comparison to those born AGA.Our data suggest that being born SGA induces early metabolic disorders, further amplified by the weight gain with time when adults, both probably resulting from fetal programming. Moreover, the modest increase in IR contrasts with the constant and much higher prevalence of MS, suggesting that the metabolic complications are not related only to the degree of fat mass but that fetal programming strongly affects the physiology of the adipose tissue.

FC6-032 SGA & Turner

Long term effects of prematurity on glucose metabolism and body composition in this generation and the next

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Background: Very premature(<32 weeks gestation) children and young adults have been shown to have reduced insulin sensitivity (S_I). It is unclear whether less premature and older adults have alterations in glucose metabolism and body composition. Furthermore, despite evidence of intergenerational effects in low birth weight models there is no data in the term offspring of preterm parents.
Objectives: Evaluate (i) S_I, insulin secretion and body composition in prematurely born adults (<37 weeks gestation) as compared to those born at term. (ii) S_I and body composition in their healthy prepubertal children.
Methods: Preterm and term adults aged 34-38 years and their healthy offspring aged 5 to 10 years were recruited. S_I was assessed using either a sampled intravenous glucose tolerance test (children) or hyperglycaemic clamp (adults) and DEXA scans used to measure body composition.
Resuts: Expressed as Mean (SEM). 52 adults participated (31 preterm, mean gestational age 33.3 weeks). Compared with those born at term those born preterm had reduced S_I (19±2.5 vs 36.3± 5.2 $mg.kg^{-1}min^{-1}mu.1^{-1}x$ 100; p=0.002, increased first phase insulin secretion (56.1± 8.5 vs 25.3± 3.7 mu/L;P=0.003), second phase insulin secretion (75.2± 10.7 vs 36.2± 4.3; p=0.005) and increased total body fat (34.3± 1.6 vs 29.5± 1.95 %; p=0.05) especially abdominal fat (41.98± 1.7 vs 33.2± 2.1 %; p=0.003). There was a gender effect with preterm men being much fatter. Compared to control men of a similar height preterm men were on average18 kg heavier.
61 children participated (37 of preterm parent). Children of preterm parents had similar S_I to those of term parents (12.6± 1 vs 14.6± 1.7 $10^{-4}/min^{-1}$ mU/l; p=0.32) but had increased total body fat (22.7± 1.5 vs 18.2±1.6%; p=0.05) and abdominal fat (27.4± 2.1 vs 19.9±2.2%; p=0.02).
Conclusions: Even mild prematurity results in substantial reduction in insulin sensitivity as well as increased total and abdominal fat mass during mid-adulthood. There is no evidence of impaired beta cell function. Offspring of preterm parents have alterations in body composition suggesting intergenerational inheritance.

FC6-033 SGA & Turner

Genetic association analysis of 10 candidate genes in a large multinational cohort of SGA children and children with idiopathic short stature (NESTEGG)

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Background:
The multinational Network of European Studies in Genes in Growth (NESTEGG) is an international growth genomics project, focussing on small for gestational age (SGA) and idiopathic short stature (ISS) phenotypes, compared to healthy controls. We hypothesized that minor genetic changes (polymorphisms) in several growth-related genes might explain part of the phenotype of children born SGA or children with ISS.
Objective:
To study 226 SNPs in10 genes involved in placental development, pre- and/or postnatal growth and glucose metabolism: *GH1, GHR, IGF-I, IGF-IR, STAT5A, STAT5b, MAPK1, MAPK3, PPARg* and *genes in the INS region*.
Design/Patients:
Seven hundred controls and 1275 cases with their parents were recruited, from 4 countries. Subjects were included when born SGA with or without catch-up growth to a normal height or born appropriate for gestational age with ISS or normal height, based on local references. All were white Europeans (all grandparents and parents must have been born and living in Western Europe), born after 30 or more weeks of gestation with an uncomplicated postnatal period and had signed the informed consent form. Genotypes were determined using the Taqman allelic discrimination assay.
Results:
We found many significant associations between parameters of placental development, pre- and/or postnatal growth, glucose homeostasis and blood pressure and polymorphisms (and haplotype blocks) in the 10 candidate genes, which have not been previously reported. This suggests that these polymorphisms might explain part of the phenotype of children born SGA and with ISS. The vast majority of the SNPs with highly significant associations had not been previously studied.
Conclusions:
Our findings suggest that polymorphisms in the candidate genes *GH1, GHR, IGF-I, IGF-IR, STAT5A, STAT5b, MAPK1, MAPK3, PPARg* and *INS region* might explain part of the phenotype of children born SGA (with or without catch-up growth) and children with ISS.

FC6-034 SGA & Turner

Evaluation of liver stiffness using transient elastography (fibroscan) in young adults with Turner syndrome

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Transient elastography (TE) is a novel, non-invasive technique, recently used to assess hepatic fibrosis in patients with chronic liver disease by measuring liver stiffness. It is widely validated in patients with chronic hepatitis C. TE has never been used in TS patients although in this setting of patients liver involvement is a frequent finding and its etiology is still unclear. We included in our cross-sectional study 22 TS girls with a mean age of 20.9 years (range 13.6 – 40) and full pubertal development (spontaneous or pharmacological). All patients, at fasting, underwent TE and the following biochemical tests: glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamil transferase (GGT), alkaline phosphatase (ALP), cholesterol, triglycerides, HDL, bilirubin, platelet count, serum albumin, prothrombin time. Our results showed: 6/23 TS patients (26 %) had elevated aminotransferase levels, the average stiffness value in the entire study group was 4.5 ± 1.8 kPA, being significantly higher in patients with elevated aminotransferase levels than in those with normal liver function (6.0 ± 2.9 vs 4.0 ± 0.9, p = 0.024). No significant differences in terms of average stiffness was found among the patients with either monosomy (4.2 ± 1.1 kPA) or mosaicism (4.6 ± 1.7 kPA) or structural anomalies of X chromosome (5.2 ± 3.2 kPA). We found a strong correlation between TE and AST (r = 0.558, p = 0.007), GGT (r = 0.817, p = 0.0001), BMI (r = 0.517, p = 0.014), insulin (r = 0.536, p = 0.032), triglycerides (r = 0.688, p< 0.0001), HDL (r= - 0.435, p = 0.049). BMI didn't differ between TS patients with normal or elevated liver enzymes (23.8 ± 4.3 vs 25.2 ± 4.4, p = 0.520).

Conclusions: although liver biopsy is the gold standard method for assessing liver fibrosis, it is an invasive, very expensive procedure that can also have life-threatening complications and for this reason cannot be used in all the patients and cannot be considered a useful follow-up tool.

According to our results, TE correlates with aminotransferase levels in young individuals with TS and then can be used for selecting among individuals with elevated aminotransferases those who deserve more invasive tests, as well as liver biopsy, in order to better staging the etiology of liver involvement. TE may also be used to monitor the progression of liver dysfunction in TS patients without exposing them to significant clinical risks and invasive procedures.

FC6-035 SGA & Turner

Partial anomalous pulmonary venous return (PAPVR) in adolescent and young adult Turner syndrome (TS) patients: prevalence and hemodynamic significance as determined by cardiac magnetic resonance imaging (CMR)

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Background: Using echocardiography, the prevalence of PAPVR in women with TS has been estimated to be up to 3.5%, more than 1000X the normal population. Recent data in adult TS patients, utilizing CMR, suggests a prevalence of 13- 25%. Our primary objective was to determine the prevalence and hemodynamic significance of PAPVR in adolescents and young adults with TS utilizing both echocardiography and CMR. In addition, we sought to determine if there is an association between PAPVR and TS phenotype.

Methods: Medical records of 39 adolescent and young adult TS patients who underwent CMR and echocardiographic evaluation were reviewed for cardiac anatomy and functional measurements. Additional TS features, including karyotype, were obtained from our clinical database of TS patients. Continuous and categorical variables were compared using unpaired t-tests and chi-square tests, respectively.

Results: PAPVR was diagnosed in 7 (18%) subjects, six newly diagnosed by CMR following normal echocardiogram. Aberrant drainage of either the right or left upper pulmonary veins was seen in all PAPVR subjects, in addition to at least a portion of the right middle lobe vein in three. In one subject, PAPVR was associated with clinically significant enlargement of the right ventricle (RV:LV volume ratio = 1.8:1) and a pulmonary:systemic blood flow ratio (Qp:Qs) of 1.9:1, prompting surgical repair. In the other PAPVR subjects, Qp:Qs ranged from 1.24 – 1.62:1 and right:left ventricular end-diastolic volume ratios (RV:LV EDV) of 1.05-1.66, with no intervention pursued. Shown in Table 1, ventricular volume ratios and Qp:Qs were different for those subjects with and without PAPVR; age, height standard deviation, and right ventricular ejection fractions (EF) were not statistically different. Furthermore, karyotype, peripheral lymphedema, neck webbing, and the presence of renal malformations were not predictive of the presence of PAPVR.

	PAPVR (n=7)	Normal Pulmonary Veins (n=32)	P value
Age	15.4±3.4	19.4±6.8	0.14
Height SD	-1.59±0.71	-1.45±0.95	0.72
RV EF (%)	66.7±9	69.7±6.9	0.48
Qp:Qs	1.5±0.2	1±0.1	0.0007
RV:LV EDV	1.4±0.2	1±0.1	<0.0001

Discussion: The prevalence of PAPVR is high in the TS population. PAPVR appears independent of karyotype and phenotype, and may be hemodynamically significant. Careful screening, and appropriate cardiac referral and management are indicated.

FC6-036 SGA & Turner

Determinants of medical care for young women with Turner syndrome: a population-based study

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Turner syndrome is associated with reduced life expectancy. Lifelong follow-up is strongly recommended, but follow-up during the transition between pediatric and adult care has been little evaluated.

Objective. To evaluate the medical follow-up of a population-based cohort of young adult patients.

Design. Questionnaire study.

Setting and Patients. A national cohort of 568 women, aged 22.6±2.6 years (range, 18.3-31.2), six years (mean) after stopping growth hormone treatment (StaTur cohort).

Main Outcome Measure(s). Proportion of patients with adequate follow-up at seven medical assessments (blood pressure, blood glucose concentration, determinations of thyroid hormones, liver enzymes and lipid concentrations, audiometry and heart imaging) over four years and its determinants.

Results. Most participants were followed by gynecologists or general practitioners. Medical assessments were performed in 16% (audiometry) to 68% (lipid level determinations) of participants, with little consistency in individual patients. Only 20 of 568 (3.5%) patients underwent all assessments in the four-year period. Multivariate analysis identified the type of physician as the only factor consistently associated with follow-up, which was better with endocrinologists than with other physicians. Other variables associated with at least one adequate follow-up assessment were paternal socioeconomic class, education level, number of Turner syndrome disease components, size of the medical center following the patient in childhood, and physical health dimensions of SF-36 scores.

Conclusions. By contrast with the intensive medical follow-up in childhood, follow-up was grossly inadequate during the transition phase. During this phase, patients should be sent to physicians specializing in Turner syndrome and particular attention should be paid to patients with lower levels of education from families of low socioeconomic status. Although recommendations for the transition between pediatric and adult care have been made for several endocrine diseases of childhood, this is the first evidence-based analysis of the determinants of actual transition.

FC7-037 Pancreas & Glucose Regulation

Successful islet autotransplant permits normal glucose regulation without severe hypoglycemia in pediatric patients undergoing total pancreatectomy for chronic pancreatitis

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Background: Total pancreatectomy (TP) may be indicated for definitive treatment of chronic pancreatitis refractory to medical interventions. The goal of simultaneous islet autotransplant (IAT) is to prevent or minimize post-surgical diabetes. Evidence from the Diabetes Complications and Control Trial suggests that residual beta-cell function in type 1 diabetes allows for tighter glycemic control at lower insulin doses with fewer hypoglycemic episodes and lower risk of microvascular complications. The goal of this prospective study is to determine the impact of the IAT on postsurgical glucose regulation in pediatric patients undergoing TP.

Methods: Insulin use and islet function was assessed in 10 patients (age 5- 18 years) before TP and IAT and at 3, 6, and 12 months postoperatively. Labs include hemoglobin A1c (HbA1c) levels and a 2-hour mixed meal tolerance test (MMTT, 6mL/kg Boost HP, maximum 360 mL). Continuous variables were compared using non-parametric tests (Wilcoxin rank sum).

Results: Patients received a mean of 3,805 ± 1,922 islet equivalents/ kg body weight (IE/kg) at the time of transplant. Of these 10 IAT recipients, 5 became insulin independent at a mean of 4.5 ± 4.2 months posttransplant, although low dose glargine was subsequently resumed in 1. The only 2 preadolescents (age 5 and 9 years) achieved insulin independence. Patients requiring no insulin or minimal insulin (<0.25 u/kg/day) maintained a normal HbA1c level in the absence of severe hypoglycemic episodes. Those patients requiring 0- 0.25 u/kg/day of insulin had a lower mean HbA1c level and trend towards greater C-peptide response to MMTT compared to patients on >0.25 u/kg/day (table 1).

Insulin use at 6-12 months:	0- 0.25 u/kg/day	>0.25 u/kg/day	p-value
N=	6	4	
IE/kg	4,840 ± 1,380	2,250 ± 1,590	0.07
HbA1c (%)	5.7 ± 0.3	8.6 ± 2.3	0.05
Delta C-peptide (ng/mL)	3.2 ± 1.0	0.4 ± 0.2	0.12

Conclusions: In conclusion, certain diabetes with complete insulin dependence would have resulted if these patients had undergone pancreatectomy alone. IAT allowed good metabolic control in the majority. Consistent with our previous findings that insulin independence rates are highest in preadolescents, the 2 youngest patients in this series both discontinued insulin after IAT. Endogenous insulin secretion likely promotes tight glycemic control in these patients while protecting them from severe hypoglycemia.

FC7-038 Pancreas & Glucose Regulation

Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism

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Background: Congenital hyperinsulinism (CHI) is a clinically heterogeneous condition. Mutations in seven genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1 and HNF4A*) are known to cause CHI. The phenotype and prevalence of the genetic subgroups in a large cohort of patients has not been studied previously.

Aim: To characterise the clinical and molecular aspects of a large cohort of patients with CHI.

Methodology: 300 patients with biochemically confirmed CHI were recruited. Detailed clinical information was collected prior to genotyping. *ABCC8 and KCNJ11* genes were sequenced in all patients with diazoxide unresponsive CHI. Mutations in the *GCK, GLUD1 and HADH* genes were sought in patients with diazoxide responsive CHI with hyperammonaemia (or leucine sensitivity (*GLUD1*)), raised 3-hydroxybutyryl-carnitine (*HADH*) or positive family history with delayed presentation (*GCK*). If no mutations were identified and in all other patients with diazoxide responsive CHI; *ABCC8, KCNJ11 and HNF4A* genes were sequenced.

Results: Mutations were identified in 140/300 patients (46.6%). Mutations in the *ABCC8/KCNJ11* were the commonest genetic cause identified (n=112,37.3%). Among diazoxide unresponsive patients (n=96), mutations in these two genes were identified in 81(84.3%); of whom 51 patients had recessively inherited mutations while four patients had novel dominantly inherited mutations. A paternal mutation in the *ABCC8/KCNJ11* genes was identified in 25 diazoxide unresponsive patients; of whom 15 had confirmed focal disease, 5 had diffuse disease, 1 had atypical disease and the histological differentiation is not known in the remaining four patients. Among the diazoxide responsive patients (n=204), mutations were identified in 59 patients. These include mutations in the *ABCC8(n=28), KCNJ11(n=3), GCK(n=2), HNF4A(n=10), GLUD1(n=13) and HADH*(n=3). No mutations were identified in 145(71%) patients in this group.

Conclusions: This is the largest group of patients with CHI that has been systematically phenotyped and genotyped. A genetic diagnosis was possible in only 46.6% of patients with mutations in the *ABCC8* gene being the commonest cause. The vast majority of patients with diazoxide responsive CHI had no mutations identified suggesting other novel mechanisms of insulin secretion. Understanding the genetic aetiology of CHI in this large cohort of patients will provide novel insights into pancreatic beta-cell physiology and have implications for hypoglycaemia and diabetes mellitus.

FC7-039 Pancreas & Glucose Regulation

New insights into glucose metabolism in cystic fibrosis (CF)

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We aimed to assess the evolution towards CF related diabetes(CFRD), verifying if changes were related with age, genotype, and/or severity of the disease, and establish if insulin-resistance(IR) contributed to its onset. We evaluated relationships between markers of insulin-sensitivity and adiponectin, resistin, IL-6, TNF-α and IGF-I.

119 pts were enrolled and subdivided in 3 age-groups (prepubertal N:59,aged 5-12 yr; pubertal N:17, 12-17,4 yr; adult N:43, 19-43,3yr), in 3 groups based on genotype (homozygote F508del N:47, heterozygote F508del, N:47, other N:25) and in 2 groups based on Schwachman-Kulczycki clinical score (excellent/good, N:75; moderate, N:44).

94 normal-weight healthy controls (aged 4-36,3yr), comparable for sex and age were enrolled.

All pts underwent a 5 point OGTT, and 57 also an IVGTT for the calculation of first phase (FPIR) and acute insulin responses (AIR). Pts and controls had baseline blood samples taken for glucose and insulin, pts for C-peptide, and Glycated Hb. HOMA-IR, QUICK-I, Fasting Glucose Insulin ratio(FGIR), and insulinogenic index were calculated as indexes of IR and pancreatic β cell function. Cytokines and IGF-I were assayed in serum using specific research assays.

The homozygous pts had a higher chance of developing impaired glucose tolerance (51%) and CFRD, a significantly lower FPIR, increased Glycated Hb although normal, decreased HOMA-IR, QUICK-I, and insulinogenic index. Peak insulin concentrations (OGTT) were increased from puberty onwards. Pts had significantly higher resistin, IL-6 and TNF-α, while adiponectin was unchanged compared with controls.

HOMA-IR correlated with FPIR (R:+0,45) and IGF-I (R:+0,42). FPIR correlated with QUICK-I (R:-0,61), insulinogenic index (R:+0,64), and IGF-I (R:+0,37). AIR correlated with FGIR (R:-0,6), and C Reactive Protein

(R:+0,41). Resistin correlated with IL-6 (R:+0,6), and FEV1 (R:-0.43).
In conclusion, age, inflammatory status and severity of the disease did not have a clear impact on insulin deficiency whereas genotype did. This suggests in addition to recent in vitro data a possible alteration in insulin secretion. A non-classical IR from puberty onwards was observed which could suggest unknown insulin signal transduction defects. Indexes of insulin sensitivity were related with IGF-I, resistin, and IL-6 suggesting anyway an effect of both the IGF system and inflammation.
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FC7-040 Pancreas & Glucose Regulation

Pigmentary hypertrichosis and insulin dependent diabetes mellitus (PHID) is caused by mutations in the *SLC29A3* gene encoding the human equilibrative nucleoside transporter-3 protein (hENT3)

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Background: PHID syndrome has been recently described as a unique syndrome characterised by **Pigmented H**ypertrichosis; non immune mediated **Insulin depended D**iabetes mellitus (DM). Other clinical features of the syndrome include pancreatic exocrine insufficiency, short stature and pubertal delay. The history of consanguinity in the parents and the similarity of clinical features in the patients are suggestive of an autosomal recessive inheritance pattern.
Aim: To identify the genetic basis of PHID syndrome in six patients from five unrelated families.
Methods: Homozygosity mapping was performed in all 5 families followed by candidate gene sequencing in our cohort of 6 patients. Functional studies were performed on the diseased fibroblasts followed by knockdown of drosophila dENT3 protein.
Results: Single nucleotide polymorphism microarray genotype analyses from the patients identified a single common 1.4Mb region of shared homozygosity within cytogenetic band 10q22.1. Five loss of function mutations were found in *SLC29A3* gene at chromosome 10q22.1 (3 missense, 1 frameshift and 1 nonsense). The *SLC29A3* gene encodes for a highly conserved protein (hENT3) which is essential for nucleotide synthesis by salvage pathways in cells that lack de novo pathways and are required for the cellular uptake of cytotoxic nucleosides used in cancer and viral chemotherapy. Functional studies from diseased fibroblasts revealed a 34% reduction in the concentration of hENT3 mRNA and defect in cellular trafficking of residual protein compared to control fibroblasts. Ubiquitous knockdown of the Drosophila ortholog of hENT3, dENT3 induced scutellar bristle phenotype similar to previously reported in knockdown of the ortholog of *islet* gene and a reduction of cell size/number through interactions with the insulin signalling pathway.
Conclusion: Inactivating mutations in the human *SLC29A3* gene cause a novel Mendelian disorder associated with insulin dependent DM and pigmented hypertrichosis. Mutations in *SLC29A3* lead to alterations in cell size/number possibly via the insulin signalling pathway. Given the marked phenotypic heterogeneity of the PHID syndrome other patients with unexplained syndromic forms of DM may have mutations in *SLC29A3.* Further studies are required to understand the genotype and phenotype correlations in this syndrome and to determine the physiological role of normal and mutated hENT3 protein in humans.

FC7-041 Pancreas & Glucose Regulation

A novel syndrome of partial lipodystrophy and ketosis-prone diabetes due to a homozygous mutation in *CIDEC*

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Introduction and aims: Genetic lipodystrophies, characterized by a significant lack of subcutaneous adipose tissue, are an unusual cause of insulin-resistant diabetes in pediatrics. To date, at least 7 different loci have been identified (*AGPAT2, BSCL2, CAV1, LMNA, PPARG*, *AKT2 and ZMPSTE24*), but the causative gene remains unknown in a significant proportion of cases. We aimed to exclude a new candidate gene, *CIDEC*, as a cause of partial lipodystrophy. It is predominantly expressed in white adipocytes and encodes one of the newly identified lipid droplet-associated proteins required for the precise regulation of lipid traffic into and out of these organelles.
Methods and Results: The coding regions and splice junctions of *CIDEC* were amplified and sequenced in 139 probands with unexplained partial lipodystrophy. A homozygous nonsense mutation (c.556G> T, p.Glu186X) in *CIDEC* was found in a Hispanic female who presented with diabetic ketoacidosis at 14 years. At presentation, she was noted to have partial lipodystrophy with muscular lower limbs and severe acanthosis nigricans. Pancreatic antibodies were absent. Within the next 18 months she developed severe dyslipidemia, which ultimately caused two episodes of acute pancreatitis and diabetic ketoacidosis. A total body MRI confirmed the absence of lower limb and femorogluteal fat pads and the preservation of visceral, neck and axilliary fat as well as an enlarged steatotic liver. Leptin and adiponectin levels were correspondingly low. She has recently developed persistent microalbuminuria and hypertension. An adipose tissue biopsy from the patient revealed numerous multilocular white adipocytes. Functional *in vitro* studies showed that, in contrast to wild type CIDEC, the mutant protein does not localize to lipid droplets. No mutation was identified in the remaining patients.
Conclusion: We describe the first patient with partial lipodystrophy and diabetes secondary to a loss of function mutation in *CIDEC*, demonstrating that CIDEC is required for the formation of unilocular white adipocytes and optimal energy storage in adipose tissue in humans.

FC7-042 Pancreas & Glucose Regulation

The classical intracellular targets of leptin, SOCS3 and PTP1B, may modulate insulin signaling in the rat hypothalamus

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Leptin and insulin regulate energy homeostasis and body weight at the hypothalamic level. Obese subjects generally show hyperleptinemia and hyperinsulinemia, and are often resistant to the effects of both hormones. Elucidation of their signal transduction mechanisms has demonstrated that intracellular cross-talk occurs in response to these two hormones at the hypothalamic level, although the precise mechanisms linking leptin and insulin resistance remain poorly characterized. Our aims were to investigate the influence of chronic

leptin infusion on insulin signaling in rat hypothalamus and to analyze the eventual existence of shared intracellular mediators for insulin and leptin resistance. Adult male Wistar rats were treated ICV for 14 days with either saline or leptin (12 μg/day) followed by an acute injection of either insulin (10 mU) or saline, and were killed 15 minutes later. This resulted in the following 4 groups (n=5): (I) chronic saline, acute saline (II) chronic leptin, acute saline, (III) chronic saline, acute insulin and (IV) chronic leptin, acute insulin. Immunoblotting was used to determine protein levels of the β chain of the insulin receptor (IRβ), protein tyrosine phosphatase 1B (PTP1B) and suppressor of cytokine signaling 3 (SOCS3) in the hypothalamus. The associations between IRβ and IRS-2 and between IRS-2 and the regulatory subunit of PI3K (p85) were studied by immunoprecipitation. Leptin increased PTP1B levels ($p<0.05$), decreased the association between IRβ and IRS, and blunted the insulin-induced increase in IRβ ($p<0.05$). Insulin increased and leptin decreased the association between IRβ and IRS2, with the combination resulting in a significant decrease in this association ($p<0.05$). Both leptin and insulin increased the association between IRS2 and p85 ($p<0.05$) and this effect was not additive. Insulin reduced SOCS3 levels ($p<0.05$), but this reduction was lower when administered after leptin infusion ($p<0.05$ versus insulin). In conclusion, leptin could reduce the initial steps of insulin signaling by inhibiting PTP1B. Furthermore, reduction of SOCS3 levels could potentiate the interaction between IRS2-p85.

FC8-043 Adrenal

Newborn screening for congenital adrenal hyperplasia (CAH) using tandem mass spectrometry (MS/MS) as a primary screen

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Introduction: A method for newborn screening (NBS) for Congenital Adrenal Hyperplasia (CAH) using MS/MS was developed for the Province of Manitoba, Canada, a province that has over 20 years of experience screening for CAH using different technologies.

Methods:17-OH-progesterone (17-OHP), androstenedione, and cortisol were eluted from 3/16" DBS using a TLX-2 Turbulent Flow System (Thermo Fisher), and analyzed by an API-4000 MS/MS (Applied Biosystems) as the primary screen for CAH, not a second tier test for confirmation. Results were compared to the DELFIA immunoassay screen for 17-OHP to evaluate performance.

Results: 2722 sequentially collected samples were analyzed both by immunoassay for 17-OHP, and for three steroids by MS/MS. 3,1% of samples analyzed by immunoassay screen positive and require further investigation. In contrast, 18/2,722 samples by MS/MS had 17-OHP concentrations above the cutoff of 25 nmol/L, a total of 0.7%. When the steroid ratio was also calculated for these 18 samples, only 3 had a ratio of greater than 2.5. These were confirmed true positive CAH cases. The referral rate for further investigation of the 2,722 samples was 0.11% when samples were analyzed by MS/MS, a 95% reduction in the follow up rate compared to the immunoassay.

Conclusions: The three steroid NBS assay for CAH using MS/MS as a primary screen is rapid, sensitive, and performs significantly better than the DELFIA immunoassay. Measuring 3 steroids by MS/MS as a primary screen for CAH eliminated the need for a second tier test to rule out CAH. Future work will compute clinical test performance on larger numbers of participants and analyze economic outcomes.

FC8-044 Adrenal

Novel P450c17 mutation H373D causing combined 17α-hydroxylase/17,20-lyase deficiency

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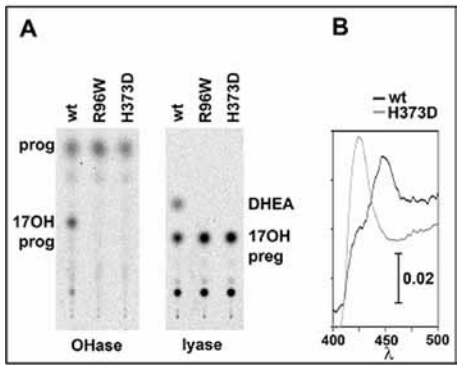
Combined 17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare form of CAH, typically presenting with hypertension and sexual infantilism. It is caused by defects in cytochrome P450c17, encoded by the *CYP17A1* gene. P450c17 catalyzes 17α-hydroxylation of pregnenolone (Preg) to 17-hydroxypregnenolone (17OH-Preg) and of progesterone (Prog) to 17-hydroxyprogesterone (17OHP); its 17,20 lyase converts 17OH-Preg to DHEA, but not 17OHP to androstenedione (JBC 273: 3158, 1998). A 14-yo 46,XX female presented with sexual infantilism and hypertension. She had K=3.3 mEq/L and undetectable PRA. An ACTH stimulation test showed very high corticosterone and 18-OHcorticosterone, slightly elevated Preg, Prog and DOC, and low 17OH-Preg, 17OHP, cortisol, 11-deoxycortisol, aldosterone and DHEA (Table). The coding regions of *CYP17A1* were amplified by PCR and sequenced, showing compound heterozygosity for R96W and H373D. When re-created in P450c17 cDNA expression vectors and transfected into COS-1 cells, neither mutant had detectable activity, as assayed by conversion of radiolabeled Prog to 17OHP and 17-Preg to DHEA, analyzed by TLC (Panel A). Prior work (JBC 268:25811, 1993) indicated that the related mutant H373L could not bind heme, as assessed by CO-induced difference spectra. Membrane preparations from *E. coli* expressing the H373D mutant vector produced a CO-induced absorption peak at 420 nm, whereas the wild-type produced a peak at 450 nm, indicating no heme binding (Panel B). Thus H373D abolished enzyme activity because of a lack of heme, probably due to protein misfolding. 17OHD should be considered in any patient with sexual infantilism and low-renin hypertension.

Steroids ng/dl	Basal	Post-ACTH	nl girls
Cortisol (μg/dl)	<1	<1	3-21
DOC	36	68	2-34
11-Deoxycortisol	<10	15	20-155
Corticosterone	13,900	37,300	70-1,860
18OHcorticost	423	894	2.4-10.5
Aldosterone	2.8	4.3	3-35
Preg	320	325	20-140
Prog	42	73	<10-26
17OH-Preg	42	45	10-186
17OHP	<10	<10	3-90
DHEA	70	75	31-345
Testosterone	4.3	4.9	<3-10



FC8-045 Adrenal

Loss of salt-wasting in classical congenital adrenal hyperplasia (CAH) secondary to aldosterone-independent sodium conservation

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Introduction: The final renal regulation of Na balance occurs in the distal nephron where Na is absorbed from urine through the apical epithelial Na channel (α-ENaC), a channel whose activity is regulated by aldosterone. Aldosterone production is impaired in patients (pts) with severe mutations in the CYP21A2 gene who have the salt-wasting (SW) form of CAH due to 21-hydroxylase deficiency (21OHD). Many pts with documented SW in infancy outgrow their need for supplemental Na and mineralocorticoid. When Na deprived, these pts conserve Na without increasing aldosterone. This clinical observation suggests that pts with CAH adapt by upregulating an aldosterone-

independent renal Na absorptive pathway. We have begun to explore this using a mouse model of CAH with targeted deletion of the CYP21A2 gene encoding the steroid 21-OH enzyme in the adrenal gland.

Objective: To test the hypothesis that apical α-ENaC is absent in the distal nephron and Na balance is achieved in the kidney of CAH mice by upregulation of the Na-K-2Cl cotransporter (NKCC2) in the thick ascending limb of Henle.

Methods: Mutant H2aw18 mice, homozygous for deletion of the 21OH gene (CYP21-/-), generally die by postnatal day 10 (d-10) from SW crises but can survive if treated with corticosterone and fluorocortisone. In this study, cryosections of kidneys from <9-d old untreated and 1 month (mo) treated CAH mice and age-matched wild type (WT; CYP21+/+) mice were labeled with anti-α-ENaC and anti-NKCC2 and examined by indirect immunofluorescence (IF) microscopy. Whole kidneys from these animals were analyzed for message encoding α-ENaC and NKCC2 by real time PCR.

Results: IF of sections from kidney revealed labeling with α-ENaC in a subset of tubules in <9-d old mutant and WT mice (n=2) as well as in 1 mo WT mice (n=3). No immunodetectable apical α-ENaC was observed in 1 mo mutant mice (n=3). Labeling with anti-NKCC2 was observed in both the mutant and WT mice early in life (n=2) and at 1 mo (n=3). There was no statistical difference between steady state abundance of mRNA encoding α-ENaC or NKCC2 in whole kidney of both <9-d and 1 mo WT and mutant mice.

Conclusion: In preliminary studies of a mouse model of CAH, targeted deletion of the CYP21A gene is associated with the absence of immunodetectable apical α-ENaC yet robust NKCC2 expression at 1 mo of age. We speculate that Na balance is achieved by upregulation of Na reabsorptive pathways proximal to the aldosterone-sensitive distal nephron.

FC8-046 Adrenal

Current management, morbidity and genotype-phenotype correlation in adults with congenital adrenal hyperplasia – analysis of the United Kingdom congenital adrenal hyperplasia adult study executive (CaHASE) cohort

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Congenital Adrenal Hyperplasia (CAH) is the most common inborn endocrine disorder and is associated with significant morbidity. In contrast to childhood management no established consensus exists for treatment of adults with CAH due to paucity of data from cohorts of meaningful size. We contacted 380 adult CAH patients under the care of 17 endocrine tertiary care centres; the expected UK prevalence is 4000 adults with CAH. 203 patients (53%) agreed to participate in the cross-sectional study [138f, 65m, median age 34 (range 18-69) yrs]. Cross-sectional assessment comprised medical history, physical examination, fasted blood sampling and psychometric evaluation with validated quality of life (SF-36, HADS) and sexuality (MFSQ-9, IIEF-5) questionnaires; bone mineral density was measured by DXA (n=77). Clinical and biochemical results were compared to age-weighted 2006 Health Survey for England (HSE) data, SF-36 results to the UK SF-36 Reference Cohort (n=14,430). 166 patients had classic CAH, 37 non-classic, two live as 46,XX males. Genotyping by multiplex ligation-dependent probe amplification, multiplex minisequencing and direct DNA sequencing was carried out in 153 patients; 88% carried a common *CYP21A2* mutation on both alleles. In addition, 6 rare known and 5 novel mutations were identified. Patients were classified into established *CYP21A2* mutation groups Null (22%), A (37%), B (23%), C (22%) and D (6%). Glucocorticoid (GC) therapy consisted of hydrocortisone (26%), prednisolone (46%), dexamethasone (19%) or a combination (10%); 2% received no GC. Raised renin without fludrocortisone treatment was found in 6, 15, 26, and 12% of patients in groups Null, A, B, and C, respectively. Compared to HSE, CAH patients were shorter (m 6, f 8cm; P<0.0001) and women had higher BMI (32.0 vs 26.7 kg/m2, P<0.0001). 41% were obese, 37% overweight and 10% had metabolic syndrome. Osteopenia was present in 40%, osteoporosis in 7%. 37% of premenopausal women were oligo/amenorrhoic. Most women had never been pregnant (Null 94%, A 88%, B 63%, C 50%). Most men never fathered a child (Null 87%, A 81%, B 90%, C 60%). SF-36 results showed significant impairment in all domains. Sexuality scores were similar to those in 60-yr-old controls. In conclusion, health status in adult CAH patients under the care of specialist centres is significantly impaired. Future studies will determine whether a therapeutic approach tailored to distinct mutation groups could help to optimize management.

FC8-047 Adrenal

Mutant PAPSS2 in a patient with premature pubarche – disruption of DHEA sulfation as a novel monogenic cause of androgen excess

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Premature pubarche has been described as an early sign of the polycystic ovary syndrome. Both conditions are characterized by androgen excess, however, underlying pathogenetic mechanisms remain elusive. Dehydroepiandrosterone (DHEA) sulfotransferase, SULT2A1, inactivates the androgen precursor DHEA to its sulfate ester DHEAS, thereby preventing the conversion of DHEA to active androgens. SULT2A1 requires the universal sulfate donor PAPS for catalytic activity. Here we investigated a girl presenting with premature pubarche at 6 years of age, followed by the development of polycystic ovary syndrome with acne, hirsutism and eventually secondary amenorrhea at 13 years. Circulating androgen precursors (DHEA, androstenedione) and active androgens (testosterone, dihydrotestosterone) were elevated. By contrast, serum DHEAS was below the limit of detection (0.40 µmol/L), suggestive of impaired DHEA sulfation. We carried out genetic analysis of index case and parental DNA by direct sequencing of the key components of the DHEA sulfation system, SULT2A1 and isoforms 1 and 2 of PAPS synthase, PAPSS1 and PAPSS2. We identified compound heterozygous PAPSS2 mutations in the affected patient, a paternally inherited missense mutation, T48R, and a maternally inherited nonsense mutation, R329X. Neither mutation was recorded upon sequencing of a healthy control cohort (n=100), excluding that the identified mutations represent polymorphisms. In vitro reconstitution of the human DHEA sulfation system employing bacterially expressed human SULT2A1 and wild-type and mutant PAPSS2 confirmed the disease-causing nature of the mutants, with 6.0±0.6% residual activity for the missense mutation and abolished activity for the nonsense mutation. In silico analysis demonstrated that the missense mutation affects a highly conserved residue located within the p loop of the PAPSS2 APS kinase domain, an area crucial for function, whereas the nonsense mutation results in early truncation of the ATP sulfurylase domain of PAPSS2. Real-time PCR analysis of RNA extracted from normal human tissue revealed high levels of PAPSS2 and SULT2A1 expression in the adrenal and the liver, i.e. the major sites of DHEA sulfation, but only low levels of expression in the ovary. These findings establish mutant PAPSS2 as a monogenic cause of androgen excess and indicate a crucial role for DHEA sulfation as a gate keeper

of human androgen synthesis, with impaired sulfation resulting in increased androgen activation.

FC8-048 Adrenal

Functional characterisation of a missense mutation (p.Y59D) of MRAP which leads to familial glucocorticoid deficiency type 2

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Familial Glucocorticoid Deficiency (FGD) is a rare autosomal recessive disorder characterised by ACTH resistance resulting in isolated glucocorticoid deficiency with preserved mineralocorticoid secretion. Mutations of the ACTH receptor (melanocortin 2 receptor, MC2R) or the melanocortin 2 receptor accessory protein (MRAP), FGD types 1 & 2 respectively, account for approximately 45% of cases. To date all reported MRAP mutations result in either an absent or severely truncated protein, consequently FGD2 children present early in infancy.

We report a family of 5 Palestinian children, of consanguineous parents, 3 of whom are affected with FGD. The proband was diagnosed aged 5yrs following a severe exacerbation of asthma. His family history revealed a brother (aged 15y) and sister (aged 20y) who were both severely pigmented with high ACTH and low cortisol levels. The brother had no significant medical history but the sister suffered a hypoglycaemic seizure in infancy and subsequently developed cerebral palsy, neither sibling had been treated for their FGD.

PCR amplification and sequencing of *MC2R and MRAP* in affected individuals revealed a novel homozygous missense mutation c.175T>G; causing substitution of the tyrosine residue with an aspartic acid residue at position 59 (p.Y59D). The mother and one unaffected sibling were heterozygous at this position. Paternal DNA was unavailable.

This mutation was functionally characterised in HEK 293 cells transfected with wild type MC2R and wild type or mutant MRAP and a cAMP luciferase reporter plasmid. ACTH stimulated the production of cAMP in a concentration dependent manner. The dose response curve was shifted to the right in the mutant bearing cells (EC_{50} wild type $1.21x10^{-8}$M, mutant $1.83x10^{-7}$M) implying the Y59D mutant has an impaired ability to traffic MC2R, or support its signalling response to ACTH.

In summary this is the first report of a missense mutation in MRAP. This mutation leads to a phenotype with later onset FGD2 than seen previously for MRAP truncation mutants. Consistent with this the characterisation of the mutant MRAP demonstrates the mutant has impaired rather than absent function.

FC9-049 GH and IGF-1 Therapy I

Analyses of treatment variables for patients with childhood craniopharyngioma – results of the multicenter prospective trial KRANIOPHARYNGEOM 2000 after three years of follow-up

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Background: Controversies surround various treatment variables for patients with childhood craniopharyngioma such as growth hormone (GH) replacement, which some believe can exacerbate recurrence/progression. In the multicenter prospective trial **KRANIOPHARYNGEOM 2000** (www.kinderkrebsinfo.de/kranio2007) we analyzed the impact of risk factors on the incidence and time course of relapses after complete resection and tumor progressions after incomplete resection in patients with childhood craniopharyngioma.

Methods: Multivariate analyses of risk factors (age at diagnosis, degree of resection, irradiation, GH-treatment and gender) and descriptive analyses of overall (OS) and event-free survival (EFS) rates were performed in 117 patients from Germany, Austria and Switzerland, recruited prospectively during 2001 and 2006 and evaluated after 3 years of follow-up.

Results: We observed a 3-year-OS of 0.97 and a 3-year-EFS of 0.46, indicating high recurrence rates after complete resection (CR) (n=47; 3-year-EFS: 0.64) and high progression rates after incomplete resection (IR) (n=64; 3-year-EFS: 0.31). The risk of an event decreased by 80% after CR compared to IR (hazard-ratio=0.20; p<0.001). Irradiation had protective effects on EFS: irradiated patients had an 88% lower risk of recurrence/progression compared to patients without/before irradiation (hazard-ratio=0.12, p<0.001). GH treatment had no impact on 3-year-EFS rates. There was a trend (p-value of the score-test: p=0.065) towards lower EFS in patients diagnosed with craniopharyngioma at young age.

Conclusions: Tumor recurrences/progressions are frequent and occur early after initial treatment of childhood craniopharyngioma. A radical resection preserving the integrity of hypothalamic structures appears optimal at original diagnosis. Irradiation was efficient in preventing recurrences/progressions. GH treatment had no impact on high recurrence/progression rates observed during short-term follow-up. Currently, the appropriate time point of irradiation of a residual tumor is analyzed in a mulicenter randomized tral (**KRANIOPHARYNGEOM 2007**).

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FC9-050 GH and IGF-1 Therapy I

Abnormal MRI in GH-deficient patients: association with clinical presentation, hormonal status and 1st year GH response

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Magnetic resonance imaging (MRI) is used to support a diagnosis of GH deficiency (GHD). Patients (pts) from the observational study GeNeSIS with MRI abnormalities (MRI-an, n=826) were compared with pts with known genetic mutations causing GHD (n=99) and pts with idiopathic GHD (IGHD, n=4664). Clinical presentation, hormonal status and 1st year GH response were examined. Baseline characteristics (median) between pts with MRI-an or genetic mutations and IGHD differed significantly with the exception of sex ratios between pts with MRI-an vs. IGHD (p=0.77); girls with MRI-an were significantly younger (p=0.004) but their height deficit relative to target height was not significantly different from that of boys (p=0.149), unlike pts with IGHD.

Baseline Parameter	MRI-an	Genetic Defect	IGHD
CA at diagnosis (y)	4.8*	5.3*	10.0
BA SDS	-2.9*	-3.7*	-2.3
Ht SDS	-2.9*	-3.9*	-2.5
Target Ht SDS	-0.1*	-0.3**	-0.6
Max GH (µg/L)	2.4*	1.5*	6.7
IGF-I SDS	-3.8*	-5.3*	-1.9
IGFBP-3 SDS	-2.4*	-5.3*	-0.3
1st y delta Ht velocity SDS	3.9*	4.6*	3.0
Sex ratio F:M (%)	33:67	42:58	32:68

*p<0.001 by ANOVA, MRI-an vs. IGHD or Genetic Defect vs. IGHD;
**p=.006 by ANOVA, Genetic Defect vs. IGHD

Additional hormonal deficiencies were frequently found (39%) in pts with MRI-an (TSH>ACTH>LH/FSH>ADH). 20% of pts with septo-optic dysplasia (SOD, n=145) and/or those with single central incisor or midline palatal

defects (n=52) also had components of the classic triad (pituitary aplasia/hypoplasia, ectopic posterior pituitary [EPP] and thin/interrupted pituitary stalk) on MRI, suggesting a more complex pattern of developmental defects in these subgroups. Few pts with MRI-an (excluding SOD pts) had all 3 features of the classic triad. EPP and pituitary aplasia were strong predictors of pituitary dysfunction, and pts with EPP had a significantly younger age at diagnosis, lower height SDS, and lower maximum stimulated GH, IGF-I and IGFBP-3 values than pts with pituitary hypoplasia. **Conclusion:** Stratification of GHD pts by presence and type of MRI abnormalities appears useful for guiding genetic analyses, for family counseling, and for management of pts, particularly with respect to additional hormonal deficits. Skewed sex ratios in pts with MRI-an may indicate that male fetuses are more vulnerable to environmental insults and/or are genetically susceptible because of as yet unidentified genes on the sex chromosomes involved in hypothalamic pituitary development.

FC9-051 GH and IGF-1 Therapy I

Identification of a novel mutation (L229P) in the GHR gene in a cohort of Pakistani patients with growth hormone insensitivity and severe growth retardation

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Background: Growth hormone insensitivity (GHI) is characterized by severe post-natal growth retardation, normal to elevated serum concentrations of GH and low serum levels of insulin-like growth factor (IGF)-I. Genetic defects most associated with GHI have been mutations in the extracellular domain (ECD) of the growth hormone receptor (GHR), a cell surface receptor that binds and mediates GH action.
Objective: Evaluate the cause of short stature and GHI in a cohort of Pakistani patients.
Clinical Cases: We evaluated 4 patients (1 male 4year and 3 females 1.2, 1.6 and 7.6 years) with suspected GHI. They presented with severe growth retardation and clinical phenotype suggestive of GHI such as, hypoplastic nasal bridge(4), blue sclera (2), shallow orbits(4), high pitch voice(3) and sparse hair(3). On investigations all patients showed failed IGF generation tests and one showed excessively low Serum IGF levels after receiving growth hormone for a year.
Results: Molecular analysis of the GHR gene from the patients identified a number of missense mutations previously reported, as well a novel missense mutations in exon 7. The parents of patient with novel mutation were not related, and were heterozygous for the mutation. Exon 7 (with Exon 6) encodes for subdomain 2 of the extracellular domain of GHR, a region important for GH-induced receptor dimerization and GH-induced rotation in initiating GHR signal transduction. The homozygous point mutation, a T->C transition altering Leucine(ctc) to Proline(ccc) at residue 229 (mature peptide), is predicted to totally disrupt the GHR structure as L229 is the first residue of the 6th ß-sheet within subdomain 2, and it is well established that prolines are incompatible with ß-sheet structures. Reconstitution studies in HEK293 cells demonstrated that mutant recombinant human GHR:L229P was expressed and correctly localized, results consistent with the normal level of GHBP detected in the serum from the patient.GH-induced activation of the STAT5b and MAPK signaling pathways was, however, abrogated.
Summary: We report identification of a novel homozygous mutation in subdomain 2 of the intracellular domain of the GHR, identified in one of the patient among this cohort. The mutation, while minimally affecting GHR expression, abrogated GH-induced GHR signal transduction, and suggested that GH-induced dimerization and/or rotation was defective. The biological consequences are GHI, IGF-I deficiency and severe growth retardation.

FC9-052 GH and IGF-1 Therapy I

Reduced IGF-1 complex formation alters mesenchymal stromal cell fate

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IGF-1 has an important role for bone remodeling, and age-related osteoporosis is accompanied by an increase in marrow adiposity and reductions in serum IGF-1 levels. To determine the relationship between low serum IGF-1 levels and bone marrow adiposity, we evaluated the adipogenic potential of marrow-derived mesenchymal stem cells from IGF-1 deficient mice.
We employed 8-16 week old liver-specific IGF-1 deficient (LID), IGFBP-3 knockout (BP3KO) and ALS knockout (ALSKO) mice and assessed marrow gene expression, osteogenic cell differentiation and marrow adipogenic potential.
ALSKO and BP3KO mice exhibited an increase in the number of osteoblast-like cells in culture. However, we found that ALSKO mice had a significant decrease in the number of osteoclasts in culture and reduced c-fms+ and B220+ cells in marrow. In contrast, BP3KO mice exhibited an increase in the number of osteoclasts in cultures, and an increase in CD11b+ and c-fms+ cells in the marrow. Likewise, osteoblasts from ALSKO mice failed to induce osteoclastogenesis from non-adherent cells isolated from control mice.
Marrow expression of C/EBPα, a marker of differentiated adipocytes, increased in ALSKO, LID and BP3KO. Adipogenic media added to MSC cultures revealed a significantly greater number of differentiated adipocytes in cultures from ALSKO mice compared to controls. Our results indicate that impairment of IGF-1 complex formation in marrow alters cell fate by increasing the adipogenic-potential of MSCs and modifying osteoclastogenesis.

FC9-053 GH and IGF-1 Therapy I

Genome-wide abnormalities in parental inheritance patterns and DNA methylation in Silver-Russell syndrome

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Silver Russell Syndrome (SRS; OMIM 180860) is a heterogeneous condition characterised by pre- and post-natal growth failure and associated with variable dysmorphic features including triangular facies and body asymmetry. SRS has previously been linked to 2 genetic abnormalities: aberrant methylation at the 11p15.5 locus in 40% and maternal uniparental disomy (UPD) of chromosome 7 in 10% of cases. Up to 50% of children currently have no identified (epi)genetic cause for their clinical phenotype. This study aimed to investigate the possibility that there may be additional regions of UPD elsewhere in the genome and if these regions are associated with changes in methylation and expression of imprinted genes.
Patients with clinical SRS were identified in the UK and Netherlands using previously published diagnostic criteria (SGA without catch up growth plus 1 or more major features including hemihypertrophy or triangular facies). Using Illumina 370 HumHap SNP arrays, 10 parent-child trios were analysed. These patients were also studied for genome wide methylation changes using Illumina GoldenGate Methylation chips.
Our results demonstrate a number of regions with apparent inconsistencies in Mendelian inheritance not previously associated with SRS across the entire genome in all 10 patients. 9/10 patients had ≥ 2 small regions of significant UPD. This was not seen in 6 HapMap trios genotyped on the same SNP platform, (public dataset). These regions were confirmed using highly informative microsatellite markers. They do not occur in a control population of patients born small-for-gestational age (SGA) without features of SRS (n=6) or normal

siblings (n=6). In addition, multiple imprinted regions were noted to have abnormal methylation patterns across the genome in several SRS patients (14/16) compared to controls. Pyrosequencing confirmed aberrant methylation in a selected number of loci (6, 7, 11 and 19). Furthermore, qPCR analysis of an imprinted gene within the chromosome 6 locus (PLAGL1) demonstrates that expression is affected by hypomethylation.
In conclusion, a novel, extensive and variable pattern of UPD has been identified in 9/10 of children with SRS. Furthermore, a proportion of patients appear to have abnormal methylation of multiple imprinted genes associated with aberrant expression.
This suggests a defect in chromosomal dysjunction may be involved in the pathogenesis of SRS, possibly disrupting methylation patterns post-fertilisation.

FC9-054 GH and IGF-1 Therapy I

Analysis of the effect of a polymorphic CA repeat in the promoter region of *IGF1* gene on the growth response to rhGH therapy in patients with severe growth hormone deficiency (GHD)

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Introduction: *IGF1* is a noteworthy candidate gene for rhGH pharmacogenetic studies. A (CA)n *IGF1* promoter polymorphism has been correlated with IGF-1 levels, birth weight and adult height. The number of repeats ranges between 10 and 24, with the most common allele containing 19 CA repeats. **Aims:** To evaluate the influence of (CA)n *IGF1* polymorphism on growth response to rhGH therapy in patients with severe GHD, as well as its interactions with *GHR and IGFBP3* polymorphisms. **Methods:** 71 patients with severe GHD, previously studied for *GHR*fl/d3 and -202 A/C *IGFBP3* polymorphisms, were genotyped for (CA)n *IGF1* polymorphism. Multiple linear regressions were performed to estimate the proper effect of each variant on 1st year growth velocity (n=71) and final height SDS adjusted for target height SDS (FH-TH SDS) after rhGH therapy (n=36). **Results**: Data at start of therapy and rhGH doses were similar among patients with different genotypes. The *IGF1* genotype did not influence the 1st year growth velocity. However, the presence of the *IGF1* 19CA repeat allele in homozygous state negatively correlated with final height SDS - target height SDS (FH-TH SDS) in multiple linear regression analysis adjusted for other covariants (p=0.01). Patients homozygous for the 19CA repeat allele presented lower FH-TH SDS (-0.6 ± 1.2) than patients with at least one different allele (+0.3 ± 1.3; p=0.06). Combined analysis of *IGF1 and GHR* polymorphisms explain 18% of FH-TH SDS (p = 0.008). A FH-TH SDS prediction model including genotype data, height SDS at puberty onset and rhGH doses exhibited a predictive power of 49%. On average, patients with at least one *GHRd3 and one IGF1* non-19CA repeat allele reached FH-TH SDS 1.5 higher than patients homozygous for *GRHfl and IGF1* 19CA repeat alleles (CI 95%= 0.3 a 2.6; p = 0.01).

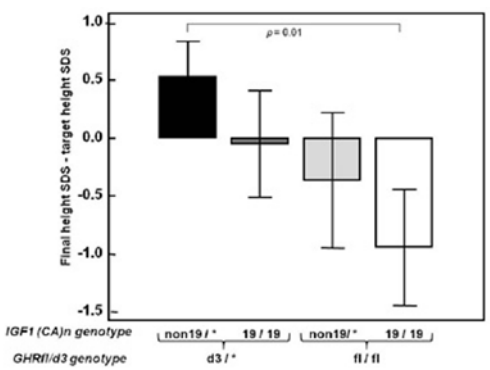

Figure1: Combined analysis of *GHR and IGF1* genotypes influencing the FH-TH SDS after rhGH therapy in patients with severe GHD.
Conclusions: We described for the first time the potential effect of polymorphic CA repeat in *IGF1* promoter on growth response to rhGH in patients in severe GHD, which is additional and independent of *GHR and IGFBP3* genotypes.

FC10-001 GH and IGF-1 Therapy II

Prader-Willi syndrome: long-term continuous growth hormone treatment and the cardiovascular and metabolic risk profile of young children

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BACKGROUND
Children with Prader-Willi syndrome (PWS) have abnormal body composition and impaired growth. Effects of long-term growth hormone (GH) treatment are unknown. The cardiovascular (CV) and metabolic risk profile of PWS children is unknown.
OBJECTIVES
1. To assess the CV and metabolic risk profile in PWS children and the effects of 2 yr of GH.
2. To investigate the effects of long-term continuous GH treatment on body composition, growth, bone maturation, and safety parameters.
DESIGN
We included 85 PWS children with a mean ± SD age of 4.9 ± 3.0 yr in a multicenter randomized controlled trial. Infants (<3 yr) and prepubertal children (3-12 yr) either received GH (1 mg/m^2•day) or were followed as controls for 1 and 2 yr, respectively. Fifty-five children completed 4 yr of GH treatment in a multicenter follow-up study (Dutch Cohort Study).
Yearly, the following data were obtained in one center: fat percentage (fat%) and lean body mass (LBM) by DXA, height, weight, head circumference, bone age, blood pressure, fasting IGF-I, IGFBP-3, glucose, insulin, HbA1c, total cholesterol, HDL, LDL, tryglicerides, Lp(a).
RESULTS
Randomized controlled trial
Fat% was elevated in 95% of PWS children. At least one CV risk factor was found in 63% of infants and 73% of prepubertal children. The metabolic syndrome was found in 5% of all children; at least one component was present in 40%. Two years of GH improved fat%SDS and HDL/LDL (p<0.0001 and p=0.04) and had no adverse effect on blood pressure, glucose homeostasis and serum lipids.
Dutch Cohort Study
Fat%SDS was lower after 4 yr of GH (p<0.0001). LBMSDS significantly increased the first year of GH (p=0.02), returned to baseline values the second year and remained unchanged thereafter. Mean ± SD heightSDS normalized from –2.27 ± 1.2 to –0.24 ± 1.2 (p<0.0001). Head circumference SDS increased to 0.07 ± 1.1. IGF-I and the IGF-I/IGFBP-3 ratio significantly increased to 2.08 ± 1.1 and 2.32 ± 0.9 SDS. Four years of GH had no adverse effect on bone maturation, blood pressure, glucose homeostasis, and serum lipids.
CONCLUSIONS
Many young PWS children have an unfavorable CV and metabolic risk profile. Short-term GH has a favorable effect on body composition and the HDL/LDL ratio. Long-term continuous GH treatment improves body composition, by decreasing fat% and stabilizing LBM, and head circumference and normalizes height without adverse effects. Thus, long-term continuous GH treatment is an effective and safe therapy for PWS children.

FC10-002 GH and IGF-1 Therapy II

Safety and efficacy of a once-a-week sustained release rGH (LB03002) in naïve children with GHD*

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Previously untreated children with GHD (N=51, boys/girls; 30/21, median age at baseline 7.3 ± 2.19 years) were randomized into 4 groups in a parallel, assessor-blinded, phase II/III study. They were treated for the 1st year with either daily rhGH 0.03 mg/kg/day or with LB03002 (LB) at any of the 3 different doses: 0.2 mg/kg/week, 0.5 mg/kg/week or 0.7 mg/kg/week. Patients treated in the 1st year with LB 0.2 or LB 0.7 were switched for the 2nd and 3rd year to LB 0.5. Patients on LB 0.5 were treated with an unchanged dose for the entire 3 years, whilst patients treated with daily rhGH for 2 years, were switched for the 3rd year to LB 0.5.

Table 1: Growth data (Height SDS)

Treatment Groups	Baseline	End of 1st year	End of 2nd year	End of 3rd year
LB 0.2	-5.00 ± 1.61	-3.97 ± 1.28	-3.10 ± 1.24	-2.56 ± 1.26
LB 0.5	-3.94 ± 0.81	-2.55 ± 0.61	-1.87 ± 0.59	-1.49 ± 0.75
LB 0.7	-4.64 ± 1.32	-3.03 ± 1.11	-2.22 ± 1.33	-1.86 ± 1.26
Daily rhGH	-4.52 ± 1.39	-3.06 ± 1.27	-2.17 ± 1.08	-1.89 ± 1.05

Sustained growth with LB was observed similar to daily rhGH. There were no safety concerns as IGF-1 and IGFBP-3 normalized (Peter *et al*, 2009). Bone age advancement and IGF-1 SDS normalization were similar between LB and daily rhGH treatment.

Over the study period a total of 319 treatment emergent AEs were reported with the majority (91.5%) being unrelated to treatment and mild in severity. The adverse event profile during the first year of treatment was the same between the 3 different doses of LB in comparison to daily rhGH. Also, comparison of the AE profile of daily rhGH over 3 years revealed no differences after the switch to sustained release. The weekly injections of LB were well tolerated and only 3 injection site reactions reported during the study with the tolerability rating 'good' occurring most frequently amongst patients.

In the LB groups growth could be predicted by a combination of IGF-1 SDS, maximum stimulated GH levels and rhGH dose (r=0.51, p=0.001). A fully recruited phase III pivotal trial utilizing LB 0.5 is ongoing. It can be concluded that in children with GHD, prolonged administration of weekly LB over the 3 years was shown to be safe and well tolerated and similar to that observed with daily rhGH.

*in cooperation with Biopartners' and LG Life Sciences' GH Study Group

FC10-003 GH and IGF-1 Therapy II

Effects of GH and nutritional therapy in boys with constitutional growth delay: a randomized controlled trial

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Background: Recent studies suggest that insufficient energy intake may contribute to the pathophysiology of constitutional delay of growth and maturation (CDGM). We conducted a study to examine the role of nutrition supplementation and its interaction with growth hormone (GH) therapy in boys with CDGM.

Methods: 20 prepubertal boys (mean ± SE: 9.3 ± 0.3y) with CDGM (defined as height or predicted adult height (PAH) <-2.0 SDS, bone age delayed by >12mo below chronologic age, BMI<25th%ile, no GH deficiency or skeletal dysplasia) were randomized to 6mo observation or daily nutritional supplementation (PediaSure; dosed at 110% of RDA to achieve ideal body weight), followed by daily GH therapy (0.3mg/kg/wk) in all for another 12mo (N=10 for each group). Height, weight, bone age, body composition (DEXA), and serum biochemistries were measured at 0, 6, 12, and 18mo. Total energy expenditure (TEE, by doubly labeled water) and energy intake (3-day food record) were assessed at 0, 6, and 12mo. T-tests compared groups and ANOVA compared data across time points.

Results: At 6mo, energy intake and TEE were unchanged compared to baseline within the Observation group but both increased within the Nutrition group, with no significant difference between groups. Six months after GH (at 12mo) the Observation/GH group had increased TEE, while the Nutrition/GH group also increased their intake along with TEE with no difference between groups (see Table 1). At 6mo, change in PAH trended higher in Nutrition vs. Observation (+2.1 ± 1.2 vs. –1.0 ± 0.9cm, p=0.055). At 12 and 18mo, both groups had increased height SDS, growth velocity, PAH, lean body mass (LBM), and serum IGF-1, but there was no better improvement in the Nutrition/GH group as compared to GH alone (see Table 2).

Table 1: Changes in Energy Intake and Expenditure (Mean ± SE)

	Observation + GH Group			Nutrition + GH therapy		
Visit	0 mo	6 mo	12 mo	0 mo	6 mo	12 mo
Intake (kcal/day)	1730 ± 137	1748 ± 135	2036 ± 131	1684 ± 107	1918 ± 125*	2019 ± 125**
Intake[a] (kcal/day)	1763 ± 138	1781 ± 136	1996 ± 149	1632 ± 120	1909 ± 117	2160 ± 129**
TEE (kcal/day)	2541 ± 157	2584 ± 167	2623 ± 213*	2064 ± 64	2504 ± 88**	2555 ± 161**
TEE[a] (kcal/day)	2195 ± 148	2319 ± 138	2330 ± 161*	2162 ± 95	2336 ± 94	2464 ± 99

*p<0.05 within group comparison with baseline, **p<0.01 within group comparison with baseline
[a]Adjusted for LBM

Table 2: Anthropometrics and Laboratory Data (Mean ± SE)

	Observation + GH Group				Nutrition + GH therapy			
Visit	0 mo	6 mo	12 mo	18mo	0 mo	6 mo	12 mo	18mo
Height SDS	-2.0 ± 0.1	-2.0 ± 0.1	-1.6 ± 0.1**	-1.4 ± 0.1**	-1.9 ± 0.2	-1.8 ± 0.2	-1.5 ± 0.2**	-1.1 ± 0.2**
Growth Velocity (cm/yr)	4.6 ± 0.4	5.5 ± 0.5	9.7 ± 0.5**	8.7 ± 0.5**	5.0 ± 0.4	6.2 ± 0.7	9.7 ± 1.1**	9.9 ± 1.1**
PAH (cm)	164.9 ± 1.3	165.9 ± 2.5	170.7 ± 2.2**	171.1 ± 1.5**	169.3 ± 1.4	171.3 ± 2.0	174.2 ± 2.2*	174.8 ± 1.7**
BMI%	20.7 ± 6.1	26.9 ± 6.9	24.9 ± 7.0	25.4 ± 6.5	15.3 ± 5.5	20.7 ± 8.8	23.1 ± 8.2	23.5 ± 9.4
LBM (kg)	18.9 ± 0.7	19.8 ± 0.6**	22.3 ± 0.8**	24.6 ± 0.8**	17.4 ± 1.1	18.3 ± 1.2**	21.2 ± 1.5**	22.7 ± 1.6**
% Fat Mass	19.7 ± 1.0	21.4 ± 1.4**	16.1 ± 0.7**	16.0 ± 0.8**	19.9 ± 1.9	20.9 ± 1.6	17.4 ± 1.0**	17.7 ± 1.1*
Glucose (g/dL)	90.0 ± 34.5	83.5 ± 20.2	92.8 ± 8.2	139.4 ± 40.4	72.9 ± 15.5	175.4 ± 64.1	144.4 ± 35.8	128.2 ± 52.6
IGF-1 (ng/mL)	187 ± 41	253 ± 46	466 ± 178*	638 ± 154*	180 ± 45	306 ± 93	708 ± 181**	695 ± 124**

*p<0.05 within group comparison with baseline, **p<0.01 within group comparison with baseline
No significant differences were observed between the groups

Conclusions: Dietary supplementation with liquid nutrition was associated with increased energy intake and TEE only within the Nutrition group. Both groups grew better on GH, albeit comparably, whether added nutrition was provided or not. This suggests that GH therapy is superior to added nutrition in enhancing growth in CDGM.

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FC10-004 GH and IGF-1 Therapy II

Final height of growth hormone deficient patients treated before one year of age: a therapeutic model

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Children diagnosed with growth hormone deficiency (GHD) and treated before one year of age provide a dramatically homogeneous population and an exceptional opportunity for assessment of GH treatment effects. We report on auxological data and final height from a cohort of 49 children presenting with isolated or multiple pituitary hormone deficiency treated early in infancy and until growth completion.

Subjects and methods: We included all the children who were diagnosed with GHD before one year of age, whose treatment began between 1977 and 1992 and thus attained adult height. Collection of data was facilitated by the obligation of a central agency (Association France Hypophyse) approval to prescribe GH treatment from 1973 to 1997. Final height (FH) data from 49 patients (45 MPHD) were obtained in a cohort of 59 patients.

Results: 20 girls (age at diagnosis: 6.2 +/- 3.6 months) and 29 boys (4.8 +/- 3.6 months) were treated during 16 +/- 1.8 years with GH. They are presently 22.5 +/- 4.3 years old.

table 1

	Birth height (z-score)	Diag-nostic height	after 3 years of GH	after 5 years of GH	after 10 years of GH	after 15 years of GH
Girls	-1.3 +/- 1.3	-3.1+/- 2.1	-1.6+/- 1.8	-1.3+/- 1.6	-.5+/- 1.7	-.8+/- 1.4
Boys	-.5+/- 1.4	-2.3+/- 1.5	-.8+/- 1.6	-.7+/- 1.2	-.1+/- .9	-.4+/- 1
Total	-.8 +/- 1.4	-2.6 +/- 1.8	-1.2 +/- 1.7	-1+/- 1.4	-.3+/- 1.3	-.6+/- 1.2

table 2

	Final height (z-score)	Midparental height (MH)	Δ between FH and MH	Total height gain
Girls	-.6+/- 1.5	-.6+/- .7	.1+/- 1.2	2.5 +/- 1.9
Boys	-.2+/- 1.1	-.3+/- .9	.1 +/- 1.0	2.1 +/- 1.7
Total	-.4+/- 1.3	-.4+/- .8	.1+ /- 1.1	2.3 +/- 1.8

Puberty occurred at a median age of 12.2 +/-1.5 and 13.7+/-1.5 respectively for girls and boys. Growth during puberty was disappointing and height gain (z score) was null between 10 years of treatment and final height.

Conclusion: When growth hormone treatments is early instituted and maintained during growth completion, patients presenting with GHD normalize their growth, and reach an adult height in the normal range, near familial genetic potential.

These data highlight the need of early GHD diagnosis and treatment. Nonetheless, a treatment optimization before and throughout puberty seems essential to overcome the reduction of height gain during adolescence.

FC10-005 GH and IGF-1 Therapy II

Final and near-final adult height in patients with severe primary insulin-like growth factor-I deficiency (IGFD) after long-term treatment with recombinant human insulin-like growth factor-I (rhIGF-I)

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Background: Treatment with rhIGF-I stimulates linear growth in children with severe primary IGFD due to GH insensitivity syndrome (GHIS). The effect of this therapy on adult height (HT) was previously assessed in only a few patients (1). We now report experience with 16 patients with severe primary IGFD treated with rhIGF-I until final or near-final adult HT.

Methods: The analysis was done in 9 males and 7 females treated with rhIGF-I who attained final or near-final adult HT (defined as a HT when the estimated bone age was ≥13.5 years [females] or ≥15.5 years [males]). The etiology of their severe primary IGFD was GH receptor abnormality (Laron syndrome, LS) in 9, clinical GHIS in 2, and GH gene deletion with growth-attenuating antibodies in 5 patients. Patients received a mean of 112 µg/kg rhIGF-I twice-daily for a mean of 9.9 years. Five patients also received GnRH-analog therapy. Adult HT was predicted using LS growth charts, assuming growth at an average rate for LS in the absence of treatment.

Results: Mean age at the start of therapy was 7.2 years (range 1.7 to 15.2). Mean baseline HT SD score (US CDC norms) was -7.1 (range -12.1 to -3.4). Mean HT SD score at last analysis was -5.2 (range -8.5 to -1.5). Mean BMI SD score at baseline was -0.6 (range -2.3 to 1.3), and was 0.5 (range -1.3 to 1.9) at last analysis. As a result of rhIGF-I therapy, the mean estimated gain in HT up to the time of near-adult HT was 13.2 cm (range -0.4 to 23.4) and the mean estimated gain in adult HT was conservatively estimated as 10.5 cm (range -2.9 to 22.3). In terms of the norms for LS, the mean baseline HT SD score was -0.1 and the mean increase was 1.2. None of the predictor variables analyzed (eg, age at start of therapy, sex, diagnosis, total dose, treatment duration, GnRH-analog use, baseline HT SD score) correlated with treatment response. There were no new safety signals identified in these patients, a subset of those previously reported (1).

Conclusions: Long-term therapy with rhIGF-I substantially improves the adult HT of extremely short patients with severe primary IGFD. However, most patients did not experience enough catch-up growth to bring their HTs into the normal adult range. We believe that suboptimal treatment adherence in some patients as well as decreased autocrine/paracrine IGF-I production, not corrected by rhIGF-I administration, explains the inability to completely eliminate the HT deficit.

Ref. 1. Chernausek SD, et al. *JCEM* 2007;92(3):902-10.

FC10-006 GH and IGF-1 Therapy II

Preliminary results of a phase II, randomized, open-label, active-treatment controlled trial of rhGH and rhIGF-1 combination therapy in prepubertal children with non-severe primary IGF-1 deficiency

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Introduction: Both GH and IGF-1 are essential for normal growth in children; a deficiency in either may limit growth. A previous 1-yr study of untreated children with IGF-1 deficiency (IGFD) found persistent low IGF-1 levels and annualized height (HT) velocities (aHVs) that were inadequate to increase HT SDS (ICE 2008 #OP007). Treatment of such children with rhGH may require very high doses (>100 µg/kg/d) to raise IGF-1 levels to the upper half of the normal range (*JCEM* 2007;92:2480-6) and rhIGF-1 BID can suppress GH secretion leading to the hypothesis that rhGH/rhIGF-1 combination therapy (combo) may outperform both monotherapies.

Methods: This ongoing randomized study evaluates the efficacy and safety of combo versus rhGH monotherapy in 100 treatment-naïve prepubertal children with HT SDS <-2, IGF-1 SDS <-1, and a stimulated GH ≥10 ng/mL. Subjects receive morning injections of either rhGH alone (45 µg/kg once-daily) or combo (45 µg/kg rhGH and 50, 100 or 150 µg/kg rhIGF-1 as two injections).

Results: As of February 2009, 81 subjects were enrolled and included in the safety analysis (baseline mean age 9.1 yr; range 5.2-13.6 yr; mean HT SDS -2.5). aHVs based on 6-mo data were available for 42 subjects (Table) and these data suggest that combo may be associated with robust catch-up growth.

Table. 6-mo data

rhGH/rhIGF-1 doses (µg/kg, once-daily)	Mean aHV (cm/yr)	6-mo change in HT SDS	Average near-peak IGF-1 SDS
45/0 (n=12)	9.7	0.39	-0.2
45/50 (n=10)	11.1	0.53	2.3
45/100 (n=9)	11.8	0.53	2.5
45/150 (n=11)	12.7	0.62	3.0

The most common adverse event was headache (26% of rhGH and 37% of combo subjects). There were no on-treatment fasting (n = 79) or non-fasting (n = 202) blood glucose values <50 mg/dL. Two combo subjects had intracranial hypertension and ceased therapy; both resumed therapy without recurrence. Two subjects reported hair loss and two subjects had generalized urticaria with positive skin test reactions to rhIGF-1, but not to rhGH. There were mild (<2x), transient increases in mean serum AST and ALT levels at 2 and 4 wk; one rhGH subject had elevations of both ALT and γ-glutamyl transferase. Relative changes in bone age and HT will be assessed at one year.

Conclusion: Preliminary results for combo suggest that aHV is accelerated in short children with non-severe Primary IGFD. This treatment should remain a research endeavor until adequate efficacy and safety data are compiled and evaluated.

FC11-007 Obesity I

Gender-dimorphic prevalence of fatty liver in obese children and adolescents: role of body fat distribution, sex steroids and insulin resistance

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Background: Non-alcoholic fatty liver disease (NAFLD) is a key component of obesity related metabolic risk in adulthood as well as in childhood and adolescence. Less information is available on the gender-specific prevalence of NAFLD in obese adolescents, its associations to pubertal development and predictive factors.

Methods: 532 obese caucasian children and adolescents (291 girls) aged 8-19 years underwent screening for hepatic steatosis fatty by ultrasound. Glucose metabolism was evaluated by oral glucose tolerance testing. Further laboratory tests included measurements of serum lipids, adiponectin, CRP, and sex steroids.

Results: The prevalence of hepatic steatosis in the study cohort was 28%. Most subjects were affected by mild (16.4%) to moderate steatosis hepatis (10.3%) according to ultrasound criteria (steatosis I° and II°). The prevalence of hepatic steatosis was significantly higher in in boys (58.9%) than in girls (17.2%) and showed an increase in the group of postpubertal boys vs. a decrease in postpubertal girls. Severity of hepatic steatosis was associated with decreasing insulin sensitivity, higher 2h-postchallenge plasma glucose, lower adiponectin levels, higher triglycerides, increased visceral fat and higher blood pressure independent of age, gender and BMI z-score. Three factors, termed "insulin resistance and visceral fat", "body fat distribution and inflammation", and "steroid hormones" were extracted from the set of investigated parameters by principal component analysis. Stepwise logistic regression analysis adjusted for age, BMI z-score, and pubertal stage revealed significant associations of simple steatosis with "insulin resistance and visceral fat" and "body fat distribution and inflammation" in both genders and additionally with "steroid hormones" in girls. The risk for steatosis hepatis with concomitantly elevated ALT (steatohepatitis) was associated only with "insulin resistance and visceral fat" in girls, and with all three factors including "steroid hormones" in boys.

Conclusion: NAFLD shows a gender-dimorphic prevalence pattern in obese adolescents with disproportionately more boys affected than girls and is associated with an adverse metabolic profile. Our results suggest significant associations of steatosis hepatis and steatohepatitis with markers of visceral obesity and insulin resistance in both genders, and additionally gender-specific associations with parameters of body fat distribution and sex steroids.

FC11-008 Obesity I

Effect of weight loss on insulin sensitivity, liver steatosis, sCD36 and hsCRP in obese Danish children

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Objective. Obesity, an increasing problem in Danish children, is associated with reduced insulin sensitivity, liver steatosis and arterial inflammation. Soluble CD36 (sCD36) is a newly discovered protein in plasma, and it has been proposed to be a marker of insulin resistance in obesity and type 2 diabetes and the associated fat accumulation in liver and arterial walls. The aim of this study was to examine the effect of weight loss on 1) insulin sensitivity, 2) markers for liver steatosis, 3) sCD36 and high sensitive CRP (hs-CRP), in obese Danish children.

Methods. One hundred and sixteen (51 males) Caucasian obese children with mean (SD) age 12.2 (1.4) years, and body mass index (BMI) for age more than 3 SD, were examined before and after a 10 weeks intervention in a Weight Loss Camp. Examinations included anthropometry with calculation of BMI, ultrasonography of the liver (US), glucose tolerance test, and blood samples for alanine aminotransferase (ALAT), sCD36 and hs-CRP. The insulin sensitivity index (ISI – HOMA) was calculated as: 22.5 / (insulin (mU/L) x glucose (mmol / L)). ISI-HOMA in 10 normal weight children was 2.1 (1.6 – 2.4).

Results. Median (range) BMI was reduced during the 10 weeks intervention from 27.5 (21.6) kg/m^2 to 24.5 (19.8) kg/m^2, $p < 0.001$. None had type 2 diabetes, but 3 children had impaired glucose tolerance. Median (range) ISI-HOMA increased from 0.7 (3.5) to 1.0 (3.1), $p < 0.001$. At inclusion 20 children had elevated ALAT, and liver steatosis by US was found in 30 children. ALAT and liver steatosis by US decreased significantly during intervention, $p < 0.05$ and $p < 0.001$. Soluble CD36 correlated significantly with BMI at inclusion in the study, p=0.014. Soluble CD36 and hs-CPR decreased significantly following weight loss, p=0.002 and p<0.001, respectively.

Conclusion. In obese Caucasian children overt type 2 diabetes and glucose intolerance are rare whereas reduced insulin sensitivity and liver steatosis are often found. Weight loss increases insulin sensitivity, and reduces liver steatosis markers and the low-grade inflammation marker hs-CRP. For the first time sCD36 was found to be reduced following weight loss. Every effort should be done to avoid obesity in children, as obese children have early markers for later type 2 diabetes, non alcoholic steatohepatitis and arteriosclerosis.

FC11-009 Obesity I

Meal related glucose excursions associate with endothelial dysfunction in obese youth

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In adults impaired postprandial glycemia is implicated in the etiology of cardiovascular disease. To this end we examined the relationship between glycemic excursions by continuous glucose monitoring (CGM) and measures of endothelial dysfunction in obese youth with impaired/borderline glucose tolerance (OBIG) and lean normo-glycemic controls (LC). Furthermore we assessed if pharmacological targeting of postprandial glycemia with acarbose in OBIG would reduce CGM readings to levels of LC and possibly improve endothelial function (EF).

Methods:

19 (BMI 41.6 ± 8.7 kg/m², age 15.1± 1.2 years) OBIG and 11 LC (BMI 22.9 ± 1.7, age 21.9 ± 2.7 years) underwent 72 hrs of CGM. 12/19 OBIG were treated with acarbose (50mg thrice daily) for 6 weeks; afterwards repeat CGM was obtained. Three parameters of CGM were calculated: 72 hr mean glucose, % of glucose values ≥140mg/dl (%≥140) and mean daily maximal excursion (DME). Data was analyzed comparing OBIG and LC at baseline and post acarbose. Non-invasive tests of EF such as reactive hyperemia –peripheral artery tonometry (RH-PAT) and flow mediated vasodilation (FMD) were measured and correlated to glucose profiles on CGM. Changes in EF with acarbose treatment as well as baseline differences between OBIG and LC were also analyzed.

Results:

At baseline OBIG had higher mean glucose on CGM (108 ± 3 vs 97 ± 3 mg/dl, p = 0.02), higher DME (100 ± 40 vs 48 ± 5 mg/dl; p=0.001), higher %≥140 (8 ± 10% vs 0.8 ± 0.5%; p = 0.04) than LC. In those taking acarbose mean glucose and DME was reduced but remained different to LC (mean glucose, p=0.045; DME, p=0.02). However % ≥140 were reduced to levels of LC (2.0 ± 1 %; p=0.37). EF as assessed by FMD was closely correlated to 72 hr mean glucose on CGM (r = 0.5, p = 0.02). FMD did not show a similar relationship. RH-PAT (p= 0.0009) demonstrated significant differences in EF between OBIG and LC. There were no significant differences in EF for the OBIG group after 6 weeks of acarbose.

Conclusions**:** Along the spectrum of glucose tolerance, meal related glucose excursions correlate with EF as assessed by RH-PAT. This suggests that postprandial glucose elevations in obese youth may be toxic to their vasculature. The reduction in % glucose excursions >140 mg/dl on CGM with short term acarbose treatment indicates that modest glucose excursions can be targeted in obese youth. Longer treatment duration appears necessary to improve ED.

FC11-010 Obesity I

Effects of meals high in carbohydrate, protein, and fat on ghrelin and peptide YY secretion in prepubertal children

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Background: Ghrelin and peptide YY (PYY) are two hormones produced by the gastrointestinal tract that have effects on appetite. Little is known about their secretion in response to meals high in individual macronutrients in prepubertal children.
Objective: We assessed serum concentrations of these hormones in response to meals high in carbohydrate, protein, and fat. We hypothesized that protein consumption would lead to the most sustained suppression of ghrelin and elevation of PYY.
Methods: This was a cross-sectional study at one tertiary care center. Subjects were 7-11 year old healthy normal weight (NW) and obese (OB) volunteers. There were 9-10 children per group per phase of the study. Following an overnight fast, the subjects ate breakfast at 0800 h. Blood samples for total ghrelin and total PYY were taken at baseline, 30 minutes, and hourly from 0900 to 1200 h. We assessed post-prandial ghrelin suppression and PYY elevation, as well as changes in reported hunger and satiety, following the three test meals.
Results: Following the high protein meal, ghrelin declined gradually in both groups over the study period without subsequent increase, whereas ghrelin suppressed rapidly to a nadir 60 minutes after the high carbohydrate meal in both NW and OB children, followed by rebound. Similarly, after the high protein meal, PYY concentrations increased steadily over the course of the morning in both groups without decline, whereas PYY levels peaked 30 minutes after the high carbohydrate meal in both NW and OB subjects with subsequent decline. Ghrelin and PYY responses to the high fat meal were somewhat intermediate between that observed with high carbohydrate and high protein. Area under the curve (AUC) analysis revealed significantly greater PYY response to the high protein meal than the carbohydrate or fat meals only within the OB group. The OB children reported higher hunger and lower satiety after the high carbohydrate meal compared to the NW subjects, whereas appetite ratings were similar between the groups after the high protein and high fat meals. Additionally, within both OB and NW groups, the high protein meal led to lower AUC hunger and higher AUC satiety than the high carbohydrate or fat meals.
Conclusions: The patterns of secretion of ghrelin and PYY in our study of pre-pubertal children suggest they may play a role in the effectiveness of high protein/low carbohydrate diets in promoting weight loss.

FC11-011 Obesity I

Treatment of hypothalamic obesity by laparoscopic truncal vagotomy: early experience

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Hypothalamic obesity (HO) is a rare but devastating complication of brain tumors and their treatment. Death of hypothalamic neurons due to the tumor, surgery, or radiation results in organic leptin resistance, mimicking the starvation response; resulting in reduction of sympathetic tone (promoting energy conservation and poor quality of life) and increase in vagal tone (promoting appetite, insulin hypersecretion, and energy storage). HO is resistant to lifestyle intervention and most pharmacotherapeutic agents. We are conducting a compassionate use trial of laparoscopic truncal vagotomy without pyloromyotomy (LTV) in patients with HO. LTV has advantages of ease of surgery, lack of foreign body placement, no anatomic alterations, and low cost. The UCSF Committee on Human Research approved this protocol and approach.
Four subjects (ages 7—15), 3 with craniopharyngioma and 1 with pituitary adenoma, s/p neurosurgery with intractable weight gain of 8 —25 kg/yr for 1.5 — 4 yr post-op, and with mean BMI 43.1 kg/m² and BMI z-score +2.80, have been recruited thus far. These subjects were receiving low-dose dextroamphetamine with improved alertness, but with salutary effects on weight; they were maintained on this medication. Each subject underwent oral glucose tolerance testing prior to surgery, and each exhibited insulin hypersecretion. Under general anesthesia, at the esophagogastric junction, the peritoneal covering was incised, the esophagus isolated, and the anterior and posterior vagal trunks coursing over the cardia of the stomach were doubly clipped and 1.5 cm segments excised. Subjects were discharged within 24 hours.
Subjects have been followed for 2—11 months post-operatively. All patients report decreases in hunger and improvements in stamina. Initial weight response ranged from -6.0 kg — +0.1 kg in the first 2 months post-op, with BMI changes of -6.0, -3.4, -3.1 and 0.0 kg/m² and BMI z-score changes of -0.10, -0.21, -0.05, and -0.06 in 2, 6, 6, and 11 months post-op, respectively. Side effects have been infrequent, consisting of occasional diarrhea and mild burping, and appear to be self-limited. Other side effects traditionally associated with vagotomy, e.g. dumping syndrome, have not occurred, due to the absence of pyloromyotomy.
Our early results suggest promise of LTV in treating HO. However, more subjects and longer follow-up will be required before this procedure can be recommended.

FC11-012 Obesity I

Resistance to the effects of leptin replacement therapy of immunological origin in children with Berardinelli Seip congenital lipodystrophy (BSCL)

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Objective: Leptin replacement has been introduced in the early 2000's for the treatment of the metabolic disorder induced by lipodystrophy and the very first results in adult diabetic patients lead to improvement of insulin sensitivity and reduction or cessation of the anti-diabetic treatment. A positive response was also recently reported in 6/7 non diabetic children with BSCL during a 4-month proof-of-concept trial which prompted us to hypothesize that in such patients leptin replacement therapy could improve or reverse the early complications of the disease.
Research design and methods: A second trial was implemented in 8 patients (6 boys, 2 girls, 5 to 16 years) for 28 months to test for the long-term efficiency and tolerance of such a therapy. Efficacy criteria were: decrease in serum TG, decrease in liver volume and increase in insulin sensitivity (≥30% each). The number of improved criteria defined the response as total (3/3), partial (1 or 2/3), or negative (0/3). Leptin doses were initially at 0.06 mg/kg/d and further increased up to 0.12 mg/kg/d in the case of lack of response. Anti-leptin antibodies were measured with a sensitive homemade radiobinding-assay. Neutralizing effect was assessed in vitro by measuring percent of pycnotic nuclei in primary culture of fetal neurons incubated with an apoptotic agent (NMDA), seumr from patients with negative response and with or without leptin.
Results: By contrast to the first trial, a negative or partial response to treatment has been observed in the majority (5/8) of the patients even when leptin dosage was increased. A displaceable leptin binding that we identified as being a complex antigene-antibodies was detectable in all patients after 2 months of treatment. At month 28, binding was higher in the patients with a negative response than in the total responders (62.4+/-28 vs 34.5+/-4.41 % respectively) and paralleled both the increase in leptin dosage and serum leptin concentrations contrasting with the lack of clinical response. Co-incubation of fetal neurons with serum from patients with negative response inhibits the neuroprotective effect of leptin.
Conclusion: Children with BSCL, who hardly secrete any leptin, develop an

immunological long-term resistance to leptin therapy in relation with the production of neutralizing antibodies against leptin. This immunological reaction counteracts the previously reported beneficial effects of leptin administration in BSCL.

FC12-013 Puberty

Recent changes in pubertal timing in healthy Danish boys: association with body mass index. The COPENHAGEN puberty study

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Objective In the early 1990's a population-based study (NHANES III) renewed focus on possible trends towards earlier age at puberty. However, due to lack of compatible data on pubertal onset in boys no conclusions can be made at present. In addition, the relationship between pubertal timing and adiposity in boys remains controversial. The aim of the present study was to evaluate secular trends in pubertal onset during the recent 15 years and its relation to BMI in boys. **Methods and Materials** A total of 1528 boys aged 5.8 to 19.9 years participated (n = 824 in 1991-93 (1991-cohort) and 704 in 2006-08 (2006-cohort)). Genital and pubic hair stages as well as testicular volume by orchidometry were evaluated. Blood samples were analysed for LH, FSH, Testosterone and SHBG. **Results** Onset of puberty, defined as age at attainment of testicular volume above 3 ml, occurred significantly earlier in the 2006-cohort (11.66 years (11.49 – 11.82), mean, (95% CI)) than in the 1991-cohort (11.92 years (11.76 – 11.82), p = 0.025). Higher LH, but not testosterone, levels were found in the 10 to 14 year old boys from the 2006-cohort compared to similarly aged boys from the 1991-cohort (p = 0.024). However, the earlier pubertal onset as well as the higher LH levels disappeared after adjustment for BMI. BMI Z-score increased significantly from the 1991-cohort (0.044 (-0.016 – 0.104)) to the 2006-cohort ((0.290 (0.219 – 0.361), p < 0.001). **Conclusions** Estimated age at onset of puberty declined significantly during the recent 15 years. This decline was associated with the coincident increase in BMI. The effects of adiposity on pubertal timing still merit further attention in boys.

FC12-014 Puberty

KiSS 1 polymorphisms analysis in a cohort of patients with disorder in the timing of pubertal onset shows association between 3' UTR polymorphisms and central precocious puberty

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GPR54 and its ligand, Kisspeptin, were recently described as key hypothalamic factors that trigger puberty. The description of several loss of function mutations of GPR54 in familial isolated Hypogonodatropic Hypogonadism (IHH) cases as well as one mutation of GPR54 in one case of central precocious puberty (CPP) suggest that polymorphisms of *KiSS1* might also be associated with both conditions. This hypothesis has been tested in a group of 92 IHH or 25 CPP compared to two control groups T1 and T5 composed by 40 healthy boys and girls tanner stage 1 (T1) or stage 5 (T5) matched in age. The 5' UTR, the coding sequence and the 3' UTR of *KiSS1* have been sequenced after PCR amplification of blood extracted DNA. To precisely define allelic variants, PCR products were sub-cloned from 5 patients and 4 controls. 8 single nucleotide substitutions were observed in coding as well as non coding sequences. 7 were already described as SNP or observed in both affected patients and controls. One point mutation leading to a H90D substitution and one insertion of 20 nucleotides within the 3'UTR (569Ins20) were observed, each in a single IHH patient. Haplotype construction in 109 patients and 40 controls defined only 6 haplotypes (H1 to H6) with strong linkage disequilibrium between polymorphisms of exon 3. The Association analysis showed that frequencies of two 3' UTR polymorphisms were higher in CPP than in control groups or IHH ($p<0.05$). This analysis extended to haplotypes based on exon 3 polymorphisms showed a higher frequency of haplotype H3 in CPP than in control groups ($p<0.05$). This difference remains significant ($p<0.05$) with haplotypes restricted to the 3'UTR polymorphisms. Functional analysis of the 3' UTR polymorphisms was performed in MDA-MB-231 cells transfected with a plasmid encoding a reporter gene under the control of a strong promoter and the 3' UTR of *KiSS1*. A significantly higher luciferase activity was observed in cells transfected with Haplotype H1 plasmid when compared to H2, H3 and H4. A significantly higher luciferase activity was observed in SK-N-SH cells transfected with a 569Ins20_3' UTR plasmid when compared to the wild type 3' UTR plasmid. However, identical luciferase activity was observed in MDA-MB-231 cells.

This analysis tends to show that genetic determinism of pubertal onset is associated to *KiSS1* 3'UTR polymorphisms in Caucasians. However, molecular mechanisms involved in this process seem to be complex and might be cell specific.

FC12-015 Puberty

TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in human puberty

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The production of LH and FSH from pituitary gonadotrope cells is largely controlled by the pulsatile delivery of gonadotropin releasing hormone from a functionally interconnected group of secretory neurons in the hypothalamus. Hypothalamo-pituitary-gonadal axis is active *in utero* and for the first few months of life before entering a state of quiescence. The mechanism of reactivation of the GnRH pulse generating system at the early second decade of life is an enduring enigma of human biology.

In this study, nine multiplex consanguineous families from Turkey affected by normosmic idiopathic hypogonadotropic hypogonadism (nIHH) were subjected to genome-wide SNP analysis, and within each family regions of homozygosity common to all affected individuals were identified. In three families, homozygosity at a locus on chromosome 4 segregated with nIHH. Alignment of all six affected members of the three families refined a 2.74 MB genomic region encompassing 20 known or predicted genes. Of those genes, we selected TACR3 for further analysis. TACR3 encodes NK3R, the receptor for Neurokinin B, a tachykinin peptide known to be highly expressed in hypothalamic neurons. And, on sequencing we found homozygous nonsynonymous mutations (G93D and P353S) in the coding sequence of TACR3. In yet another multiplex family in which TACR3 mutations had been excluded, we identified two autozygous regions that were common to the two affected family members but not to their unaffected sister. Although these regions encompassed around 120 genes, TAC3, on chromosome 12 encoding Neurokinin B, the preferred ligand for the neurokinin-3 receptor was the most biologically plausible candidate. On sequencing TAC3, we identified a homozygous missense mutation (M90T), which properly segregated with nIHH within the family.

Deleterious nature of the mutations in both genes were confirmed by demonstrating impaired calcium signaling in a heterologous expression system.

In summary, we have identified loss-of-function mutations in either neurokinin B or its receptor in four multiplex families affected by normosmic idiopathic

hypogonadotropic hypogonadism. These findings establish that neurokinin B action *via* the NK3R is necessary for normal development of human puberty.

FC12-016 Puberty

Fertility induction with HCG and FSH in males with hypogonadotrophic hypogonadism is more rapid and more effective when used in adolescence

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Background: Past methods of pubertal induction for hypogonadotrophic hypogonadism (HH) in males have utilised androgen alone. Persistence of prepubertal gonadotrophins occurs. Spermatogenesis remains immature, currently unknown if this is detrimental to fertility. Spermatogenesis is slow in onset when gonadotrophin deficient adult men are treated with gonadotrophins. Mean sperm concentration at conception is reported as >5 x 10^6 / ml. An imprinting effect of first spermatogenesis occurs, with reduced time to fertility in 2nd and future treatment cycles. It is unknown whether FSH is required for spermatogenesis in adolescent males.
We hypothesized that pubertal induction with HCG and rFSH in boys with HH would result in effective testicular growth and normal spermatogenesis over a time span similar to that of natural puberty. We compared past treatment outcomes using gonadotrophins in adult males for 1st fertility induction with similar treatment of adolescent males with HH.
Methods: Eleven males aged 13-23, with HH were given human Chorionic gonadotrophin (HCG)1500 units x 2/ week, by subcutaneous injection, with the addition of 150 – 300 unit x 3/ week of recombinant FSH after three months. Underlying diagnoses were isolated HH (7), congenital hypopituitarism (3) or past hypothalamic –pituitary lesion (1). Outcomes were compared to those of 5 men with similar conditions treated with gonadotrophins for the first time at age 26-30 years.
Results: For treated men aged 26 – 30 years, first treatment cycle resulted in spermatogenesis at 22–24 months and time to conception 20 – 41 months. Combined testicular volumes were 8–14 ml at conception. In contrast, adolescent boys had rapid increase of combined testicular size, 8 -30 ml, 6 months after initiation of treatment. Inhibin B rose to within the normal adult range for spermatogenesis (48 – 251 pg/ml) by 9 months, but was variable (15-53). Spermatogenesis was achieved in 3 males so far, 9 months after treatment commenced (study ongoing).
Conclusions:HCG and rFSH to treat HH in adolescent males results in normal linear growth, normal pubertal progress and testicular growth over a time span similar to that of normal puberty. Spermatogenesis is seen at 9 months after onset of treatment. Testicular imprinting during adolescence reduces treatment time and is likely to reduce duration of later fertility induction in adulthood.

FC12-017 Puberty

Parenthood in adult female patients treated for Hodgkin's disease during childhood and adolescence

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Background: Infertility is one of the long-term complications following intensive chemo- and radiotherapy in adult female patients treated for Hodgkin's disease (HD). Little is known about fertility and pregnancy rates in patients treated for HD during childhood and adolescence. We describe the number of children observed in long-term survivors of HD diagnosed and treated before 18 years.
Patients and Methods: 589 female patients were treated between 1978 and 1995 in the 5 successive DAL-studies HD 78 – HD 90. Median ages at diagnosis and at last follow-up were 13.8 years (range 2.9-17.9 years) and 28.5 years (6.7-43.1 years) respectively. Median follow-up was 14.8 years ranging from 0.1 to 28.0 years. 508 patients are in 1st CCR. Information about children was obtained by questionnaires and personal follow-up.
Results: 289 children were reported by 175 female patients until September 2008. The number of children varied from 1 – 6 per mother. The median age at the birth of the first child was 26 years (range 17-38 years) at a median follow-up of 12 years (3-27 years) after the diagnosis of HD. Generally, fewer children (63%) were born to HD patients between the ages 20-30 years when compared to the general German population. Preliminary data indicate that more children (109%) were born to mothers after the age of 30, when the birth rate in the general population already declines.
No difference in birth rates was noted if patients had received 0 (49 %), 2 (51 %), 4 (52 %) or 6-8 cycles (51%) of procarbazine with cumulative doses of 0, 3000 mg/m², 6000 mg/m², 9000-12 000 mg/m². The percentage of birth rates was not affected by radiation doses of 18-50 Gy applied to the chest (31.0%) or 5-50 Gy applied to the abdomen (30.1%), but was considerably decreased to 15.7% in women who had received 19.5-40 Gy to the pelvic area.
Conclusion: Long-term follow-up in female patients with HD documents a slightly lower birth rate between 20-30 years and a higher birth rate thereafter. Birth rates are apparently not affected by chemotherapy (DAL studies HD78 – HD 90), doses of chest or abdominal irradiation, but by pelvic radiation doses of 19.5-40 Gy.

FC12-018 Puberty

The effects of the suppression of puberty and the consecutive addition of cross-sex hormones on brain activation during mental rotation in transsexual adolescents: an fMRI study

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The VUmc gender team for children and adolescents assesses early-onset transsexuals on a regular basis. Little is known about aetiological aspects of transsexualism. Sex differences in cognition, gender identity and sexual orientation may all reflect sex-related neuroanatomical differences in the human brain. In transsexual people a reversal of the sex difference of the hypothalamus has been observed.
We hypothesize that in early-onset transsexualism the brain develops to some extent in the direction of the desired sex. The present study focuses on brain activation and performance during a complex cognitive skill depending on the manipulation of mental representations (mental rotation task) which reflects the most robust sex differences. Neuroimaging studies have consistently reported activation of the parietal cortex during performance on mental rotation tasks. We present functional MRI data using an adapted version of the 3D mental rotation test of Vandenberg and Kuse. Scans were acquired using a Philips Intera 3.0 Tesla MRI scanner.
We included 14 male-to-female (mean age 15.3 years) and 15 female-to-male transsexual adolescents (mean age 16.2 years) on GnRH analogues only. 12 mtf's were at least one year on estrogens (mean age 19.03 years) and 20 ftm's were at least one year on testosterone(mean age 19.2 years) when scanned. In addition, 38 female controls (15 mean age 15.6 and 23 mean age 19.0 year for both group representatives resp.) and 31 male controls (14 mean age 15.9 and 17 mean age 19.2 years resp.) were included.
Analysis of response accuracy revealed significant performance differences between control males (mean accuracy 54.7%) and females (mean 41.4%) (p=0.02). The transsexual adolescents showed performance in accordance with the desired sex, although these differences did not reach significance. Robust activation of the volumes of interest in the parietal lobe (Brodmann area's 39, 40, 7a and 7b) was seen on individual and group level.

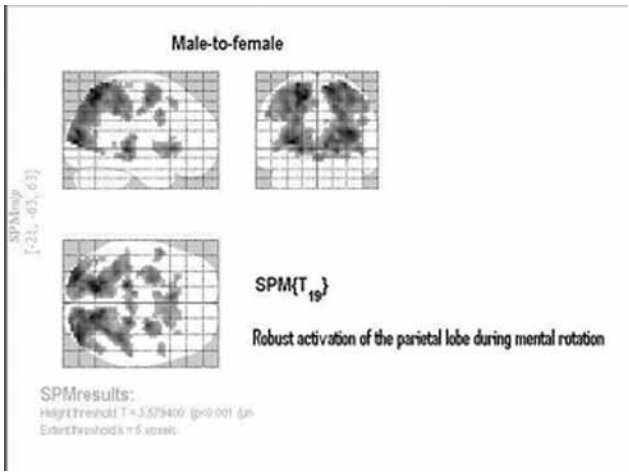

Fig. 1 Group activation of mtf's during mental rotation: activation of the parietal lobe
Group comparisons and the effects of the addition of cross-sex hormones on activation will be shown.

FC13-019 Diabetes Session I

The use of real-time continuous glucose monitoring to correct hypoglycaemia unawareness in adolescents: initial data from a randomised trial

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Hypoglycaemia unawareness (HU) carries a significant risk of severe hypoglycaemia. HU is associated with impaired glucose counterregulation, in particular impaired adrenaline response to hypoglycaemia: hypoglycaemia associated autonomic failure (HAAF). Although scrupulous avoidance of hypoglycaemia can restore autonomic symptoms of hypoglycaemia and counterregulatory responses, this is difficult to achieve. This study was designed to determine whether a real-time continuous glucose monitoring system (CGMS), with preset alarms at specific glucose levels would prove a useful tool to achieve avoidance of hypoglycaemia and thus reverse HAAF.
To address this question, adolescents with type 1 diabetes and HU were randomised to either standard therapy (standard group) or to use real-time (Medtronic Minimed Paradigm® REAL-Time System) CGMS (CGMS group) for 4 weeks. Both groups were advised to strictly avoid hypoglycaemia with conventional testing at least 6 times daily to maintain blood glucose levels between 6-10mmol/L. In addition, the CGMS group wore a subcutaneous sensor with preset low alarms at 6mmol/L and advised to institute standard hypoglycaemia therapy below this level. A hyperinsulinaemic hypoglycaemic clamp (nadir glucose level 2.8mmol/L) was performed at baseline and after the intervention to assess hypoglycaemic symptom and hormone responses.
To date, 5 subjects in the standard group (mean age 15.0±0.8yrs, mean HbA1c 7.9±0.3%) and 6 subjects in the CGMS group (mean age 13.4±0.7yrs, mean HbA1c 8.0±0.3%) have been studied. As expected, at baseline the adrenaline response to hypoglycaemia was blunted and there was no difference between subjects randomised to standard or CGMS groups (403±217 vs. 368±247nM). Following the intervention, there was a greater adrenaline response in the CGMS group (371±284 vs. 840±341nM, standard vs. CGMS group respectively). This represents a greater percentage rise in adrenaline during hypoglycaemia following therapy in the CGMS group (-32±19% vs. 189±67%, standard vs. CGMS group respectively, p=0.026). Following the intervention, there was no deterioration in glycaemic control in the standard or CGMS group (HbA1c 7.9±0.4% vs. 8.3±0.3%, p=0.474). These preliminary results suggest an improved adrenaline response during hypoglycaemia following strict avoidance of hypoglycaemia with real-time continuous glucose monitoring and alarm system. This may prove a useful clinical tool to reverse hypoglycaemia unawareness.

FC13-020 Diabetes Session I

Hypoglycaemia does not change the threshold for arousal from sleep in adolescents with type 1 diabetes

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Nocturnal hypoglycaemia is a significant problem for children and adolescents with type 1 diabetes mellitus (T1DM). The counterregulatory hormone response to hypoglycaemia is blunted both in patients with T1DM and healthy subjects during sleep. It is not known whether the threshold for arousal from sleep is also modified by hypoglycaemia. To address this question we compared the acoustic arousal threshold from sleep during hypoglycaemia and euglycaemia in adolescents with T1DM.
Adolescents with T1DM were studied on 2 occasions: under hypoglycaemic and euglycaemic conditions. During the hypoglycaemia night, subjects underwent an hyperinsulinaemic hypoglycaemic clamp with nadir glucose level of 2.8mmol/L. Hypoglycaemia was initiated during stage 2 sleep and maintained during stage 3/4 or slow wave sleep. During the euglycaemia night, blood glucose was maintained at 5.5mmol/L utilising the same clamp technique. The acoustic arousal threshold was determined during the first cycle of slow wave sleep. Sleep was monitored using standard polysomnography with electroencephalogram (EEG) to determine sleep stage. The equipment for arousal testing was designed locally and consisted of a sound source and an array of speakers mounted to a frame above the subject's head. Sounds of increasing intensity, from 50 to 120dB with 5dB increments were played. The acoustic arousal threshold was defined as the sound intensity required to provoke arousal from slow wave sleep. Arousal was defined by standard criteria, including brief muscle arousal, increase in electromyographic activity and a change to low-amplitude mixed-frequency EEG activity with increased proportions of alpha-waves (8-13Hz). Repeat measurements were taken once slow wave sleep was re-established. Reproducibility of technique was validated in 4 healthy adolescents.
Seven subjects (mean age 14.2±0.8yr, mean HbA1c 8.1±0.3%, duration of diagnosis 2.5±0.5yr) completed both study nights. Arousal was only noted during acoustic testing and did not occur during hypoglycaemia alone. The acoustic arousal threshold during slow wave sleep was similar under both conditions: 79±8dB during euglycaemia and 71±6dB (p=0.311) during hypoglycaemia. There was no difference in arousal threshold during hypoglycaemia between subjects with a counterregulatory hormone response (n=4) and those without. In adolescents with T1DM, hypoglycaemia does not appear to impair arousal from slow wave sleep induced by an external auditory stimulus.

FC13-021 Diabetes Session I

Discontinuation of insulin pump treatment in children, adolescents and young adults. A multi-center analysis based on the DPV database in Germany and Austria

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Background and aims: Insulin pump therapy is well established in the treatment of children and adolescents with type 1 diabetes. Most studies focus on outcome parameters like HbA1c, hypoglycemia and quality of life, whereas few reports address patients who discontinue pump therapy. This survey focuses on the discontinuation rate of insulin pump treatment in the pediatric and young adult age group.
Materials and Methods: The prospective multi-center DPV (electronic diabetes patient documentation system) database has been established since 1990 and is broadly used in Germany and Austria. All pump users among the participating centers documented since 1995 were included in this analysis.
Results: 11 710 patients with type 1 diabetes were recorded as treated with insulin pumps. In total 463 patients (4%) switched from insulin pump treatment to multiple daily injections (MDI). In the group of patients who stopped with pump treatment, the mean duration of pump therapy was 1.7 years (SE +/- 0.06 years), 60.5% were female. Subdivided into age groups, the discontinuation rate was lowest in the age group <5 years (0.1%), followed by the groups aged 5-10 years (0.3%), 15-20 years (0.8%) and > 20 years (0.8%). The group aged 10-15 years showed the highest rate of discontinuation (2%).
Conclusions: The discontinuation rate of insulin pump therapy is in general low (4%). The younger the patient at the time of initiating insulin pump treatment, the lower is the discontinuation rate. The highest rate was seen in adolescents aged 10-15 years. Girls stopped insulin pump treatment more often than boys (60.5% vs. 39.5%).

FC13-022 Diabetes Session I

The RealTrend study: effect of continuous glucose monitoring on metabolic control in addition to pump therapy in poorly controlled type 1 diabetic patients

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Background and aims: Efficacy of insulin pump augmented with continuous glucose monitoring (CGM) versus Continuous Subcutaneous Insulin Infusion with standard self-monitoring of blood glucose has not yet been determined**.**
Materials and methods: In this randomized, controlled, multi-center trial, 132 adults and children with type 1 diabetes, insufficiently treated with multiple daily insulin injections (A1c≥ 8%) were assigned to a 6 months treatment in one of 2 study arms: PRT arm, fitted with the Paradigm REAL Time System (Medtronic insulin pump with integrated CGM), or CSII arm, fitted with an insulin pump and conventional blood glucose self-monitoring. In the PRT arm, patients wore glucose sensors for 9 training days prior to baseline HbA1c. HbA1c change between baseline and study end served as a primary endpoint in the 2 study arms. Secondary endpoints included hyper- and hypoglycemia parameters measured by CGM: average glucose, time spent above and below hyper- and hypoglycemia limits, and respective area under the curve.
Results: HbA1c was analyzable for 115 patients (46 children, 69 adults) of the full analysis population (FAS) and improved between baseline and study end in the two groups (PRT, n=55, -0.81%±1.09; CSII, n=60, -0.57%±0.94; p=0.087). A per protocol (PP) analysis of 91 patients (35 children, 56 adults) who wore sensors over 70 % of the time (as required by the inclusion criteria) showed a significant difference in A1c reduction between groups (PRT; n=32; -0.96%±0.93, CSII, n=59; -0.55%±0.93, p=0.004). Ancillary analyses revealed a significant decrease in HbA1c levels between the screening visit and the end of the study (PRT -1.14±1.21, p<0.001; CSII group -0.57±0.91, p<0.001), as well as a significant difference in favor of the PRT group (p=0.006) for the entire study population as well for the per protocol population (PRT -1.23±1.08, p<0.001; CSII -0.55±0.90, p<0.001; inter-group comparison: p<0.001). In PRT group, CGM hyperglycaemia parameters decreased in line with HbA1c, without increased hypoglycaemia.
Conclusion: In both FAS and PP populations HbA1c decreased in both study arms after treatment was changed from MDI to CSII or PRT, but improved significantly more in the PRT group when patients wore the CGM more than 70% of the time versus CSII.

FC13-023 Diabetes Session I

An in vitro paradigm for diabetic cerebral oedema and its therapy – a critical role for taurine and water channels

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BACKGROUND: The etiology of diabetic ketoacidosis (DKA) related cerebral oedema (CE) remains enigmatic. *In vivo* data from children with T1DM and DKA suggests a role for idiogenic osmoles such as taurine, an essential neuro-osmoregulator, in its pathogenesis. Neuroimaging and spectroscopy studies confirmed neuronal swelling and neurochemical changes such as taurine peaks in the fronto-medial, basal ganglia and parietal subcortical neurons (Ref).
OBJECTIVE: Our aim was to investigate the role of taurine in the development of neuronal swelling using our established *in vitro* model for DKA–CE, the SH-SY5Y neuroblastoma cell line. A high glucose, hypertonic environment mimicking DKA was established and subsequent responses to fluids (hypo- and isotonic), mimicking "fluid therapy", were examined. Under conditions of increasing glucose and sodium concentrations in culture medium followed by "fluid therapy", cell morphology, taurine production and release were examined (via HPLC and radiolabelled taurine tracer). Responses of the volume sensitive organic anion channel (VSOAC - a well recognized channel for taurine fluxes) and aquaporin receptors were examined, using specific blockers.
RESULTS: Intracellular production and accumulation of taurine was demonstrated in a graded fashion, concomitant with the degree of hypertonicity, acting as a protective neuro-osmolyte in a high glucose hypertonic, DKA-like environment. When such cells were then treated with hypotonic fluids, neuronal swelling occurred, but was mitigated in part by rapid cellular extrusion of taurine (for which we identified expression of the taurine receptor regulatory gene TonEBP). Inhibition of the rapid efflux of taurine by blocking VSOAC during hypotonic fluid therapy led to neuronal cell swelling. Aquaporin 4 & 9 receptors were identified, a novel finding in this neuronal cell line. These receptors were upregulated in increasingly hyperosmolar environments and blocking such receptors during "treatment" also led to neuronal swelling. Blocking both VSOAC and Aquaporin receptors led to further neuronal swelling.
CONCLUSION: We provide novel in vitro evidence for the role of taurine, aquaporin receptors and the VSOAC in the development of neuronal swelling such as occurs in CE following therapy for DKA with hypotonic fluids.
Ref: Cameron FJ, Insights into the acute cerebral metabolic changes associated with childhood diabetes. Diabet Med. 2005 May;22(5):648-53.

FC13-024 Diabetes Session I

ZnT8, GAD 65 and IA-2 autoantibodies in type 1 diabetes children at diagnosis and 3-5 years after the diagnosis, stratified for residual beta cell function

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Objective. Diabetes specific antibodies and residual beta cell function (RBF) after diagnosis of type 1 diabetes(T1DM) are not well characterized in children. The beta cell associated zink transporter antigen ZnT8, has newly been described, and autoantibodies against ZnT8 may increase the predictive value of autoimmune mediated C-peptide loss and T1DM. In the future immune intervention to reduce autoimmunity and preserve RBF might be an option. The aim of study was to examine GAD65, IA-2 and ZnT8 autoantibodies(Ab) at diagnosis and 3-5 years after the diagnosis of DM and to examine the association between these Abs and RBF.

Methods. 263 children (130males) with mean(SD) age 13,2(3,2) years and T1DM for 3-5 years were included. GAD65, IA-2 and ZnT8 (both ZnT8R and ZnT8W) Abs were assessed at diagnosis and 3-5 years after the diagnosis. RBF was assessed by meal stimulated C-peptide (MCP) after 3-5 years.

Results. At onset the distribution of antibodies was: GAD65Ab - 91%, IA2Ab - 78%, ZnT8Ab - 74%. 86% were positive for 2 or 3 Abs. 1 patient had only ZnT8Ab, and 6 patients were Ab negative.

After 3-5 years the distribution of antibodies was: GAD 65Ab - 73%, IA-2Ab - 68%, ZnT8Ab - 61%. 74% were positive for 2 or 3 Abs. 9 patients had only ZnT8Ab, and 24 patients were Ab negative.

Patients with RBF (MCP>10 pmol/L) had a significant lower decrease in GAD65Ab and IA2Ab compared to patients with no RBF. The same association was not found for ZnT8Ab, neither ZnT8RAb nor ZnT8WAb.

Antibodies and residual beta cell function							
		GAD65			IA-2		
	n	At onset	After 3-5 years	Change	Onset	3-5yrs	Change
Non-RBF (<10 pmol/L)	140	177	95	-83	141	61	-80
RB F (>10 pmol/L)	123	168	114	-54	121	68	-53
	Mann-Whitney-U p =	ns	ns	0,001	ns	ns	0,016
		ZnT8R			ZnT8W		
		Onset	3-5yrs	Change	Onset	3-5yrs	Change
Non-RBF	140	186	66	-120	141	67	-74
RBF	123	167	70	-97	155	95	-60
	MWU p =	ns	ns	0,66	ns	ns	0,53

(Concentrations U/mL)

Conclusions. Many children diagnosed with T1 DM have measurable residual beta cell function after 3-5 years DM duration. The highest mean antibody levels 3-5 years after the diagnosis were found in children with RBF. GAD65Ab and IA2Ab decreased significantly less in the RBF group compared with the non RBF group. ZnT8Ab was associated with RBF. If immune modulation with preservation of beta cells become an option, this might be an offer to children with high GAD65Ab, IA2Ab and RBF even after 3-5 years of DM.

FC14-025 Disorders of Sexual Differentiation (DSD)

R-spondin1 is expressed during human ovary development and augments β-catenin signaling

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Background: Human testis development starts from around 42-44 dpc with a transient wave of SRY expression followed by up-regulation of testis specific genes and distinct morphological, paracrine and endocrine events. Although anatomical changes in the ovary are less marked, a sub-set of ovary specific genes are also expressed during this time. The thrombospondin-like factor R-spondin1 (*RSPO1*) is expressed in early mouse ovary and causes ovarian transdifferentiation into testicular tissue in a knockout murine model. Similarly, patients with disruption of *RSPO1* have 46,XX testicular DSD (formerly "XX males") or 46,XX ovotesticular DSD (formerly "true hermaphrodites"). These effects of R-spondin1 are thought to be mediated via the canonical Wnt-signaling pathway following paracrine secretion of the R-spondin1 peptide.

Aims & Methods: We aimed to study the role of R-spondin1 in normal early human gonad development and in the ovotestis of a patient with a mutation in *RSPO1* (c.286+1G>A) and to use this naturally occurring mutant R-spondin1 to study the interaction of this protein with β-catenin signaling in human cell systems. Ethical approval and consent was obtained for all human studies.

Results: 1) *RSPO1* expression increases in the ovary during critical stages of human gonadal determination (42-63 dpc) but remains unchanged in the developing testis. 2) The ovotestis from a patient with altered R-spondin1 showed decreased β-catenin and WNT4, consistent with down regulation of ovarian pathways. 3) Transfection of wild-type *RSPO1* cDNA resulted in weak dose-dependent activation of the β-catenin responsive TOPFLASH reporter (maximum 1.8 fold). Co-transfection of *CTNNB1* (encoding β-catenin) with *RSPO1* resulted in dose-dependent synergistic activation of this reporter (maximum 10 fold). No activation was seen with mutant *RSPO1* vector. Similar synergistic activation was obtained using mouse R-spondin1 peptide. 4) R-spondin1 showed strong nuclear/nucleolar localization in several different cell lines when assessed using immunocytochemistry, tagged protein (HA-, GFP-), or western blot analysis of nuclear/cytosolic extracts. Exogenous R-spondin1 peptide showed internalization and nuclear localization in some cases.

Conclusions: *RSPO1*/R-spondin1 is a critical regulator of ovary development in humans and may function as a tissue-specific amplifier of β-catenin signaling to oppose testis development. Its nuclear role may be more important than previously thought.

FC14-026 Disorders of Sexual Differentiation (DSD)

Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the *CBX2* gene

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The process of sexual differentiation is central for reproduction of almost all metazoan. Nevertheless, factors and mechanisms regulating this process are not completely known. Insights in this cascade derived mainly form animal models and cases of patients in whom the genetic and somatic sex do not match. We took advantage of both these two tools and identified the human homologue of the M33 knock-out mouse model. M33 deficient mice have male-to-female sex reversal with perfectly normal female gonads and genitalia in 50% of the cases, but are invariably sterile. We demonstrated a loss-of-function double heterozygote mutation state in a 46,XY girl with normal ovaries at histology, normal uterus and external female genitalia, accidentally diagnosed because of a discrepancy between prenatal karyotype and phenotype at birth. Functional studies demonstrated that the mutated CBX2 does not properly bind to and regulate the expression of target genes essential for sex development such as SRY,

Steroidogenic Factor 1, Wilm-Tumor gene 1, SOX9, DAX1 and WNT4. Our data identify CBX2 as essential for normal human male gonadal development, suggest that it lies upstream of SRY in the human sex development cascade and identify a novel autosomal recessive cause of defect of sex development.

FC14-027 Disorders of Sexual Differentiation (DSD)

Molecular genetics of secondary amenorrhea: functional analysis of an heterozygous variant of FOX-L2 gene (G187D) supports its involvement in non-syndromic premature ovarian failure

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In human, FOX-L2 mutations cause the Blepharo-Phimosis-Epicanthus inversus Syndrome (BPES) which associates palpebral abnormalities to amenorrhea due to premature ovarian failure (POF).

In a screening program of genetic causes of secondary amenorrhea related to POF, we identified an heterozygous variant of FOX-L2 : c.560 G>A, in a young woman with POF and XX caryotype. The gene variant identified lead to the amino acid substitution pGly187Asp FOX-L2 in a highly conserved fragment. This variant was absent in 110 control chromosomes.

The potential deleterious effects of this amino-acid change was firstly based on the SIFT software which calculates the probability of a substitution of being deleterious. Moreover, the changes in polarity induced by the substitution of Glycin by Aspartate may interfere with the post-translational modification process and perturbs ovarian-specific protein interaction. From a functional perspective, FOX-L2-G187D was found to partly activate the promoter of FOX-L2 itself and the FOX-L2 specific artificial promoter. Whereas the transcriptional capacity of the FOX-L2-G187D was significally lower than that of normal, the FOX-L2-G187D mutant was able to strongly activate a reporter construct driven by the promoter of Osr2 (odd-skipped related 2 transcription factor), suggested to be a crucial target of FOXL2 in the craniofacial region. This is compatible with the absence of BPES in our patient.

Our results support the implication of FOX-L2 variants in non-syndromic POF and confirm the regulatory interaction between FOX-L2 and OSR-2, whose perturbation might contribute to the palpebral phenotype in BPES. We suggest that systematic genetic screening of FOX-L2 mutations would be of interest in non-syndromic POF, to improve genetic counselling and to better understand the molecular etiology of adolescent amenorrhea.>

FC14-028 Disorders of Sexual Differentiation (DSD)

Two new heterozygous SF1 gene mutations presenting phenotypic variability in 46,XY DSD siblings and low ovarian reserve in affected fertile 46,XX subjects

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Steroidogenic factor-1 (SF-1) is a nuclear receptor that plays crucial roles on adrenal and gonadal development, reproduction, and male sexual differentiation. In humans, mutations in SF-1 gene have been reported to cause gonadal dysgenesis with or without adrenal failure, in 46,XY individuals. Primary ovarian insufficiency has been recently described in 46,XX affected patients. We describe two new heterozygous SF-1 gene mutations in two unrelated families with a history of 46,XY disorders of sex development (DSD) and 46,XX ovarian insufficiency.

Family 1 had two 46,XY siblings with different degrees of prenatal masculinization. One, raised as a male, had perineal hypospadias but normal testosterone and gonadotropin levels during early infancy and late adolescence. However, he had subnormal testicular volume (10 cc) and low inhibin B levels. The other, raised as a female, had pronounced undermasculinized external genitalia, very low testosterone, müllerian remnants and severe testicular dysgenesis (gonadectomy). In both siblings, a W279X heterozygous mutation of the SF1 gene was found. This new mutation predicts the loss of a large part of the ligand binding site and the AF2 domain of the SF-1 protein. In family 2, four of six siblings had 46, XY DSD. The oldest one was referred to us at 12 years of age, raised as a female, presenting pre- and postnatal partial virilization. Mild testicular dysgenesis was found after gonadectomy. Two of the other three siblings (raised as males) had severe hypospadias but one had a mild form. One of the formers had normal puberty with slightly increased FSH levels. The others have not reached pubertal age. A 22-year-old, 46,XX sister, had normal sexual development but increased FSH levels. The mother entered menopause at 37 years of age. In these five siblings and in their mother, an Y183X heterozygous mutation was detected. This new mutation predicts the loss of the whole ligand binding site and the AF2 domain of the SF-1 protein.We concluded that an extreme phenotypic variability can be observed in siblings with the same mutation. Spontaneous puberty close to normal was found in 46,XY siblings raised as males. However, according to previous reports, the relative low increment of serum FSH levels in the presence of small adult testes might suggest partial hypogonadotrophic hypogonadism. Low ovarian reserve and preserved fertility in 46,XX subjects can be observed in heterozygous SF-1 gene mutations.

FC14-029 Disorders of Sexual Differentiation (DSD)

A gain of function mutation in the mastermind like domain 1 *gene* (MAMLD1) discloses a new pathway in the etiology of 46,XY disorders of sex development (DSD)

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MAMLD1 has been shown to be implicated in the etiology of penoscrotal hypospadias. To date, 2 loss-of-function *nonsense* mutations have been identified in Japanese patients with hypospadias. **Objective:** To screen *MAMLD1* for mutations in a large cohort of Brazilian patients with undetermined 46,XY DSD. **Patients and Methods:** We evaluated 50 patients in which defects of testosterone synthesis, 5 alfa-reductase 2 deficiency and partial androgen insensitivity were previously excluded. Seven of them had been previously gonadectomized and reared in the female sex. The entire coding region and the splicing sites flanking regions of *MAMLD1* were amplified and sequenced from genomic DNA. Two microsatellite markers flanking the gene were used to analyze founder effect. Transactivation function of *MAMLD1* was analyzed by a luciferase assays. COS-1 cells seeded in 6-well dishes were transiently transfected with luciferase reporter vector (p-Hes1, p-Hes3, p-Hes5 and p-Hes7), expression vector for *MAMLD1* (WT and Mutant) and pRL-CMV vector as an internal control. All experiments were performed in triplicates and repeated 3 times. **Results:** We identified a novel mutation, the p.H274Q on exon 3 in 4 unrelated patients (2 sporadic and 2 familial cases). Two patients underwent gonadectomy in infancy and were raised as girls. Pelvic ultrasound showed a uterus in one of them. The other two male patients had micropenis, cryptorchidism and perineal hypospadias. This mutation was absent in 150 Brazilian controls. The transactivation activities of the mutant were 2.5 folds higher than the WT with p-Hes7 and 2.0 folds higher with p-Hes3. No founder effect was demonstrated in these families. **Discussion**: The new mutation is located in a highly conserved region of *MAMDL1* which is essential for male genitalia development. HES is a family of transcriptional repressor of Notch signaling. Hes genes display an oscillatory expression pattern and control the timing of biological events, such as somite segmentation. Both absence and persistent Hes7 expression leads to somite fusion. Therefore, overexpression of *Hes* genes should impair the normal male differentiation by continuous suppression of downstream genes involved in male sex development. **Conclusion**: This is the first report of a gain of function mutation in *MAMLD1* in patients

with 46,XY DSD and the presence of Müllerian derivatives. Furthermore, a novel mechanism for involvement of MAMDL1 in the etiology of 46,XY DSD is suggested.

FC14-030 Disorders of Sexual Differentiation (DSD)

Epigenetic abnormalities of the androgen receptor gene in hypospadia

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Hypospadia affects approximately one in 250 live male births, with a progressively increasing incidence in Europe and North America. The exact etiology remains unknown in the majority of cases, although hypospadia has been associated with some genetic syndromes or defects involving androgen receptor (AR) abnormalities. To assess whether defects in the AR function contributing to the development of hypospadia are present in children with this abnormality, CAG length and AR gene methylation in peripheral blood leukocytes (PBLs) and foreskin tissues from 20 patients with isolated hypospadia were analyzed. The same parameters were examined in 20 age-matched (3.5±0.6 *vs* 5.0±1.0 yr, mean±SE) control children undergoing surgical procedure for circumcision. **MATERIALS AND METHODS:** Genomic DNA was isolated from PBLs and foreskin tissue obtained from patients with hypospadia and normal control children. The number of CAG repeats was determined by PCR amplification. Methylation analysis was performed by Real-Time PCR after sodium bisulfite treatment. Two different fluorogenic probes (MGB) recognizing the bisulfite-converted methylated and un-methylated DNA, were used, respectively, and the percentage of methylation *vs* baseline was calculated. Protein content was obtained after lysis of the cells/tissues. The AR expression was analyzed using an anti-AR (1:1000) specific polyclonal antibody. **RESULTS:** The mean number of CAG repeats both in PBLs (22.6±1.6 *vs* 21.6±0.9, mean±SE) and foreskin tissues (21.6±1.1 vs 21.9±1.0) was similar in patients with hypospadia and controls. Conversely, AR gene methylation in foreskin tissues from patients with hypospadia was higher than in normal children (methylation percentage: 69.3±2.7 *vs* 50.8±4.0, mean±SE; P=0.006). No differences in PBLs AR gene methylation was detected between the patient and control group (methylation percentage: 98.2±0.5 *vs* 96.4±1.4; P=0.28). In line with these results, the AR expression analyzed by Western blotting in foreskin tissue of hypospadic patients, was lower than in controls (OD: 32510.0±4719.7 *vs* 95650.6±4422.8, mean OD±SE; P<0.001). **CONCLUSIONS:** the AR gene in target tissues from patients with hypospadia is more methylated than in control children, resulting in a decreased expression of the AR. This epigenetic alteration of the AR gene might be involved in the pathogenesis of hypospadia. Further studies aimed at clarifying the mechanisms responsible of these abnormalities are warranted.

FC15-001 Obesity II

Early adiposity rebound is associated with metabolic status representative of metabolic syndrome at 12 years of age: a cohort study

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Background

The age of adiposity rebound (AR), when body mass starts to rise after infancy, is thought to be an origin of obesity in later life. In order to elucidate whether the early origin of obesity is related to the future occurrence of metabolic syndrome, we investigated the relationship between the timing of AR and the later metabolic consequences including small dense LDL (SDLDL), which is a metabolic marker of insulin resistance, at 12 years of age. We also investigated whether the difference of body mass index (BMI) between the age of 1.5 years and 3years related to the future occurrence of metabolic syndrome.

Methods

A total of 271 children (147 boys and 124 girls) who were born in 1995-1996 were enrolled in the study. Annual measurements of BMI from 4 months to 12 years were carried out prospectively. We calculated the age at AR, defined as the age which the lowest BMI occurred during this period. The subjects were divided into 6 groups according to the age at AR as follows: group 1: AR below 2 years; group 2: 3y; group 3: 4y; group 4: 5y; group 5: 6y; group 6: over 7y. At 12 years of age, SDLDL, other lipids, lipoproteins, blood pressure (BP) and atherosclerotic index (AI) were measured in each group. We also divided the subjects into 2 groups according as the BMI at 3 years is higher than at 1.5 years or not. We compared levels of metabolic parameters at 12 years old in each group.

Results

Children who exhibited an earlier AR were associated with the highest BMI value at 12 years of age. The cumulative prevalence of SDLDL is 8.4% in children in whom AR occurred before the age of 5 years and 2.5% in those in whom AR occurred after 6 years. The earlier AR was associated with a lipoprotein phenotype representative of insulin resistance, consisting of elevated triglyceride and apolipoprotein B (Apo B) and decreased HDL-cholesterol levels in boys and elevated Apo B in girls. The earlier AR also related to elevated AI at 12 years. The odds ratios of hypertension in boys and AI in girls at 12 years are high in the group in which the BMI at 3years is higher than at 1.5years.

Conclusion

The present longitudinal population-based study indicates that children who exhibit AR at a younger age predispose towards the future development of metabolic syndrome. Thus, identifying high-risk children may be possible if we pay attention to early AR.

FC15-002 Obesity II

Subcutaneous and visceral fat are determinants of bone density measures in obese adolescent girls

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Background: Fat mass, particularly regional fat distribution, is increasingly being recognized as a determinant of bone mineral density (BMD). Elevated leptin and adiponectin may be deleterious to bone, and low BMD has been linked to a chronic inflammatory state. We have previously demonstrated that subcutaneous adipose tissue (SAT) is an important determinant of high sensitivity (hs) CRP, whereas visceral adipose tissue (VAT) determines inflammatory markers such as s-ICAM and E-selectin in obese adolescents.

Objectives: Our objectives were to examine whether SAT and VAT are determinants of BMD and bone mineral apparent density (BMAD) in obese adolescent girls. We hypothesized that VAT would be an inverse and SAT a positive determinant of BMD, mediated by adiponectin, leptin and inflammatory cytokines.

Subjects and Methods: We studied 30 adolescent girls (15 obese and 15 normal weight) 12-18 years old, matched for maturity (bone age), race and ethnicity. We assessed regional fat distribution (SAT, VAT) using MRI, and total fat and BMD using DXA. Fasting levels of leptin, adiponectin, hsCRP and s-ICAM were obtained.

Results: Mean BMI SDS was 3.7±1.5 in obese subjects and 0.1±0.4 kg/m^2 in controls. Total fat, VAT, SAT, leptin, hsCRP and s-ICAM were higher in obese girls ($p<0.05$), and adiponectin trended lower but was comparable. BMD Z-scores were higher in obese girls. SAT and VAT correlated positively with BMD, with SAT being the stronger predictor ($p<0.03$) within obese girls and for all subjects on univariate regression. In contrast, on multivariate analysis after controlling for SAT, VAT was a *negative* predictor of (i) lumbar BMD, BMAD, BMAD Z-scores, and whole body (WB) BMD, BMC/height and BMC/height Z-scores in obese girls, and of (ii) WB BMD and BMC/height for the group as a whole. In a stepwise regression model after including SAT, VAT, adipokines and cytokines in the model, within obese girls, SAT and VAT remained positive and negative predictors respectively of almost all BMD measures, leptin inversely predicted lumbar BMD and BMAD, and sICAM inversely predicted WB BMD Z-scores. For the group as a whole, SAT positively predicted all BMD measures, sICAM and leptin were negative predictors of lumbar BMD and BMAD, and VAT an inverse predictor of WB BMD and BMC/height.

Conclusion: VAT is an independent and inverse determinant of bone density measures, and this association may in part be mediated by adipokines and a chronic inflammatory state.

FC15-003 Obesity II

Body composition in 48 morbidly obese adolescents one year after laparoscopic gastric bypass (AMOS study)

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Background: Severe obesity in the adolescence is rapidly increasing. Gastric bypass surgery for morbid obesity in adolescents has been suggested, though one might have concerns to such an aggressive weight loss intervention during growth.
Methods: A sub-group of forty-eight morbidly obese adolescents (18 boys/ 30 girls), aged 13–18 yrs, drawn from the nationwide Swedish study (Adolescent Morbidity Obesity Surgery study, AMOS) underwent laparoscopic gastric bypass. DXA was perfomed before and after 1 yr postoperatively.
Results: Mean (SD) age at baseline was 16.9 (1.1) and after a year 18.1 (1.1) yrs. BMI decreased from 44.5 (4.9) to 30.9 (4.3) over the first year ($p<0.001$). The total Fat mass (FM) was preoperatively 65.0 (10.6) kg and decreased to 37.5 (11.7) ($p<0.001$). Lean tissue mass (LTM) was 61.2 (11.3) kg and 51.8 (11.5) kg after one yr ($p<0.001$). This suggests a 42% decrease for FM and 15 % decrease in LTM. BMD gr/cm^2, expressed as z-score in relation to a gender and age-matched-control was +1.9 and +0.8 after one year ($p<0.001$). Bone mineral content (BMC) increased from 3.04 (0.5) to 3.3(0.6) kg ($p<0.001$).
Conclusion Laparoscopic gastric bypass due to morbid obesity in the adolescents leads to dramatic weight loss characterized by predominant loss of body fat. Lean tissue was well preserved, especially in boys. BMD decreased from supra-normal to normal levels over two years while BMC continued to increase despite surgery, which suggest that peak bone mass had not yet been achieved.

FC15-004 Obesity II

The fat mass- and obesity-associated *FTO* gene is expressed in human placenta and is related to birth weight and placental visfatin

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Background and aims: The fat mass- and obesity-associated (*FTO*) gene has been found to contribute to obesity risk in adult subjects. The loss of *FTO* in mice leads to postnatal growth retardation with reduction of both fat and lean mass. Visfatin, a newly described adipokine, is strongly expressed in fetal membranes. In adipose tissue, the expression of visfatin correlates with that of *FTO*. Our aim was to assess whether the *FTO* gene is expressed in human placenta and whether it correlates with fetal growth and placental visfatin.
Materials and methods: Human placentas from 83 Caucasian women with uncomplicated pregnancies were obtained at delivery after informed consent. Clinical variables of the newborns were assessed. *FTO*, visfatin and housekeeping genes (TATA box binding protein and succinate dehydrogenase complex, subunit A) were quantified in placental tissue by real-time PCR using TaqMan probes. A relative quantification was used using the delta-delta Ct formula.
Results: *FTO* was highly expressed in human placenta and was independently and directly related to newborn's weight and length (both $p<0.001$) and to the fetal-to-placental weight ratio ($p<0.05$). *FTO* gene expression was also directly related to visfatin gene expression ($p<0.0001$).
Conclusion: For the first time to our knowledge, we describe that the *FTO* gene is expressed in human placenta and is directly associated with measures of fetal growth and to placental visfatin. The role of the *FTO* gene in the regulation of body weight is herein suggested to include prenatal development.
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FC15-005 Obesity II

Serum HMW adiponectin, AdipoR1, the adaptor protein, APPL1, and the GTPase Rab5 in primary adipocyte cultures of lean and obese prepubertal children in relation to insulin sensitivity

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Introduction:Childhood obesity is associated with glucose intolerance and Diabetes Mellitus Type II. Adiponectin, an insulin-sensitizer, stimulates glucose utilization through activation of its receptors AdipoR1 and AdipoR2. The adaptor protein, APPL1, binds to these receptors and facilitates insulin-stimulated translocation of the glucose transporter Glut4 in adipose tissue via the GTPase Rab5.Aim: To study the expression levels of AdipoR1, APPL1 and Rab5 in obese and lean pre-pubertal children in association with Adiponectin and Insulin.Methods: Primary cultures of pre- and mature adipocytes were developed from routine surgical biopsies of subcutaneous abdominal adipose tissue from 17 obese (BMI ≥ 95%) and 36 healthy lean pre-pubertal children (BMI <85%) in 2 age groups (group A: 2 months-7 yrs and group B: 8-12 yrs). The expression of AdipoR1 APPL1 and Rab5 was studied at the mRNA level (mR) by RT-PCR and AdipoR1 and APPL1 were studied at the protein level (Pr) by western immunoblotting. HMW Adiponectin (HMW) and serum Insulin were measured by ELISA. HOMA-IR was calculated in all subjects. Results:1)HMW was decreased in both the older lean and obese children when compared to the younger lean and obese, but it was increased in the younger obese in comparison to all the children. 2)The Pr of AdipoR1 was significantly decreased in the mature adipocytes of the obese children in both age groups as compared to their respective lean ($p<0.05$). 3)APPL1 showed no significant differences between the older lean and older obese children. The mR and Pr of APPL1 though, were increased ($p=0.005$) in the mature adipocytes of the younger obese children in comparison to the younger lean. 4)Rab5mR was significantly decreased in the mature adipocytes of the older and younger obese children in comparison to their respective lean. 5)Insulin and HOMA-IR were increased in the older obese and lean as compared to the younger children. Conclusions:1)The decreased HMW and increased insulin and HOMA-IR in the older lean pre-pubertal children may pre-empt the "physiologic" insulin resistance of puberty. 2)The decreased expression of AdipoR1Pr and Rab5mR together with the decreased HMW in the older obese pre-pubertal children though, may facilitate glucose intolerance. 3)The normal insulin sensitivity of the very young obese children may be due to the protective increase in HMW and APPL1 despite the decreased AdipoR1 and Rab5.

FC15-006 Obesity II

Sirt1 is involved in resveratrol-stimulated changes in human adipocytes

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Objectives:
Calorie restriction (CR) leads to retardation of the aging processes and to longer life in many organisms. This effect of CR can be mimicked by resveratrol,

a sirtuin-activating compound present in grapes and red wine. One main effect of CR in mammals is a reduction of body fat from white adipose tissue.
We have shown earlier that resveratrol influences human fat cell number and function. In this study, we sought to identify the underlying molecular mechanisms.
Methods:
Using a lentiviral system we generated human SGBS preadipocytes and adipocytes stably expressing Sirt1 shRNA. Proliferation, adipogenic differentiation, de novo lipogenesis, and secretory functions were studied.
Results:
Resveratrol inhibited human preadipocyte proliferation and conversion into mature adipocytes. These effects were abrogated in SGBS preadipocytes stably expressing Sirt1 shRNA. Resveratrol inhibited de novo lipogenesis by down-regulating glucose tranporter-4 (Glut-4) expression. This inhibitory effect was not influenced by down-regulation of Sirt1 in SGBS adipocytes. Furthermore, resveratrol influenced the endocrine function of human pre- and adipocytes, e.g down-regulated secretion of IL-6 or IL-8. Sirt1 was differentially involved in the regulation of adipokine expression.
Conclusions:
Our data suggest that resveratrol might influence adipose tissue size by several mechanisms involving Sirt1. Furthermore, by influencing the endocrine function of human pre- and adipocytes, resveratrol might positively interfere with the development of obesity associated co-morbidities.

FC16-007 Diabetes Session II

Complication screening at 2-5 year diabetes duration in adolescents

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Current Australian and ISPAD guidelines for type 1 diabetes recommend screening for complications to commence after 2 years duration in adolescents, whilst other guidelines recommend screening after 5 years. In this study, we examined early complications in patients with type 1 diabetes, screened between 2-5 years duration, at the Children's Hospital at Westmead.
Method: Complication status was assessed in adolescents with type 1 diabetes of 2-5 years duration (n=821, 55% female, median age 14.4yrs, range 11-17yrs). Retinopathy, assessed using 7-field stereoscopic fundal photography, was defined as presence of microaneurysms or haemorrhages (≥21, according to Airlie House classification). Early nephropathy was defined as elevated mean albumin excretion rate (AER) ≥7.5ug/min, and microalbuminuria as AER ≥20ug/min on ≥2/3 timed overnight urine collections. Peripheral nerve function was measured by thermal and vibration threshold at the foot. Independent variables associated with complications examined using logistic regression were HbA1c, gender, age, duration, BMI centile, systolic and diastolic blood pressure (BP), number of injections and cholesterol.
Results: The prevalence of retinopathy was 9%. 4/691 had moderate to severe background retinopathy (grades 31 and 41). 22% had elevated mean AER, 3% had microalbuminuria and 22% had peripheral nerve abnormalities. 34% of patients were overweight (BMI ≥85thpc). Girls had higher median BMI than boys (78th vs 71st pc, p<0.0001).

Complication outcome		Age		p-value
	11-13 yrs	13-15 yrs	15-17 yrs	
Retinopathy	9 /151 (6%)	27/265 (10%)	29/275 (11%)	0.26
Mean AER ≥7.5ug/min	27/173 (16%)	67/286 (23%)	71/280 (25%)	0.045*
Micro-albuminuria	4/172 (2%)	10/283 (4%)	7/275 (3%)	0.69
Peripheral nerve abnormality	48/177 (27%)	58/311 (19%)	69/317 (22%)	0.093
Median HbA1c	8.2% (IQR 7.7-9.5)	8.5% (IQR 7.8-9.5)	8.6% (IQR 7.7-9.5)	0.34

Retinopathy was associated with higher diastolic BP(OR 1.012, 1.001-1.023). Elevated mean AER ≥7.5ug/min was associated with age (OR 1.17, 1.03-1.32), duration(OR 1.34, 1.05-1.72) and systolic BP(OR 1.02, 1.004-1.035). Peripheral nerve abnormalities were associated with higher BMI centiles(OR 1.008, 1.000-1.077).
Conclusion: Early diabetes complications are found in adolescents with less than 5 years duration, supporting the value of early screening. Further interventional studies would help determine the potential modification of the natural history.

FC16-008 Diabetes Session II

Deterioration in vascular function during puberty in adolescents with type 1 diabetes

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Children with Type 1 diabetes (T1DM) have vascular endothelial and smooth muscle dysfunction as measured by flow mediated dilatation (FMD) and glyceryl trinitrate mediated dilatation (GTN) [1]. Puberty is known to be a critical time for the development of later vascular disease. We aimed to evaluate vascular function longitudinally in the peri-pubertal age in T1DM and healthy children.
Eighty children (44 males, aged 13.2 ± 2.6 years) including 52 T1DM children without retinopathy or microalbuminuria (mean diabetes duration of 6.4 ± 3.8 years and HbA1c of 8.8 ± 1.3 %) and 28 healthy aged matched children were assessed at 0, 18 and 36 months as a part of an ongoing study. At each visit FMD, GTN, high sensitive C-reactive protein (hsCRP), lipids, glucose, HbA1c, blood pressure, height and weight were measured.
In the T1DM children there was a significant decline in GTN over 36 months (p=0.009) but not in FMD (Table 1). Change in GTN was independent of change in other variables that deteriorated (BMIz score and HbA1c). There was no significant change in lipids over time. In the healthy children FMD and GTN did not change over time (Table 2).

Table 1. Vascular function over time in T1DM

	Initial visit (n=52)	18 months (n=46)	36 months (n=15)	p
Age (years)	13.8 (2.2)	15.1 (2.1)	15.9 (1.5)	< 0.001
BMI z score	0.6 (0.7)	0.7 (0.7)	0.8 (0.6)	0.01
FMD (%)	5.2 (4.5)	4.8 (3.8)	3.0 (3.3)	0.19
GTN (%)	21.2 (8.7)	18.9 (5.9)	16.2 (5.4)	0.009
hsCRP (mg/L) *	0.9 [0.2-14]	0.9 [0.2-9]	0.7 [0.3-13]	0.56
HbA1C (%)	8.8 (1.3)	9.3 (1.6)	8.9 (1.2))	0.03
Insulin dose (unit/kg/day)	1.1 (0.3)	1.2 (0.4)	1.3 (0.3)	0.03

Mean (SD), * geometric mean [range]

Table 2. Vascular function over time in healthy children

	Initial visit (n=28)	18 months (n=22)	36 month (n=17)	p
Age (years)	12.7 (3)	14.7 (3.2)	15.7 (3.1)	< 0.001
BMI z score	0.3 (0.9)	0.1 (0.8)	0.2 (0.8)	0.87
FMD (%)	6.1 (4.6)	7.4 (5.9)	6.2 (4.6)	0.60
GTN (%)	28.6 (6.6)	26.8 (9.9)	27.1 (7)	0.47

Mean (SD)

Deterioration over time in smooth muscle function appears to be an earlier marker of vascular disease progression than endothelial function in adolescents with T1DM. Decline in smooth muscle function was independent of deterioration in diabetes control and weight gain.
1. Pena et al. J Clin Endocrinol Metab 91; 4467-71. 2006

FC16-009 Diabetes Session II

Polymorphisms in endothelial nitric oxide synthase influence folate status and predict endothelial response to folate in children and adolescents with type 1 diabetes or obesity

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Endothelial nitric oxide synthase (eNOS) is a key enzyme in maintaining vascular tone. Endothelial dysfunction precedes development of diabetes complications. Folate normalises endothelial function in children with diabetes but not obesity[1-3] and directly affects eNOS function[4]. We therefore hypothesize that eNOS polymorphisms affect endothelial function and response to folate. We aimed to determine the effect of the a-deletion/b-insertion (eNOS4) and Glu298Asp polymorphisms on: 1) vascular function and folate status; 2) endothelial response to folate during intervention trials, in children with diabetes or obesity.

We studied 251 subjects (130 male): 145 with type 1 diabetes (age 13.8±2.7), 57 with obesity (13.4±2.2), 49 controls (14.4±3.4). The diabetes and obese subjects participated in 3 separate randomized controlled trials[1-3] evaluating the effect of folate on endothelial function - 85 diabetes and 28 obese subjects received folate 5 mg/day. Endothelial and smooth muscle function were assessed using flow- and GTN- mediated dilatation (FMD/GTN), together with auxology, BP, red cell + serum folate, homocysteine, HbA1c, fasting lipids and glucose, at baseline and at 8 weeks of intervention. Polymorphisms were assessed with PCR and RFLP analysis using published primers.

FMD and GTN were lower in subjects with diabetes or obesity than controls. 298Asp (p=0.05) and eNOS4 deletion (p=0.02) were more common in the obese group. At baseline in all subjects, red cell folate was higher with 298Asp homozygosity (p=0.04), serum folate was higher with eNOS4 deletion (p= 0.04), but neither polymorphism influenced FMD or GTN. eNOS4 insertion had a beneficial effect on change in FMD during folate therapy.

Change in FMD(%) with folate by eNOS4 polymorphism*

	Insertion	Deletion	P
Diabetes	7.4 [2.4 - 10]	3.6 [-1.6 - 5.9]	0.01
Obesity	1.0 [-2.8 - 6.1]	-2.3 [-6.9 - 3.0]	0.05
Combined	5.6 [1 - 9.4]	1.7 [-3.8 - 5.0]	0.001

*(Median[IQR])

We have shown for the first time that eNOS polymorphisms influence folate status and predict endothelial responsiveness to folate in intervention trials in children with diabetes or obesity. We speculate eNOS4 may also explain variation in response to folate for cardiovascular outcomes (MI/stroke) in prevention studies in adults.

Refs: 1)Pena et al. J Pediatr 2004;144:500. 2)MacKenzie et al. Pediatr 2006;118:242. 3)Pena et al. Diabetes Care 2007;30:2122. 4)Stroes et al. Circ Res 2000;86:1129.

FC16-010 Diabetes Session II

Modifiable risk factors for complications in young adults with type 1 diabetes: a follow-up study

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AIM: Evidence suggests that risk factors other than A1C play an important role in the incidence and progression of microvascular complications. We explored potential modifiable risk factors in a group of young adults who attended our service and were transitioned to adult care.

METHODS: This study included 152 young adults (69 M) with T1D from a group screened between 1997-2002 (T1) and invited for follow-up in 2007-2008 (T2). Complications screening included retinopathy assessment by 7-field fundal photography and albumin excretion rate (AER) from 3 timed overnight urine specimens. Non-participants had higher A1C levels (8.9 ± 1.4 vs. 8.6 ± 1.5, p=0.003) and no other baseline differences were obsereved. Multiple regression models were used to evaluate factors associated with microvascular complications at T1 and T2.

RESULTS: Median age [IQR] at T1 was 15.1 years [13.5-16.8] and 23.5 years [21.0–25.6] at T2. Duration of diabetes at T2 was 15.4 years [12.5–19.5] and follow-up 8.2 years [7.0-9.5]. Despite a decrease in A1C from 8.6% to 8.0% (p<0.001), retinopathy increased from 31% to 53% of subjects (p<0.001) and early elevation of AER (>5ug/min) increased from 49% to 63% (p =0.03). Baseline BMI and A1C predicted prevalent retinopathy at T2 (OR, 95%CI: 3.02, 1.24-7.34; 1.32, 1.00-1.73). Baseline HbA1c (OR 1.35, 1.02-1.80) and systolic BP (OR 1.05, 95%CI 1.01-1.09) predicted incidence and progression of retinopathy at T2. BMI increased between T1 and T2 (p<0.001) and males had a higher prevalence of overweight and obesity than females (58.8% vs. 47.3%) at T2.

CONCLUSIONS –Despite improved glycaemic control, rates of retinopathy, early elevation of AER and prevalence of excess weight increased. BMI, A1C and BP are modifiable risk factors which predict retinopathy. This suggests that in young people with T1D glycaemic control alone may not suffice to ameliorate complications and other therapeutic avenues may need to be explored.

Characteristics of 152 subjects pts at T1 and T2

	T1	T2	P
A1C (%)	8.6 ±1.5	8.0 ± 1.4	<0.001
Systolic BP Z score	0.52 ± 0.83	0.25 ± 0.89	0.001
Diastolic BP Z score	0.57±0.70	0.58 ± 0.85	0.85
BMI (kg/m2)	22.4 ± 3.3	25.7 ± 4.1	<0.001
Retinopathy	31/145	80/151	<0.001
Log mean AER (ug/min)	0.77 ± 0.38	0.84 ± 0.35	0.05
Early elevation AER (>5ug/min)	67/137	82/131	0.03

FC16-011 Diabetes Session II

Permanent neonatal diabetes (PNDM/MDI) in Italy: results of multiple gene screening in 41 patients

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BACKGROUND/METHODS. Permanent neonatal diabetes/Monogenic diabetes of infancy (PNDM/MDI) is a rare form of diabetes with onset within 6 months of birth. Forty-one patients have been diagnosed by our group with PNDM/MDI and therefore screened for mutations in KCNJ11, INS and ABCC8 genes, which are frequently associated with this condition. Selected patients were screened for GCK, FOXP3 and NGN3 genes mutations. All genes were examined by DNA direct sequencing.

RESULTS. Twenty mutations were identified in the KCNJ11 gene (48.7%),

including V59A, a novel mutation in a patient with DEND syndrome, 9 in the INS gene (21.9%), 3 in ABCC8 (7.3%), and 1 in the GCK gene (2.4%). Among the 8 remaining patients: No mutation was found in 4 of them when the initial three genes were sequenced (PNDM/MDI "X"), while in 3 patients analysis of ABCC8 gene is still pending. A male patient with very low birth weight (990 g) who died of severe diarrhea was negative to the search of mutations in FOXP3 (causing IPEX syndrome) and NGN3 genes.
The four PNDM/MDI "X" probands presented only diabetes with onset between 27 and 180 days from birth. Their birth weight was close to 50th centile (3000-3400 g), similar to patients with INS mutations (median 3055 g +227), and different from patients with KCNJ11 (median birth weight 2455 g +350) or ABCC8 mutations (range 1850-2900 g).
CONCLUSIONS. We conclude that: 1) molecular genetic diagnosis can be reached in ~80% (maybe more) of patients clinically diagnosed as PNDM/MDI when KCNJ11, INS and ABCC8 genes are sequenced and 2) pathophysiology of disease of PNDM/MDI "X" patients could be similar to that observed in patients with INS mutations, which seem to affect fetal insulin secretion less (higher birth weight) than mutations of KATP channel.

FC16-012 Diabetes Session II

Patients with rare KCNJ11 mutations exhibiting variable developmental delay and response to glyburide treatment identified through a neonatal diabetes registry

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Permanent neonatal diabetes (NDM) has a monogenic cause in nearly 2/3 of cases diagnosed under 6 months of age and in a smaller fraction of those diagnosed later in infancy. NDM caused by heterozygous activating mutations in the genes (KCNJ11 or ABCC8) encoding the 2 subunits of the ATP-sensitive potassium channel will usually respond to treatment with oral sulfonylureas in lieu of insulin and may also exhibit neurodevelopmental disabilities. Although genetic diagnosis can lead to dramatic improvement in diabetes control and quality of life, many cases likely remain unidentified.

Subjects with diabetes diagnosed under a year of age were recruited through our IRB-approved website, http://NeonatalDiabetesRegistry.org to participate in comprehensive longitudinal survey data collection and gene sequencing. Mutations were confirmed in a CLIA-certified laboratory.

Of 69 subjects currently enrolled in the Registry, we report those with novel or extremely rare mutations in KCNJ11 about which previous clinical information including response to sulfonylureas is limited. A boy with the previously unreported R50P mutation has been treated with glyburide since his diagnosis shortly after birth and continues to exhibit significant developmental delay. A boy with Y330C transitioned off insulin at the age of 12.5 months with subsequent improvement in his developmental delay. A girl with V59A also exhibited significant developmental delay with slow progress after transition to an unusually high dose of glyburide (2.2 mg/kg/day). A mother and 2 daughters with E179A exhibit a moderate degree of cognitive impairment and recently transitioned successfully to glyburide. A girl with G53D was treated empirically with glyburide just after birth, prior to a genetic diagnosis that was uncovered only through inclusion in the Registry. Importantly, her lack of significant developmental delay by 18 months is in contrast to three published G53D cases with significant neurological impairment.

A web-based registry is an effective means for identifying and surveying patients with NDM and provides a foundation for prospective clinical studies, including careful longitudinal neurodevelopmental assessment. Involvement in such a national registry is critical for uncovering variability among patients with rare mutations that may be related to differences in treatment or other clinical characteristics.
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FC17-013 Thyroid

Hes1 gene is involved in normal thyroid development: *hes1* knock-out mice display thyroid hypoplasia

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In the mouse thyroid gland, the thyroxine-producing follicular cells derive from the endodermal thyroid diverticulum, from embryonic day (E) 8.5 and onwards. The calcitonin producing C-cells arise from the ultimobranchial bodies beginning at E11.5. After fusion of the anlagen at E13.5, the thyroid proliferates and differentiates. Further genes involved in thyroid development remain to be defined. As Notch signaling and Hes1 have been shown to be involved in endocrine pancreas and pituitary development, we reasoned that this might also be the case for thyroid development.
Objectives: This study aimed to determine 1) the expression profile and 2) the function of hes1 in the developing mouse thyroid.
Methods: Expression of *hes1* was investigated by RT-qPCR and immunohistochemistry in micro-dissected embryonic (from E13.5 to E18.5) and in adult WT mouse thyroids. Thyroids of *hes1*$^{-/-}$ and WT mice were analyzed by immunohistochemistry in order to quantify thyroid area, morphology and cellular composition in E9.5, E11.5, E13.5, 15.5 and E16.5 embryos using antibodies against hes1, nkx2.1, pax8, T4, mash1 and calcitonin. Proliferation was analyzed by BrdU expression and apoptosis by TUNEL experiments.
Results: In WT mice, *hes1* mRNA expression is upregulated from E13.5 to E18.5 as compared to adult thyroid. Fusion of the median anlage and the ultimobranchial bodies was delayed in *hes1*$^{-/-}$ mice by 3 days (E16.5 vs. E13.5 in WT). In *hes1*$^{-/-}$ mice, the thyroid area (nkx2-1 positive) was significantly smaller (-40 to -60%) compared to WT at all investigated stages. Within the hypoplastic thyroids we found a significantly decreased calcitonin (-65% vs. WT) and T4 (-78% vs. WT) labelling surface if normalized to the nkx2.1 positive surface. Apoptosis and proliferation ratios were similar in wild-type and *hes1*$^{-/-}$ thyroids from E11.5 to E16.5. The number of nkx2.1 positive progenitor cells in the median anlage of *hes1*$^{-/-}$ at E9.5 and in the ultimobranchial bodies at E11.5 were markedly decreased compared to WT (P<0.05). This might be the mechanism leading to thyroid hypoplasia.
Conclusions: During thyroid development hes1 is required 1) for maintenance of follicular cell and C-cell progenitors, 2) for correct fusion of the median and the lateral anlagen, and 3) for adequate endocrine function of follicular cells and C-cells.
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FC17-014 Thyroid

Functional RNA editing of the DUOX2 gene

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DUOX2 is the oxidase that generates H_2O_2 in the thyroid, a key step in the synthesis of thyroid hormone. Known regulators of DUOX2 function are TSH-induced cAMP signals, intracellular calcium and cytokine Interferon-g. RNA editing is a posttranscriptional process that overwrites the encoded information of an RNA molecule to modify gene function or regulation. Most edited nucleotides are within intronic or untranslated regions. Only 13 mammalian genes have been discovered so far where editing results in an amino acid change. Hence, studies are scarce on the biological significance of single amino acid changes on the activity of "edited" proteins.
We identified a discrepancy between genomic (gDNA) and complementary (cDNA) DNA sequences of human DUOX2 at nucleotide position 74, where adenine (74A) is found in gDNA, and guanine (74G) in cDNA. This modification changes asparagine at position 25 (25N) into serine (25S). With the exception of chimpanzee, 25N/S is not conserved in DUOX2 mammalian orthologs. In cDNA from human thyroid (n=5), liver (n=2), kidney (n=3), skeletal muscle

(n=3) and fibroblasts (n=2) only 74G was identified. Both 74A and 74G were present in cDNA from lung (n=2) and whole-blood (n=2), suggesting cell type-specific or incomplete editing in certain tissues. In transfected cells, edited 25S-DUOX2 generated 60% more H_2O_2 than unedited 25N-DUOX2, and immunoblots also suggested higher protein levels of 25S- vs 25N-DUOX2.
In conclusion, a functionally critical position (N/S site) of the human DUOX2 oxidase is controlled by adenine to inosine (A-to-I) RNA editing. This editing seems a relatively recent achievement in mammalian evolution. RNA editing is complete in thyroid and 4 other tissues but only partial in lung and blood cells. In the thyroid, 25S-DUOX2 represents a more efficient generation of hydrogen peroxide, a rate-limiting step in thyroid hormonogenesis.

FC17-015 Thyroid

Genetic congenital hypothyroidism: who's at risk, and how do we identify them?

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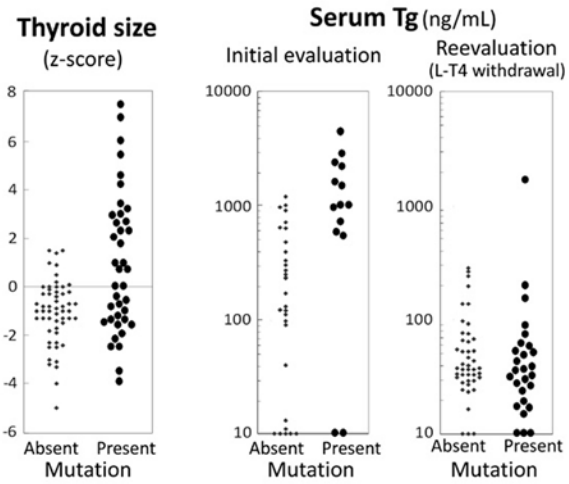
A subset of patients with congenital hypothyroidism (CH) shows Mendelian inheritance (*i.e.,* genetic CH). Family history and goiter are conventional predictors, however, the accuracy of prediction has been unknown. This study was conducted to define prediction accuracy of three putative predictors of genetic CH, namely family history, goiter (thyroid size, ≥2 SD), and abnormal serum thyroglobulin (Tg)(>1000 or ≤10 ng/dL without athyreosis).
Subjects and Methods: The subjects were 169 Japanese CH patients with information on thyroid morphology. They were enrolled regardless of phenotype so as to minimize selection bias. For 126 subjects, serum Tg was measured before starting treatment or with discontinuing treatment. We sequenced all coding regions for *TSHR, PAX8, TITF1, FOXE1* and *NKX2-5*. Several common mutations (*e.g., T354P of SLC5A5*) were also analyzed. If a patient had either goiter, high ^{123}I uptake (>40%), high $KClO_4$/KSCN discharge rate (>10%) or high serum Tg (>200 ng/mL), we additionally sequenced *TPO, SLC5A5, SLC26A4, TG, IYD, DUOX2* and *DUOXA2*. We examined prediction accuracy of three predictors by determining sensitivity and specificity of each predictor alone or in combination.
Results: Family history, goiter, and abnormal serum Tg were observed in 12, 17 and 16 subjects, respectively. We found 45 subjects having genetic CH: mutations were found in *TSHR*, n=14 (biallelic 6, monoallelic 8); *PAX8, n=4; TG, n=5; TPO, n=2; DUOX2*, n=17 (biallelic 10, monoallelic 7); and in two genes, n=3. Each of family history, goiter and abnormal serum Tg had high specificity (98%, 100% and 98%, respectively) but had low sensitivity (20%, 38% and 36%, respectively). If they were combined, the sensitivity was raised to 60%. The predictors failed to detect 18 mutation carriers, including *TSHR* mutations (n=10); *PAX8* mutations (n=2); and *DUOX2* mutations (n=6).



Conclusions: Near 100% specificity of the predictors indicates that each of them is enough powerful to identify patients at risk for genetic CH. Sensitivity of these predictors was low, however, it could be improved if they were combined, implying that the predictors worked complementarily.

FC17-016 Thyroid

Altered hippocampal functioning in adolescents with congenital hypothyroidism on associative memory tasks

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Rationale: Despite markedly improved outcome in congenital hypothyroidism (CH) due to early treatment following newborn screening, many children still exhibit a variety of subtle specific cognitive deficits. One area of particular difficulty concerns their memory functioning, particularly on tasks known to involve the hippocampus such as learning and remembering word pairs, places, and events. Extensive research on animals has found that adequate levels of pre- and postnatal thyroid hormone are necessary for normal hippocampal development. Although we have previously shown hippocampal malformations in CH reflecting reduced volumes and widths, altered chemical composition, and microstructural differences, and that these abnormalities were correlated with their memory deficits, their hippocampal functioning was not directly examined. This study therefore compared CH and typically developing adolescents using functional magnetic resonance imaging (fMRI) with two associative memory paradigms shown in adults to activate the hippocampus. Methods: Studied were 11-14 year olds with CH identified by newborn screening and age-matched controls, who underwent two sessions on a 1.5T scanner. In one session, they were assessed with a verbal associative memory paradigm and in the other, a visuospatial associative memory paradigm involving location and object-pair judgments. Data were processed and analyzed using SPM5. Results: Preliminary findings based on 9 CH and 12 controls indicated that although groups did not differ in overall memory performance, activation during correct recognition was more diffuse and bilateral in CH than controls. On the verbal task, CH showed increased activation in left and right posterior hippocampus (p = .018 and .032, respectively); on the visuospatial task, CH showed increased activation in left and right middle hippocampus on location trials (p=.002 and p=.007), and in left middle and right posterior hippocampus on object-pair trials (p=.013 and p=.027). Conclusions: Early thyroid hormone insufficiency in children with CH may alter how they process aspects of associative memory in the hippocampus and suggest use of compensatory hippocampal mechanisms to support these memory functions.

FC17-017 Thyroid

Papillary thyroid cancer is commonly detected when routine ultrasound screening is used for evaluation after radiation exposure during childhood cancer treatment

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Background
Lifelong surveillance for long-term and late effects of childhood cancer treatment is mandatory. Direct or scatter radiation (XRT) from cranial or chest XRT is associated with increased risk of differentiated thyroid cancer. Current COG long term follow up guidelines suggest annual thyroid palpation, proceeding to ultrasound (US) and fine needle aspiration biopsy (FNA) if a nodule is palpated.
Introduction
Currently we commence routine thyroid US screening 2 years after XRT exposure, repeated every 2 years if no lesion is seen. Where a nodule <5mm is present, ultrasound is repeated in 6 months. If >5mm, FNA followed by total

thyroidectomy and central node clearance occurs, if cancer or suspicion thereof is reported. Prior to 5 years ago surveillance was less frequent.
Aims
To audit our thyroid surveillance in a paediatric and young adult cohort and identify the frequency of malignant thyroid disease after paediatric XRT exposure.
Methods
Retrospective audit of all XRT recipients attending the RCH oncology clinics, patients identified through Late Effects Clinic records (MZ).
Results
Between 1994-2009, 201 XRT exposed patients were identified: cranial (CXRT) 101, cranio-spinal (CSXRT) 70, total body irradiation (TBI) 34, plus local XRT 12. Mean(SD) age at first malignancy diagnosis was 6.4(3.9) years; neurologic 121 (57.6%), haematologic 60 (28.6%), other solid tumours 20 (9.5%). Thyroid US was performed in 153 (76%), 24% were deceased or followed elsewhere. Thyroid screening was commenced at 6.0(4.8) years after completion of XRT. Mean age at latest US was 17.3(5.9) years.
Thyroid US was abnormal in 54 (35%), suspicious findings on US ± FNA led to thyroidectomy in 22, of whom 13 (41%) [8.5% of screened cohort] had papillary cancer, invasive in 6. Only one nodule was palpable (benign adenoma). Median age at thyroid cancer diagnosis was 17 (range 9-35) years, occurring 9 (6-26) years after XRT. Radiation exposure was direct (CSXRT or TBI) in 8, scatter (CXRT) in 3 and both (CXRT & TBI) in 2. Subtle US changes were present in 32 who remain under surveillance.
Conclusions
Routine thyroid US surveillance after childhood XRT exposure is an effective screening stratagem to detect clinically significant ± invasive cancer. As 8.5% of our cohort had thyroid cancer, we believe this is a common and serious problem, more easily treated if detected early. Thyroid palpation is not sufficiently sensitive to detect malignant lesions and we recommend regular US surveillance.

FC17-018 Thyroid

Dissimilar hepatotoxicity of propylthiouracil and methimazole in pediatric patients

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OBJECTIVE: To assess patterns of liver injury associated with the use of propylthiouracil (PTU) and methimazole (MMI) in the United States. METHODS: United States Food and Drug Administration (FDA) Adverse Event Reports (AER) databases were examine using the Multi-item Gamma Poisson Shrinker (MGPS) data mining algorithm. The MGPS uses a Bayesian model to calculate adjusted observed:expected ratios of drug–adverse event associations (Empiric Bayes Geometric Mean [EBGM] values) in huge drug safety databases. RESULTS: PTU had the highest adjusted reporting ratios for both Severe Liver Injury (EBGM 14.8, 90% confidence interval [CI] 10.2–20.7]) and Mild Liver Injury (EBGM 4.85, 90% confidence interval [CI] 3.2–7.3]) and this was seen in the age group <17 years. The EBGM values for PTU decreased with age. The highest EBGM values for MMI were with Mild Liver Injury in the age group of >=61 years (EBGM 5.13, 90% CI 3.65–7.18), The EBGM values for MMI decrease with age. CONCLUSIONS: PTU is associated with a risk of hepatotoxity, especially in the pediatric population. MMI hepatotoxicity is also observed, although MMI hepatotoxic events are generally less reported and less severe than those associated with PTU, and occur in adults. No MMI-associated hepatotoxic events are reported in children.

LB-FC18-001 Late Breaking Submissions

Development of a murine model for the study of pheochromocytoma (pheo)

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We have previously shown that IGF-1R expression is increased in malignant compared to benign pheo suggesting a potential role for the IGF/IGF-1R system in the pathogenesis of the disease. Murine models of tumor development are useful tools; unlike breast or colon cancer, the use of murine models for the study of pheo has been less explored. Aim: In order to understand the influence of IGF/IGF-1R on the biology of pheo, we aimed to generate a murine model by using a cell line derived from pheo occurring in heterozygous neurofibromatosis knockout mice (MPC cells) in a no immunodeficient strain of mice. Materials and Methods: MPC cells were grown in complete RPMI medium (5%FBS, 10%HS). Western Blot: MPC cells were seeded in 6 well plates and allowed to reach 60% confluency, starved and stimulated with 20nM of rhIGF-1 for 5´ to 15´. 500/50ug of protein extracts were used for Immunoprecipitation or SDS-PAGE respectively. Membranes were probed for IGF-1R, PY20, Total AKT, pAKT, total ERK, p42/44. Proliferation assays: $3x10^5$ or $8x10^5$ cells were seeded in 24 well plates in complete or low serum (0.5%FBS,1%HS)RPMI respectively, with or without (w/wo) 100nM or 50nM rhIGF-1 for 7 days. Medium was replaced every 48 hs, and cells were counted on days 1, 3, 5 and 7. Eight weeks old C57B6 mice were injected sc (right flank, n=10,10^6cells) or iv (tail vein, n=5, 10^6cells) and monitored for weight, and tumor growth.On week 14 mice were sacrificed and tumors dissected, snap frozen and fixed for IHC analysis.Results: After 5´ incubation with rhIGF-1,IGF-1R, AKT and ERK were phosphorylated as revealed by western blot analysis. MPC cells proliferate in complete medium w/wo rhIGF-1. On day 5, there was an increased number of cells upon rhIGF-1stimulation compared to basal proliferation (2.1 ± 0.2 vs $1.5\pm0.3x10^5$,p=0.03). On Low Serum medium, MPC cells proliferation was blunted while it was stimulated by rhIGF-1 compared to basal condition (1.5 ± 0.4 vs $2.7\pm0.2x10^5$,p=0.009). 10/10 injected sc mice developed sc tumors from week 5. On week 14 tumors had reached 2cm diameter.3/5 iv injected mice developed hepatic tumors. Histology was performed and in all cases pheo were confirmed. Conclusions: MPC cells have an intact IGF-1R that is activated by rhIGF-1, stimulating their proliferation *in vitro*. When injected sc or iv in normal C57B6 mice, MPC cells grow and form tumors that have pheo histology. Therefore, we have developed a new murine model for the study of pheochromocytoma biology.

LB-FC18-002 Late Breaking Submissions

Neurogenin 3 deficiency is a novel cause of permanent neonatal diabetes and severe congenital diarrhea

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Background Despite the recent advances in the understanding of the molecular basis of neonatal diabetes, approximately 30% of the cases remain undiagnosed. Neurogenin 3 plays an essential role in pancreatic islet development. *Neurog3*-null mice fail to develop mature islets and die from diabetes within the first few days after birth. Although *NEUROG3* is therefore a good candidate gene for monogenic diabetes, no mutations have been identified in patients with maturity-onset diabetes of the young (MODY). In contrast, homozygous hypomorphic missense mutations have been recently associated with a rare form of congenital malabsorptive diarrhea due to the lack of enteroendocrine cells ("enteric anendocrinosis"), which highlights the role of *NEUROG3* in enteroendocrine cells development. We hypothesized that null mutations in *NEUROG3* might be responsible for the disease in a patient with permanent neonatal diabetes and severe congenital diarrhea.
Case report The patient was born at 37 weeks gestation with intrauterine growth retardation [birth weight: 1910 g (-3.1 SDS), length: 43 cm (-3.2 SDS), head circumference: 32 cm (-1.7 SDS)] and presented with non-ketotic hyperglycemia and undetectable C peptide during the first day of life. As soon as enteral feeding was introduced, she developed severe malabsorptive diarrhea that has required long-term home parenteral nutrition. Abdominal CT scans repeatedly showed mild intestinal dilation and a macroscopically normal pancreas. Intestinal mucosa structure appeared normal but enteroendocrine cells were absent. No other significant clinical abnormalities have been identified.
Methods The single coding exon of *NEUROG3* was amplified and sequenced

from genomic DNA. The mutant protein isoforms were functionally characterized by using in-vivo chicken endoderm electroporation.
Results Two different heterozygous point mutations in *NEUROG3* were identified in the proband [c.82G>T (p.E28X) and c.404T>C (p.L135P)], each being inherited from an unaffected parent. In-vivo functional studies indicated that the mutant isoforms are biologically inactive. No mutations were identified in a further 30 patients with isolated permanent neonatal diabetes from unknown cause.
Conclusion NEUROG3 deficiency produces a rare novel subtype of syndromic permanent neonatal diabetes. This finding confirms the main role of *NEUROG3* in islet development and function in humans.

LB-FC18-003 Late Breaking Submissions

Mechanism of hyperinsulinemic hypoglycemia in SCHAD deficiency: studies of insulin dysregulation in SCHAD-/- mice

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Several children have been reported with congenital hyperinsulinism (HI) associated with recessive, inactivating mutations of a mitochondrial β-oxidation enzyme, short-chain 3-OH acyl-CoA dehydrogenase (SCHAD). To investigate the mechanism of insulin dysregulation in this disorder, we studied mice with a global knockout of the enzyme.
In vivo, SCHAD-/- mice had a phenotype consistent with SCHAD-HI children, including reduced random (98±6 vs. 151±6 mg/dL, $p<0.01$) and fasting (70±4 vs. 112±12 mg/dL, $p<0.01$) levels of plasma glucose and elevated plasma levels of a short-chain fatty acid intermediate, 3-OH-butyryl-carnitine (0.61±0.08 vs. 0.16±0.02 μM, $p<0.01$) compared to controls. Isolated SCHAD-/- islets showed similar responses compared to controls with glucose stimulation, either alone or in the presence of long-chain and medium-chain fatty acids. However, SCHAD-/- islets were hypersensitive to stimulation of insulin release by a complete mixture of amino acids compared to controls which had no response. Amino acid hypersensitivity was confirmed by a leucine ramp stimulation with a decreased threshold and a greater maximum response. These findings resembled the abnormalities associated with activating mutations of glutamate dehydrogenase (GDH) that cause HI. Specific activation of GDH was further shown in SCHAD-/- islets by a two-fold increase in [U-^{14}C]-glutamine oxidation compared to controls and an abnormal positive cytosolic calcium response to amino acid that could be blocked by inhibition of GDH with epigallocatechin-gallate.
Since fatty acid intermediates did not appear to play a role in the insulin dysregulation of SCHAD-/- islets, we examined the possibility of direct protein-protein interactions between SCHAD and GDH. Pull-down experiments with liver mitochondrial extracts showed that GDH bound to SCHAD. Studies of GDH enzymatic activity in liver showed no differences. However, in extracts of isolated islets, with submaximal stimulation by 10 μM ADP, GDH from SCHAD-/- mice showed a two-fold reduction in the Km of the enzyme for its substrate, alpha-ketoglutarate, and a 50% increase in enzyme efficiency (Vmax/Km).
These data in SCHAD-/- mice suggest that SCHAD protein regulates amino acid stimulated insulin secretion in normal β cells by binding with and inhibiting GDH enzyme activity. Loss of this regulatory "moon-lighting protein" function of SCHAD may provide an explanation for hyperinsulism in children with SCHAD deficiency.

LB-FC18-004 Late Breaking Submissions

A heterozygous mutation of the IGF-1 receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation

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Background: Mutations in the insulin-like growth factor-1 receptor (*IGF1R*) gene can be responsible for intrauterine and postnatal growth disorders.
Objective: We here report on a novel mutation in the *IGF1R* gene in a female patient. The aim of our study was to analyse the functional impact of this mutation.
Patients: The girl was born with a birth length of 47 cm (-1.89 SDS) and a birth weight of 2250 g (-1.90 BMI-SDS). Despite of high IGF-1 levels (+1.83 to +2.17 SDS) she presented no postnatal catch-up growth. Clinical examination revealed microcephaly and cognitive developmental retardation.
Results: Denaturing HPLC screening and direct DNA sequencing disclosed a heterozygous missense mutation resulting in an amino acid exchange from valine to glutamic acid at position 599 (V599E-IGF1R). A hydrophobic amino acid at this position in the extracellular Fibronectin III 2a domain is highly conserved among various species. R- cells transfected with V599E-IGF1R demonstrated no phosphorylation of the mutated receptor and downstream proteins (Akt) after stimulation with IGF-1. Flow cytometry of transfected COS-7 cells revealed a lack of cell surface expression of V599E-IGF1R. By means of confocal laser scanning microscopy complete retention of V599E-IGF1R in the endoplasmic reticulum was shown.
Conclusion: The V599E-IGF1R mutation interferes with the receptor's trafficking path thereby abrogating proreceptor processing and plasma membrane localisation. Diminished cell surface receptor density solely expressed from the patient's wild type allele is supposed to lead to an insufficient IGF-1 signalling. This results in intrauterine and postnatal growth retardation of the affected patient. The reported retention of the nascent IGF1R in the endoplasmic reticulum is a novel mechanism of IGF-1 resistance.

LB-FC18-005 Late Breaking Submissions

Mutational screening of children born SGA reveals an unexpectedly high percentage of patients carrying sequence aberrations within the IGF1 receptor gene

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Background. Per definition three percent of all newborns are born small for gestational age (SGA). A subgroup of them presents with IGF1 resistance and failure to catch up growth. Although most cases has to be regarded as idiopathic mutations within genes coding for proteins of the IGF – IGF1 receptor (IGF1R) system have recently been identified in a few individuals.
Objective. To evaluate the frequency of sequence aberrations affecting the *IGF1R* gene in a cohort of children born SGA that fail to thrive with or without IGF1 resistance.
Results. 64 patients (38 boys, 26 girls) presenting with birth lengths between -1.4 SDS and -8.9 SDS, IGF1 levels between 2.8 SDS and -2.2 SDS, and no major additional comorbidities were recruited from the CrescNet database Leipzig and external collaboration partners. All coding exons of the *IGF1R* gene were PCR amplified, prescreened for sequence aberrations by means of denaturing HPLC followed by direct sequencing of suspicious PCR fragments. In addition, the occurrence of copy number variants of the *IGF1R* gene was investigated by MLPA. 9 unrelated patients were identified to carry heterozygous aberrations affecting the *IGF1R* (14.1 %; 7 females, 2 males). All aberrations

represent novel point mutations or exon deletions. Five of them are supposed (due to premature peptide chain termination, 3 cases) or have been shown (by functional characterization, 2 cases) to interfere with the receptor's functionality. The affected patients presented with elevated IGF1 levels (range 0.8 to 1.9 SDS). Generally, after initiation of GH therapy the increase in growth velocity of all affected patients identified in our lab so far bearing IGF1R mutations (including two previously reported cases) was below the average of the entire SGA cohort [ΔSDS (growth velocity), median 0.58 (n=8) vs. 1.33 (n=53), respectively].

Conclusion. The comparably high fraction of monogenic aberrations in a polygenic regulated trait such as growth control highlights the unique role of the IGF1R in governing somatic growth. The relevance of the higher prevalence of affected girls and the observed poor response of most mutational carriers to GH deserves further investigation in larger cohorts. Basically, gene targeted resequencing approaches and CNV analyses employed in this study along with genome wide screenings represent a valuable tool to identify additional factors involved in growth regulation and eventually to leverage individualized therapeutic strategies.

LB-FC18-006 Late Breaking Submissions

Intravenous infusion of potent non-IGFBP-3 binding analog of humanin increases insulin sensitivity via activation of hypothalamic STAT-3

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Humanin (HN), a novel mitochondrial peptide, has been recognized for its neuro-protective role against AD-related neurotoxicity and analogs of HN have been shown to be more potent than native HN. We showed earlier that in addition to its neuro-survival effects, HN regulates both hepatic and peripheral insulin action. We also showed that binding of HN to IGFBP-3 tempers the effects of central HN on insulin action.

Here, we examined the effects of a peripheral intravenous infusion of a potent, non-IGFBP-3 binding HN analog, HNGF6A. We studied awake, unstressed and chronically catheterized Sprague Dawley rats under hyperinsulinemic-euglycemic clamps. IV administration of HNGF6A (100 ug total, 25 ug bolus and 75 ug over 6 hours) dramatically improved insulin sensitivity. To identify if these effects are mediated through the hypothalamus, we infused either a STAT-3 Peptide inhibitor (PI) or a scrambled peptide ICV (controls) along with IV HNGF6A infusion. With IV infusion of HNGF6A, glucose infusion rate (GIR,) during the hyperinsulinemic clamp was significantly higher in the presence of a scrambled peptide ICV compared to STAT-3 PI ICV (Fig A, $p<0.01$). Among the HNGF6A IV group, hepatic glucose production was significantly lower in the group that received no ICV infusion or scrambled peptide ICV (Fig B, $p<0.01$). The effect of IV HNGF6A on HGP was negated ($p<0.01$) and the effects on glucose uptake were attenuated ($p<0.05$) in the presence of STAT-3 PI ICV. In primary hepatocytes, HN did not have any direct effect on hepatic glucose production or on STAT-3, further suggesting that the actions of HN on liver are centrally mediated. We conclude that HNGF6A has potent effects on insulin action that is mediated through STAT-3 activation in the hypothalamus. HN and HN analog HNGF6A could provide a potential therapeutic option for the treatment of insulin resistance and type 2 diabetes.

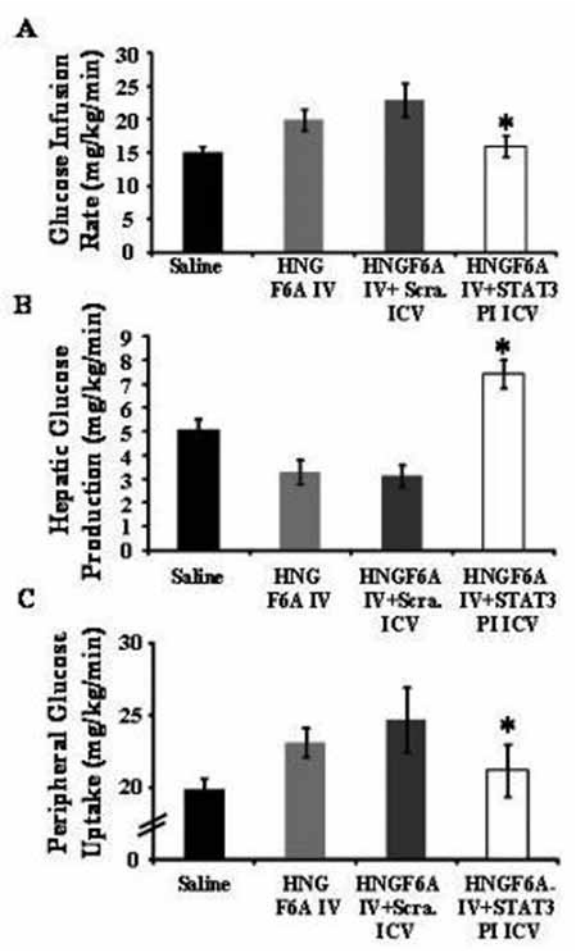
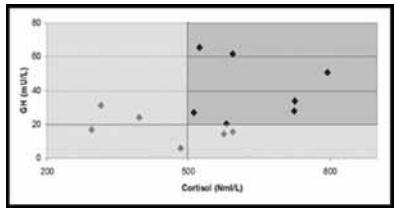


Figure for LB-FC18-006

Poster Presentations



In the 5 children undergoing second testing, one subject having had a borderline appropriate cortisol response on first testing, failed to exhibit a normal response in the later test.
DISCUSSION: These neuroendocrine findings in children following TBI are similar to those reported in adults and indicate that routine testing should be undertaken in those cases of TBI previously admitted to PICU.

PO1-001 Adrenal I

Neuroendocrine sequelae of traumatic brain injury in childhood

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BACKGROUND: Neuroendocrine dysfunction is a potential complication of traumatic brain injury (TBI). Data on pituitary function in children following TBI is limited. We have therefore undertaken a prospective, longitudinal, observational cohort study of pituitary function in children following TBI.
METHOD: Children aged 5 to 16 years, admitted to the Paediatric Intensive Care Unit (PICU) at Addenbrookes Hospital (Cambridge, UK) with moderate to severe TBI (GCS< 8) from 06/2006 to 07/2007 were potential subjects. Exclusion criteria were: pre-existing endocrine, growth or metabolic disorders; ongoing cardiac, respiratory / hepatic or renal disorder; or, severe neurological disability, or epilepsy. After consent, children were followed up in the first and second years after TBI. Neuroendocrine function was evaluated using endocrine dynamic tests including Glucagon and Thyrotropin Releasing Hormone (TRH) stimulation tests, and single measurements of prolactin.
RESULTS: To date, 13 children have had the dynamic assessment on the first year, and 5 also have had a second assessment in the second year. There were 7 (54%) boys and mean age at the time of injury was 12.3 ± 2.7 years. The average weight and height of the subjects at the time of the first assessment was 52±17.8 Kg, and 154±13.8 cm, respectively. The mean prolactin level was 319 ± 288 U/L for girls and 126 ± 60 U/L for boys. All except for one subject had prolactin level within the normal range. There were 4 children with abnormal TSH response to TRH stimulation. In regard to the cortisol and growth hormone response to glucagon stimulation, 62% of the children had an abnormal response. Four children had peak cortisol response <500 nmol/L and 4 children had growth hormone response <20mU/L.

PO1-002 Adrenal I

Insulin sensitivity and ultrasound evaluation of intima media thickness at common carotids, carotid bulbs, femoral and abdominal aorta arteries in adolescents with congenital adrenal hyperplasia

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Background: Increased intima-media thickness (IMT) is considered as a measure of subclinical atherosclerosis and a predictor of both myocardial infarction and stoke. Reduced insulin sensitivity and increased IMT were demonstrated in young adults with classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) (Sartorato et al., JCEM 2007, 92: 1015-8).
Objective: To evaluate peripheral insulin sensitivity (HOMA model) and IMT of the abdominal aorta (AAo), right and left common carotids (CCR-L), carotid bulbs (CBR-L) and common femoral (CFR-L) arteries in 18 adolescents (10 males) with either classical (9 cases) or non-classical CAH (mean age 16.2 +/- 2.2 yrs, range 13.3-20.0). Patients data were compared with the ones of 16 age and BMI-matched controls.
Results: Homa insulin resistance index was significantly higher in CAH patients than in controls (2.3 +/- 1.6 vs 1.1 +/- 0.8; $p < 0.0025$). IMT of all the investigated arteries was significantly higher in CAH and this difference was especially evident when AAo was considered (1.2 +/- 0.4 vs 0.8 +/- 0.1 mm; $p < 0.0005$).
Conclusions: If compared with age and BMI-matched controls, CAH patients are probably exposed to an increased risk for cardiovascular disease, as suggested by our data. This risk was not significantly different in the patients with either classical or non-classical form.

PO1-003 Adrenal I

Estradiol, independently of ERβ, and progesterone inhibit 3βHSD2 gene expression in adrenal cells

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In vitro and in vivo studies showed that estrogens might increase adrenal androgen production, suggesting that estrogen may modulate the Δ5 pathway of steroidogenesis. We have recently shown that ERβ, but not ERα, was expressed in the zona reticularis (ZR) of post-adrenarche human adrenal cortex. In addition, cP450 aromatase was localized in the zona glomerulosa and adrenal medulla (AM) (Baquedano et al, JCEM 2007), suggesting that estrogens, produced locally in AM, would play a role in ZR functional differentiation through ERβ. It has been reported that Progesterone Receptor levels were high

in adrenal glands (de Cremoux P et al, Endocr Relat Cancer 2008). To provide a clue for a possible role of estrogens and progesterone (P) in adrenarche, we studied the effect of estradiol (E_2) on 3βHSD2 expression and steroidogenesis in the human adrenocortical cell line, H295R and in primary human adrenal cell cultures. In dose response experiments with E_2 (0.01 to 1 μM; 24h), only the 1 μM dose increased the DHEAS/Cortisol ratio on basal and 8Br-AMPc-stimulated H295R cells (98 and 72% respectively, $p<0.05$). E2, (1 μM, 24hs) also stimulated significantly DHEAS production in primary human adrenal cell cultures.

By real time RT-PCR, 3βHSD2 was significantly down-regulated by E_2 (1 μM) and P (100 nM) at the transcriptional level in H295R cells. Inhibition was maximal after 6h (42% ± 3.9 and 61% ± 1.2, respectively) and returned to basal levels after 24h. The effect of E_2 was not inhibited either by ICI 182,780 (10 μM) (estrogen receptor inhibitor); or after suppression of ERβ by siRNA technique. Interestingly, the inhibitory effect of E_2 (1 μM, 6h) on 3βHSD2 mRNA levels was even higher in cells transfected with ERβ siRNA or in cells pre-treated with ICI 182,780. According to previous reports, our results confirmed that E_2 regulates DHEAS production in human adrenal cells. However, this study shows, for the first time, that E_2 and P favored the Δ5 pathway by inhibiting 3βHSD2 mRNA expression. Even though the mechanism remains to be elucidated, these results show that the decrease of 3βHSD2 expression might not be mediated by ERβ.

PO1-004 Adrenal I

An MLPA synthetic probe set for the detection and mapping of deletions in the *NR0B1 (DAX1)* locus enables to explain phenotypic differences in patients with congenital adrenal hypoplasia

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X-linked adrenal hypoplasia congenita (AHC) is a rare developmental disorder of the adrenal cortex characterized by primary adrenal insufficiency (AI) and hypogonadic hypogonadism (HH). It is caused by deletions or point mutations of the *NR0B1 (DAX1)* gene. AHC can be associated with glycerol kinase deficiency (GKD), Duchenne muscular dystrophy (DMD) and mental retardation (MR), as part of a contiguous gene deletion syndrome. *IL1RAPL1* defects have been shown to be responsible for MR in several cases. The aim of this study was to use MLPA to characterize the genotype in patients with *NR0B1* deletions and to correlate this to their divergent phenotypes.

Five patients with AHC were selected for this study. All patients manifested with severe salt loss during infancy. Patient #1588 was 6 yrs of age and showed AI combined with mild MR. Patients #16 and #98 were adults and suffered from AI and HH; no mental deficits were reported. Patient #912 was an adolescent suffering from AI, GKD, DMD, HH, short stature, seizures and mental problems. Patient #2966 was an infant with apparently isolated AI. A *NR0B1* deletion was detected previously with various techniques as cause for AHC. An MLPA synthetic probe set was designed to fine map the deletions, with probes within the *IL1RAPL1, MAGEB1, NR0B1, CXorf21, GK, MAP3K7IP3 and DMD* genes.

A deletion of the *IL1RAPL1, MAGEB1, NR0B1* genes was detected in patient #1588, explaining his MR phenotype. Patient #16 has a deletion of *MAGEB1, NR0B1* and *CXorf21,* while patient #98 has a deletion of *NR0B1* exon 1, thus confirming their isolated AHC phenotype. Patient #912 has a deletion affecting *IL1RAPL1, MAGEB1, NR0B1, CXorf21, GK, MAP3K7IP3 and DMD* genes; his mental phenotype may be aggravated by the *IL1RAPL1* deletion. A deletion of *MAGEB1, NR0B1, CXorf21, GK, and MAP3K7IP3* was identified in patient #2996, thus revealing his risk for metabolic crises.

In conclusion, MLPA analysis is a valuable tool to detect *NR0B1* and the contiguous gene deletions in patients with AHC. The analysis has shown a good genotype-phenotype correlation. It is especially helpful for the detection of *IL1RAPL1* deletions causing mental retardation as no clinical markers for this aspect of the disease are available. MLPA has also the advantage to identify female carriers that, depending on the extension of the deletion, have a high risk to have children with MR, AHC, GKD and DMD. Some female carriers could even present a disease phenotype due to skewed X inactivation.

PO1-005 Adrenal I

Cortisone reductase deficiency (CRD) – a rare cause of premature pubarche revealed by GC-MS urinary steroid profiling

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In CRD hepatic regeneration of cortisol from cortisone does not occur due to a failure of the oxoreductase 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD). Clinical characteristics - resulting from increased cortisol clearance and androgen excess - are signs of hyperandrogenism such as premature pubarche, excess body hair growth (hirsutism), acne, as well as oligomenorrhea and infertility in females.

A 7-year old caucasian girl of a nonconsanguineous family presented at our endocrine outpatient clinic with a 8-months history of premature onset of pubic (Tanner P2, B1) and axillary hair, as well as increased height velocity (SDS +0.57). On presentation, she had a height SDS of +0.53, the midparental target height was +0.26 SDS and her weight SDS was -0.10. She was born at term with normal weight and length. She is the second oldest of four siblings. The family history was uneventful.

Her bone age (Greulich/Pyle) was accelerated (8 10/12 years). Serum androgen levels were mildly elevated (androstenedione 64 ng/dl; DHEA 609 ng/dl; testosterone 29 ng/dl). LHRH testing ruled out central precocious puberty. Ultrasonography of the adrenals was normal. 21-hydroxylase deficiency, 3β-hydroxysteroiddehydrogenase deficiency, 11β-hydroxylase deficiency were excluded by a GC-MS urinary steroid profile. However the urinary steroid profile showed elevation of C19-steroids (androsterone 1014 μg/ 24hr (P95: 297 μg/24hr); etiocholanolone 290 μg/24hr (P95: 269 μg/24hr). Cortisone metabolites were elevated (e.g. THE 5035 μg/24hr (P95: 3245 μg/24hr)). Cortisol metabolites were normal (e.g. THF + aTHF 921 μg/24hr (P75-90). The ratio 5alpha-tetrahydrocortisol + tetrahydrocortisol / tetrahydrocortisone (5alpha-THF + THF / THE) - reflecting 11β-HSD activity - was clearly reduced with a ratio of 0.18 (normally >0.5).

Conclusion: The girl presented with the typical meatbolic pattern of cortisone reductase deficiency consisting in high cortisone metabolites, a low ratio between cortisol and cortisone metabolites and elevated androgens.

The differential diagnosis of premature pubarche is complex. GC-MS urinary steroid profiling is highly advantageous because this unbiased metabolomics approach allows for the delineation of all disorders of steroid metabolism in this entity. This case reminds of 11β-HSD-type 1 deficiency presenting a rare but important differential diagnosis of premature pubarche.

PO1-006 Adrenal I

13-year old Turkish female with obesity and hirsutism caused by adreno-cortical tumor

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Introduction: Adrenocortical tumors (ACT) are extremely rare in childhood. While children under 4 years most often present with virilization as the only symptom, additional Cushing's syndrome is typical in adolescents. Tumor size is important for assessment of malignant potential.

Case report: A 13 year old turkish adolescent was referred to hospital for evaluation of hirsutism which began with onset of puberty one year ago. Weight gain 26 kg during the last 16 months. No Menarche. Physical examination: abdominal obesity, hirsutism, deep voice, acne, acanthosis at the neck, no striae, breast

B3-4, pubes P5, no clitoromegaly.
Results: Steroid profiling showed extremely elevated DHEA-S (23.428 (n:562-1.985) ng/ml), testosterone (100 (n:7-30) ng/dl) and androstendione (738 (n:28-137) ng/dl) in plasma. Free cortisol in urine was elevated (172 (n:15-85) µg/m²/d). Plasma-cortisol was in the upper normal range (243 (n:50-250) ng/ml). B and DOC were 4 (n:0-4,93) ng/ml and < 0.02 ng/ml, respectively, B/DOC ratio was > 200 (n: (8.9-125)). ACTH was suppressed (3 (n:10-60) pg/ml) as well as basal and stimulated LH and FSH (< 0,5 mIU/ml). Plasma renin activity was normal (1,7 (0,9-7,6) ng/ml/h). Ultrasound and MRI showed an adrenal tumor on the left side (75x89x70mm), no metastases.
Clinical course: Hemiadrenalectomy on the left side was performed and a tumor of 272g was completely removed. Histological examination showed low mitotic count, no atypical mitotic figures, suspicion of capsule infiltration but no evidence for capsule penetration. While no clear histological distinction between adenoma and carcinoma could be done, tumor weight and size strongly suggested malignant potential. 2 ½ years after diagnosis there is no evidence for recurrence. The patient lost 20 kg, menstruation is nearly regular and hirsutism declining.
Conclusion: Despite hirsutism and obesity are common among female adolescents, the Paediatric Endocrinologist should keep in mind the differential diagnosis of an ACT. Steroid profiling demonstrating elevated adrenal androgens leads to the diagnosis. Classification of tumor dignity in childhood is difficult. High B/DOC ratio supports the benign histology and the course until now (1) despite high tumour weight. Long-term follow-up for all patients is mandatory.
(1) HG Dörr et al. 1987, Cancer 60:1625-1629

PO1-007 Adrenal I

Non classical congenital adrenal hyperplasia: to treat or not to treat? Which treatment?

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Is a treatment necessary in prepubertal patients suffering from non classical 21-hydroxylase deficiency (NC21OHD)? If yes, should this treatment be hydrocortisone (HC)? Should an antiandrogen [cyproterone acetate (C)], alone or combined to HC, be used?
Aim: A retrospective study to evaluate 1) Criteria of treatment 2) Final height (FH).
Patients: 43 prepubertal children (13 boys) from 34 families with 1-24 ACTH stimulated 17-hydroxyprogesterone (17OHP) peak level > 30 nmoles/L and molecular abnormalities. According to their initial treatment, 4 groups were defined: HC alone, C alone, combined HC+C or not treated (NT). All the treated patients defined a TT group.
Results: At diagnosis, 72% of the patients had symptoms of hyperandrogenism. In addition, familial testing, newborn screening and fortuitous circumstances led to the diagnosis. At diagnosis, mean chronological age (CA) was 6.9 ± 3.1 (0.1 to 12) years (mean±SD and range), height (H) was 1.3±1.4 SDS (-2.4 to 3.5) and 1.3±1.2 SDS (-2.3 to 3.9) compared to target height (TH). Bone age (BA) was 1 year older than chronological age in 73% of the patients and than height age (HA) in 28%. The 17OHP peak was 138±55 nmoles/L (42 to 239) and the ratio of the testosterone level on the upper testosterone level for age and sex (T/Tmax) was 1.3±0.9 (0.3 to 4.0). Twenty eight patients achieved their FH.

At diagnosis(n)	**NT**(15)	**TT**(28)	HC(16)	C(6)	HC+C(6)
Age (y)	**5.6±4.3**	**7.6±1.9***	7.2±2.1	8.4±1.7	7.8±1.5
H (SDS)	**0.6±1.6**	**1.7±1.2***	1.7±1.3	1.4±0.7	2.1±1.5
H-TH (SDS)	**0.8±1.4**	**1.5±1.0***	1.7±1.1	1.0±0.5	1.6±1.2
BA/CA	**1.0±0.2**	**1.3±0.1***	1.3±0.1	1.2±0.1	1.4±0.2
BA/HA	**1.0±0.2**	**1.1±0.1***	1.1±0.2	1.1±0.0	1.1±0.1
T/Tmax	**0.6±0.3**	**1.6±1.0***	1.9±1.1	1.0±0.5	1.6±0.7
At Final Height(n)	(7)	(21)	(10)	(1)	(10)
FH (SDS)	**-0.8±1.1**	**-0.1±0.7***	-0.2±0.7	0.5	0.0±0.8
FH-TH (SDS)	**0.0±0.9**	**-0.2±0.7**	-0.2±0.9	0.0	-0.2±0.5

*significantly different from NT

Conclusions: In this study, a treatment was used only for symptomatic patients with elevated testosterone levels and/or BA advance. Cyproterone acetate can be an alternative, alone or combined with hydrocortisone, for the most symptomatic patients, to avoid high doses of hydrocortisone. Final height compared to target height was similar in treated or not treated groups.

PO1-008 Adrenal I

Precocious pubarche: height, bone age and its relationship with target height at diagnostic time

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OBJECTIVE: to evaluate height in children with idiopathic precocious pubarche at diagnostic time and its relationship with target height and bone age.
METHODS: This is a historical retrospective study reviewing records of patients with idiopathic precocious pubarche from March 1994 to August 2008. Variables evaluated: age at first medical visit, sex, age at onset of pubarche, height and its relationship to target height and bone age at diagnostic time. Precocious pubarche was defined as onset of pubic hair before eight years of age in girls and nine years of age in boys.
RESULTS: We analyzed 81 records – 72 (88.89%) girls and 09 (11.11%) boys. The average ages at first visit were 6.17±2.11 years in girls and 8.12±0.7 years in boys, and the height standard deviation (SD) was 3.07±2.43 and 2.34±1.99, respectively. The onset ages of pubarche were 4.60±2.05 years in girls and 6.77±1.06 years in boys. Twenty five girls (34.72%) were within the target height at diagnostic time and forty seven were outside this range – 46 (63.89%) were above and 01 was below the target height (p=0.36). Eight boys were above and one was at target height (p=0.04). Nineteen girls (26.39%) had bone age at +2 SD average for age, fifty one (70.83%) had bone age compatible and two had delayed bone age (p= 0.58). Five boys had advanced bone age and four had compatible bone age for chronological age (p=0.007).
CONCLUSIONS: Precocious pubarche was more frequent and earlier aged onset in girls than boys. The time between the onset of symptoms and the first medical visit was 1.5 years in average. Increased height was observed at diagnosis as compared to target height in most patients, although there was no relevant advance in bone age.

PO1-009 Adrenal I

Familial case of pheochromocytoma due to Von Hippel Lindau (VHL) gene mutation

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We report a familial case of VHL syndrome presented with bilateral multiple adrenal pheochromocytomas and spinal cord hemangiomas. VHL syndrome is an autosomal dominant disorder associated with multiple tumour growth. Patient 1 manifested hypertension at the age of 10 years, bilateral pheochromocytoma was diagnosed. Bilateral excision of the tumours and partly adrenal resection was provided. At the age of 22 mild hypertension recurred. At the age of 35 neurological symptoms manifested: recurrent paresis of the right arm and leg. At the age of 38 during clinical examination relapse of multiple bilateral pheochromocytomas and spinal cord hemangiomas were diagnosed. Urine normetanephrine was markedly elevated – 4086 mcg/day (N 35-445), metanephrine was within normal ranges. ^{123}I-MIBG scintigraphy revealed chromaffine tissue in the location of adrenal tumours. Surgical management of pheochromocytomas was provided. No paragangliomas were detected in spite of the fact that 27 years have past since manifestation of the disease. Radiological surgery for spinal cord angiomas was chosen as the next step of the therapy. Our patient's daughter manifested symptoms of tachycardia, episodes of sweating and mild hypertension at the age of 13 years. Ultrasound and computer tomography detected bilateral pheochromocytoma, urine normetanephrine was significantly elevated, ^{123}I-MIBG scintigraphy revealed chromaffine adrenal tumours. The girl underwent bilateral adrenalectomy.
VHL syndrome was suspected based on clinical presentations – multiple bilateral pheochromocytomas, isolated noradrenalin overproduction, spinal cord angiomas and autosomal dominant inheritance. The diagnosis was confirmed

by previously described R161Q *VHL*-gene mutation. According to the diagnosis of VHL syndrome both patients were screened for cerebellar and retinal angiomas, tumour of endolymphatic sack, renal neoplasms and other described components of the syndrome. None of them were found. Both of our patients undergo annual examination for VHL syndrome components. Conclusion: Phenotype of VHL sydrome in this case was characterized by the absence of paragangliomas during 27 year follow up. VHL patients demand careful follow up for the whole life expectancy, genetic verification is of a great value for correct therapeutic and surgical treatment.

PO1-010 Adrenal I

Long term follow up of adult male patients with classic congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency: outcome in 23 affected male patients

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CAH is a continuum challenge for patients and physicians. This study reports fertility outcome in CAH male patients.
Patients and Methods : 23 patients (21-62 yr) with identified mutation on the *CYP21* gene were recruited; 12 had the salt wasting form (SW) and 11 had the simple virilizing (SV). Each patient received for at least 2 years a constant twice a day glucocorticoid (GC) and mineralocorticoid substitution. Patient gave written informed consent, and underwent clinical examination, hormonal investigation (2 -3 hours after morning treatment) performed 3 successive times, testicular ultra sonography and adrenal CT-Scan. **Results:** the 2 groups of patients (SW vs SV) did not differ in age (34.6±9.2 vs 40.3±14.4 yr), height (166 ±10 vs 166 ± 6 cm) and BMI (24.4±3.1 vs 23.4±3.1 kg/m^2). Overweight was observed in 3 patients (2 SV/1 SW) and obesity in 1 SW. Doses of GC converted to hydrocortisone equivalent were similar in both groups (19.70±9.58 in SW vs 13.98±7.82 mg/m^2/day in SV, p=0.13). Fludrocortisone doses were significantly higher in SW than in SV patients (37.56±22 vs 17.57±17.53 mg/m^2). The age of puberty differ significantly between SW and SV patients (3.0±1.3 vs 9.9±2.7 yr, p=0.0017). Mean levels of 17-OHP and ACTH did not differ between groups. As defined by a 17-OHP level over 75 nmol/l, 5 SW had poor control vs 3 SV patients. In contrast, 3 patients from each group were overtreated (17-OHP < 6 nmol/l). When D4-androstenedione (D4-A) levels were analyzed, 1 SV and 3 SW patients appeared overtreated. The correlation between D4-A and 17-OHP levels was highly significant (r=0.88, p=0.01). Stable adrenal hyperplasia on CT-Scan was noticeable in 7 SW vs 9 SV patients, with macronodular aspect in 3 SV and 1 SW patients during a 5 yr follow up. Bilateral testicular adrenal rest tumors (TART) were detected in 10/23 patients (8SW, 2SV). Interestingly, the presence of TART was not associated to poor hormonal control, testosterone (p=0.46) or inhibin B levels (p= 0.087) or to the presence of adrenal hyperplasia. Testicular hypotrophy (v<10 ml) was noted in 6 SV patients. Decreased levels of testosterone was found in 11/23 patients (6SW and 5SV). 5 SW and 7 SV patients fathered children. In patient with no fathering desire, semen analysis was normal in 3 SW and 1 SV. However, 2 SW and 2 SV patients have azoospermia.
Conclusions these results advocate further prospective studies for preserving normal reproductive function in CAH male patients.
PHRC # 2003.309

PO1-011 Adrenal I

Late presentation, milder phenotype, of a novel CYP11B2 gene mutation in a Pakistani toddler with aldosterone synthase deficiency type 2 (ASD 2)

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Background: Aldosterone synthase (ASD) deficiency is a rare, recessively inherited disorder, caused by a mutation in the CYP11B2 gene, which presents during infancy with failure to thrive, hypotension, hyponatremia and hyperkalemia.
Aldosterone synthase catalyzes the last three steps (11beta-hydroxylation, 18-hydroxylation, and 18-oxidation) required for aldosterone synthesis. Two biochemical forms of ASD have been described i.e. Type1 and 2. In both types, corticosterone is increased and aldosterone is markedly decreased to undetectable, while 18-hydroxy-corticosterone is increased only in Type 2.
Case report: A 13 month old female, of Pakistani descent, the product of a consanguineous marriage, was discovered to have hyponatremia 120-135 mmol/L [normal (N) 135 -147] and hyperkalemia 5.5-7.3 mmol/L [N 3.5 -5.3] during the work up for failure to thrive. Normal cortisol 22.4 ug/ml [N 4.3-22.4], ACTH level 7 pg/ml and 17-hydroxyprogesterone 11ng/L [1-4 yrs: N 4-115] excluded 21-hydroxylase deficiency. The low aldosterone 3ng/dL [N 2-37] and high PRA (Plasma renin activity; 143 ng/ml/hr: N1-3yr <10] pointed to an ASD. Steroid precursors when measured, revealed high 18-hydroxy-corticosterone 518 ng/dL [1-5 years: N 7-155] and high normal corticosterone 2625ng/dl [1-40 years: N 160-2040]. The 18-OH-corticosterone to aldosterone ratio of 171 [type 1<10; type 2>100] established the diagnosis.
Urinary steroid profile was characteristic with a peak for 18-OH-THA (18-hydroxy-tetrahydro-11-dehydrocorticosterone, the 18-OH-corticosterone metabolite) with undetectable THAldo (tetrahydro-aldosterone). A high ratio of 18-OH-THA to THAldo was suggestive of ASD 2. The sum of corticosterone metabolites over the sum of the cortisol metabolites was elevated 0.3(normal mean 0.1, upper limit of normal 0.2), another characteristic of ASD.
Sequence analysis of the CYP11B2, revealed a novel homozygous mutation of Q276R (c.827A>G) in exon 5, which was also carried by each of her parents (as heterozygotes).Treatment with 9 alpha-fluorocortisone normalized growth and PRA levels.
Conclusion: The late presentation and milder phenotype of our case is unique, illustrating that the mutant enzyme retained a significant amount of activity in vivo or there was mineralocorticoid biosynthesis via an alternative pathway to delay disease presentation. Furthermore, this is the first reported case in literature of ASD 2 in a child of Asian origin

PO1-012 Adrenal I

Bone age maturation in congenital adrenal hyperplasia (CAH): a French retrospective cohort of 497 children

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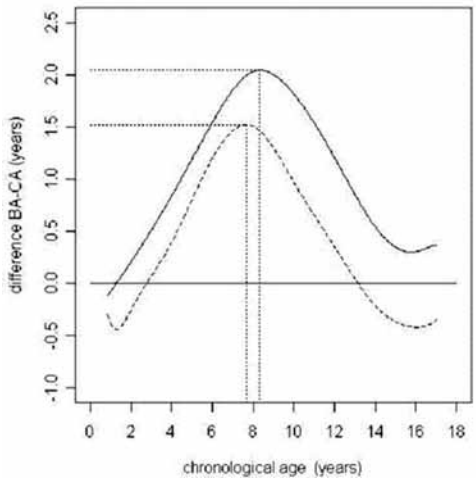
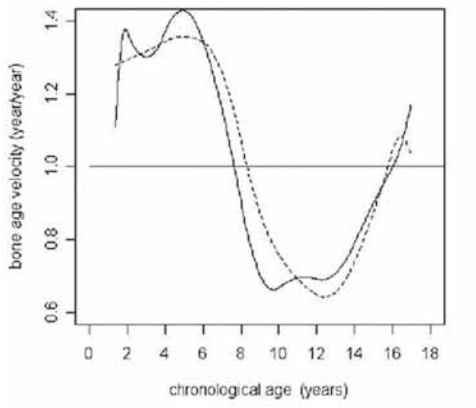
[10]CHU Limoges, France; [11]CHU Marseille, France; [12]CHU Montpellier, France; [13]CHU Nancy, France; [14]CHU Nice, France; [15]Hôpital Robert Debré, Paris, France; [16]CHU Saint Etienne, France; [17]CHU Strasbourg, France; [18]CHU Toulouse, France; [19]Hôpital Necker Enfants Malades, Paris, France; [20]CHU Lille, France

Appropriate dosage of glucocorticoids is essential for regular bone maturation in CAH-children. We study correlation between bone age (BA) and chronological age progressions in CAH-patients, specifically in Salt Wasting (SW) form. We led a French multicentre retrospective cohort study. Children born between 1970 and 1991, genotyped and followed-up by pediatric centres were included. Patients were not enrolled when CAH was associated with chronic disease or concomitant long-term treatment (such as GH). Based on centralized genotyping, patients were classified in SW, simple virilizing (SV) or non classical form. Retrospective X-rays for BA, clinical and growth data were collected in patient's file. All X-rays were re-evaluated by a single expert, using Maturos® software. A mixed linear regression based on maximum likelihood method was used to develop a model of bone maturation taking into consideration gender, genotype and age at treatment initiation. Four hundred ninety seven CAH-patients were enrolled retrospectively in 30 centres. 4973 X-rays were available corresponding to 409 patients. Among them, 180 were salt wasters treated early (82 boys, 98 girls).



Mean difference between BA and chronological age in boys (full line) and girls (doted line) with SW form of CAH.



Mean BA maturation velocity curve in boys (full line) and girls (doted line) affected by SW form of CAH

Figure 1 illustrates that early-treated boys and girls develop progressive excessive BA maturation, with a maximum of 2 years in boys and 1.5 year in girls. BA maturation velocity is accelerated in both boys and girls until the age of 8 (figure 2).

In our French CAH-cohort, BA maturation was not adequally controlled despite early conventional substitution treatment. Further analyses will assess the impact on this advanced BA maturation on lost in adult height.

PO1-013 Adrenal I

Growth charts in congenital adrenal hyperplasia (CAH): a French retrospective cohort of 497 children

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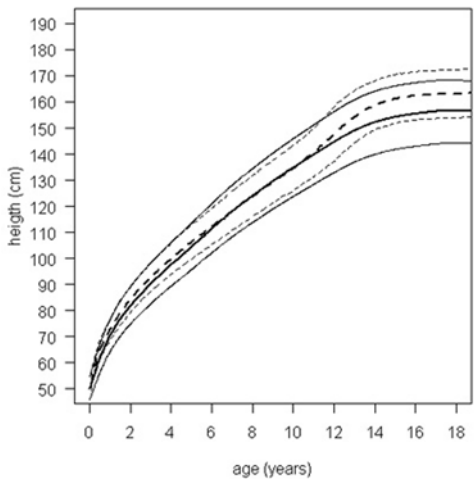
CAH remains often responsible for small adult stature. In contrast to Turner or Prader-Willi syndrome, no specific growth chart are available for CAH-children.

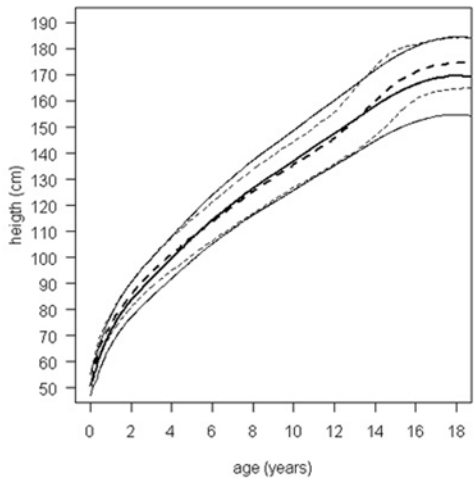
We build growth charts in classical form of CAH.

We led a French multicentre retrospective cohort study. Children born between 1970 and 1991, genotyped and followed-up by paediatric centres were included. Patients were excluded when CAH was associated with chronic disease or long-term treatment (such as GH). Based on centralized genotyping, patients were classified in salt wasting (SW), simple virilizing (SV) or non classical form. Retrospective available clinical and growth data (measured every 6 months) were collected in the patient's file. Only data on children treated before one year were used. Gender-related growth charts were built using the LMS (Box-Cox Cole and Green) method and exact measurement of age. The mean height values were modelled from birth to 20 years.

Four hundred ninety seven CAH-patients were enrolled in 30 centres. Among them, 256 patients were treated before the age of 1, representing 220 SW (94 boys, 126 girls) and 36 SV (12 boys, 24 girls). They all received hydrocortisone and fludrocortisone. 2494 and 3278 height measurements were available for boys and girls respectively.



Mean height at different ages, 5th and 95th percentiles for CAH-females (full lines) compared to French female reference population (doted lines) (Sempé, 1997).



Mean height at different ages, 5th and 95th percentiles for CAH-males (full lines) compared to French male reference population (doted lines)
In our CAH cohort of 256 early-treated SW and SV cases, growth charts show that mean childhood's heights are rather similar to those of French population. However, a limitation in pubertal spurt results in a mean lost in adult height equal to 7.2 cm in both males and females.

PO1-014 Adrenal I

Neonatal screening for congenital adrenal hyperplasia: results of the national screening program 1996-2003

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Neonatal screening for congenital adrenal hyperplasia (CAH) aims at preventing death due to neonatal adrenal crisis or misdiagnosis resulting in incorrect gender assignment or severe early hyperandrogenism. Screening for CAH remains controversial due to the easy clinical diagnosis in the vast majority of affected females and to the high false positive rate in premature infants. In France, CAH screening using 17-hydroxyprogesterone (17OHP) measurement on blood collected on filter paper at d3 has been introduced as part of the national screening program in 1995.
We report on the analysis of the results during the years 1996-2003. All positive screening tests were collected, as well as the follow-up measures performed to reach a final diagnosis. For cases of CAH, patient's charts were analyzed to evaluate the contribution of neonatal screening to the diagnosis. The severity of the disease was evaluated clinically and by CYP21B genotyping.
A total of 6 059 001 infants were screened, resulting 15 439 positive screening tests (1/393 or 0.25%). Of the infants with positive screening tests, 366 cases of CAH were identified, yielding a positive predictive value of 2.37% or 1/42. The positive predictive value decreased from more than 50% above 40 weeks of gestation to 14% at 37 weeks and <<1% below 34 weeks. Children born before 36 weeks of gestation contributed less than 5% of cases of CAH and more than 89% of false positives.
Neonatal screening was pivotal for the diagnosis of 52% of cases, whereas in the other half the diagnosis was already known when the screening results were communicated. Molecular analysis of CAH cases confirmed classical (salt wasting or simple virilizing) 21-hydroxylase (21-OH) deficiency in 90%, non classical 21-OH deficiency in 1%, 3-beta-OHD deficiency in 1%. In 8% of cases, novel mutations in the 21-OH gene precluded molecular classification, but clinical presentation was that of classical CAH in most cases. Last, the sex ratio (M/F) was 1.27, strongly skewed towards males.
We conclude that neonatal screening for 17OHP levels can efficiently detect CAH in infants at term but will contribute to the diagnosis in about half of the cases. In premature infants, the rarity of the disease, the high false positive rate and the medical management in neonatal units renders this screening test poorly efficient. This population-based study, the largest performed so far, will allow further analysis of the effectiveness of neonatal screening for CAH.

PO1-015 Adrenal I

A novel splice mutation (IVSds3+3insTA) in the melanocortin 2 receptor accessory protein that leads to skipping of exon 3

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Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disease resulting from adrenal unresponsiveness to ACTH. This potentially fatal condition is characterized by glucocorticoid deficiency in the absence of mineralocorticoid deficiency. Patients present by early childhood with hyperpigmentation, hypoglycaemic episodes, seizures or failure to thrive. Mutations in the ACTH receptor (MC2R) account for ~25% of cases (FGD type 1). More recently, MRAP (melanocortin 2 receptor accessory protein) mutations were shown to cause a further ~20% of cases (FGD type 2). MRAP is a single transmembrane protein that plays an important role in MC2R trafficking from the endoplasmic reticulum to the cell surface for receptor function.
We report a patient of Indian origin, who was diagnosed with FGD in the first few days of life. He presented with hypoglycaemic seizures and was noted to have generalized hyperpigmentation and normal male genitalia. Biochemical investigations revealed hypocortisolaemia (cortisol 0.223 µg/dl; NR 1-23 µg/dl) and elevated plasma ACTH (170 pg/ml). Serum electrolytes, aldosterone and plasma renin activity were normal. Peak cortisol following a standard synacthen test was 0.018 µg/dl. He responded to hydrocortisone treatment and continues on a dose of 20 mg/m^2/day.
PCR amplification and sequencing of the coding exons of *MC2R and MRAP* revealed a novel homozygous splice mutation (IVSds3+3insTA). To demonstrate that this mutation affected RNA splicing the mutated exon and flanking intronic sequences was introduced into the intron of a well-characterised splicing reporter, AdML-Par. Using an *in-vitro* splicing assay we were able to demonstrate that the IVSds3+3insTA mutation resulted in skipping of exon 3.
In conclusion we have identified a novel *MRAP* mutation where disruption of the intron 3 splice site results in a transcript with a foreshortened open reading frame encoding a prematurely terminated translation product. This protein (if produced) would lack the transmembrane domain that is essential for MC2R interaction. We predict that this would cause complete loss of ACTH response, hence explaining the early presentation seen in the case.

PO1-016 Adrenal I

The novel IVS1 +2T-C, L157Q (CTG®CAG), and R358X (CGA®TGA) mutations in the CYP 17A1 gene causes amenorrhea, hypokalemia, and hypertension

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17 α–hydroxylase deficiency (17OHD) is an autosomal recessive disorders that produces an excess of mineralocorticoids and sexual differentiation abnormalities. It characterized by cortisol deficiency and impaired androgen synthesis. We describe a 18 years old Taiwanese girl presented with 46,XX karyotype and complete female genitalia, absent pubertal development (Tanner stage A1 B1P1), and **amenorrhea**. She had an episode of proteinuria and hypertension. Clinical evaluation and series of endocrine test were followed by genomic DNA from the peripheral leukocytes of patient and her family for sequencing of the CYP17A1 gene.
Laboratory tests showed high levels of FSH, LH and ACTH, very low cortisol, 17-hydroxyprogesterone and estrodiol, associated with hypokalemia and low renin. Glucocorticoid replacement with spironolactone and premarin had improved the symptoms. A total of three mutations, IVS1 +2T-C, L157Q (CTG®CAG), and R358X (CGA®TGA), were identified by polymerase chain reaction amplification and sequencing of the eight CYP17A1 exons. The splicing donor site mutation in the first intron (IVS1 +2T-C) was also identified in the mother. Two other mutations, including a L157Q in exon 3 and a R358X in exon 6 were not found in the mother. These two mutations were putatively originate from the father, who passed away with no available samples. R358X introduce premature stop codon prior to the heme binding cysteine, which was reported earlier, and are predicted to completely inactivate the encoded P450c17 protein. The splicing site mutation may cause cryptic mRNA splicing with result of aberrant protein. Both IVS +2T-C and L157Q are novel mutations and further functional studies are needed.

PO1-017 Adrenal I

Blood pressure in the first year of life in children with congenital adrenal hyperplasia

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Introduction: Current treatment of congenital adrenal hyperplasia (CAH) in the first year of life in our centre consists of initially administration of both glucocorticoids and mineralocorticoids independent of the type of CAH. This treatment strategy is successful in preventing salt wasting but may lead to hypertension in the first months of life. Studies on the incidence of hypertension in CAH patients in the first year of life are missing.
Objective: We retrospectively evaluated blood pressure in the first year of life in patients with a classical form of CAH.
Patients and Methods: 24 children were included, all born after the introduction of the Dutch neonatal CAH screening program. All children were treated with a fixed dose of hydrocortisone and variable doses of fludrocortisone in the first year of life. Retrospectively blood pressures, fludrocortisone dosage, serum renin and 17-hydroxyprogesterone (17-OHP) levels in the first year of life were evaluated. Blood pressure values were compared to reference values for males and females in the first year of life. Correlations between blood pressure in CAH-patients and serum renin levels, serum 17-OHP levels and the dosage of fludrocortisone were calculated.
Results: After the 8th week of life, systolic and diastolic blood pressure were not significantly elevated in both males and females. In the first eight weeks of life relatively more children had blood pressure values above the 50th percentile, although statistical analysis could not be performed because of the small number of patients included and rapidly changing reference values. In week 0-8 77.8% of systolic blood pressure values measured in females and 73.3% of systolic blood pressure values measured in males were above the 50th percentile. No significant correlations between blood pressure and serum renin levels, serum 17-OHP levels and fludrocortisone dosage were found.
Conclusions: In CAH patients blood pressure values are not significantly elevated in the first year of life. However, in the first 8 weeks of life relatively more children had blood pressure values above the 50th percentile without any correlation between blood pressure and serum renin levels or fludrocortisone dosage. Further prospective studies are necessary to evaluate the blood pressure in the first weeks of life in CAH patients in more detail.

PO1-018 Adrenal I

Short stature and Cushing's disease

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Cushing's disease (CD) is the most common cause of endogenous Cushing's syndrome in children and adolescents and represents a rare cause of short stature. A 14 yr-old boy came to our attention for progressive weight gain and short stature. Birth length and weight were normal; clinical history was negative for use of glucocorticoids. At examination, height was 140 cm (3th centile), weight was 37.7 kg (10th centile). Tanner stage was: G2, PH 3, testis 3 ml. Hypothyroidism and growth hormone deficiency were excluded. A marked increase of urinary free cortisol, a nonsuppressible serum cortisol after Liddle 1 test and an elevated ACTH value confirmed the diagnosis of ACTH dependent Cushing's syndrome. Pituitary MRI showed a left microadenoma and an other right focal area of lesser enhancement. Therefore, bilateral inferior petrosal sinus sampling (BIPSS) with CRH stimulation was performed to obtain an accurate preoperative localization of the adenoma: the interpetrosal sinus ACTH gradient indicated lateralization of ACTH secretion to the left side. The patient underwent transsphenoidal surgery with selective microadenomectomy, with an immediate ACTH decline in the postoperative phase. Histology confirmed the diagnosis of corticotrophic pituitary adenoma. Glucocorticoid replacement therapy was instituted. Clinical examination demonstrated a rapid catch-up growth (10th centile), with a normalization of body mass index and an adequate pubertal development.
This is a rare case of pediatric Cushing's disease: one of the most reliable indicators of hypercortisolism in these patients is growth failure associated with weight gain while laboratory data and pituitary MRI are very important tools to confirm the clinical suspicion. In our case, BIPSS was necessary to lateralise the site of ACTH production, because of the co-existence of an ACTH secreting microadenoma and a pituitary incidentaloma. Transsphenoidal surgery allowed a successful remission of hypercortisolism, with a dramatic improvement of auxological parameters.

PO1-019 Adrenal I

Are guidelines for glucocorticoid coverage being followed in patients with adrenal insufficiency during periods of stress?

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BACKGROUND: Failure to adapt glucocorticoids during stress puts patients with adrenal insufficiency at risk of death. OBJECTIVES: This retrospective study was performed to evaluate whether stress management protocols were being followed in children with adrenal failure and to search for evidence of acute adrenal failure (AAF) linked to inappropriate care. PATIENTS AND METHODS: Patients followed for primary adrenal insufficiency (PAI: n=102) or secondary adrenal insufficiency (SAI: n=34) by the Endocrinology Service between 1973 and 2007 were included. All hospitalizations subsequent to the initiation of glucocorticoid treatment, both urgent (n=157, 73% for infections) and elective (n=90) were examined. We recorded clinical evidence of AAF, parental glucocorticoid management prior to admission and details of glucocorticoid prescription and administration by the emergency department and ward medical teams. Data were analyzed over three time periods because of significant changes in health care personnel/delivery: <1990, 1990-1997 and 1998-2007.
RESULTS: For urgent hospitalizations, subgroup and time period did not influence the proportion of patients hospitalized. 45% of PAI and 38% of SAI (p=0.55) patients had at least 1 hospitalization. A small number of patients (PAI=7, SAI=2) required multiple admissions (maximum/patient: PAI=13, SAI=7). The use of stress glucocorticoid doses by parents increased significantly after 1997 (p<0.05), although still only 47% had increased glucocorticoids prior to hospitalization. Similarly, glucocorticoid stress doses were more frequently administered in the emergency department after 1990 (p<0.05), reaching 65% of urgent hospitalizations. Upon arrival, patients with signs and/

or symptoms of AAF (58 urgent hospitalizations, PAI=51; SAI=7) decreased from 56% in 1990-1997 to 27% after 1997 (p<0.01). 24% of all hospitalizations were marked by sub-optimal adherence to glucocorticoid stress protocols, with rare but significant clinical consequences (2 cases of AAF with no long-term morbidity and 1 death in a child with septo-optic dysplasia and hypopituitarism).
CONCLUSIONS: Despite an increased use of glucocorticoid stress dose protocols by parents and physicians, patients remain at risk of morbidity and mortality from AAF. This risk may be minimized with a more conscientious application of glucocorticoid stress doses, but other patient-specific risk factors may also be implicated.

PO1-020 Adrenal I

Prepubertal Cushing's disease: diagnosis, management and therapeutic outcome

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Cushing's disease (CD) is rare in prepubertal children and remains a challenge for diagnosis and management. We review the diagnostic features and therapeutic outcome of prepubertal CD patients treated in a single centre.
Seventeen prepubertal patients (mean age 9.7 yr; range 5.7-14.1) fulfilled standard diagnostic criteria for CD. There was a male preponderance (13M (75%), mean age 9.5 yr; 4F (25%), mean age 10.6 yr). All patients had excessive weight gain, change in facial appearance and growth failure. Excessive virilization was common occurring in 14/17 (82%). Normal suppression of serum cortisol to <50 nmol/l during low-dose dexamethasone suppression test (LDDST; 0.5 mg, 6hly for 48 h) did not occur in 94% (16/17) patients. Suppression of serum cortisol to <50% of baseline during high-dose DST (2mg, 6hly for 48h) occurred in 87% (12/14). Administration of CRH (1mcg/kg or 100mcg IV) induced an increase in serum cortisol in all patients and was calculated as the percentage change in serum cortisol after CRH. This response was significantly exaggerated in all seventeen patients (mean change 113%; range 30-438%). Radiological evidence of a pituitary corticotroph adenoma on MRI was seen in only 59% (10/17) and concordance of the pituitary image with findings at transsphenoidal surgery (TSS) was poor (31%, 5/16). Bilateral simultaneous inferior petrosal sinus sampling (BSIPSS) was performed in 11 subjects without complications and gave excellent prediction of the site of adenoma. An inter-petrosal sinus gradient ≥1.4 after 100mcg CRH indicating lateralization was present in 82% (9/11). There was good concordance of site of adenoma from BSIPSS with findings at TSS (91%, 10/11). Post TSS, the corticotroph adenoma was confirmed histologically in 53% (8/15) of patients. TSS alone achieved cure (cortisol <50 nmol/l post-operatively) in 44% (7/16) of patients. The cure rate by TSS following BSIPSS was 64%. Of the patients who were not cured by TSS alone, 89% (8/9) were cured by second-line direct external pituitary irradiation (45 Gy).
Prepubertal Cushing's disease has distinctive features with increased frequency in males, abnormal auxology and excessive virilization. The cortisol response to IV CRH administration was particularly exuberant and contributed to the diagnosis. BSIPSS was more helpful than pituitary imaging in localisation of the microadenoma and was associated with improved cure rate by TSS.

PO1-021 Adrenal I

Adrenal function of extremely premature infants in the first five days after birth

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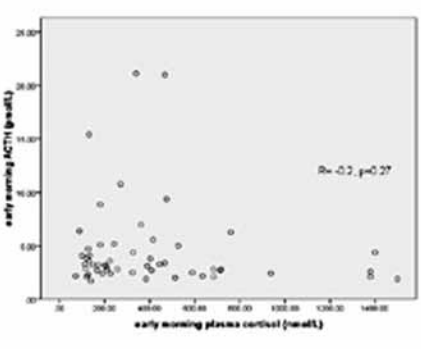
Background
There are limited data on adrenal function of extremely premature infants during the early days after birth.when a diminished cortisol response to Corticotropin Releasing Factor (CRF) has been reported. The relationship between plasma ACTH and cortisol is central to the integrity of the hypothalamic-pituitary-adrenal (HPA) axis; yet, there are no studies examining this in prematurity. The aim of this study was to examine the relationship between early morning plasma cortisol and ACTH concentrations during the first 5 days after birth in infants born at less than 28 weeks' gestation and to identify any independent factors factors that determine plasma cortisol levels in these infants.
Method
We prospectively studied early morning plasma ACTH and cortisol concentrations in infants born below 28 weeks' gestation during the first 5 days of birth. Plasma cortisol was measured without extraction, using DPC Immulite®2000 using a solid phase 2 site chemiluminescent immunometric assay. ACTH was measured using a radioimmunoassay. The relationship between cortisol and ACTH was determined. Multiple regression analysis was used to examine the relationship between plasma cortisol and clinical risk index for babies (CRIB) score, antenatal dexamethasone, mode of delivery and gestation.
Results
There were 95 infants (53 males) of mean gestation 25.3 ±1.3 SD (range 23-27^{+6}) weeks. Mean birth weight was 809 ±17.0 grams. Mean plasma cortisol was 400.5 ±42.6 nmol/L and mean plasma ACTH was 4.5 ±0.5 pmol/L. Early morning plasma ACTH did not correlate with early morning plasma cortisol (see Figure).



Multiple regression analysis showed that gestation was the only independent determinant of early morning plasma cortisol concentration (beta coefficient = -0.4, p=0.04).
Conclusion
The relationship between early morning plasma ACTH and plasma cortisol is either not established or is impaired in infants of less than 28 weeks' gestation in the first five days after birth. Plasma cortisol level is mainly determined by gestation and is not directly related to illness severity, antenatal steroids or plasma ACTH in these infants in the first 5 days after birth.

PO1-022 Adrenal I

A method comparison for a newborn screening program (NSP) for congenital adrenal hiperplasia (CAH)

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Introduction: The implementation of a NSP with a given methodology requires the careful establishment of its cut off values. The Argentine government provided with an ultramicroanalytical system by TecnoSUMA (TS), Cuba to develop a nationwide NSP for 5 medical conditions. The experience with UMELISA 17OHP Neo kit of TS revealed discrepancies with other methods and made it difficult to apply different cut off levels according to gestational age or birth weight to deal with premature babies. Our first findings revealed the need of using a cutoff for term babies which is markedly higher compared with that used by an alternative technology (DELFIA, Perkin Elmer). Objectives: To analyze differences with methodology DELFIA and to evaluate their causal factors. Materials and Methods: a) Linear and Passing & Bablok regressions between TS and DELFIA were made in 844 blood samples in filter paper. b) Samples from an International Quality Program (CDC, Atlanta. USA) for low, medium and high levels of 17OHP were evaluated. c) In order to corroborate the concentration of TS and DELFIA standards as a cause of the discrepancies, they were assayed by ELISA Siemens Enzaplate Neo 17αOHP and HPLC and the results were compared with the content informed by the manufacturers. TS results were corrected according to HPLC and compared with DELFIA results. An analysis of Bland Altman was made using ratio plots and the analytical CVs for the two assays to estimate the 95% prediction interval. Results: TS method shows higher values than DELFIA, with the following linear correlation TS = 31,5531 + 1,2213*DELFIA p<0,0001 r:0,607. Passing and Bablok regression showed a significant deviation from linearity TS = 0,8543 + 4,3478 * DELFIA (p<0.01). Mean Bias % of the quality controls were for DELFIA: 21.4% (CV% interassay: 14.3%) whereas for TS: 62.4% (CV% interassay: 21.2%). The mean Recovery (%) of DELFIA and TS standards according to HPLC tests were 100.6% and 55.8%, respectively; coincident to the recoveries obtained by ELISA: 90.1% and 56.3%. Conclusions: The collected data are consistent with an overestimation in the calibration of the standards. Its adjustment tends to harmonize the differences, but a bias persists in a way which is not imputable to the calibration of the standards. The overestimated standards in TS methodology imply a narrower working range of concentrations than expected which must be taken into account when trying to quantify samples obtained from preterm babies.

PO1-023 Adrenal I

Two siblings with triple A syndrome and novel AAAS mutation presenting as hereditary polyneuropathy due to early neurological dysfunction

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The triple A- syndrome (Allgrove syndrome) is an autosomal recessive disorder characterized by adrenal insufficiency, achalasia and alacrimia. Impairment of autonomous, central and peripheral nervous system function are usually discovered in late childhood and adolescence with progressive deterioration. The disease is caused by mutation in the AAAS gene on chromosome 12q13 encoding the nuclear pore protein ALADIN.
We report on sister and brother who presented with signs of neurological dysfunction already in early childhood initially diagnosed as having hereditary polyneuropathy. Both patients presented with delayed developmental milestones, with inability to stand unsupported until the age of 14 months. They started walking at the age of 18 and 19 months, respectively, with frequent tripping, falls and inability to walk on toes. Both children showed weakness and hypotrophy of distal muscles of the lower extremities indicating peripheral motor neuropathy. Since the younger sister had adrenal crisis at the age of 3 years, the older brother was evaluated at the age of 5 years and latent adrenal insufficiency was discovered. As both of the siblings had alacrimia, hyperkeratosis of palms, cutis anserina and nasal speech, diagnosis of triple A syndrome was considered.
Sequencing of the AAAS gene identified compound heterozygous mutation consisting of a novel mutation in exon 9 (c.887C>A, p.Ser296Tyr) and a previously described p.Ser236Pro (c.787T>C transition) missense mutation in exon 8 in both siblings.
Although it is known that there is no consistent correlation between genotype and phenotype in triple A syndrome, if new patients with this rare mutation could be discovered, it would be of interest to search for signs of early neurological dysfunction similar to the ones found in our siblings.
In patients with early neurological dysfunction and developmental delay, triple A syndrome should be considered, and adrenal function tests should be performed regularly to prevent life-threatening adrenal crises.

PO1-024 Adrenal I

Testicular adrenal rest tumors in adolescents boys with classical congenital adrenal hyperplasia: radiologic and histological investigation

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Testicular adrenal rest tumors (TART) are uncommon finding in classical congenital adrenal hyperplasia (CAH) in adolescence. We present two adolescents boys with TART.
Case1: A 9 $^{9/12}$-year-old boy with classical CAH presented at last visit with bilaterally irregular testicular masses. The physical examination was unremarkable except a penile size of 14x3.5 cm, and pubic hair of Tanner V. Testicular volumes were 15 to 20 ml bilaterally; they were irregular with palpable firm masses. On USG, both testes were lobular with heterogenic parenchyma containing multiple uneven sized hypoechoic nodules with blurred margins. Testicular volume was increased for his age (right 40x23x18 mm, left 37x23x17). On MRI the intratesticular masses were homogenous and hypointense relative to testicular parenchyma on T1-weighted images in the right testis. TART was not ruled out by radiological investigations and bilateral testicular biopsies were done. At operation, both testes were irregular in contour. Irregularity was more prominent in the lower half of the right testis. Wedge biopsies involving both poles were taken bilaterally. Histopathology revealed proliferated large, polygonal, eosinophilic cells which formed the testicular mass. There was arrested spermatogenesis, hypo-spermatogenesis and thickness of the basal membrane of the tubules. After the diagnosis of TART was confirmed high-dose dexamethasone therapy was started. On the 10th month of therapy testicular masses were reduced in size.
Case 2:A 15 $^{9/12}$–year-old boy with classical CAH admitted to the clinic for routine visit. On the admission irregular masses were found on both testes. Penile size was12x3.7 cm and pubic hair was Tanner V. Scrotal USG showed heterogeneous and lobular parenchyma with a 20x11 mm mass on the right testes, and heterogeneous parenchyma with two 6x5 mm and 4x3 mm sized masses in the left testes. On MRI there was a 15mm diameter nodule, isointense relative to testicular parenchyma on T1-weighted images in posterior of the right testis. A similar 8 mm diameter mass was found in the posterior of the left testis. TART was diagnosed and high dose dexamethasone was started. On the 13 th month of therapy testicular mass disappeared. He is currently under medical therapy.
Conclusion: TART is a benign lesion by itself. However it can cause testicular irregularity and damage with resultant infertility. Its diagnosis and appropriate medical management are therefore paramount importance.

PO1-025 Adrenal I

The effect of ketoconazole on Cushing syndrome on a girl presenting McCune Albright syndrome: a case report

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The GNAS1 activating mutation in McCune Albright syndrome (MAS) may cause hyperthyroidism, gigantism, peripheral precocious puberty, café-au-lait skin spots, polyostotic fibrous dysplasia of the bone, renal phosphate wasting and, in rare cases, Cushing syndrome (CS). Treatment for ACTH-independent CS is controversial and satisfactory control is seldom achieved. Herein we report the case of an eleven year-old girl who, at the age of eight, began to present impairment of linear growth and typical signs of CS. Thelarche and pubarche were also present. Baseline gonadothropins and estradiol were at prepubertal range. GnRH test was negative for central puberty. ACTH was undetectable (< 5pg/ml; normal range from 0 to 46 pg/ml); serum cortisol showed no circadian rhythm, and free urinary cortisol (FUC) was high (691.84ug/24h; normal range from 92.4 to 473 ug/24h), and its level rose to over 50% after Liddle test (paradoxal response). An abdominal computed tomography (CT) revealed moderate adrenal bilateral enlargement. PRKAR1A mutation was not found on chromosome 17, neither were any signs of Carney Complex, excluding the diagnosis of primary pigmented nodular adrenocortical disease (PPNAD). A bone scintigraphy showed fibrous bone dysplasia was found on her skull, thus leading to the diagnosis of MAS. Clinical control of CS was obtained with the administration of ketoconazole, beginning with 200mg/day, and then increased to 400mg/day. The patient presented with signs of adrenal insufficiency (hypocortisolism) one month after ketoconazole was initiated, and therefore the use of corticoid (hydrocortisone 60 mg/m2/day) became necessary until clinical and laboratory normalization. After the first year of treatment, BMI decreased from 24 to 20.8 kg/m2, and blood pressure became normal. FUC was 109.59 ug/24h. Ketoconazole may be an option to avoid adrenalectomy, which has been the current treatment in such cases so far.

PO1-026 Adrenal I

Paraganglioma type 4 (PGL 4) caused by a mutation in the SDHB complex: first description of a case in a child

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Introduction: Paragangliomas are extremely rare tumours, occurring mainly in adults. During childhood they are associated with rare familial syndromes such as von Hippell-Lindau, MEN 2a and NF1. Data on their prevalence and treatment are scarce. Recently genetic testing of these patients has been developed and therefore the interest on these tumours is increasing. Four types of paraganlioma syndromes have been described, 3 of which are associated with specific mutations. We present the course of the disease and the results of genetic testing of a child with paraganglioma type 4.

Case description: A 9 yr old boy was admitted to the intensive care unit with symptoms of hypertensive encephalopathy after a prolonged episode of convulsions and electrolyte disorders (hyponatremia and hypokalemia). He had a history of headaches, night sweats and anxiety in the last year. Laboratory and imaging investigations showed an abdominal paravertebral tumour, 4.0x4.5x3.0 cm in size, excreting catecholamines. The tumor was laparoscopically extracted, histology was suggestive of a paraganglioma with no signs of malignancy.

During the next few months of follow-up the patient was asymptomatic. Eighteen months later the boy complained again of headaches, dizziness and night sweats. MRI scan of the abdomen showed two masses (2.5x2.0x0.8 and 3.2x2.0x0.8 cm in size) next to the aorta and the mesentery, which were again removed laparoscopically. Histology showed paragangliomas with no signs of malignancy. DNA analysis showed that the patient is heterozygote for the mutation P254fsX255, a frame shift mutation in site 761 of exon 7, in chromosome 1. Paraganglioma type 4 is characterised by a mutation of the gene coding for complex II of succinate dehydrogenase (SDHB), a mitochondrial enzyme involved in Krebs cycle. The syndrome occurs in adults, at an average age of 35 years. The time between diagnosis and disease recurrence varies between 5-19 years, whereas in our patient it was only 18 months. The disease involves multifocal tumors, leading to metastasic spread in 22% of cases. Follow up every six months is recommended for investigation of disease recurrence or malignancy. Genetic testing of relatives is recommended due to the strong correlation between genotype and phenotype. Furthermore, if genetic screening of asymptomatic carriers starts at 10yrs of age, 96% of patients are likely to be diagnosed.

PO1-027 Adrenal I

Are young people with congenital adrenal hyperplasia engaging with adult endocrine care?

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Background
The challenges of organising transition from paediatric to adult services in endocrinology is recognised. An audit in the UK identified that the majority of paediatric endocrine services conduct joint clinics with adult endocrinologists to facilitate successful transfer. However there is no evidence that this model is successful in engaging the young person.
Objectives
To evaluate whether patients with congenital adrenal hyperplasia (CAH) have been successfully transitioned to adult endocrine services from a tertiary paediatric endocrine service and assess the impact of the Young Person Clinic (YPC) which has been run jointly by paediatric and adult endocrinologists within the same hospital since 2001.
Methods
All patients with CAH who had attended the paediatric adrenal clinic and were currently between 16 and 30 years of age were identified.
Results
Of the 73 patients identified, 33 were male and 51 were salt wasters. There was inadequate information on 11 patients including one patient who had died. 3/62 (5%) had been lost from the paediatric service. Of the 19 patients referred to local adult endocrine services, a letter signifying first attendance was received in 68%. Of the 40 patients referred to the adult endocrine service within the same hospital, 35% were no longer attending, the majority having been discharged following non attendance. 2 patients had never attended and had been discharged. 53 new appointments had been offered to these patients by the adult service including re-referral by their family doctor. The median duration in adult endocrine care before non attendance was 2 years (range 0-5 years) with non attendance rates increasing significantly 3 years after transfer up to 43% compared with the previous 5 years, which spanned attendance at paediatric and adult services ($p<0.001$). The introduction of the YPC had no significant impact on whether patients were likely to attend adult endocrine services.
Conclusions
Despite the introduction of the YPC, when paediatric and adult endocrinologists consult together, a similar proportion of patients with CAH are failing to engage with adult endocrine care. The risk appears higher in the second/third year after transfer when non-attendance rates increase. Transitional care needs to be improved and expanded in both paediatric and adult services to provide support during this time.

PO1-028 Adrenal I

Adrenal insufficiency due to DAX-1 mutations. Difficulties in the diagnosis

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Mutations in the DAX-1 gen are described in many patients with congenital adrenal hypoplasia (AHC) and hypogonadism. Alterations in the DAX-1 gene must be a differential diagnosis in the cases of adrenal insufficiency in neonatal period. We report two cases of adrenal insufficiency with a difficult diagnosis because their confusing symptoms and analysis results.
First child presents salt losing crisis at 25 days of age. The analysis revealed hyponatremia and hyperkalemia, ACTH 1970pg/ml (NV: 20-80), cortisol and aldosterone were decreased, and 17-OH-progesterone was normal. Abdominal ultrasounds were normal. DNA analysis of the 21-OH revealed heterozygosis Val281Leu. He began treatment with hydrocortisone, fludrocortisone and salt. Analysis results showed variable levels of cortisol and aldosterone, sometimes increased and others decreased. We studied mutations in the hMR or the ENaC genes, genes of pseudohypoaldosteronism, but they were normal. At three years old he was operated of criptorquidia. Analysis of gen DAX-1 and SF-1 were normal. At six years of age a magnetic resonance showed hypoplasic adrenal glands. We repeat a new study of DAX-1 that revealed a mutation previously undetectable.
Second boy presents a salt wasting crisis in the first week of age. ACTH was elevated (516 pg/ml), aldosterone was decreased and cortisol and 17-OH-P were normal. Abdominal ultrasounds and DNA analysis of 21-OH were normal. He was diagnosed of primary hypoaldosteronism, treated with fludrocortisone and salt. When he was 20 months old presents a new episode of salt losing with low cortisol. Adrenal autoantibodies and very long fatty acids were normal. Abdominal CT revealed hypoplasic adrenal glands, and the subsequent DAX-1 study showed the mutation G315A. Actually he is twelve years old and he presents signs of hypogonadotropic hypogonadism.
AHC is a rare form of primary adrenal insufficiency, with a variable phenotype that is difficult to identify in some patients. Some children present an adrenal crisis within days after birth while others do not develop adrenal insufficiency until they reach adulthood. One probable explanation is genotype-phenotype correlations, existence of mechanisms for persistence of fetal adrenal cortex, presence of modifying genes and/or environmental factors are among the mechanisms potentially affecting clinical presentation. It is important to suspect AHC in boys with signs of hypogonadotropic hypogonadism, like criptorquidia or micropenis.

PO1-029 Adrenal I

Virilizing adrenocortical adenoma associated with acquired anhydrosis in an adolescent girl

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Functional virilizing tumors are the most common presentation of adrenocortical adenoma (ACA) in children and adolescents. Deepening voice, hirsutism, increased muscle mass, acne, secretion and proliferation of the sebaceous glands with characteristic adult odor are typical manifestations of virilization. We report a 15.5 yo female with virilization, secondary amenorrhea, recent loss of ability to perspire, and associated decreased body odor. She had deepened voice, increased facial and body hair growth for 3 years requiring daily shaving, secondary amenorrhea for 1.5 years. Though being very athletic, she stopped playing tennis for 6 months due to feeling overheated during vigorous activity. No fainting episodes were reported.
Physical examination: Patient was athletic and muscular with BP: 130/70 mmHg, T: 36°C, RR: 16/ min, HR: 89/min, Ht 161cm (25-50p),Wt 62.5 kg (75-90p), BMI 23.9 (75-85p). She had a deep voice, generalized hirsutism and clitoromegaly. Abdomen was soft with no palpable mass. Skin was dry with minimal axillary, and body odor.
Laboratory evaluation: CT revealed a mass anterior to the right adrenal that was removed laparoscopically. Pathological diagnosis of 9x8 cm para-ACA was made. Hormonal values returned to normal 1 month after surgery.

Androgen levels before and after surgery

Androgen levels	Normal range	Before surgery	After surgery
Testosterone (ng/dl)	20-38	246	33
DHEA (ng/dl)	215-850	9888	476
DHEA-S (µg/dl)	44-248	472	53
17-OH- pregnenolone (ng/dl)	44-235	846	39
3αAG (ng/dl)	35-200	3216	224

Discussion: 3α-androstenediol glucuronide (3αAG) is produced in peripheral androgen target tissues such as skin; adrenal androgen precursors have been proposed as the prime source. Androgen receptors are identified not in just pilosebaceous units and apocrine glands but also in eccrine glands which are of critical importance for the regulation of body temperature. Our patient had dry skin, lack of perspiration, dysregulation of body temperature and absence of body odor in the presence of hirsutism and clitoromegaly.We propose extremely high levels of 3αAG (and perhaps other androgens) as the cause of anhydrosis and temperature dysregulation.
Conlusion: To our knowledge this is the first report of an ACA associated with acquired anhydrosis and temperature dysregulation which emphasizes the importance of integumentary system as an endocrine organ and support routine measurement of 3αAG in virilized patients.

PO1-030 Adrenal I

Delineation of disorders of aldosterone metabolism from spot urine in early infancy by GC-MS steroid metabolomics

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Aldosterone synthase I deficiency (18-hydroxylase deficiency, CMO I deficiency), aldosterone synthase II deficiency (18-hydroxysteroiddehydrogenase deficiency, CMO II deficiency) or pseudohypoaldosteronism are important disorders with respect to the complex differential diagnosis of salt wasting in early infancy. Gas chromatography-mass spectrometry (GC-MS) is unsurpassed in its potential for profiling – i.e. the simultaneous determination of – a maximal number of various steroids with highest specificity. We investigated spot urine samples from neonates and young infants with salt wasting (n = 19; median 0.20 yrs, range 0.05-0.51) in whom 21-hydroxylase deficiency had already been excluded by normal plasma 17-hydroxyprogesterone. Urinary steroids were profiled by GC-MS after enzymatic hydrolysis, extraction and derivatisation. 10 Patients were diagnosed with CMO I deficiency: their urinary steroid metabolome showed gross elevation of metabolites of corticosterone, predominantly tetrahydrated 11-dehydrocorticosterone (THA [µg/l]; median [range]; normal upper limit: 566 [193 – 4540]; 84), and hexahydrated 11-dehydrocorticosterone (HHA; 594 [147 – 5733]; normally undetectable); no 18-hydroxylated precursors or metabolites of aldosterone were detectable. 4 Patients were diagnosed with CMO II deficiency: in addition to elevated metabolites of corticosterone, their urinary steroid metabolome revealed increased excretion of 18-hydroxylated THA (18-OH-THA; 3074 [2076 – 4837]; 36); no metabolites of aldosterone were detectable. 5 Patients were diagnosed with pseudohypoaldosteronism: in addition to grossly elevated metabolites of aldosterone precursors, their urinary steroid metabolome was dominated by highly elevated excretion of tetrahydrated aldosterone (THAldo; 1745 [155 – 13000]; 36). **Conclusion:** Urinary steroid profiling allowed clear differentiation of

disorders of aldosterone metabolism (CMO I deficiency, CMO II deficiency, pseudohypoaldosteronism) from easily obtainable spot urine samples. This non targeted analytical approach rendered possible by GC-MS permits generation of unbiased metabolomic profiles of highest diagnostic potential.

PO1-031 Bone, Calcium I

A novel mutation in the CLDN16 gene in a Palestinian family with familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC)

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Background:
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessive renal disorder characterized by excessive renal magnesium and calcium loss, bilateral nephrocalcinosis, and progressive renal failure, due to impaired tubular reabsorption in the thick ascending limb of the loop of Henle. FHHNC is caused by mutations in the caludin-16 gene (CLDN16), which encodes the tight junction protein, claudin-16. Claudin-16 belongs to the claudin family and regulates the paracellular transport of magnesium and calcium.
We describe a novel mutation in the CLDN16 gene in a Palestinian family with FHHNC.
Clinical Data:
A 2 month old male infant, born to a consanguineous Palestinian family, presented with convulsions during hypomagnesemia, hypocalcemia & hyperphosphatemia. PTH was high 204pg/ml and fractional excretion for magnesium was 52%. 24 hours urine collection for calcium was high 14mg/kg/day & renal u/s showed bilateral nephrocalcinosis. Kidney function tests were normal. He has been treated with magnesium and thiazide with good response. A male sibling died at 12y of age due to renal failure.
Molecular Data:
Sequencing the CLDN16 gene for the patient revealed a novel missense mutation with replacement of G by A in codon 120 of exon 2 (TGC à CGC), predicting Cysteine to Arginine substitution (C120R).
Conclusion:
To our knowledge, this is the first confirmation of this diagnosis by molecular testing in a Palestinian family allowing genetic counseling and future prenatal diagnosis, in addition to early diagnosis of affected kindreds allowing early treatment & intervention.

PO1-032 Bone, Calcium I

Familiar hypophosphatemic rickets: molecular findings in eleven Argentinean families

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Introduction: human disorders of phosphate (Pi) handling and skeletal mineralization can result from inactivating mutations in PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), an X linked dominant disorder (XLH) and the most common form of heritable rickets which is fully penetrant with variable severity. Autosomal-dominant hypophosphatemic rickets (ADHR) is a rare disorder caused by mutation in FGF23 that prevents its degradation and which in contrast to XLH, displays incomplete penetrance and variable age of onset. We analyzed genomic DNA of 11 Argentinean families in order to characterize these genes.
Patients and methods: we studied 20 patients with hypophosphatemic rickets, belonging to 11 families; 14 patients (70%) were familiar. We found only one single affected patient in six families, 2 in two families, 3 in two families and 4 in one family. All patients, except two had been diagnosed in early childhood based on hypophosphatemia, increased serum alkaline phosphatase activity and radiological signs of rickets. DNA was extracted from venous blood. FGF23 was analyzed by bidirectional complete sequencing of the three exons. In PHEX all 22 exons and introns/exons boundaries were analyzed by SSCP/HD and bidirectional complete sequencing of abnormal migration pattern regions were performed
Results: FGF23 missense mutation was identified in 4 affected members of a family (G >A, R179Q, in exon 3). Three novel PHEX gene mutations were detected in 6 patients. All were familial cases except one who was sporadic. Family 1: the two affected patients, a boy and his mother presented ins G +1 of intron 3-4 in splice donor. Family 2: the three affected members, a boy, his mother and maternal aunt had a silence mutation C>A pos 11 exon 3. Family 3: a single girl with healthy parents showed another silence mutation T > G in pos. 93 exon 20.Patients with FGF23 mutation presented with rickets in infancy, osteomalacia in adulthood and isolated hypophosphatemia while the patients without the FGF 23 mutations tended to have more severe skeletal disease. Conclusions: We describe 3 novel mutations in gene PHEX. All patients with XLH presented with severe phenotype during follow-up. In contrast, we detected only one family with ADHR where we could observe different clinical manifestations.

PO1-033 Bone, Calcium I

Vitamin D status in children with celiac disease

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Introduction: Celiac Disease (CD) is one of the most frequently etiology of malabsortion syndrome in pediatric patients. CD can negative affect the mineralization of growing skeleton. The aim of this study is analyze at the moment of diagnosis of CD, the prevalence of vitamin D insufficiency or deficiency.
Population: twenty – six prepuberal (n:16) and puberal (n:10) patients were evaluated (Female 15) in clinical and biochemical aspects. DEXA lumbar spine (Hologic 4500 QDR) was performed. Results (expressed in mean ± DS) : chronological age 8,2 (5,05), weight (Z Score) -1,15 (1,32), height: - 1,4 (1,45). Two patients had autoimmune hepatitis. BMI (Z Score) : 18,01 (4,57). Bone age : 9,08 years (± 3,88, range: 2,9-17).
Laboratory: PTH: 54,54 pg/ml (± 50,46, range: 3-200), 25 OH Vitamin D(Dia Sorin RIA): 22 ng/ml (± 12,7, range: 8-48), Calcium: 9,31 mg/dl (±0,93, range: 7,7-12,6), Phosphorus : 4,95 mg/dl (± 0,65, range: 4-6,5), FAL: 629 UI/l (± 357,5, range:169-2024).Two of our patients had hipercalciuria (>4mg/kg/day). Secondary hyperparathyroidism were found in five patients (19,2 %). All of them had vitamin D deficiency. DEXA (n: 14) (mean ± SD): -0,91 (± 1,6). Two of our patients had Z Score below – 2 SD.
At the diagnosis eleven pacientes (42,3%) had vitamin D deficiency (<20ng/ml) and four patients (15,3%) insufficiency (<30 ng/ml). Positive correlation was found between PTH and Vitamin D concentrations. (p: 0.01, R= 0.561) and weight Z score and Vitamin D Levels (p: 0.05, R= 0.478).We no found correlations between vitamin D levels and BMD.
Conclusions: the patients affected with CD presented low levels of 25 (OH) D levels at the moment of the first clinical evaluation frequently. Determination of the serum levels of 25(OH) vitamin D is an important tool in the clinical management in this group of patients .

PO1-034 Bone, Calcium I

Effectiveness of once-monthly oral ibandronate treatment in adolescents with osteogenesis imperfecta

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Intravenous pamidronate has been widely used in patients with osteogenesis imperfecta (OI) and have showed to be very effective and safe, but sometimes it is not available in some countries, so we have to study other therapeutic possibilities for our patients. The objective of this study was to assess the safety and effectiveness of once- monthly oral Ibandronate in reducing pain and rate of fractures in adolescents with OI. We started an observational study in which

12 patients, 5 girls and 7 boys, with OI were included. All of them has presented at least three or more fractures per year and showed some bone deformities in lower limbs. Mean age at start of therapy was 14.8 years. Once-monthly oral ibandronate 150 mg was started. All patients had a daily oral supplement of vitamin D and calcium. Clinical evaluation was done every three months and, and bone mineral density (BMD) was measured before starting therapy and after 12 months of treatment. Treatment continued for two years. No side effects were observed during the treatment. BMD increased between 50 –130 % after the fisrt year of therapy. Bone pain was reduced in all patients and no fractures occurred during the period of treatment.
In conclusion, Once-monthly oral ibandronate appears to be a well tolerated and effective alternative therapy in patients with OI when there is no availability of intravenous pamidronate.

PO1-035 Bone, Calcium I

Effectiveness and safety of once-monthly oral ibandronate treatment in two adolescents with polyostotic fibrous dysplasia of bone

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Cyclical intravenous infusions with the bisphosphonate pamidronate has been used in treatment of fibrous dysplasia (FD) of bone, but there is no much experience with the use of oral ibandronate especially in children. Here we describe two girls with polyostotic FD. Both patients have two and four lytic lesions in femur respectively and have suffered at least one fracture in the last year that were managed with surgery and fixation. Age at start of therapy was 13 and 16 years. At the time of starting therapy they did not show any fractures. Due to difficulties to obtain the intravenous pamidronte we start therapy with once-monthly oral ibandronate 150 mg in addition to oral daily intake of vitamin D and calcium. Radiologic studies were preformed every three months. Treatment continued for two years. No side effects were observed. The first observation was pain relief after the second month. Levels of serum alkaline phosphatase and urinary collagen type I N-telopeptide were elevated at the start of therapy and showed progressive decrease continuously during the two years of therapy. Radiological findings start to show some evidence of filling of the lytic lesions after the first 18 months of treatment. None of them suffered fractures during the period of treatment.
In conclusion, once-monthly oral ibandronate therapy appears to be safe and effective in adolescents with polyostotic FD when there is no availability of intravenous pamidronate. However, more studies with more patients and for more time is necessary to confirm the beneficial effect of this therapy in such patients.

PO1-036 Bone, Calcium I

Unusual clinical presentation of an infant with familial hypocalciuric hypercalcemia: effect of pamidronate treatment

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The calcium sensing receptor (CaSR) plays a crucial role in extracellular calcium homeostasis. Heterozygous inactivating mutations of the *CaSR* gene cause a usually asymptomatic, benign form of hypercalcemia known as Familial Hypocalciuric Hypercalcemia (FHH), whereas homozygous or compound heterozygous mutations produce a severe, symptomatic hypercalcemia called severe neonatal hyperparathyroidism (SNHP).
Here, we report an infant who had a unique presentation of osteopenia and numerous pathological fractures despite only moderate hypercalemia. His workup included an initial serum calcium that was 11.4 mg/dL obtained during a sepsis workup at 3 weeks of age. At 2 months of age, serum calcium was found to be persistently elevated at 13 mg/dL, with an elevated PTH (127 pg/mL) and an inappropriately low urine calcium excretion. A skeletal survey at that time revealed diffuse demineralization along with multiple rib fractures. A baseline DXA at 2-month of age showed a BMD (L1-4) of 0.131 g/cm^2 (Z-score <-2 S.D).
In addition to conventional supportive treatment, he received a single infusion of pamidronate (0.5mg/kg) due to his pathological fractures. Serum calcium levels gradually decreased over 1 week to near-normal levels (11.5 mg/dL) but then returned to 13 mg/dL by two weeks post infusion. The patient remained asymptomatic seven months following pamidronate therapy despite persistent moderate hypercalcemia Both his weight and height improved from the 3rd%ile at 2 months of age to ~25th%ile at ten months of age. A repeat BMD was significantly improved to 0.264 gm/cm^2 (Z-score now normal).
DNA sequence analysis of the *CaSR* gene revealed two single-base mutations in exon 7 (2383C>T resulting in the amino acid change R795/W/R and 3031C>G resulting in the amino acid change Q1011Q/E). While the former has been described in association with FHH, the latter has not been shown to have significant change in the function of CaSR and is considered as a single nucleotide polymorphism.
In sum, although the severe phenotypic expression of these mutations remains unexplained and will require functional studies, we hypothesize that the SNP may alter CaSR function when there is another inactivating mutation present. Furthermore, pamidronate appears to be an effective strategy in the treatment of symptomatic hypercalcemia in association with FHH.

PO1-037 Bone, Calcium I

Predictors of nephrocalcinosis in patients with hypophosphatemic rickets

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OBJECTIVE: Nephrocalcinosis is known to be associated with conventional treatment of phosphate and calcitriol in patients with hypophosphatemic rickets (HPR). The predictors of nephrocalcinosis in these patients have remained unclear. This study investigates whether dosage of phosphate, dosage of calcitriol, serum intact PTH (iPTH) levels, and/or hypercalciuria correlate with the development of nephrocalcinosis.
METHODS: We conducted a retrospective chart review, and data were collected from sequential clinic visits of 12 patients with HPR. Data included serum calcium, serum phosphorous, serum alkaline phosphatase, serum iPTH, urine calcium, urine creatinine, dose of phosphate, dose of calcitriol, dose of diuretic, and renal ultrasound results. The renal ultrasounds were qualitatively evaluated by a single pediatric radiologist. Unpaired t-tests were used to compare the mean differences between subjects with positive and negative renal ultrasounds. The analysis evaluated mean serum test values, mean urine test values, and treatment for 1, 2, and 3 years prior to each renal ultrasound.
RESULTS: Of 12 patients, 4 were male and 8 were female. The mean age of diagnosis was 2.07 ±1.63 years and mean follow-up was 8.8 ±4.71 years. All patients were on conventional treatment with phosphate and calcitriol. Three (25%) have been on diuretics in addition to conventional treatment. Renal ultrasounds in 6 of 12 patients (50%) showed nephrocalcinosis. Nephrocalcinosis correlated significantly with mean calcitriol dose 0.03±0.01(P=0.024), mean urine calcium 8.54±10.35(P=0.0437), and mean iPTH 42.55±39.17(P=0.0029). However, the mean iPTH levels have a negative correlation with nephrocalcinosis
CONCLUSIONS: Nephrocalcinosis is a noted complication with the conventional treatment of children with HPR. There is a significant positive association of developing nephrocalcinosis with the dose of calcitriol and urine calcium levels. However, our data did not demonstrate that iPTH level is a significant risk factor for nephrocalcinosis.

PO1-038 Bone, Calcium I

Longitudinal evaluation with quantitative ultrasound of bone marrow transplantation-associated bone loss in a series of 28 children

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Transplantation-associated bone loss has been demonstrated to occur during follow-up after bone marrow transplantation (BMT) in children. Quantitative ultrasound (QUS) of the hand phalanges is considered a useful and safe tool to assess longitudinal bone status. Many factors are related to bone loss as high-dose toxicity of chemotherapy, conditioning regimen, graft versus-host disease (GVHD) and its treatment.
Our aim was to evaluate the longitudinal bone mineral status with quantitative ultrasound (QUS) of 28 (18 M) pts who underwent BMT for malignant (n.17), non malignant (n.5) haematological diseases or solid tumours (n.6). The mean age at BMT was 10,2 ± 4,6 years, 18 pts were prepubertal, the median follow-up is 6 yrs (range 3-10 yrs); pre BMT conditioning was obtained with high dose chemotherapy with (5 pts) or without (23 pts) total body irradiation (TBI). 15/28 pts underwent allogeneic BMT and 7/15 showed Graft Versus Host Disease (GVHD).
7/28 pts showed stable values of amplitude-dependent speed of sound (Ad–Sos) Z-Score, even if reduced in 3 cases (between -2 and -1 Z –score).
7/28 pts showed at least one Ad-Sos Z-Score <-2; these pts were all but one prepubertal at BMT (allogeneic in 5/7 pts) and in 4 cases received high dose corticosteroids for anti-leukaemia induction. During follow-up 3 of these pts were stable, 3 pts improved and in one case worsened: 4/7 entered normally timed puberty, 1/7 is still prepubertal, 1/7 is lost at follow-up and 1/7 started estradiol for hypergonadotropic hypogonadism. No patients showed bone fractures. During follow-up 5 pts showed improvement of Ad-Sos Z-score, from -1,35±0,8 at first control (1,6 yr after BMT) to 0,3 ±0,8 (4 yrs after BMT) with a medium gain of 1,66±0,9.
Ad-Sos Z-Score at last control is significatively reduced ($p<0,05$) compared to values before BMT (7 cases).
A negative trend in mean values at last control was found in pts with GVHD. No significative correlation was found between TBI conditioning, type of BMT and Ad-Sos Z-Score at last control.
Our data showed that bone status of BMT pts is affected particularly in those transplanted prepubertal, with allogeneic BMT and who received induction therapy with corticosteroids. Among factors related to bone loss after BMT, GVHD and its treatment should be considered.

PO1-039 Bone, Calcium I

Compensation of bone degeneration in a mouse model of muscle paralysis by whole body vibration and IGF-I

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INTRODUCTION
Bone development is affected by muscle function. In a previous study botulinium toxin A (Botox) induced muscle paralysis results in bone resorption in mice]. It was shown that high-frequency mechanical loading enhanced bone mineral density. Insulin-like growth factor-I (IGF-I) is essential for bone homeostasis and stimulates bone formation in the normally loaded bone. We examined the effect of whole body vibration (WBV) and IGF-I on bone degradation caused by localized disuse induced by muscle paralysis of Clostridium botulinum Toxin A (BTA).
METHODS
Thirty female C57BL/6N mice (16 wk of age at start of the study) were randomized into six groups (n=5 each): basic control (BC), sodium group (CON), BTA (IM), BTA+WBV (IM+WBV), BTA+IGF-I (IM+IGF-1) and BTA+WBV+IGF-I (IM+WBV+IGF-I). On day 1 of the study anesthesia was intraperitoneal administered. While anesthetized, BTA was intramuscular injected in both the right quadricep and calf. An equal volume sodium chloride solution was applied to the left hindlimb at the same sites. The IM+IGF-I and IM+WBV+IGF-I groups received daily subcutaneous injections of recombinant IGF-I into the neckfold. The IM+WBV and IM+WBV+IGF-I groups underwent WBV (25 Hz, 0.83 mm amplitude) for 10x3 min/day, 5 day/wk. The mice were sacrificed after 28 days of intervention. Femora were assessed by pQCT. Transverse sections were scanned at the distal femoral metaphysis and at the midshaft. Trabecular and cortical parameters were determined.
RESULTS AND DISCUSSION
Body mass of mice decreased within the experimental time (-0.2g/mouse). The body mass ranges changed from 21.0 g-25.2 g to 20.4 g-24.6 g. The immobilization effect by BTA decreased approximately from 90 % immobility (day 5) to 30 % (day 28).
The IM group showed a decrease in both trabecular (-20 %, $P < 0.05$) and cortical (-3 %, $P < 0.05$) bone mineral density compared to CON group. The IM+IGF-I group displayed a decrease in trabecular (-18 %, $P < 0.08$) and cortical (-3 %, $P < 0.05$) bone mineral density. The IM+WBV+IGF-I had only a lower cortical bone mineral density (-3 %, $P < 0.05$).
CONCLUSIONS
Muscle paralysis induced by botulinum toxin A results in bone degradation. Whole body vibration may compensate the bone loss caused by muscle atrophy. Our results further indicate that the absence of muscle contraction caused bone resistance to IGF-1. It has previous ly been shown that IGF-1 administration in vivo did not stimulate bone growth and formation in the unloaded bone.
Study is financially supported by IPSEN Pharma GmbH, Germany.

PO1-040 Bone, Calcium I

Low bone mineral density and high incidences of fractures and vitamin D deficiency in 52 paediatric cancer survivors

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The long-term survival rate of paediatric oncology patients is constantly increasing. Long term outcomes of survivors become an issue of great importance.
Objective: The evaluation of bone mineralization as well as the presence of fractures and of vitamin D deficiency in pediatric patients in complete remission of a solid malignant tumor. We also aimed at the identification of potential risk factors for those three abnormalities.
Patients and methods: We included 52 consecutive patients, 30 boys and 22 girls who survived a solid malignant tumor during their childhood or adolescence. Cancer was diagnosed at an average age of 9.46 $\pm$ 4.49 years. Data were collected between 0.3 to 86 months (median 13.8 months) after completion of cancer treatment. A clinical examination, nutritional assessment and laboratory workup were performed. Bone mineral density (BMD) was measured by absorptiometry. Hormonal and vitamin D deficiencies were treated. The subjects were called in again a year later for reevaluation. Among them 21 completed the second evaluation.
Results: Calcium intake was inadequate in 75% of patients and vitamin D reserves were low in 61.5%. Bone densitometry revealed an average Z-score of -0.86 $\pm$ 1.11 in the spine and -0.87 $\pm$ 0.98 in the femoral neck. BMD was low (Z-score < -1.5) at the spine in 32.7%, and at the femur in 24%, of patients. The demineralization was more severe (Z-score < -2.5) in 9.6% of subjects at the spine and 6% at the femur. Spinal and femoral BMD Z-scores correlated significantly with each other ($\rho=0.66$, $p<0.001$). Femoral BMD Z-score but not spinal BMD Z-score showed significant positive correlations with changes in

body mass index (4) and time since treatment completion (ρ=0.28, *p*=0.05); and a significant negative correlation with treatment duration (ρ=-0.37, *p*=0.018). Urinary calcium/creatinine ratio also tended to correlate with femoral BMD Z-score (ρ=0.30, *p*=0.06). Fractures were noted in 10 patients but were not correlated with BMD. In the 21 re-evaluated patients no significant improvements were found in their calcium intake, vitamin D levels or BMD Z-score. A BMD aggravation was observed in certain patients, more obvious at the spine level.
Conclusions: Survivors of childhood solid cancer have high rates of insufficient calcium intake, vitamin D deficiency, demineralization and fractures.

PO1-041 Bone, Calcium I

Malignant infantile osteopetrosis presenting with hypocalcemia due to a novel mutation of the *CLCN7* gene

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Background: Malignant infantile osteopetrosis is a disorder characterized by defective osteoclastic function leading to impaired bone resorption and resulting in dense bones with obliteration of the marrow space and compression of cranial nerves. Affected infants may present with failure to thrive, developmental delay, pancytopenia, deafness, blindness and hepatosplenomegaly.
We report an infant who presented with hypocalcemia and was found to have infantile osteopetrosis secondary to a novel mutation of the chloride channel 7 *(CLCN7)* gene.
Clinical Case: A 2 month old full term female Indian infant was evaluated for hypocalcemia noted incidentally on blood work performed during an admission for RSV bronchiolitis. Serum calcium was 5.8 mg/dl (nl 9-11), ionized calcium 0.76 mmol/L (1.20-1.38), phosphorus 3.8 mg/dl (4.5-6.7) and alkaline phosphatase 502 U/L (150-420). The child was previously healthy with no jitteriness or seizure activity. Physical examination was significant for natal teeth and nystagmus. Height and weight were appropriate for age. The infant had been receiving breast milk and formula at home. Radiographic studies were evaluated for evidence of rickets, albeit atypical in a formula fed 2 month old.
Results: Chest X-ray performed for evaluation of respiratory distress was consistent with increased bone density. A subsequent skeletal survey was significant for generalized osteosclerosis of the bony structures and thickening of the orbital fossa. Optic canals were narrow on CT scan. Additional blood work indicated an elevated intact PTH of 109 pg/ml (15-65), nl 25-Hydroxy-vitamin D of 27 ng/ml (25-80) and elevated 1,25-Dihydroxy-vitamin D of 335 pg/ml (24-86). CBC indicated thrombocytopenia (84,000) and severe anemia (Hgb 7.9 g/dl,). Sequencing of the *CLCN7* gene revealed a homozygous c.889G>A transition in exon 10 resulting in an amino acid change from valine to methionine. This change has not been previously reported.
Clinical Course: The infant was treated with calcium and high dose calcitriol. Bone marrow transplantation was performed using a matched sibling donor.
Conclusion: We have described a 2 month old infant, presenting with hypocalcemia and found to have malignant infantile osteopetrosis due to a novel mutation in a highly conserved region of the *CLCN7* gene. Although rare, osteopetrosis should be considered in the differential of neonatal hypocalcemia if initial lab studies are atypical.

PO1-042 Bone, Calcium I

Celiac disease mimicking pseudohypoparathyroidism: a phosphocalcic pitfall!

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Introduction
Celiac disease may mimick different clinical pictures, covering the endocrinological spectrum. We report the case of a young girl presenting with recurrent abdominal pain and paresthesia falsely diagnosed as type 1 pseudohypoparathyroidism.
Clinical history
A 9-year old girl presented with febrile acute abdominal syndrome and bad general state. Her history was marked by recurrent abdominal pain with normal work-up and diagnosis of constipation. She grew up regularly along the 3rd percentile, in accordance with her Hispanic origins. Abdominal ultrasound found ileo-ileal intussusceptions and large mesenteric adenopathies. Paresthesia lasting for "a long time" were described and profound total hypocalcemia (2.3 mEq/L = 4.8 mg/dL) with slight hypoprotidemia and phosphatemia in the normal to upper range were discovered. Haemoglobin was normal (14.1 g/dL) and INR elevated to 1.6. Work-up showed a very increased PTH (180 pg/mL, norm 10-70) and decreased 25OHD (4.4 ng/mL, norm 10-60). UCa/UCr was very low (0.01). Osteopenia was suspected on initial abdominal X-ray and DEXA confirmed lumbar and hip Z-scores of -4.1 SD and -2.6 SD. Thyroid function was normal. Left lens calcifications were found, but brain CT and renal ultrasound were normal. The girl was eumorphic besides discrete shortness of the 4th and 5th metacarpals. A diagnosis of type 1b pseudohypoparathyroidism was proposed and calcitriol and calcium carbonate were started with complete disappearance of abdominal signs, which unfortunately recurred soon after. After two weeks, calcemia failed to increase and a second advice confirmed celiac disease, on the basis of antitransglutaminase IgA and histological analysis of duodenal mucosa.
Conclusion
Celiac disease has to be evoked in every case of prolonged abdominal signs. Secondary malabsorption causes depletion in liposoluble vitamins and intestinal chelation of calcium, resulting in elevated INR and hypocalcemia, which can be profound and symptomatic. Osseous hyperparathyroidism and normal to upper limit phosphatemia may mimick renal PTH resistance. 25OHD levels are helpful: low in malabsorptive states, and frequently normal in PTH resistance, in absence of conjugated vitamin D deficiency. In opposition to the classical description, phosphatemia can be normal in malabsorption. Finally, normocalcemia in pseudohypoparathyroidism occurs in 7 to 10 days with calcitriol, and every delay must re-interrogate the diagnosis.

PO1-043 Bone, Calcium I

Low dose pamidronate improve bone mineral density in children with cerebral palsy

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Introduction: Cerebral palsy (CP) is a non progressive disorder of posture and movement due to an insult to the developing brain. Multiple factors may potentially adverse affect mineral metabolism in children with cerebral paisy (nutritional, hormonal, immobilization). Children and adolescent with CP are also prone to low trauma fractures.
Aim: to investigate the efficacy of low doses pamidronate in increasing bone mineral density in eight CP wheel chair patients, who previously presented bone fractures.
Patients and methods: The mean age of the patients was (mean ± SD) 8,2 (±2,01) (range: 8,3 -12.0).
All patients are prepuberal. All patients presented one or more fractures (mean ± SD) 1,82 (± 0,67).
All patients had baseline studies performed on blood and urine samples. Calcium (Ca),Phosphorus (P), Alcaline Phosphatases (AP),Albumin, PTH (Inmulite-inmunochemiluminometric assay),25 - dihydroxyviytamin D (DiaSorin-RIA) and urinary calcium. Whole body and femoral neck bone mineral density were meassured by DEXA (Hologic QDR 4500 W).All these patients received two day cycle pamidronate (0,5 mg/kg/day,max:30 mg/day)), every three months, for one year. All the patient
Stadistical analysis: to compare differences between groups (basal and after one year treatment) Mann –Whitney test was performed.
Results: The levels of serum Ca, P and AP were normal in all patients. The dietary calcium was (mean ± SD)

0,607 g ± 0,28 and calcium and vitamin D complements were use in all cases. The calcium excretion rate was normal.
Vitamin D levels in patient previously to pamidronate treatment was (mean ± SD): Vitamin D: 26, 7 (± 5,12) ng/ml. PTH levels was normal in the whole group. Biochemical test showed no significant change with treatment. Hypocalcaemia without clinical symptoms was found in two of out 32 infusion cycles. DEXA femoral neck improved after treatment.

	Before infusion	1 Year pamidronate	p value
BMD whole body(g/cm2)	0,80 (± 0,14)	0,88 (± 0,08)	n/s (0,08)
Z score BMD Whole body	- 1,61 (± 1,26)	- 1,37 (± 0,30)	n/s (0,076)
BMD Femoral neck (g/cm2)	0,32 (± 0,07)	0,48 (± 0,03)	< 0,02
Zscore Femoral Neck	- 3,6 (± 0,72)	- 2,4 (± 0,62)	< 0,02

Conclusions: low doses pamidronate were effective improving bone mineral density (femoral neck) in this group of patients, without significant adverse effects.

PO1-044 Bone, Calcium I

25-hydroxyvitamin D serum levels among primates of the *callithrix penicillata* species raised in captivity

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Introduction: Due to its taxonomical closeness and its similarity in some genetic aspects to humans, the non-human primates of *Callithrix penicillata* species are frequently used in biomedical research. Vitamin D is a hormone that participates not only in the regulation of bone and calcium metabolism, but it is also involved on the modulation of autoimmunity processes, some anti-oncogenic pathways, synthesis of natural antibiotics and it seems to modulate the expression of genes that regulate brain cells development and behavioral functions.
Objectives: to determine the serum 25-hydroxivitamin D [25(OH)D] levels among captivity raised *Callithrix penicillata* animals settled in different conditions of sunlight exposure; to study the influence of sunlight exposure periods and sex on 25(OH)D levels; to discuss a proposal of which would be the serum 25(OH)D standard range for these animals.
Methodology: we studied 84 animals of this species, belonging to two different primatology research centers. They were classified into 3 groups: free sunlight exposure (group 1, n=29); partial exposure (group 2, n=34), and no sunlight exposure (group 3, n=21). Serum 25(OH)D measurement was performed through chemiluminescence assay. Data were analyzed using the nonparametric tests Kruskal-Wallis and Mann-Whitney.
Outcomes: The obtained mean serum 25(OH)D levels were: group 1- 121.2±33.3ng/ml; group 2- 115.2±32.2ng/ml; group 3- 53.3±10.4ng/ml. There was a statistically significant difference between groups 3 and 1 ($p<0.001$) and between groups 2 and 3 ($p<0.001$), but no statistical difference between groups 1 and 2 ($p=0.605$). There was no influence of sex on the 25(OH)D levels ($p=0.563$). From these data, it is proposed a serum 25(OH)D standard level range from 104.8 to 137.1 ng/ml for captivity raised animals of this species, within a confidence interval of 95%, regardless of sex, when the measurement is performed by chemiluminescence assay.
Conclusions: This study reinforces the importance of direct sunlight exposure on 25(OH)D sufficiency. Quality of life and access to adequate vitamin D levels to these animals must be a main concern for all the institutions that house them, due to ethical aspects and also because 25(OH)D levels may have direct implications on the reliability of the biomedical researches outcomes involving these animals, once this hormone regulates many metabolic axis and pathways.

PO1-045 Bone, Calcium I

Effects of cyclical intravenous pamidronate therapy on bone markers in pediatric patients with *osteogenesis imperfecta*

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Introduction: Cyclical intravenous pamidronate (PAM) therapy in patients with *osteogenesis imperfecta* (OI) is associated to a decrease in bone fractures events and bone deformities and improvement in functional mobility. This improved clinical outcome follows the decreased bone remodeling and resorption rates induced by PAM.
Objective: To evaluate the effects of PAM on the following biochemical bone markers in OI patients after 12 months of treatment: calcium (Ca), phosphate (P), alkaline phosphatase (ALP) and intact parathyroid hormone (PTH).
Patients and methodology: This is a retrospective study comprised of 26 patients (12 girls) with OI receiving PAM cyclical therapy (cumulated dose 9mg/kg/year). It was compared the pre-treatment serum levels of Ca, P, ALP and PTH to the 12th month levels after PAM onset, using *Student's paired t* test for statistical analysis.
Outcomes: Patients' mean age at diagnosis was 2.8 ± 3.7 years (range from birth to 14.5 years); mean age at PAM treatment onset was 6.7 ± 4.8 years (range from 18 days to 15.6 years). All patients presented a decrease in fracture and bone pain events and improvement in functional mobility. Pre-treatment and 12th month values of the studied bone markers were, respectively: Ca- 2.64 ± 0.20 mmol/L and 2.61 ± 0.14 mmol/L; P- 1.74 ± 0.25 mmol/L and 1.60 ± 0.35 mmol/L; PTH- 21.90 ± 13.11 ng/L and 34.78 ± 18.70 ng/L; ALP- 649.0 ± 182.64 IU/L and 479.69 ± 196.44 IU/L. Analyzing these two time points, there was a statistically significant rise in PTH levels (mean increment: 58,8%, $p<0,01$) and decrease in ALP levels (mean decrement: 26,1%, $p<0,01$). There was no statistically significant difference in Ca and P serum levels.
Conclusion: The decrease in ALP and the increase in PTH levels reflect the diminished bone remodeling and resorption rates due to PAM. Although all the patients of this group have improved their clinical status, they need a close and long term follow-up, once the long term effects of this lower bone remodeling rate is unknown.

PO1-046 Bone, Calcium I

Quantitative UltraSonography (QUS) for the assessment of bone status in boys with haemophilia A. Comparisons with dual energy X-ray absorptiometry (DXA)

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Introduction: Recent studies demonstrate decreased bone mineral density (BMD) values in young hemophilic patients due to multiple factors, the main cause being recurrent hemarthrosis and subsequent reduced physical activity. The aim of this study was to assess bone status in boys with haemophilia A, both with Quantitative UltraSonography (QUS) and Dual Energy X-Ray Absorptiometry (DXA), and consequently to determine the degree of correlation between these two methods.
Patients and Methods: Seventeen patients with severe phenotype of hemophilia A, aged 11.87 ± 4.91 years (range: 4.94 – 17.62 years) were enrolled in this study. With regards to study methods, weight and height were measured using standard techniques, whereas Body Mass Index (BMI) was calculated as the ratio weight / height^2 (kg/m^2). For every auxological parameter, Standard Deviation Scores (SDS's) were calculated according to sex- and age-matched normal greek population. BMD at lumbar spine (L2-L4 vertebrae) was determined by DXA technique. QUS measurements (Speed Of Sound, SOS) were performed at two peripheral sites: distal third of the radius (SOS_R) and midshaft tibia (SOS_T). Z-scores were calculated according to normative data derived from sex- and age-matched Greek population. Finally, joint evaluation was performed using the Hemophilia Joint Health Score (HJHS), a validated

11-item scoring tool scale assessing six index joints (elbows, knees and ankles) ranging from 0 (normal) to 20 (heavily impaired).
Results: Mean BMD Z-score was -0.12 ± 1.08 g/cm^2, whereas 3 and 2 patients were classified as having osteopenia and osteoporosis respectively. Mean SOS_R Z-score and mean SOS_T Z-score were -0.08 ± 0.83 m/sec and -0.10 ± 1.6 m/sec, respectively. No correlation was observed between DXA values and QUS-derived measurements. No agreement was recorded between the two methods in identifying hemophilic patients at risk for osteoporosis (kappa value = -0.25, p=0.17). SOS values at the dominant side were significantly correlated to SOS values at the non-dominant side both at radius (r=-.541, p=.01) and at tibia (r=.45, p=.04). Finally, the HJHS was negatively correlated with the SOS_T Z-scores (r=-.541, p=.023), whereas it was, surprisingly, positively correlated to BMD Z-scores (r=.473, p=.044).
Conclusion: DXA detected a significant number of hemophilic children with impaired bone status; however, these findings were not confirmed by QUS measurements.

PO1-047 Bone, Calcium I

The increase of serum phosphate after GH treatment is not mediated through suppression of FGF23

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Introduction: FGF-23 has been revealed to be a major regulator of phosphate homeostasis by promoting phosphate excretion and therefore reducing serum phosphate. On the other hand, GH treatment is accompanied by an increase in serum phosphate and stimulates phosphate renal reabsorption but the underline mechanisms are not elucidated. We investigated if the observed effects of GH on serum phosphate are mediated through down-regulation of FGF23.
Methods: We studied 23 GH deficient children (13 females and 10 males), aged 8.9 years, before and after 3 months on GH treatment (0.022mg/kg/day). Serum phosphate, calcium, IGF-I, 1,25(OH)2 Vitamin D, and FG23 were measured before and after treatment as well as renal tubular maximum threshold for phosphate (TmPO4). We measured the c-terminal of FGF23 by ELISA.
Results: Serum phosphate and TmPO4 were significantly increased after GH treatment (4.99 ± 0.5 vs 4.65 ± 0.4mg/dl, p<0.001 and 5.48 ± 0.9 vs 4.87 ± 0.8, p<0.01, respectively), as well as IGF-I (204 ± 83 vs 123 ± 59ng/ml, p<0.0001), whereas 1,25(OH)2 Vitamin D was not affected (127 ± 37 vs 116 ± 28pmol/L, p>0.05). GH treatment increased significantly the serum levels of C terminal FGF23 (56 ± 16 vs 48 ± 13RU/ml p<0.01). FGF23 levels were positively correlated with TmPO4 and negatively correlated with 1,25(OH)2 Vitamin D (p<0.01 and p<0.05, respectively), and IGF-I was positively correlated with 1,25(OH)2 Vitamin D (p<0.05). **Conclusion:** GH increases serum phosphate by increasing renal tubular reabsorption. This effect of GH is not mediated through suppression of the phosphaturic protein FGF23. Instead, we observed that GH treatment was associated with increased FGF23 serum levels. This effect most likely reflects a counter regulatory mechanism secondary to the increased serum phosphate caused by GH treatment.

PO1-048 Bone, Calcium I

Hypocalcaemic seziures in adolescents with cerebral palsy

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BACKGROUND
Bone health is adversely affected in a number of ways; restrictions of physical activity, limited exposure to sunlight and medications used to treat seizures can all adversely affect Ca homeostasis and vitamin D metabolism in children with cerebral palsy (CP).
Three cases of vitamin D deficiency rickets who presented with hypocalcaemic symptoms in cerebral palsied children are reported.
Case 1: A 15 years old girl with CP was brought to our hospital because of fatigue, vomiting, constipation and carpopedal spasm. She was confined to bed and was on antiepileptic therapy (AET) for a long time. She was undernourished, had a fasciculation on her tongue and enlarged wrist. Her total serum Ca level was 4 mg/dl, P 5.7 mg/dl, ALP 380 U/L, PTH 820.8 pg/ml and 25 OH vitamin D<4 mg/L.
Case 2: A 14,3 years old cerebral palsied boy was admitted to the emergency department with generalized convulsion. Although he was given AET, he has uncontrolled seizures for the last 6 years. On the last admission he was found to have severe vitamin D deficiency and hypocalcaemia (Serum Ca 6,3 mg/dl, P 4.7 mg/dl, ALP 773 U/L, PTH 217.6 pg/ml and 25 OHD was very low to be detected).
Case 3: 16,3 years old boy on AET who was followed as known CP presented with tonic clonic fit. Frequency of his seizures increased for 3 wks. He has laboratory and radiologic evidence of rickets; hypocalcaemia and secondary hyperparathyroidism (Ca 6.4 mg/dl, P 2.7 mg/dl, ALP 478 U/L, PTH 345 pg/ml) and serum 25 OHD level was 12.8 mg/L. His wrist x-ray showed widened and irregular metaphysis and osteoporosis.
D-stoss therapy and IV calcium gluconate were commenced based on a diagnosis of nutritional rickets in all 3 patients. After normalized serum Ca level, oral Ca and vitamin D supplementation were continued.
CONCLUSION
The problem of diagnosis; rickets may present atypically in adolescence with seizures and seizures could be attributed to known CP. Patients with CP should be strictly monitorized for vitamin D deficiency and hypocalcaemia should not be overlooked.
We recommend at least 400 U/day and if necessary a higher dose vitamin D supplementation. Regular exposure to sunlight is strongly advised.

PO1-049 Bone, Calcium I

Early skeletal changes in children with newly diagnosed glucocorticoid-treated nephrotic syndrome: results of a national, multicenter prospective study

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Background: Glucocorticoids (GC) are known for their adverse effect on bone health; however, little is known about their skeletal effects following short-term, systemic use in children.
Aim: The purpose of this study was to evaluate the skeletal status in children with nephrotic syndrome (NS) within 30 days of GC initiation (time A) and 3 months later (time B).
Methods: Sixty-three children (39 boys) with newly diagnosed idiopathic NS (median age 3.9 years) were studied. Lumbar spine areal bone mineral density (BMD), second metacarpal percent cortical area (CtA) on left hand radiograph and vertebral fracture status on lateral thoracolumbar spine were assessed at time A; spine BMD was repeated 3 months later at time B. GC exposure was calculated as cumulative dose up to and including time A (which corresponded with the time of the first spine BMD, taken within 30 days of GC initiation), and up to and including time B (the time of the second spine BMD, 3 months later).
Results: Despite normal height Z-scores, the mean spine BMD Z-score was reduced compared to the healthy average at time A (-0.55±0.99, p<0.0001) and B (-0.52±1.08, p=0.001). Cumulative GC exposure was 0.96±0.6 g/m^2 at time A, increasing to 3.8±1.1 g/m^2 1.1 at time B. Multivariate analysis showed that the cumulative GC dose was inversely associated with spine BMD Z-score at time A (-0.36, 95% CI -0.78 to 0.06; p=0.09). This relationship was persistent 3 months later: for every 1 g/m^2 increase in cumulative GC dose over the first 3 months, spine BMD Z-score declined by 0.14 (95% CI -0.26 to -0.02; p=0.023). Mild vertebral fractures were evident in 3 children at time A, while

mean CtA Z-score was above average (0.20±0.8, p=0.05).
Conclusions: Spine BMD is already reduced within 30 days of GC initiation, remains low during the first 3 months of therapy, and can be associated with mild vertebral fractures. On the other hand, second metacarpal percent CtA (a cortical site) is preserved. These results suggest that even short-term GC administration can have an adverse effect on spine health in children with NS.

PO1-050 Bone, Calcium I

Prevalent vertebral compression in children with newly diagnosed rheumatic conditions: results of a national chronic illness osteoporosis surveillance program

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Background: Vertebral fractures (VF) have emerged as an important but under-recognized problem in children with inflammatory disorders. The time of VF occurrence remains unknown.
Objective: We evaluated spine health among 134 children (93 girls) with rheumatic conditions (median age 10 years; 25,75%ile IQR 6.0-14.0) who were enrolled in a national bone health research program.
Methods: Children were divided into three groups: 1) juvenile idiopathic arthritis (JIA; N=28, excluding systemic onset JIA (SJIA)); 2) juvenile dermatomyositis (JDM, N=30); and 3) other diagnoses (lupus, vasculitis, connective tissue disease, SJIA; N=76). Thoracolumbar spine radiograph and lumbar spine areal bone mineral density (LSaBMD) were performed within 30 days of glucocorticoid initiation. Genant semi-quantitation was used for vertebral morphometry. Age, pubertal stage, gender, disease activity, physical activity, calcium/vitamin D intake, back pain and height-adjusted LSaBMD Z-score were analyzed for association with VF.
Results: Thirteen VF were noted in 9 children (7% of the cohort; 3 JDM patients, 6 with other diagnoses). 6 patients had a single VF and 3 patients had 2-5 fractures. Twelve of the VF were anterior wedge morphology and 1 was a crush fracture. Fractures were clustered in the mid-thoracic (69%) and lumbar regions. Three VF (23%) were moderate (Grade 2); the rest were mild (Grade 1). For the entire cohort, mean (±SD) LSaBMD Z-score was reduced (-0.6±1.27, p<0.001) despite a mean height Z-score that was similar to the healthy average (0.02±1.0, p=0.8). LSaBMD was lowest in children with grade 2 fractures (-2.7±.0.7), compared to those with grade 1 fractures (-0.6±0.9) and those without (-0.5±1.2). Of the clinical variables tested, back pain was highly associated with an increased odds for VF (OR 10.6, 95% CI 2.0-54).
Conclusions: VF can present at diagnosis and/or soon after GC initiation in children with chronic rheumatic disorders. Back pain is a highly associated clinical feature.

PO1-051 Central Weight Regulation

Ghrelin blood levels in children with a metabolic disease complicated by anorexia

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Introduction. Grelin, an orexigenic peptide produced by the stomach, influences appetite and food intake. Ghrelin, released by the stomach, activates the GHSR on the nervus vagus and informs the brain about the feeding status of the body.
This study was set up to investigate differences in plasma levels of ghrelin in children suffering from a metabolic disorder and anorexia compared to a control group of children with a normal appetite: fasting blood samples were taken and compared.
Methods. This work was set up as a single-centre observational study. The following inclusion criteria have been applied for the selection of the study group: children age 11 years and younger, metabolic disorder, insufficient oral intake of food necessitating tube feeding.
The control group was formed by 10 children under 5 years old, hospitalized for elective surgery, not suffering from any chronic disorder, showing a normal growth rate and a normal appetite.
Ghrelin concentrations were analysed in the laboratorium of Gastroenterology in fasting blood samples using a RIA technique.
For statistical analysis of the results the Wilcoxon test was used.
Results. Patients suffering from a metabolic disorder and anorexia necessitating tube feeding have significantly lower plasma ghrelin levels compared to the control group (551 +/- 77 vs. 1067 +/- 336 pg/ml; $P < 0.10$).
Conclusion. The results of our study show that children suffering from a metabolic disease associated with anorexia have a significant low plasma ghrelin concentration in their blood. The pathophysiology of this finding is still unclear. Whether this low ghrelin is the most important underlying factor of the anorexia found in these patients or just an epiphenomenon needs further investigation.

PO1-052 Central Weight Regulation

The expanded hormonal phenotype of prohormone convertase 1/3 deficiency

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Background:
Congenital deficiency of prohormone convertase (PC) 1/3 is one of five forms of monogenic obesity involving the hypothalamic leptin–melanocortin system of energy balance. The major phenotype of PC1/3 deficiency is small intestine dysfunction and early-onset obesity. The hormonal abnormalities in the three reported cases include partial ACTH deficiency, hypogonadotropic hypogonadism, central hypothyroidism, and reactive hypoglycemia. We describe a fourth case whose phenotype includes central diabetes insipidus.
Methods:
The male proband was the 3.8 kg product of a full term gestation. Watery diarrhea started on day 3 of life and persisted despite switching to an amino acid based formula. He was hospitalized at 16 days for diarrhea and dehydration. Extensive work-up was inconclusive. He was discharged on parenteral nutrition and nasogastric feeds but continued to have multiple loose stools.
At 3 months, he developed hypoglycemia following removal of his central line for line sepsis. He was diagnosed with selective hypopituitarism (gonadotropin deficiency [micropenis (1.75 cm length, 0.7 cm width)], ACTH deficiency [hypoglycemia and low cortisol], and central hypothyroidism [low free T4 with TSH in the normal range] and started on hydrocortisone and thyroxine replacement. Monthly testosterone injections were given for 3 months.
At 9 months, he began gaining weight at an accelerated rate despite limiting the caloric intake.
Results:
1. A random insulin was low (0.2 µU/mL) and proinsulin was >200 µU/mL suggesting PC1/3 deficiency.
2. DNA analysis of his PC1/3 gene revealed a homozygous single base pair deletion which results in a frameshift, the introduction of 89 abnormal amino acids followed by a premature stop codon. The mutation is in a region of the enzyme that encodes the catalytic domain and would be expected to abolish PC1 function.
3. He developed marked polydipsia and polyuria. At 3 years and 9 months, following restriction of fluid overnight, his serum Na was 145 mEq/L, Cl 107 mEq/L and osmolality was 304 mOsm/kg. His urine SG was 1.010 and osmolality 386 mOsm/kg. He was started on DDAVP with improvement of his symptoms of polyuria and polydipsia.

Conclusion:
This patient with PC1/3 deficiency developed central diabetes insipidus, suggesting that that PC1/3 is involved in the processing of provasopressin. The endocrine phenotype of congenital PC1/3 deficiency therefore includes partial deficiency of ACTH, TRH, GnRH, insulin, and vasopressin.

PO1-053 Central Weight Regulation

SNRPN methylation analysis to detect Prader Willi syndrome in neonates with hypotonia

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INTRODUCTION. "Floppy infants" are a group of patients whose determinant signs include lack of spontaneous movement with or without muscular weakness and generalized hypotonia evident from the neonatal period. Hipotonia is classified in central and peripheral, in the first, central nervous system is predominantly affected, and in peripheral the defect is located at the motor unit. Prader Willi Syndrome (PWS) is a genetic disease caused by the loss of expression of the paternal allele in a group of imprinted genes located in 15q11-q13. It is characterized by hypotonia and feeding difficulties in early infancy, followed by excessive eating, and weight gain; being the most common genetic cause of obesity. Motor milestones and language development are delayed. Other characteristics include cognitive impairment and a distinctive behavioral phenotype, hypogonadism and characteristic facial features. The molecular diagnosis is made by methylation analysis (ms-PCR) for the specific region (*SNRPN*).
PWS incidence is 1:25,000. This value is probably underestimated, since many patients are not diagnosed in an early stage. Because of this, most patients do not receive proper managenment.
To ensure early diagnosis and therefore prevent complications such as morbid obesity and Diabetes Mellitus, it is proposed to perform ms-PCR for the diagnosis of PWS in all infants with central hypotonia.
METHOD. We studied 12 patients with central hypotonia of unknown origin, both sexes, newborn to 24 months old; patients were carefully evaluated by a geneticist and then ms-PCR for *SNRPN* was performed.
RESULTS. 5 patients (41.6%) were diagnosed PWS with the molecular study; 4 girls and a boy. The common characteristic to all of them was hypotonia and swallowing difficulty, most of them did not display any other suggestive data of PWS.
CONCLUSIONS. Early diagnosis of PWS allows to drastically improve the prognosis of these patients but it is difficult to raise a clinical suspicion before age 2 years. We concluded that the molecular test for diagnosis of PWS must be made in all hypotonic newborn with feeding difficulties or those with some other clinical criteria for PWS diagnosis.
This project was carried out with support of PAPPIT IN205608-3

PO1-054 Central Weight Regulation

A DNA-hypermethylation polymorphism in the POMC gene is associated with childhood obesity and affects a p300 binding site

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Mutations in the POMC and MC4R gene, that are embedded in the leptin-melanocortin signalling cascade of central body weight regulation, lead to severe early onset obesity. We tested the hypothesis, if in addition to classical genetic defects also epigenetic alterations of the POMC gene locus might be associated with human obesity.
Methods: We investigated the DNA methylation pattern of the POMC CpG islands by bisulfite-sequencing. Functional studies of p300 binding were performed with ChIP analysis.
Results: We first described the methylation pattern of the POMC gene locus which revealed a distinct methylation pattern at the 5´ POMC CpG island in human peripheral blood cells (PBC), which was conserved in mice PBC. In the 3´ CpG island we obtained in human PBC a sharp boundary of DNA methylation with a hypermethylated intron 2 and a completely hypomethylated exon 3. Both DNA methylation patterns were reproducible in microdissected ß-MSH positive human postmortem brain samples and PBC-DNA extracted from newborn screening cards, indicating a stable pattern, which is present directly after birth. We further analyzed the DNA methylation in PBC of 152 obese childhood patients and 90 normal weight individuals with two independent bisulfite-based methods. We found a significant hypermethylation of 10 CpG positions at the intron2-exon3 intersection in obese patients ($p<0,001$). Moreover at the first CpG position within the exon3, which is significantly hypermethylated in the obese cohort, we confirmed a binding site of the histone acetyltransferase p300 by ChIP analysis. **Conclusion**: We describe for the first time a DNA-hypermethylation polymorphism that is significantly associated with childhood obesity. The hypermethylation polymorphism is located within a p300 binding region of the 3´POMC CpG island suggesting an effect on chromatin formation and altered POMC gene expression.

PO1-055 Central Weight Regulation

Bone age advancement in children with Prader Willi syndrome (PWS)

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PWS is characterised by growth failure, hypotonia, hypogonadotrophic hypogonadism and bi-phasic body compositions with early feeding problems and failure to thrive; and late hyperphagia and obesity. Early growth failure is associated with bone age delay but in late childhood, there appears to be advanced bone age. We hypothesise that advancement of bone age in late childhood may be due to aromatisation of androgens in adipose tissue at the time of change in body composition and increased adipose tissue.
We studied children (n=27, 16 male, age range 1.4-16.3 yr) with PWS (confirmed by genetic testing) using records on our clinical database at the Mater Children's Hospital for age (CA), sex, height, weight, bone age (BA)(Greulich and Pyle), BMI, BMI SDS (CDC 2000) and CA-BA. We compared CA-BA and BMI SDS in two groups age <8y (n = 16) and >8 y (n =11). Statistical analysis for linear regression and t- test were performed using SPSS 15.
Overall there was a positive association between age and bone age advancement (r= 0.43, P= 0.025). This was not associated with the onset of puberty, GH therapy or sex hormone replacement. The mean CA-BA in <8 yr was + 0.37 (± 0.67) and was -0.70 (± 0.92) in >8 yr (P= 0.004). Mean BMI SDS was – 0.44 (± 0.71) in <8 y and +2.64 (± 1.52) in >8 y (P=0.009). Overall there was no correlation between CA-BA and BMI SDS. However the correlation between CA-BA and BMI SDS in <8y group was (r= 0.26, P= 0.146) and in the >8y group was (r= 0.62, P= 0.024). This suggests that the relationship in the 2 subgroups is due to differing factors
We conclude that children with PWS older than 8 years have advancement of BA beyond CA and it is associated with a higher BMI. BA delay in >8 y group has no relation with BMI and is due to other factors such as relative growth hormone deficiency. The positive relationship between advanced BA and BMI in >8 y group supports our hypothesis and will be the basis of a prospective cohort study to test the hypothesis that increase in adipose tissue provokes bone maturation via increased androgen activity. (Character 2217)

PO1-056 Central Weight Regulation

The assessment of thirst plasticity in patients with primary and secondary adipsic diabetes insipidus

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Introduction
Central osmoreceptors involved in thirst perception are anatomically distinct from those regulating vasopressin (AVP) production although they occupy a

contiguous area. Assessing posterior pituitary function by determining the AVP response to graded osmotic stimulation is well-described. The use of a visual analogue scale to assess thirst in young patients with established or suspected adipsia/hypodipsia may also be of value. We describe the assessment and natural history of adipsia and hypodipsia in patients with primary and secondary hypothalamic dysfunction.

Methods

7 patients with adipsia and a history of hypernatremia were assessed. Their median age at surgery or presentation with hypernatremia was 13y (range 4-15y). 5 had a history of central nervous system tumours treated surgically with subsequent cranial diabetes insipidus (4 craniopharyngioma; 1 hypothalamic glioma). 1 patient had a picture consistent with 'rapid onset obesity with hypothalamic dysfunction' and 1 had hypernatremia in association with learning difficulties and congenital hydrocephalus. Patients were assessed by infusing hypertonic saline (HSI) with thirst measured using a visual analogue scale. AVP levels were measured by an in-house radio-immunoassay.

Results

Stable sodium concentrations were maintained in all adipsic patients with a regimen involving fixed fluid intake, DDAVP, daily weights and regular assessment of capillary sodium. Thirst recovered within 3m of surgery in one patient (initially adipsic with sodium > 150mmol/L). The remaining individuals underwent HSI (aged 4 and 11y or 0.4, 0.6, 1.0 and 3.8y post surgery). Abnormal thirst responses (2 primary and 1 secondary adipsia) were associated with a loss in the linear relationship between plasma osmolality and thirst perception. 4 of 5 surgically treated patients recovered their thirst perception. All had abnormally low AVP production at raised plasma osmolalities.

Conclusions

Thirst osmoreceptors can adapt more than those regulating AVP production as they recovered in most of our patients with secondary adipsia within 12 months post surgery whereas their cranial diabetes insipidus persisted. Those patients with congenital hypothalamic disorders had abnormal thirst perception in keeping with a more permanent abnormality of their central osmoreceptors. The HSI can be particularly useful in patients known to have adipsic DI where there is suspicion of recovering thirst perception.

PO1-057 Genetics of Growth I

Atypical growth hormone insensitivity resulting from compound heterozygous mutations of the GH receptor, including a novel nonsense mutation affecting the intracellular domain

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A 2.8 yr old boy, born to non-consanguineous parents, was referred to the endocrinology service for evaluation of poor growth. His birth weight and length was normal (3381 grams and 19 inches, respectively), but at presentation, had a height of 79.4 cm (-4.07 SDS), weight, 10 kg (-3.34 SDS), and head circumference of 47 cm (6.2 percentile). At age 4.08y, height remained severely retarded (-4.18 height SDS; -3.66 weight SDS), and his bone age was determined to be 3 years. The parents were of normal stature with the father's height at 180.3 cm (+0.48 SDS) and mother's height at 154.4 cm (-1.38 SDS). Results of a GH stimulation test demonstrated GH insensitivity (GHI) in the proband. His stimulated growth hormone level using arginine and levadopamine was markedly elevated at 37 ng/ml from a normal baseline of 8 ng/ml. Serum GHBP was 1459 pmol/L (normal 267-1638 pmol/L), IGFBP-3, 0.7 mg/L (normal 0.8-3.0 mg/L), and acid –labile subunit (ALS), 3.2 mg/L (normal 1.9-10 mg/L). Serum IGF-I level, however, was below -3 SDS (16 ng/ml; normal 54-178 ng/ml). A recent MRI of the proband indicated a microadenoma and is currently being further evaluated.

The extraordinarily low level of serum IGF-I, in spite of the high-normal serum GH and normal GHBP concentrations, suggested an IGF-I deficiency due to GHI. Analysis of the GH receptor (GHR) gene was undertaken and revealed two heterozygous mutations: a previously described missense mutation in exon 7, R211H, and a novel duplication of a single nucleotide in exon 9, *899dupC*. The heterozygous R211H mutation has been associated with short stature, although the mechanism(s) remains unclear. The *899dupC*, within the critical Box 1 region of the GHR intracellular domain, is predicted to cause a frame shift from residue P282, with early protein termination 6 residues from the site of the duplication. The net effect would be disruption of GH-induced GHR signaling. We, therefore, hypothesize that the combination of these two heterozygous mutations most likely is the etiology for the severe short stature and atypical GHI observed in the proband. Recombinant human IGF-1 (rhIGF-1) 120 mcg/kg twice daily has been initiated, and growth velocity has increased to 8.2 cm per year, a response typical in children with GHI.

PO1-058 Genetics of Growth I

Tall stature and increased copy numbers of the *SHOX* gene in 221 patients with additional sex chromosomes (47,XXX, 47,XXY, 47,XYY, 48,XXYY, 48,XXXY)

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Patients with Klinefelter syndrome (47,XXY) are characterized by increased growth from early childhood, suggesting that hypogonadism in puberty and young adulthood cannot solely explain their tall stature and long extremities. The short stature homeobox-containing gene (*SHOX*) is located on the distal ends of Xp and Yp in the pseudoautosomal region 1. We suggest that part of the increased longitudinal bone growth in Klinefelter patients could be due to the expression of the additional copies of the *SHOX* gene, which escape X inactivation, although this has not been shown previously. Patients with 3 and 4 sex chromosomes could theoretically show increased stature due to a *SHOX* gene dosage effect. By a newly developed qPCR methodology we therefore quantified copy numbers of *SHOX* in a total of 317 subjects, hereof 221 tall statured patients with sex chromosome aberrations. In girls with 47,XXX karyotypes (n=18) median height SDS was +0.5 (range -0.9 to +3.2), but -0.6 (-1.9 to +0.4) in 48,XXXX girls (n=3). For comparison, girls with Turner syndrome (n=6) had a median height SDS of -2.6 (-4.1 to -1.6). In males the median height SDS in 47,XXY subjects (n=131) was +0.9 (-4.3 to +4.6), +1.1 (-1.8 to +4.9) in 47,XYY (n=31), +1.1 (-1.9 to +3.3) in 48,XXYY (n=34), and +1.4 (+0.1 to +2.7) in 48,XXXY males (n=2). In contrast, patients with 49,XXXXX (n=1) and 49,XXXXY (n=2) were shorter than controls. In 311 of 317 subjects (98.1%) the copy number of *SHOX* was in agreement with the number of sex chromosomes deduced from the karyotype, and was associated with height of subjects, except in patients with 5 sex chromosomes. In 5 out of 6 patients with discrepant results between qPCR and karyotype, results from qPCR were confirmed by MLPA suggesting either a translocation (one SRY-pos 46,XX-male with 3 *SHOX* copies), duplications (two 47,XXY with 4 *SHOX* copies), or deletions (two 47,XXY with 2 *SHOX copies) of SHOX*, respectively. In conclusion, we found a very high degree of concordance (98%) between the copy number of *SHOX* by qPCR and the expected number from karyotype. Interestingly, we found that increased copy number of *SHOX* gene was associated with tall stature in patients with 47,XXX-, 47,XXY-, 47,XYY-, 48,XXXY- and 48,XXYY-karyotypes, suggesting a *SHOX* overdosage effect on height. Future studies will evaluate the usefulness of quantifying *SHOX* gene copy numbers in tall statured patients with normal karyotypes.

PO1-059 Genetics of Growth I

Comparison of the spectrum of endocrinopathies in children with severe midline defects

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Background: Holoprosencephaly (HPE) results from a defect in the induction and patterning of the basal forebrain during the first four weeks of embryogenesis and is characterized by various degrees of incomplete separation of the cerebral hemispheres. Children with the most severe forms of HPE may have neurologic impairment, developmental delay and visual disturbances in addition to endocrine deficits. On the other hand, forebrain abnormalities and pituitary hormone deficiencies are part of the clinical spectrum of septo-optic dysplasia (SOD).
Aim and Methods: The aim was to describe the spectrum of endocrinopathies in children with HPE and compare their characteristics to those with severe SOD, as defined by the combination of optic nerve hypoplasia, pituitary hormone deficiencies and defects in the midline structures. We studied retrospectively notes of children who had presented to our department over the last ten years.
Results: We identified 22 patients with severe HPE [M:F 10:12] and compared their characteristics to those of 28 children with SOD. Children with HPE presented at a mean age of 1.4±1.3 yrs (0-4.1 yrs), with a mean height SDS of -2.08 ±1.0 (-3.40 to -0.16). Diabetes insipidus (DI) was observed in 54.5% (n=12) and occurred either in isolation (18.2%, n=4) or in combination with anterior pituitary hormone deficiencies (36.4%, n=8). Twelve patients were diagnosed as growth hormone deficient (54.5%), 40.9% (n=9) had ACTH deficiency and 18.2% (n=4) had TSH deficiency. The majority of patients with HPE had an absent (56.3%) or dysplastic (25%) corpus callosum, and more than half had an absent septum pellucidum (56.3%). As the most severe forms of SOD were included in this study, there was no significant difference in the appearance of the midline structures between the two groups. However, the incidence of DI was significantly higher in children with HPE (54.5% *vs* 18.5%, p<0.05), whilst TSH deficiency was more frequent in patients with SOD (48.1% *vs* 18.2%, p<0.05). There was no significant difference in the incidence of GHD (54.5% *vs* 59.3%, p>0.05) and ACTH deficiency (40.9% *vs* 59.3% p>0.05) in children with HPE and SOD respectively.
Conclusion: Children with severe HPE and SOD present with a variable but distinct spectrum of pituitary hormone deficiencies. The involvement of hypothalamic nuclei in children with HPE may account for the increased incidence of DI. Detailed neuro-imaging studies are necessary in order to clarify this association.

PO1-060 Genetics of Growth I

Variability in the length of the polyalanine (PA) tract of SOX3 in patients with congenital hypopituitarism is associated with variable functional and phenotypic effects

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Background: *SOX3* is a single-exon gene located on Xq27 and expressed in neuroepithelial progenitor cells. In mice, its expression in the infundibulum is important for the correct induction of Rathke's pouch morphogenesis, with loss of function resulting in defects of CNS midline structures and variable hypopituitarism We have previously demonstrated that both loss-of-function polyalanine (PA) tract mutations and over-dosage of *SOX3* as a result of gene duplication are associated with variable degrees of hypopituitarism, in association with anterior pituitary hypoplasia, absent or hypoplastic infundibulum and an undescended or ectopic posterior pituitary.
Aim: To screen a cohort of patients with congenital hypopituitarism for variability in the size of the PA tract and mutations in *SOX3*.
Results: 154 patients with either multiple pituitary hormone deficiencies (n=101) or isolated growth hormone deficiency (n=53) and an ectopic posterior pituitary were screened for mutations in *SOX3* and variability in the size of the PA tract. In addition we screened the size of the PA tract in 300 patients with SOD. We identified a 7-alanine expansion (+7PA) in SOX3 in a pedigree where two males presented with IGHD and learning difficulties. We have previously reported that this mutation is associated with panhypopituitarism and results in loss of function due to cytoplasmic aggregation of the mutant protein. Additionally, we identified a deletion of 18 bp within the same PA tract resulting in the loss of six alanine residues (del6PA), in a female patient with combined pituitary hormone deficiency including GH, TSH and gonadotrophin deficiency. Transient transfection assays revealed a 2-fold activation by the del6PA protein as compared with wild type SOX3. Mutant *SOX3* retained the ability to repress the activity of β-*catenin in vitro*, in contrast to the +7PA construct. Transfection assays of a missense variant (c.14G>A) leading to substitution of arginine by glycine (p.R5G) in a boy with hypopituitarism and a solitary median maxillary central incisor, revealed no functional compromise.
Conclusions: Mutations in *SOX3* are rare in patients with hypopituitarism and expansion of the PA tract can be associated with variable phenotypes: IGHD or panhypopituitarism, with or without learning difficulties. We have now shown that, similar to overdosage of *SOX3* due to gene duplication, PA deletions may be associated with hypopituitarism due to possible gain of function.

PO1-061 Genetics of Growth I

Genetic screening for variability in regulatory regions of *SOX2* and implications for hypothalamo-pituitary development

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Background: *SOX2* is a member of the SOX (SRY-related HMG box) family of transcription factors, and shares homology with *SOX1 and SOX3* which are members of the SOXB1 subfamily. Heterozygous, *de novo*, loss-of-function mutations in *SOX2* were initially reported in patients with bilateral anophthalmia/microphthalmia, developmental delay and male genital tract abnormalities, with variable manifestations including defects of the corpus callosum, oesophageal atresia and sensorineural hearing loss. We have identified and reported a number of *SOX2* mutations in patients with anterior pituitary hypoplasia and hypogonadotrophic hypogonadism,which highlight the role of *SOX2* in hypothalamo-pituitary development. Five regions that may be implicated in *SOX2* regulation have been identified, four upstream (4.9Kb-1Kb) and one downstream of *SOX2*, based on sequence conservation and previously published data on *Sox2* regulation.
Aim and Methods: The aim was to screen a cohort of 200 patients, who did not have changes within the *SOX2* coding sequence, for variations within these regions. As we cannot predict the phenotypes that may result from variability in regulatory regions, this cohort included patients with (a) bilateral or unilateral eye phenotype of varying severity (b) patients with an ectopic posterior pituitary, without eye phenotype, who had been initially screened for *SOX3*
Results: Direct sequencing showed that these regions were highly conserved in our cohort. However, in one patient with anterior pituitary hypoplasia, undescended posterior pituitary and GH, ACTH and TSH deficiency, we have identified a single base change (C>T) in a region approximately 4.5kb upstream of *SOX2*. We did not find this change in 100 matched controls. Transient transfection of NT2/D1 cells that constitutively express *SOX2* has shown no difference compared with the wild type (wt) construct. However, the lack of an effect may be explained by the cell- and tissue-specific regulation of *SOX2* expression. Search for transcription factor binding sites using Genomatix MatInspector, showed that this change is predicted to affect binding of transcription factors including Foxo3 and SF-1. We are investigating the consequences of this change in the regulatory region with respect to its interaction with these transcription factors.
Conclusion: This variation in a highly conserved region may provide further

insight into the phenotypic consequences of mutations affecting regulation of *SOX2* expression.

PO1-062 Genetics of Growth I

45,X/46,XY mosaicism remains undetected in some short boys because this group is not routinely karyotyped

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Girls with unexplained short stature are routinely screened for the presence of Ullrich-Turner syndrome by clinical examination, laboratory tests, and karyotyping. In this study, we performed chromosomal analysis in boys to explore the role of 45,X/46,XY mosaicism for short stature in males. Short-term effects of growth hormone treatment in male 45,X/46,XY individuals were compared retrospectively to those in female patients.
In 31 boys with unexplained short stature we performed a karyotyping. In 7 boys with a normal-appearing male phenotype we found a 45,X/46,XY mosaicism. 4 boys were treated with growth hormone (0.35-0.45 mg/kg/day) in analogy to girls with Ullrich-Turner syndrome and gonadal dysgenesis. The male patients with 45,X/46,XY mosaicism responded to short-term growth hormone treatment similarly to females with an increasing height SDS though in two boys CK levels increase to more than 1000 IU/l.
Conclusion: 45,X/46,XY mosaicism remains undetected in some short boys because this group is not routinely karyotyped. We recommend chromosomal analysis of boys with otherwise unexplained short stature who are short for their families. Growth hormone treatment should be offered to short boys with 45,X/46,XY mosaicism and a predicted adult height below the mid-parental range within clinical trials.

PO1-063 Genetics of Growth I

A novel missense mutation of the *ALSIGF* gene causing a L172F substitution in LRR6 is associated with short stature in two Swedish children homozygous or compound heterozygous for the mutation

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Ternary complex formation among IGF-I, IGFBP-3 and ALS (acid labile subunit) prolongs the half-life of IGF-I and IGFBP-3 in serum. Severe primary IGF-I deficiency with extremely low IGFBP-3 levels have been reported in children with moderate short stature resulting from ALS deficiency. To date, 14 different mutations of *IGFALS* are reported to result in lack of ALS protein expression.
We evaluated two Swedish children from non-consanguineous families, not known to be related. Case 1 was a boy born SGA at term with unremarkable medical history. At 12.5 yrs he was pre-pubertal with a height (Ht) of -2.4 SDS. He had been short since infancy. IGF-I was -5.0 SDS, IGFBP-3 -12.7 SDS, and peak GH 27.0 µg/L. Case 2 was a girl born AGA at term, previously evaluated for minor psychosocial delay. At 11.5 yrs she was pre-pubertal with a Ht of -2.5 SDS. Growth retardation became pronounced from the age of 4 yrs. IGF-I was -6.3 SDS, IGFBP-3 -13.5 SDS, and peak GH 19.4 µg/L. She had a poor response to 1 yr of rhGH therapy. At 13.7 yrs she was Tanner B2.
Using anti-ALS antibodies (Santa Cruz), serum from both cases displayed a 75 kDa band (mol wt of ALS) on WIB; their parents and controls displayed bands at similar size and intensity while GHD and Laron patients displayed fainter bands. Functional assays with 125-iodine labeled IGF-I demonstrated lack of ternary complex formation in sera from both cases but not in serum from their parents or controls. Addition of rhIGFBP-3 did not rescue ternary complex formation. Case 1 was compound heterozygous with a L172F substitution in the Leucine Rich Repeat (LRR) 6 due to a novel missense mutation in the *IGFALS* gene (paternal) and a previously reported S195_197Rdup. Case 2 was homozygous for the same novel L172F mutation. To further investigate whether we had identified the first *ALSIGF* gene mutation resulting in expressed, non-functional ALS protein, control WIB using anti-ALS antibodies (DSL) and ALS immunoassays (Isoterix) were performed. Surprisingly, immunoreactive ALS was non-detectable in both cases. In accordance, the 75 kDa protein immunoprecipitated with Santa Cruz anti-ALS antibodies and identified by N-terminal sequencing had no sequence homology with ALS.
We have identified two cases of a novel missense mutation in the *ALSIGF* gene affecting LRR 6 by L172F substitution. The lack of ternary complex formation associated with short stature is caused by lack of expression/secretion of the ALS protein like in previous reports.

PO1-064 Genetics of Growth I

The comparison of somatic development in children and adults with Down syndrome (DS)

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Background: DS is one of the most common chromosomal disorders with somatic growth impairment.
Aim: To determine whether there is a difference in physical development between children and adults with DS.
Material and methods: 117 patients (97 children) age from 3 months to 52 years. Average age: adults 29.8 ± 11.03 years, children 6.9 ± 5.4 years. A questionnaire concerning the course of gravidity and evaluation of development was given to the minders.
Patients were divided into age groups: 0-3, 4-6, 7-9, 10-12, 13-24, 25-36 months, 4-6, 7-9, 10-12, 13-15, 16-18 and >18 years (adults). Anthropometric measurements of patients were taken and results compared. The results were stated in SDS as the age range was large.
The SDS values of height, body weight, BMI, head and chest circumferences (HC and ChC) were referred to the Polish centile cards. For children with DS all values but BMI were referred to the American centile cards.
Results: According to the Polish standards the average height-SDS was; in children(A) -2.03 ± 1.97 and in adults(B) -3.6± 1.53; The adults are significantly shorter than children. Some of the adults have hypothyroidism as none had received L-thyroxin supplement during childhood. Children with hypothyroidism receive hormonal supplement. BMI-SDS was significantly higher in (B) (4,48 ± 3,92) than in (A) (0,98 ± 2,55). All adults are obese. Body weight-SDS of (A) was -0.77 ± 2.25, and of (B) 0.75 ± 2.98; All patients revealed minor head circumference according to the standards: (A) -2.99 ± 1.54, (B) -2.63 ± 1.43. Chest circumference SDS in (A) was 1.26 ± 4.92 and in (B) 3.47 ± 3.74. The strongest deviation was stated at infants 0-3 months, children at puberty and adults.
According to DS American standards body weight in 18% of patients was above 90th centile and in 4% below 10th. Height in 27% of patients was above 90th centile and 7% below 10th.
The recorded differences in development according to the Polish standards were greater then to American Both, children and adults, are shorter according to the Polish standards but higher according to the American.
Conclusions:

- Retardation and dissonance of development in DS patients increase with age. Final height is significantly lower than expected.
- Therapy progress in DS children may improve their development.
- Centile cards for Polish DS patients are needed as there is a large discrepancy between standards for Polish children and standards for American DS children.

PO1-065 Genetics of Growth I

The distribution of exon 3-deleted/full-length growth hormone receptor polymorphism in Turkish population

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Background: The exon 3 – deleted / full length (d3/fl) GH receptor polymorphism (d3/fl- GHR) has been associated with responsiveness to GH therapy in some children.
Aim: To evaluate the distribution of d3/fl GHR polymorphism in Turkish population.
Subjects and Methods: The study included 528 (61 F/ 407 M) healthy adults. Mean (SD) age was 30.9(9.5) years (range 18 to 57). GHR exon 3 isoforms were studied by simple multiplex PCR method. Height and IGF-1 and IGFBP-3 values were also evaluated and expressed as SDS (national standards).
Results: The results of GHR polymorphism and height SDS, IGF-1 SDS and IGFBP-3 SDS are seen in the Table. There was no difference between genders in GHR isoforms as seen in Table.

Some clinical and laboratory findings in GHR exon3 genotype groups, mean(SD) values are given.

	fl/fl	d3/d3	fl/d3
Male n(%)	163(34.9)	121(26.1)	182(39.0)
Female n(%)	20(32.8)	18(29.5)	23(37.7)
Height SDS	-1.4(4.6)	-1.4(4.9)	-1.2(4.3)
BMI SDS	-0.8(1.1)	-1.4(4.9)	0.8(1.1)
IGF-1 SDS	-0.9(1.2)	-0.6(1.8)	-0.7(1.3)
IGFBP-3 SDS	-0.7(1.6)	-0.5(1.9)	-0.1(1.7)

Conclusions: This study presents the results of GHR polymorphism in Turkish population as a reference for further studies. The distribution was similiar to European populations. There were no correlations between heights of the individuals and GHR isoforms except for higher IGFBP-3 in fl/d3 group. Whethere this implies an abnormality needs further investigations.

PO1-066 Genetics of Growth I

Genome-wide screening for DNA methylation defects and genetic imbalances in Silver-Russell syndrome

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Background: growth failure and dystrophy in Silver-Russell syndrome (SRS) are associated with hypomethylation of ICR1 on 11p15 (40% of cases) or with maternal uniparental disomy of chromosome 7 (UPD(7)mat) (10%). The cause of the 11p15 epimutation is not known, nor the genetic defect(s) associated with the 50% left unexplained. We aimed to screen the whole genome for DNA methylation defects, microdeletions and microduplications associated with SRS.
Patients: gDNA prepared from blood lymphocytes was available from 40 children with SRS defined by the presence of intrauterine growth retardation, lack of catch-up growth, and at least two of the criteria: typical face, relative macrocephaly, and skeletal asymmetry. Conventional karyotyping was normal and UPD(7)mat absent in all cases. 20 children carried an ICR1 hypomethylation on 11p15.
Methods: DNA methylation profiling was performed with the Illumina HumanMethylation27 BeadChip® which screens 27,578 CpG sites from 14,495 human genes. DNA samples from 9 SRS-children (4 with 11p15 epimutation) were compared with DNA samples from 2 SGA-children and an enzymatically fully methylated DNA sample. For the detection of submicroscopic imbalances the 40 DNA samples were analyzed using the Affymetrix GeneChip® Human Mapping 500K Array Set. The detected imbalances were surveyed in respect to gene coverage and possible overlaps with registered copy number variations (CNVs) by online databases query.
Results: overall frequency distribution of methylation values was similar in SRS patients and controls. A general DNA hypomethylation could not be observed for any patient studied. In addition, no common previously unknown, specific DNA methylation defect was detected in the idiopathic SRS group. We identified numerous CNVs, however, a common imbalance in either epimutation carriers or idiopathic SRS patients was not detected. One idiopathic patient carried a pathogenic variant overlapping with the recently published 12q14 microdeletion.
Conclusions: our data show that ICR1 hypomethylation on 11p15 in SRS is not accompanied by a genome-wide DNA hypomethylation. There was no evidence for the common presence of additional specific DNA methylation defects. In addition, array data did not provide evidence for the presence of a common submicroscopic imbalance in SRS. The detection of one patient with a microdeletion illustrates the necessity to include molecular karyotyping in the genetic analysis of SRS.

PO1-067 Genetics of Growth I

Growth hormone insensitivity and immunodeficiency: mutation in the STAT5b gene

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Growth hormone insensitivity (GHI) can be caused by defects in the GH receptor or in the post-receptor signaling pathway. STAT5 proteins are components of the GH and interleukin 2 family of cytokine signaling pathway. Six patients from five families with five different homozigotic mutations of the STAT5b gene have been reported to have similar phenotypes to those of patients with GHR defects, but with wild-type receptors. The reported patients, however, showed several immunological aspects with no clear genotype-phenotype linkage.
We describe a new case of homozygous nonsense mutation in the gene for STAT5b with several clinical characteristics of GH insensitivity (height: -5.3 SDS) and history of inmunologycal dysfunction in a 12-year-old girl. She was born at 35 weeks with 1650 g to Argentine parents and was adopted soon after birth. The first manifestation of immunological dysfunction was a neonatal sepsis. Since her earliest months of life she presented poor weight gain, severe growth failure, and generalized eczema. Other features observed were chronic diarrea, keratitis, herpes zoster, hemorrhagic varicella, autoimmune thyroiditis, and dyshidrotic ectodermal dysplasia. She had early history of recurrent pulmonary infections developing biopsy-proven lymphoid interstitial pneumonia as chronically activated cells expression and that constitutively expressed cytotoxic T lymphocyte- associated protein4(CTLA4) indicating the concept of general functional T-cell defect in the IL-2 signaling pathway. Then the pulmonary fibrosis evolved to progressive respiratory failure and death.
Serum-stimulated max GH level was 12.4 ng/ml. Serum level IGF1 0.8ng/ml (DSL IRMA-6.4SDS) was very low without response to IGF1 generation test. IGFBP3 and ALS levels were strikingly reduced.
The sequencing of genomic DNA for the STAT5b gene demonstrated a single nucleotide change, a homozygous C-T transition, which resulted in a nonsense mutation at codon 152 (R152X). This mutation is the same as another one already described in an Argentine patient in the 5 exon, which encoded the coiled-col domain, very close to the NH2 terminal-domain, and determines one immunodetectable protein absence.
The fact that we found the same mutation in two unrelated patients with this infrequent pathology suggests that we should consider a founding effect. In patients with these clinical characteristics and immunological alterations, possible mutations in STAT5b gene should be investigated.

PO1-068 Genetics of Growth I

Ring chromosome 15 syndrome presenting with an unusual growth pattern – case report

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-Introduction: Ring chromosome 15 [r(15)] is a rare chromosomal anomaly leading to a syndrome in which an important short stature is attributed to the deletion of the gene that codes for the insulin-like growth factor 1 receptor (IGF-1R, locus 15q26.3).
-Objective: To report a 10-year-old patient with r(15) syndrome who presented a growth pattern not common to the syndrome.
-Case report: 10-year-old boy, born at term (gestational age 42 weeks) but small for gestational age (SGA): weight 2.9 kg and lengh 46 cm. His is son of non-related healthy parents. Due to dysmorphic features (microcephaly, triangular face, café-au-lait spots, short lengh, cryptorchidism) he was referred to genetic evaluation in the first month of life and it was found the karyotype 46,XY,r(15). Father's karyotype is 46,XY and the mother's is 46,XX with a structural anomaly in one allele of the chromosome 2. He did not experience catch-up growth, keeping growth bellow the 3rd percentile during infancy and childhood, but sustaining a normal growth velocity since 5 years of age (4.5 to 6.0 cm/year), being the z-score of height between -2.19 and -2.94 standard deviations. He developed cognitive and motor disabilities, which are part of the syndrome. He also presented delayed bone maturation (in average bone ages were 3 years younger than chronological age). The predicted final height (176 cm) was near the lower limit of the familial target height range (185.0 ± 10 cm). Laboratory evaluations showed a normal IGF-1 level for bone age (234ng/mL, references: 57-316ng/mL) and two non-responsive pharmacological stimulated somatropin release tests (insulin-induced hypoglicemia and clonidine). Other causes of short stature were ruled out.
-Comments: In this case, both the normal growth rate and IGF-1 serum levels are not compatible with IGF-1 resistance, neither characteristic of growth hormone deficiency, even after two non-responsive somatropin release stimulus tests. Although he presents many signs of r(15) syndrome, his growth pattern corresponds to a child born SGA who did not catch-up and it suggests he does not present the IGF-1R gene deletion, which depends on the extent of euchromatic loss before the ring formation. It is discussed if it would be appropriate for him to receive recombinant somatropin therapy, once the degree of his mental and motor impairments demands for special care needs and continuous assistance.

PO1-069 Genetics of Growth I

Isolated growth hormone deficiency type II caused by a novel intronic mutation

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The human growth hormone (GH) gene, *GH1*, comprises five exons that are constitutively spliced to produce a full-length, 22-kDa protein that accounts for the majority of circulating GH. Aberrant splicing of wild-type transcripts gives rise to at least five smaller isoforms, the most abundant of which are a 20-kDa isoform and a 17.5-kDa isoform. The 17.5-kDa isoform exerts a dominant-negative effect by preventing secretion of wild-type GH. Patients with inherited mutations that increase the levels of the 17.5-kDa exhibit isolated GH deficiency type II (IGHD II), an autosomal dominant form of GHD. Common characteristics of IGHD II include short stature due to impaired bone elongation and, in severe cases, anterior pituitary hypoplasia with concomitant disruption of the anterior pituitary hormone axis.

Patient : Girl of 3y 4m of age with important growth delay. She was born at term. Gemelar pregnancy. Delivery was by cesarean section. Birth weight: 2360 gr (SDS -2.18) Birth lenght: 43 cm (SDS -3.85). She presented with neonatal hypoglycemia and jaundice. Current: Phenotype concordant with GH deficiency. She also presents convergent strabism, clinodactyly and subtle psychomotor delay. Auxology: Target height: 159.2 cm (SDS: -0.35), height: 84.1 cm (SDS: -3.56), growth velocity <P3, BA: 1y 6m. S1P1. Karyotype: 46XX. Biochemical data: TSH: 2.5 mUI/L,T4L:1.04 ng/mL, Cortisol: 13 µg/dL, Prol: 8.7 ng/mL, IGF1: 25 ng/mL, IGFBP3: 1.1 µg/mL. Two GH stimulatory test showed a GH peak of 1.5 and 2.3 ng/mL respectively. MRI: Normal. Her twin is healthy without growth impairment.
Genetic analysis: DNA was extracted from leukocytes by standard procedures. *GH1* gene was PCR amplified and the coding as well as intron-exon boundaries regions were directly sequenced. The study was performed in parents and sibling too.
Results: The proband was a heterozygous carrier of the novel mutation c.291+27G>A in intron 3. Parents and sibling did not presented the mutation which indicated that it probably occurred as a *de novo* event.
Conclusion: The presented mutation is probably affecting the normal mRNA processing because it is located in an intronic splicing enhancer where other mutations have been reported associated with IGHD II. Therefore, the c.291+27G>A mutation could be considered as pathological.

PO1-070 Genetics of Growth I

A novel mutation (p.R511W) in *IGF1R* gene in a child born small for gestational age without catch-up growth

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Background: Recently, IGF-1 insensitivity caused by *IGF1R* mutations was identified as one of the causes of growth impairment in children born small for gestational age (SGA) who did not present catch-up growth in postnatal life.
Objectives: to study *IGF1R* gene of children born SGA suspected to have IGF-1 insensitivity.
Patients and methods: Twenty-five children born SGA without catch-up growth were selected. IGF-1 insensitivity was suspected by the presence of IGF-1 levels above mean of normal reference values. Total RNA from peripheral blood leukocytes were obtained followed by cDNA synthesis. The *IGF1R* cDNA was amplified using specific primers followed by direct sequencing. Allelic variants found in cDNA were confirmed by direct sequencing of genomic DNA.
Results: We identified one child carrying a nucleotide substitution in heterozygous state at the first nucleotide of codon 511 in *IGF1R* exon 7 (c.1531C>T), resulting in an arginine to tryptophan substitution (p.R511W). This variation was not observed in 306 alleles from normal height control individuals born adequate for gestational age. Inagaki *et al.* (JCEM 2007, 92: 1542) described a mutation in the same residue that changes arginine for glutamic acid. R511 residue is conserved among different species and is located in the proximity of the first disulfide bond at cysteine 544 between the two alfa-subunits of the IGF-1R protein. The patient is a girl born at term after an uneventful pregnancy, weighing 2.2 kg (-2,5 SD) and presented poor growth after birth: at 5.8 years of age her height was 100.5 cm (-2.3 SD), weight 13.7 kg (BMI SDS = -1.5) and her head circumference SDS was -2. Psychomotor development was normal. Basal GH levels ranging from 0.3 to 3.8 µg/L and GH peak after stimulation with clonidine of 12.1 µg/L. IGF-1 and IGFBP-3 levels were in the upper normal range. She was treated with growth hormone (50 µg/kg/day) and her growth velocity increased from 4.6 to 8.2 cm/y in the first year of therapy. During rhGH therapy IGF-1 levels increased from 257 µg/L (1.3 SD) to 497 µg/L (3.7 SD). Her mother, who was also born SGA, is 144 cm tall (height SDS of -3) and carries the same mutation. The father who has normal height and two siblings that presented normal growth have only wild type *IGF1R* alleles.
Conclusion: A new mutation in *IGF1R* gene (p.R511W) associated with pre and postnatal growth impairment was described. Mutations in *IGF1R* gene emerge as a relevant cause of intrauterine growth retardation.

PO1-071 Genetics of Growth I

Unusual inheritance and phenotype in a family with SHOX gene deletion

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The pseudoautosomal regions (PAR) are homologous sequences of nucleotides on the X and Y chromosome. Genes that reside in the PAR escape X-inacitvation. In normal individuals the SHOX gene is found in two copies in the PAR1 of both sex chromosomes (Xp22.3/ Yp11.3) and the expression of both of these copies is essential for a normal activity of SHOX. The phenotypes associated with haploinsufficiency of SHOX gene vary widely from isolated short stature to Leri-Weill dyschondrosteosis (LWD) with short stature, mesomelia and Madelung deformity. LWS occurs more frequently in females than in males and females usually show a more severe phenotype than males.

We report a family with unusual inheritance and unusual clinical presentation of SHOX gene deletion. The index patient is a 15.3 years old boy with disproportionate short stature (153.9 cm, -2.64 SDS) with mild Madelung deformity. His father shows normal height (187.6 cm, +1.2 SDS), but disproportionate stature with shortening of his legs. A 11.3 years old sister presented also with normal height (141.0 cm, -1,29 SDS), but marked Madelung deformity.

MLPA identified a SHOX gene deletion in the patient, his father and sister. To characterize the deleted region on the X or Y chromosome FISH analysis was performed and showed the gene deletion on Yp11.3 in the index patient and his father and on Xp22.3 in the affected sister.

The heterogeneous phenotype in this family, especially in the father, may arise as a consequence of background genetic effects, for example the presence of modifying genes.

The unusual inheritance can be explained by a meiotic crossing over of the SHOX gene region between the X and Y chromosome. Crossing over between the sex chromosomes is restricted to the PAR. The PAR pairs and recombines during meiosis like autosomes, but the recombination activity in PAR is extremely different between sexes. PAR1 creates a male-specific recombination "hot spot" with a recombination rate that is about 20 times higher than the genome average. In our family the recombination involving the SHOX gene led to a uncommon transmission of a Y linked SHOX gene deletion in the father to a X linked SHOX gene deletion in the daughter. In summary, our results indicate the importance of an extensive clinical examination of all members in a family with SHOX gene deletion. Depending wether the defect is inherited by the father the involved sex chromosomes should be identified by FISH analysis.

PO1-072 Genetics of Growth I

A role of infants' daycare centers (DCC) on infantile growth pattern

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Many infants attend DCC from a very young age and during the critical age of infancy childhood transition (ICT) when the GH-IGF1 axis sets in and that was shown to affect adult height.

Hypothesis: ICT would be negatively affected in infants attending daycare. We therefore studied the association between DCC attendance age and ICT.

Patients and Methods: The study was performed in 3 DCC representing an average socioeconomic status. Exclusion criteria were: prematurity, LBW and chronic illness. The research group consisted of 123 infants, 59 females and 64 males with a birth weight of 3207±345 and 3397±444 g, respectively, p<0.01. The study group was divided into group A: attended DCC from age <6 m (9 M, 10 F), group B: DCC from age 6-12 m (15M, 19F) and group C >15 m (40 M, 30 F). ICT was estimated according to the Karlberg's ICP model charts.

Results: Each month of delay in the ICT resulted in a loss in length of 0.32 SDS in F and 0.34 in M. The average ICT age was 10.8±1.7 month in F and 11.7±1.7 in M, p<0.004. Group B had the latest ICT 11.8±1.6; M but not F lost a mean 0.64 SDS in lengths as compared with M in group A (p<0.017). Group A had the earliest transition at the age of 10.8 ± 2.0 m in both F and M. In group C the ICT age was 11.3 ±1.7 m; F in this group had earlier transition than M 10.5 ±1.3 versus 11.8 ±1.7 m p<0.001. While there were no difference in F between the three groups, a significant differences were found between M in group A vs. B p<0.017, and A vs. C p<0.03. No correlation was found between birth weight and ICT age. A positive correlation was found in M between infantile height SDS and the age of ICT transition p<0.05.

Conclusions: ICT in M is negatively affected by DCC attendance, whereas F are not. The most vulnerable period to attend DCC is age 6-12 m. Earlier or later DCC attendances are to be preferred. We speculate two mechanisms to explain the impact of DCC attendance at age 6-12: recurrent infections after fading of maternal immunity, and mother's separation at an age when GH dominance sets in.

PO1-073 Genetics of Growth I

Auxological implications of heterozygous *IGFALS* mutations

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Background/Aims. To date, 14 novel mutations of *IGFALS* in 17 patients (13 families) have been reported. This cohort is highly divergent in ethnicity and countries of residence. The phenotype of homozygous *IGFALS* mutations is well described, although it may be influenced by ascertainment bias. The effect of heterozygous *IGFALS* mutations on phenotype is less known. The present study was undertaken to evaluate the impact of heterozygous expression of *IGFALS* mutations on stature. **Patients/Methods.** Patient information was derived from an IGFALS Registry (n=55). The Registry includes patients who carry homozygous or compound heterozygous *IGFALS* mutations (n=17) and family members, who were either heterozygous carriers (HC, n=30), or homozygous wild type (WT, n=8). Height at diagnosis was expressed as SDS (HSDS) for US references. In the whole cohort means were calculated for 3 groups. Within each family in whom height data of one or more WT individuals was available the effect on stature of two mutant alleles versus one; one mutant allele versus WT; and two mutant alleles versus WT was calculated. The differences in HSDS were then pooled and further evaluated. **Results**. Out of 11 families, 2 (18%) were consanguineous, and 9 (82%) were non-consanguineous; data on 2 families were not available. Two children were adopted. Among 14 *IGFALS* mutations 8 (57%) were homozygous, and 6 compound heterozygous. According to the type of mutation, 9 (64%) were missense, 2 (14%) in-frame insertions, 2 (14%) frameshifts with premature stop codons, and 1 (7%) a nonsense mutation. In the whole cohort mean ±SD HSDS in patients was -2.36±0.84 (below -2 SDS in 65%); in HC -0.83±1.34 (below -2 SDS in 26%) and in WT -1.02±1.04 (below -2 SDS in 12.5%). The difference in mean HSDS between HC and WT was -0.90±1.51 (n=12, 4 families); between patients and HC: -1.46±0.86 (n=14, 10 families); between patients and WT: -2.13±0.83 (n=3, 3 families). **Conclusions.** Heterozygosity for *IGFALS* mutations results in approximately 1.0 SD height loss in comparison with wild type, while homozygosity gives a further loss of 1.5 SD, suggestive for a gene-dose effect. Further studies involving a larger cohort are needed to evaluate the impact of heterozygous *IGFALS* mutations not only on auxologic characteristics, but also on the GH/IGF system.

PO1-074 Genetics of Growth I

A novel *GLI2* mutation L788fsX794 associated with combined pituitary hormone deficiency and polydactyly without holoprosencephaly

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Introduction: Congenital combined pituitary hormone deficiency (CPHD) was associated with defects in genes involved in the development of the pituitary gland. GLI2 is a transcription factor, downstream of the Sonic Hedgehog pathway, acting in the initial phase of pituitary gland organogenesis. Patients with heterozygous GLI2 loss-of-function mutations present defects of anterior pituitary formation, hypopituitarism, holoprosencephaly and postaxial polydactyly with a wide phenotype variability and incomplete penetrance within families. Hormonal data and pituitary MRI of these patients have not been published.
Objective: Study *GLI2* gene in a large cohort of patients with congenital CPHD.
Material and Methods: DNA was isolated from peripheral white blood cells of 140 patients with CPHD. The molecular analysis of the *GLI2* gene consisted of PCR using intronic primers (17 fragments) followed by automatic sequencing.
Results: We identified a heterozygous 7 bp deletion in exon 13 of *GLI2* (c.2362_2368del) in a female patient with CPHD. This mutation results in a frameshift causing a premature stop codon (p.L788fsX794) that predicts a truncated protein lacking almost 800 amino acids corresponding to the C-terminal activator domain of GLI2 protein. The patient was first evaluated in 1989 at 7 yr with height SDS of -5.4, postaxial bilateral polydactyly and past history of seizures of difficult control since the 1st year of life. Hormonal response to the combined test (insulin 0.05 U/kg; TRH 200 µg; GnRH 100 µg iv) demonstrates severe pituitary GH, PRL, ACTH and TSH deficiencies. MRI disclosed an extremely hypoplasic anterior pituitary, ectopic posterior pituitary lobe, stalk interruption, right cerebral hemiatrophy but no holoprosencephaly-like abnormalities. At the age of 16 yr, she did not develop puberty and presented undetectable levels of LH and FSH, characterizing the diagnosis of hypogonadotrophic hypogonadism. Her mother, who also carries the same mutation, is apparently healthy, has polydactyly, height SDS of -1.0 and normal fertility. A maternal first-degree female cousin also presents postaxial polydactyly, short stature and primary amenorrhea. The family history suggests an autosomal dominant trait with incomplete penetrance.
Conclusion: We expand the spectrum of *GLI2* mutations describing a novel frameshift mutation p.L788fsX794 resulting in loss of most of the C-terminal activator domain in a patient with CPDH and polydactyly without holoprosencephaly.

PO1-075 Genetics of Growth I

Identification of a heterozygous IGF-1 gene splice mutation

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A young male was born at 42 weeks with a normal birth weight (3040g) and length (47 cm). Slow post-natal growth was noted, with significant growth retardation from age 1 y. At age 9.1 y, the subject's height was 111.1 cm (-4.0 SDS) and weight was 20.6 kg (-2 SDS). His father had normal stature (185.4 cm, +1.2 SDS) but the mother was significantly short (137.2 cm, -4.0 SDS). Severe short stature could be traced back several generations on the mother's side, suggesting an autosomal dominant inheritance pattern. The clinical phenotype of the patient was otherwise unremarkable.
A GH stimulation test with arginine/clonidine was normal (baseline 0.82 pmol/L; peak 15 pmol/L, stimulated). Serum GHBP was normal (642 pMol/L, normal range, NR, 267-1638) as was IGFBP-3 (2.4 mg/L, NR 2.0-4.8) and ALS (13 mg/L, NR 4.2-13). The serum IGF-I level, however, was below -2 SDS (115 ng/ml, NR 123-275). Transcription of the *IGF-1* gene results in several pre-propeptide isoforms, but the mature IGF-1 protein is encoded by only two exons. The four domains of mature IGF-I, designated B, C, A and D, are encoded by exon 3 (part of domain B) and exon 4 (all remaining domains). In addition, exon 4 encodes for 16 amino acid residues of domain E, whose function remains unclear. Sequencing of the IGF-I gene (exons 1, 3, 4 and 6) revealed a novel heterozygous donor splice site mutation at the exon 4-intron 4 junction. The *402+1G>A* transition would abolish the donor site of intron 4 in the aberrant allele. Consequences could include read-through into intron 4, with protein termination after the addition of 76 new amino acid residues, or aberrant splicing events excising exon 4. Since the IGF-I protein is encoded predominantly by exon 4, excision would result in mature IGF-1 being expressed only from the normal allele. This could, in part, explain the moderately low serum IGF-1 detected. However, in the first documented report of an *IGF-1* mutation (Woods, et al, 1995), the parents, who were heterozygous for the exon 4/5 deletion, were of normal stature, although heights for both were below the mean (-1.8 SDS and -1.4 SDS for father and mother, respectively). We thus entertain the possibility that the abnormal IGF-I peptide generated by this novel mutation may act in a dominant negative manner by interfering with IGF1R signal transduction. This hypothesis would be consistent with the familial transmission of short stature in this pedigree.

PO1-076 Genetics of Growth I

Novel compound heterozygous *IGFALS* mutation associated with impaired postnatal growth and low circulating IGF-I and IGFBP-3 levels

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Up to 90% of circulating IGF-I is carried bound up in 150kDa ternary complexes with IGFBP-3 and ALS, an 85kDa glycoprotein that modulates IGF-I bioavailability by increasing its circulating half-life and preventing a quick plasma clearance. ALS synthesis is postnatally stimulated by GH, as are both IGF-I and IGFBP-3. Up to 10 different mutations in the ALS encoding gene, *IGFALS* (16p13.3), have been reported in recent years in patients with impaired postnatal growth presenting with very low IGF-I and IGFBP-3 levels, as well as delayed puberty onset, basal hyperinsulinaemia or microcephaly, in some cases.
We present the case of a male Spanish proband with postnatal growth deficit, born at term (BW: 2.87 kg; height not documented) from non consanguineous parents, whose main clinical characteristics at diagnosis are summarized in the enclosed table. Hormonal tests revealed severely decreased circulating IGF-I and IGFBP-3 as well as peak GH levels in GH-stimulation tests of 69 ng/ml (basal, 2.3 ng/ml) after exercise, and a IGF-I generation test that failed to increase IGF-I circulating levels. Physical examination at diagnosis noted plagiocephaly, discrete craniofacial disproportion and joint hyperlaxity.

Chron. age (years)	Bone age (years)	Height (cm) (SDS)	Weight (kg) (SDS)	IGF-I (ng/ml)	IGFBP-3 (ng/L)
4.1	2.6	96 (-2.14)	12 (-2.0)	< 25	<0.5

The *IGFALS* coding sequences, intron-exon boundaries and known regulatory regions were screened by DNA sequencing. *IGFALS* analysis revealed two compound heterozygous mutations in the proband: N276S, a previously described missense mutation at a conserved residue, inherited from the mother, and a novel frameshift mutation, c184-185insG, that generates a premature termination codon (E35fsX51), inherited from the father.
The N276S mutation has been recently identified in two non-related Spanish cases of primary ALS deficiency, thus suggesting that this mutation may be frequent in the Spanish population. Both cases were also associated with postnatal growth delay and severely decreased IGF-I and IGFBP-3 levels. The E35fsX51 mutation is predicted to produce a severely truncated protein, hence both proband´s alleles generate non-functional ALS molecules. The increasing number of recently reported inactivating *IGFALS* mutations indicates that ALS

deficiency should be considered as a possible cause of postnatal growth deficit in patients presenting with low IGF-I and IGFBP-3 levels in the absence of GH deficiency.

PO1-077 Genetics of Growth I

Prevalence of idiopathic short stature in a pediatric population attending endocrinology clinics in Spain

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Introduction: Short stature is one of the most common concerns presenting to pediatric endocrinologists. Idiopathic short stature (ISS) is defined as 2 standard deviations (SD) or more below the corresponding mean height for a given age, sex and population group and where the ethiologic disorder is unknown. Some studies have explored the prevalence of ISS among children that attend pediatric endocrinologists offices, though findings can hardly be extrapolated to the Spanish population.
Objectives: To determine the frequency of ISS among the children population attending pediatric endocrinology clinics in Spain. To describe the frequency of other causes of short stature.
Design and Methods: Twenty one centers participated in this transversal epidemiological study. Patient data was collected during a single visit including demographics, clinical history, blood and hormonal analysis and any additional procedure required for obtaining a diagnosis according to normal clinical practice.
Results: A total of 363 children have been evaluated in the study (188 female and 175 male). The mean chronological age (SD) was 7.7 (3.7). Ninety five children had a diagnosis of ISS (26.2%; 95%CI 21.72-31.01). The frequency of other causes of short stature are described in Table 1.

Table 1. Frequency of other short stature diagnostic categories. Number of patients (%).

Familial short stature	130 (35.8)
Constitutional delay of growth and development	85 (23.4)
Small gestational age (Intrauterine retarded growth)	83 (22.9)
Malnutrition	5 (1.4)
Short stature with psychosocial origin	2 (0.6)
Endocrine diseases (ED): Isolated growth hormone deficiency	74 (20.4)
ED: Insensitivity to growth hormone	3 (0.8)
ED: Hypothyroidism	1 (0.3)
ED: Others	8 (2.2)
Chronic diseases (CD): Chronic renal failure	1 (0.3)
CD: Others	10 (2.8)
Osteochondrodysplasias: Others	1 (0.3)
Genetic abnormalities (GA): Turner syndrome	2 (0.6)
GA: Others	5 (1.4)

Conclusions: Prevalence of ISS in the pediatric population attending the endocrinology clinics due to short stature in Spain is greater than expected according to previous studies in different geographic regions. Familial short stature, constitutional delay of growth and development, intrauterine retarded growth and growth hormone deficiency are the most frequent diagnosis among the population of the study.

PO1-078 Genetics of Growth I

Genetic and environmental influences on age of the infancy-childhood transition (ICT)

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Short–term predictive adaptation to low energy stores modifies the timing of transition from infancy to childhood (ICT), when GH-IGF-1 axis sets, resulting in short stature when ICT is delayed (DICT).
Hypothesis: Environmentally influenced timing of ICT is transmitted genetically to future generations. We therefore studied sibs, mono- and di-zygotic twins for the age of their ICT.
Methods: The subjects were 268 boys and girls, including 72 pairs of twins: 31 monozygotic (MZ), 41 dizygotic (DZ), 32 pairs of siblings (SB) and 60 unrelated children (UC) who were measured for body weight, length and head circumference over the first two years of life. ICT was estimated according to the Karlberg's ICP model charts without correcting for GA. We implemented a model-based variance component analysis, utilizing a likelihood ratio test of the hierarchical submodels to choose the best fitting and most parsimonious model of the ICT phenotype inheritance. The general model included the following variance components: additive genetic, common environment effects shared by sibs (regardless whether they twins or regular sibs) and shared by only twins, plus residual component.
Results: Intra-pair correlation for age of ICT increased with genetic affinity from r=0. 61 for SB, r=0.67 for DZ twins to r=0.94 for MZ twins, while no correlation was found for UC r=0.02. Variance decomposition analysis established two main components in familial covariation in ICT: one is attributable to the putative genetic factors explaining ~52% of the total variation of the phenotype, and the second one is the common sibs' environment, explaining additional 37% of the variance. The rest is an unexplained residual (random environment effects specific for an individual) component. However, when the dependent variable ICT was adjusted for length at birth (BL), the genetic component became undetectable, and the residual phenotype variance was attributable to common sib (51.6%) and twin (20.1%) environment, and the rest (27.%) to random environmental effects.
Conclusions: Putative genetic factors explain ~52%% of the timing of the ICT trait variance unadjusted for BL. The common sibs environment explains additional ~37%. However, after adjustment for BL the main governing factors of ICT variation became a common environment (71.7% in total), suggesting that ICT and BL share the same genes.

PO1-079 Genetics of Growth I

Two novel mutations in the promoter region of the growth hormone gene (GH1) in familial isolated growth hormone deficiency

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Introduction: Short stature associated with isolated GH deficiency (GHD) is both sporadic and idiopathic, but between 5 and 30% have an affected first degree relative consistent with a genetic etiology. GHD is diagnosed either by subnormal GH serum concentrations after provocation of GH secretion (classic GH deficiency, cGHD), or by subnormal 24h spontaneous GH secretion (GH neurosecretory dysfunction, GHND). Children with cGHD and GHND have severe growth retardation and respond to exogenous human GH (hGH) therapy with significant catch-up growth. The proximal promoter region of the hGH1 gene is highly polymorphic. 16 SNPs have been described and manifested in a total of 40 haplotypes, some of them affecting GH1 transcriptional activity.
Objective: The aim of this study was to explore the GH1 gene and its promoter region for possible mutations which could induce defective GH expression in familial GHD. **Patients and Methods:** We studied 2 families. Family I: 1 girl with GHND [A1, height-SDS (H-SDS):-4.4], 1 girl with cGHD (A2, H-SDS:-3.6), 2 male siblings with normal stature (A3, A4), and their parents

(mother A5, H-SDS:-1.94 and father A6, H-SDS:-0.4). There was phenotypic variability between siblings. Family II: 1 boy with cGHD (B1, H-SDS:-2.9), 1 girl with cGHD (B2, H-SDS:-2.8), and their parents (mother B3, H-SDS:-0.8 and father B4, H-SDS:-2.7). Patients of the two families responded well to exogenous hGH replacement. Genomic DNA was extracted from lymphocytes by standard methods. The GH1 gene and its promoter were PCR amplified and sequenced. **Results:** 1) A new heterozygous mutation (-461G>A relative to the GH1 translational start site) was found in the promoter region of the GH1 gene in GHND patient A1 and her mother A5. 2) A new heterozygous mutation (-546G>C) was found in the promoter of GH1 gene in GHD patient B1 and his father B4. 3) 2 new SNPs (-93insG and -95insG) were identified in A1, A3, A5 and B4. 4) No mutations were identified in the GH1 gene. **Conclusions:** The 2 new mutations in the GH1 promoter may affect GH expression since nucleotide -461G contains the prediction binding sites for the head domain transcription factors, PAX (2,4,6) and ETS-1, and nucleotide -546G contains the prediction binding site for the transcription factor ETS1 which may activate the GH1 promoter alone or with the transcription factor Pit-1. Whether these new mutations affect GH function needs to be clarified with GH functional studies.

PO1-080 Genetics of Growth I

Mutation screening of *HMGA2 and CDK6* genes in Dutch patients with isolated growth hormone deficiency

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In the last two years several Genome-Wide Association independent studies have identified multiple loci robustly associated with adult height in normal population. Candidate gene studies in short stature patients are a complementary approach to implicate genes in the molecular pathways that are important in human growth.
In the Netherlands, the incidence of Isolated Growth Hormone Deficiency (IGHD) is estimated to be 1 in 3500 to 1 in 10.000 live births and in the vast majority of patients the genetic cause is unknown. In order to assess whether novel genes possibly involved in the phenotype of IGHD patients with unknown genetic defects, we selected *HMGA2 and CDK6* genes, identified in at least three GWA studies, to perform genetic mutation screening.
HMGA2 is an excellent candidate for being involved in height-related syndromes; severe mutations in this gene alter body size in mice and humans. *CDK6* is a member of the cyclin-dependent protein kinase (CDK) family, important regulators of cell cycle progression.
Patients and methods: Genetic analysis was performed in 105 Caucasian IGHD patients from the Dutch HYPOPIT study. We used direct sequencing approach to analyse the coding region of exons and exon-intron boundaries of *HMGA2 and CDK6* genes. When novel variants were found, a healthy Dutch controls was genotyped for these variants using TaqMan SNP Genotyping Assays to determine its nature as mutation or a polymorphism.
Results: In five patients we identified an unknown heterozygous intronic variant in the *HMGA2* gene (IVS3+6 T>A), that could affect the donor splice site of exon 3. This variant was also present in healthy Dutch controls (2,7%), and therefore probably this novel polymorphism is not related with IGHD. Few minor genetic variations outside the coding regions with unknown functional effect were also found in *HMGA2* gene.
In the *CDK6* gene we found an missense SNP (rs35654944; D110N) in one IGHD patient. This variant was also present in healthy Dutch controls (1,1%), thus we consider its functional impact is small. In addition, one novel synonymous exonic variant in heterozygosis c.546G>A (T182T) was found in one patient. The base substitution is located close to acceptor splicing site of exon 5 and *in silico* analysis revealed that this variant may alter splicing.
Conclusion: Our study demonstrates that IGHD is probably not caused by mutations in coding regions of *HMGA2 and CDK6* genes.

PO1-081 Genetics of Growth I

Age at diagnosis and mortality in 47,XXX syndrome

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Background: Little is known concerning the phenotype of females with 47,XXX and the consequences hereof.
Aim: To describe the age at diagnosis and total mortality in all persons diagnosed with 47,XXX in Denmark.
Method: Using the Danish Cytogenetic Central Registry we identified all females diagnosed with 47,XXX during 1965-2008. Hereby we identified 153 persons, whereof 26 were deceased. Approximately 2.6 million women were at risk yearly. We divided the women into three subgroups according to their karyotype (Table). In the Statistics Denmark we identified up to 100 controls per index-person, all matched on gender and age (year and month), furthermore we retrieved date and causes of death. All controls were alive on day of diagnosis.
Statistics: Age at diagnosis, median year of birth, and median year of diagnosis in the subgroups were analyzed using Kruskal-Wallis. Time at risk was calculated from date of birth. Total and cause specific mortality were compared in 47,XXX persons and their controls using Kaplan-Meyer plot and log-rank analysis.
Results: We identified a reduced number of diagnosed females with 47,XXX compared to expected based on historic chromosome surveys. Data in the three subgroups are shown with medians and ranges.

Karyotype	Number of cases	Median age (yrs) at diagnosis (range)	Median year of birth (range)	Median year of diagnosis (range)
47,XXX	83	3.1 (0.0-65.0)	1983.0 (1913.4-2008.9)	1989.2 (1966.2-2008.9)
46,XX/47,XXX	37	35.8 (0.0-73.2)	1948.5 (1895.7-1984.3)	1983.3 (1963.6-2000.4)
Others	33	17.4 (0.0-66.4)	1973.9 (1909.8-2003.5)	1992.4 (1963.3-2005.5)
Total	153	16.8 (0.0-73.2)	1973.8 (1895.7-2008.9)	1987.7 (1963.3-2008.9)

There were no significant differences in the three subgroups; however, age at diagnosis was surprisingly delayed. Mortality in 47,XXX persons was significantly increased with a median survival of 77.8 years, compared to 84.3 years in controls, with a log-rank analysis corresponding to $p<0.0001$.
Preliminary data suggest that a significantly increased number of deaths were due to cardiovascular cause, $p<0.02$.
Conclusion: Few females with 47,XXX are diagnosed. Diagnosis is delayed and mortality is increased. The syndrome has yet to be described in greater detail

PO1-082 Genetics of Growth I

The degree of H19 hypomethylation in children with Silver-Russel syndrome (SRS) is not associated with the clinical severity score (CSS) and the response to growth hormone (GH) treatment

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Background: Hypomethylation of the imprinting control region 1 (ICR 1) at the IGF2/H19 locus on 11p15 is linked to Silver-Russel syndrome (SRS).
Objectives: We tested the hypothesis that the severity of the phenotype in SRS patients depends from the clinical severity score (CSS)(Bartholdi et al. 2009). In addition, we compared the CSS with the growth response on the growth

hormone (GH) treatment.
Methods and results: Methylation analysys was performed by bisulfite treatment of DNA samples, radiolabelled PCR amplification, and digestion of the PCR products using restriction enzymes.Four SRS patients were clinically scored (CSS). Two out of four SRS patients (50%) had hypomethylation of one allele.
Results: All four patients had high CSS (12, 13, 13 and 13). Nevertheless, only two of them had hypometylation of one H19 alellae (044, and 0.39; normal 0.46 to 0.83). Two of them had ventricular septal defects, but only one had H19 hypomethylation. All children had low birth length and weight (>3SD), a classic facial phenotype, hemihipertrophy (2.5 cm thinner left arm/leg in comparison to the right ones), shorter leg in 3 children, and striking thinness (BMI >16). All children were short (-5.5 SD), one child had hypopituitarism (GH, thyroxin, sex steroids). GH treatment increased growth velocity, but with the exception of the hipopituitary patient, all others grew on the third percentile.
Conclusions: Only the patient hypopituitarism had a better growth (height 174 cm). Since two SRS patients with high CSS (12 and 13) had no methylation defect it seems that the methylathyon status did not influence the clinical expression or growth in those children.

PO1-083 Genetics of Growth I

Polymorphic variability in steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) related to growth and blood pressure in children

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Background: Steroidogenic factor-1 (SF-1, *NR5A1*) is a nuclear receptor that plays a central role in many aspects of adrenal, gonadal, pubertal and metabolic function. SF-1 is also emerging as an important regulator of ventromedial hypothalamic development and programming, and post-natal obesity has been reported in Sf1 knock-out mice rescued by adrenal transplantation. Although incidental reports of weight changes and hypertension in patients with disruptive SF-1 mutations exist, the influence of a potentially hypofunctional p.G146A polymorphism in SF-1 on childhood growth and blood pressure is unknown. This variant has been reported to be associated with micropenis and undescended testes in a Japanese cohort, but its prevalence and associations in other populations is not reported.
Aims: We studied the association of a non-synonymous coding polymorphism in SF-1 (p.G146A) (rs1110061) with longitudinal growth and blood pressure in two cohorts of Caucasian mothers and children.
Methods: Genotyping for the p.G146A polymorphism in SF-1 was performed by restriction digestion (*Sph*I) or Taqman analysis in the UCL Fetal Growth Study (UCLFGS) cohort (473 children at birth, 233 at 3 years) and in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (8413 children at birth, 765 at 3 years, 5365 at 9 years).
Results: The frequency of the p.G146A polymorphism was lower than previously reported (heterozygous frequency 7.8% in UCLFGS and 2.2% in ALSPAC) but occurred in Hardy-Weinberg equilibrium. In the UCL cohort, heterozygosity for the p.G146A variant was associated with a reduction in placental weight (634 (126) g vs 679 (130) g, $p<0.05$), increased preterm delivery (20.8% vs 7.4%; Chi-square, 5.58; p=0.03; OR 0.30, 95% CI 0.11-0.86) and higher BMISDS (0.62 (1.68) kg/m^2 vs -0.03 (0.93) kg/m^2, $p<0.001$) and shorter stature (length SDS -0.28 (1.54) vs 0.25 (1.0), $p<0.05$) at 3 years of age. However, no significant differences in these parameters were observed when the larger ALSPAC cohort was analyzed, and no significant association with blood pressure was seen.
Conclusion: Although initial data suggested that a polymorphism in SF-1 might be associated with important parameters of pregnancy and post-natal growth in humans, it is likely that the p.G146A variant does not have a major influence on these variables within a Caucasian population. The influence of this polymorphic variant in other populations, where it is more prevalent, remains to be determined.

PO1-084 Genetics of Growth I

A novel "D244H" missense mutation in the growth hormone receptor, identified in a patient with severe growth retardation, is a splicing mutation resulting in premature protein termination

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Background: Mutations in the growth hormone receptor gene (*GHR*) often lead to severe post-natal growth retardation, GH insensitivity (GHI) and IGF-I deficiency (IGFD). The majority of the mutations are located in the extracellular domain of the GHR protein, which can be proteolytically cleaved and circulate as GH binding protein (GHBP). Lack of detectable GHBP is indicative of aberrant GHR expression, consistent with Laron Syndrome.
Objective: To evaluate the cause of severe growth retardation in a young female patient from a consanguineous pedigree. The parents, of Turkish ethnicity, had normal statures of 173 cm (-1.2 SDS, Turkish standard) and 153 cm (-1.5 SDS), for father and mother, respectively.
Case Report: The proband was normal sized at birth with a birth length of 48.5 cm, weight of 3200g, and head circumference of 43 cm. At presentation, age 15 months, she had a height of 63 cm (-5 SDS), weight of 7.5 kg (-2.3 SDS). Physical examination was, otherwise, unremarkable. Repeated basal serum GH measurements were high (>40 ng/ml; 32 ng/ml). Serum IGF-I (8.5 ng/ml; normal, 13-100), IGF binding protein (IGBP)-3 (126 ng/ml; normal, 365-1294) and acid labile subunit (ALS; 0.59 mg/L; normal, 5.6-16), in contrast, were markedly low. Significantly, serum GHBP was well below normal (120 pMol/L; normal, 431-1892). At age 3.25y, the patient had a height of 73 cm (-6 SDS) and weight of 10.5 kg (-2.5 SDS). Recombinant IGF-I therapy was initiated at a dose of 120 ug/kg/day, but was reduced to 80 ug/kg/day after development of hypoglycemia/hyperglycemia. Growth velocity improved to 8 cm/year.
Results: The abnormally low GHBP level in the proband was indicative of an aberrant *GHR* gene. Analysis of the *GHR* gene identified a novel homozygous mutation in the last nucleotide (784) of exon 7; the parents, as expected, were heterozygous for the mutation. Nucleotide 784 is the first nucleotide of aspartate, D244 (which is part of the proteolytic cleavage site), but is also part of the donor splice site for intron 7. Analysis of the *GHR* mRNA (primary dermal fibroblast derived from the patient) indicated that the *784G>C* transversion resulted in an mRNA lacking exon 7 sequences, and the fusion of exons 6 and 8 led to a frameshift with predicted early protein termination.
Conclusion: Analysis of primary cultures from a patient with severe short stature, GHI and IGFD, permitted the elucidation of a novel and severe splicing homozygous *GHR* mutation.

PO1-085 Genetics of Growth I

Guanine nucleotide binding protein alpha subunit (Gsα) signalling in children with short stature and shortening of the metacarpals IV and V

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Objective: GNAS is an imprinted region on chromosome 20q13 generating the α subunit of the stimulatory G protein (Gsα). Gsα inactivating mutations may cause Albright Hereditary Osteodystrophy (AHO), a phenotype with short stature and brachydactyly. Maternal inherited Gsα mutations cause pseudohypoparathyroidism type Ia (PHPIa), characterised by AHO phenotype. In these patients brachydactyly and shortening of the 4th and 5th metacarpals are common features. Therefore, disproportionate shortening of the lengths of the metacarpals and phalanges in relation to height was evaluated in children with short stature. Gsα activity and the GNAS gene were further analysed in suitable patients.
Methods: A radiograph of the left hand was available from 774 patients with short stature (height SDS <-2) previously evaluated in our department. Measurements of the lengths of the metacarpals (1-5) and phalanges (distal, medial and proximal) were performed. Coding mutations of the GNAS gene were analysed by sequencing of the exons 1-13. A platelet aggregation test was used

to investigate Gsα function (Freson K. et al., J Clin Endocrinol Metab, 2008). Results: 58 patients (ISS n=35, GHD n=7, UTS=4, TS n=3, SGA n=9) and 4 PHPIa patients presented shortenings of the metacarpals 4 and 5 <-2 SDS with a ratio to height SDS >1; (mean height SDS = -2.3 ± 1.38; mean MC SDS = -2.98 ± 1.67). Sequencing of the GNAS gene was performed in 22 patients. Coding mutations in the GNAS gene were detected in only 2 of 4 PHPIa patients, but all of them had a reduced Gsα activity (mean 48.3 %). No coding mutations of the GNAS gene were detected in the short stature patients. However, in 4 of these patients a reduced Gsα activity was measured (mean 56.82 %). 3 out of 4 PHPIa and 2 out 4 short stature patients with reduced Gsα activity presented a TT genotype of the T393C polymorphism in the GNAS gene. Conclusions: Shortening of the metacarpals IV and V can be found in children with various types of short stature and may be linked to GSA dysfunction as part of its broad phenotypic spectrum. Coding mutations of the GNAS gene were detected in 2 of 4 PHPIa patients, but not in short stature patients. Epigenetic defects of the GNAS were reported in patients with reduced Gsα activity. Therefore, examinations of the GNAS gene in short stature patients with disproportionate shortening of the metacarpals should be expanded in order to detect epigenetic alterations.

PO1-086 Genetics of Hormone Excess

Abdominal SDHB hereditary paraganglioma

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Paragangliomas (PGLs) are rare tumours arising from extra-adrenal chromaffin cells. PGLs are exceptional in the pediatric age. Three of the four genes (A,B,C,D) encoding the mitochondrial succinate dehydrogenase (SDH) complex have been related with the disease. Carriers of *SDH-B* mutations are prone to develop malignant and extraparaganglial neoplasias.

Patient study: A 16 year-old-male presented with a 5 months history of weight loss, palpitations, headache, sweating and hypertension coincident with exercise. He showed elevated blood pressure (185/125mmHg), and heart rate (102 bpm). Normal height (163 cm, p34), BMI (21, -0,4DS) and pubertal status (Tanner V). Thyroid hormone profiles, PTH and calcitonin were normal. Further laboratory tests showed:

	Plasma levels		Urinary levels	
		Normal		Normal
Metanephrines	23 pg/ml	100-150	23	74-297
Normetanephrines	>1000 pg/ml	<200	1000	105-354
Epinephrine	92 pg/ml	30-85	123	<18
Norepinephrine	17307 pg/ml	300-650	2792	<76
Dopamine	46 pg/ml	10-150	1137	<390
VMA			33	2-9
5-HIIA	6.8 mg/24	<10		
Aldosterone	1113 pg/ml	40-300		
Renine	170 pg/ml/h	3-33		

CT, MRI and MIBG scanning revealed a large abdominal left para-aortic mass of 47 x 43 x 33 mm.

His paternal grandmother was known to have hypertension and sweating crises, and a paternal uncle died from a mediastinal neoplasm at the age of 19.

Four weeks before surgery the patient was started on adrenoceptor blockers: phenoxybenzamine and atenolol. Pathology of the tumour showed no signs of malignancy. One week after surgery, blood pressure and plasma/urine catecholamines and metanephrines returned to normal, and so remained at 1-year follow-up control.

Molecular analysis: Sequencing, gross deletion analysis and PCR amplification of patient's genomic DNA identified a 16 Kb-long deletion affecting exon 1 of the *CHD-B* gene, coincident with a previously described Spanish founder defect.

Patient's father (54 years old) and two brothers (aged 25 and 22) also carried the deletion. However, their MRI, CT and plasma/urine catecholamines and metanephrins were normal.

Conclusions: We report a pediatric PGL case caused by a large deletion in *SDH-B*. Tendency to malignization of this genotype makes family genetic screening essential, especially given the low clinical penetrance described for this genotype, which is further supported by our familial study.

PO1-087 Genetics of Hormone Excess

A case of pseudohypoparathyroidism type Ia with undescended testis

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Pseudohypoparathyoidism type Ia (PHP Ia) is caused by autosomal dominant mutations in the GNAS1 gene and reduced activity of the adenylyl cyclase stimulating protein Gs alpha.

We report about a 9 year old boy who developed generalised epileptic seizures. MRI of the brain demonstrated the presence of basal ganglia calcifications. His history showed nervous hyperexcitability with paresthesia, bronchospasm. Height SDS -0.64, Weight SDS 0.99**,** BMI SDS 1.65. The clinical examination showed a round face with deep nasal bridge, subcutaneous calcifications, mental retardation, shortness of metacarpophalanges, bilateral undescended testis (in the sonography of the abdomen both testis were located within the inguinal canal; right and left testicular volume 1ml). He showed resistance (988 pg/ml, reference range 15-65) to parathyroid hormone (PTH), high TSH levels (10.94 µIU/ml), reduced levels of plasmatic calcium (Ionised calcium 0.95 mmol/l, total calcium 1.93 mmol/l). LH, FSH and testosterone were within normal ranges for the prepubertal age group.

The Gs alpha protein activity in erythrocyte membranes was analyzed and activity was reduced (59.9% normal range: 85-115%).

Molecular analysis of GNAS1 Gene identified a heterozygote insertion of guanine in the coding region 337 of exon 12, leading to diagnosis PHP Ia. Treatment consists of oral calcium and vitamin D derivates supplementation. An orchidopexy was done. Anamnesis of family members: mother and grandmother showed brachydactyly, short stature (mother 136.5 cm, grandmother 144 cm), early onset of puberty (menarche age by mother 10 yrs., grandmother 11 yrs.), treated hypothyroidism (mother). A mutation and reduced activity of Gs alpha protein (67.5%) was found in the patient's mother. Concerning the grandmother neither an analysis of the Gs alpha protein activity nor a molecular analysis of GNAS1 Gene was done.

PO1-088 Genetics of Hormone Excess

A case of multiple target organ defects form of pseudohypoaldosteronism type 1

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Pseudohypoaldosteronism type 1(PHA-1) is a rare genetic disease characterized by neonatal hyperkalemia, hyponatremia, metabolic acidosis, and elevated levels of aldosterone and renin. PHA-1 includes at least two clinically distinguishable entities with either renal or multiple target organ defects (MTOD). Here, we report a MTOD form of PHA-1 in a newborn presented with life-threatening salt wasting.

A nine-day-old male was referred to our hospital for evaluation of jaundice and salt wasting. On physical examination, his weight 2.9 kg, height 48 cm, blood pressure was 57/22 mmHg, heart rate 52/min, systemic jaundice, dehydratation, cutis marmoratus, acrocyanosis, and hypotonicity. There was no family history of consanguinity. Laboratory data on admission showed marked hyponatremia of 105 mmol/L, hyperkalemia of 10.9 mmol/L, and metabolic acidosis. Urinary sodium wasting was 85 mmol/L and the ratio of calcium/creatinine 0.2. His serum glucose was 98 mg/dL, BUN 34 mg/dL, creatinine 0.7 mg/dL, total bilirubin 20.7 mg/dL. Complementary laboratory investigations revealed high serum aldosteron (>3000pg/mL) concentration and plasma renin activity (130

ng/mL/h, N: 2.4 – 3.7). Quantitative sweat test showed elevated cloride concentration (130 mmol/L). At presentation, surrenal insufficiency was suspected and glucocorticoid therapy was given. Hyponatremia and hyperkalemia were corrected. After complementary laboratory investigations we concluded MTOD PHA-1 and dietary sodium supplementation (10 mEq/kg/d), low-potassium diet, kayexalate were recomended.
PHA should be considered in the differential diagnosis of a salt wasting syndrome in infants.
The fundamental abnormality in MTOD PHA-I is a loss-of-function mutation in the alpha or beta subunits of the epithelial sodium channel (ENaC), resulting in defective sodium transport in many organs containing the ENaC. Genetic analysis will be planned for our patient.

PO1-089 Genetics of Hormone Excess

Anesthesia method during placement of once-yearly histrelin implant for treatment of central precocious puberty

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Objective: To assess the need for general anesthesia during placement of a once-yearly GnRHa implant in children with central precocious puberty (CPP). Background: The Consensus Statement on the Use of Gonadotropin-Releasing Hormone Analogs in Children (*Pediatrics* 2009) indicated the histrelin GnRH agonist subcutaneous implant provides 12 months of therapeutic suppression in children with CPP, thereby eliminating the need for monthly injections. While adults receive a similar histrelin implant for a different indication under local anesthesia, there is question as to the need for general anesthesia during the implant placement procedure in children. We evaluated the data from 31 children who received the implant to determine the type of anesthesia used and to assess the need for differing methods based on patient and practitioner preferences. Methods: 31 children (29 female), age 4 to 10 years, (13 previously treated with GnRHa and 18 treatment naïve) were enrolled and received a histrelin implant according to Phase III protocol. The implants remained in place for 12 months, and were replaced at 12 month intervals for continuation of therapy. Results: Each study site tended to use one method of anesthesia for all patients enrolled. One site used only local anesthesia with distraction (6 pts), three sites used conscious sedation techniques (5 patients), one used either local (1 pt) or general anesthesia (1 pt), and the remaining four sites used general anesthesia (18 pts). There were no reported differences in the study between local and general anesthesia with respect to patient tolerability or practitioner difficulties with the implant procedure, and all procedures utilizing local anesthesia for the initial implant were conducted using local anesthesia for re-insertion. Conclusions: Absent a comorbid condition which in the judgment of the practitioner may indicate the use of general anesthesia, the use of local anesthesia with distraction during the implant procedure may be appropriate for most children receiving the once-yearly histrelin implant for treatment of CPP.

PO1-090 Genetics of Hormone Excess

Retrospective analysis in 12 persistent hyperinsulinemic hypoglycemia infants

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Objective To retrospectively study persistent hyperinsulinemic hypoglycemia infants clinical manifestation and treatment results. **Methods** 12 persistent hyperinsulinemic hypoglycemia infants were retrospectively analyzed. **Results** 6 female and male respectively, aged 1 day to 1year, all full term infants. 58%(7/12) patients appeared hypoglycemia symptom within one month age. The main symptoms included convulsion, cyanosis, lethargy, refusing sucking milk, irritability and sweating. The laboratory findings included persistent non ketons hypoglycemia and hyperinsulinism. Plasm glucose after fast 2-3 hours were 0.2-2.6mmol/l, at same time serum insulin were 10.1-99.3mIU/l, C peptide was 1.4->7.7ngl/l, Serum insulin:plasm glucose ratio was 0.43-3.56, All patients had good response to glucagon test. 7 patients were given diazoxide therapy, only 4 patients could keep blood glucose normal in most time during diazoxide therapy. 1 patient was given subtotal pancreatectomy and blood glucose was normal in most time after operation. 6 patients had psychomotor retardation and 3 patients were lost contact during 4 months to five years follow up. **Conclusion** Symptoms varied in hyperinsulinemic hypoglycemia infants, blood sugar, insulin levels and urine ketons were keys in diagnosis. Diazoxide therapy was effective in some patient. Subtotal pancreatectomy was effective.

PO1-091 Genetics of Hormone Excess

Succinate dehydrogenase mutations are present in pediatric and adult wild-type gists occurring in patients without associated paraganglioma

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Pediatric gastrointestinal stromal tumors (GIST) are rare mesenchymal neoplasms of the gastrointestinal tract arising from the interstitial cells of Cajal. The majority of GIST tumors are associated with activating mutations of the KIT or PDGFRA proto-oncogenes. However, 85% of pediatric GIST patients do not have these mutations, and are termed wild-type. Therefore, other genetic mechanisms are likely to be associated with pediatric GIST. The Carney-Stratakis syndrome, first described in 2002, is characterized by the presence of paragangliomas (PGLs) and GIST that are inherited in an autosomal dominant manner with incomplete penetrance. Germline mutations of genes encoding subunits B, C, and D of the mitochondrial enzyme succinate dehydrogenase (SDH) are present in the majority of patients with the Carney-Stratakis syndrome. SDH is a component of the mitochondrial electron transport chain; mutations in this gene are thought to cause increased susceptibility to cancer through loss of function of tumor suppressor activity of the wild-type gene. The frequency of patients who present with a GIST and SDHx mutations in the absence of a coexisting PGL, or family history suggestive of PGL, is still under investigation.
Methods
21 patients with wild-type GIST with a median age at diagnosis of 19 yrs (range 5-41) seen at the NIH in 2008 and 2009 were included in the study. Genetic testing for mutations in SDHB, C, and D was performed at Mayo Medical Laboratories, Rochester MN. All patients provided written informed consent.
Results
6 out of 21 (29%) of patients with wild-type GIST were found to have germline mutations of the SDHB (4), SDHC (1), or SDHD (1) genes. Five of these were missense mutations and one was a splice-site mutation.

c.DNA nomenclature	Amino acid change	Gene	Type of mutation
c.725G>A	p.Arg242His	SDHB	missense
c.405+1G>A	-	SDHC	splice site
c.600G>T	p.Trp200Cys	SDHB	missense
c.274T>A	p.Ser92>Thr	SDHB	missense
c.34G>A	p.Gly12Ser	SDHD	missense
c.380T>G	p.Ile127Ser	SDHB	missense

Conclusions
Our results show that SDHx mutations are frequently present in pediatric and young adult patients with wild-type GIST. We recommend that genetic testing for an underlying mutation in SDHx be performed in all pediatric patients with GIST and in all adult patients with wild-type GIST. Patients with the mutation, and any relatives who are carriers of such mutations, should also be monitored for the development of PGLs.

PO1-092 Genetics of Hormone Excess

Succinate dehydrogenase mutations are strongly associated with paraganglioma of the organ of Zuckerkandl in pediatric and adult patients

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Extra-adrenal pheochromocytomas, also known as paragangliomas (PGLs), may arise from a collection of para-aortic paraganglion cells around the origin of the inferior mesenteric artery extending to the level of the aortic bifurcation. These masses of chromaffin cells near the sympathetic ganglia along the abdominal aorta are known as the organ of Zuckerkandl. Mutations of the mitochondrial enzyme succinate dehydrogenase (SDH) subunits B, C, and D give rise to hereditary PGL syndromes. While an association with mutations in SDH genes has been found in patients with mediastinal PGLs, a specific analysis of organ of Zuckerkandl PGLs has not been performed. 14 patients with primary PGLs of the organ of Zuckerkandl with a median age at diagnosis of 22.5 yrs (range 9-71), seen at the NIH, U of Texas M.D. Anderson CA Ctr., and Dana-Farber CA Inst. between 1989 and 2009 were included in the study. Genetic testing for mutations in *SDHB, C, and D* was performed. 10 out of 14 (71%) of patients with PGLs of the organ of Zuckerkandl were found to have mutations of the *SDHB (8), or SDHD* (2) genes. 6 of these represent missense mutations, 1 an insertion, 3 deletions. 2 patients have pending results while 2 have no mutations found. All of the patients developed metastatic disease. Initial patient symptoms, biochemical phenotype, details of primary tumor, and patient outcome will be presented. Our results show that SDHx mutations are prevalent in pediatric and adult PGLs of the organ of Zuckerkandl. In addition, PGLs of the organ of Zuckerkandl are strongly associated with noradrenergic phenotype and aggressive behavior. The identification of SDHx mutations in patients with organ of Zuckerkandl PGLs has important implications for patient care and screening of family members. Patients with PGLs of the organ of Zuckerkandl should be screened for SDHx mutations; in addition asymptomatic carriers of an SDHx mutation among the relatives of affected patients may benefit from tumor screening for early PGL detection.

Gender, Age	Gene with mutation	Biochemistry
M,49	SDHB	U:NE,DA,NMN
M,20	Pending	not done
F,33	-	P: NE,ChA, U:NE,NMN
M,9	SDHB	P:NMN
F,9	SDHB	P:NMN,NE,DA
M,15	SDHB	U:NMN,NE,DA; P:NMN,NE
M,34	SDHB	P:NE,NMN
F,18	-	P:NMN,ChA
F,26	SDHD	P:NE
M,24	Pending	P:NE U:NE,NMN
M,58	SDHB	-
F,71	SDHB	P:NMN,MN,NE,EPI
M,21	SDHD	P,U:NE,NMN
M,11	SDHB	P:NE,NMN U:NE,NMN

P= plasma U= urine, NE= norepinephrine EPI= Epinephrine NMN= normetanephrine, ChA= chromogranin A, DA= dopamine

PO1-093 Genetics of Hormone Excess

Case reports of premature adrenarche in children with Prader Willi syndrome

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PWS is a chromosomal disorder characterised by short stature, obesity, hypogonadotrophic hypogonadism and ACTH deficiency. We report the development of premature adrenarche in 3 girls with PWS (genetically confirmed), receiving GH (4.5 to 6.5 mg/m2/wk) who presented at 7 y with pubarche and acne without thelarche or menarche. Before this the chronological age - bone age difference (CA-BA) decreased from -0.04, +0.40 and +1.63 at 3y to -0.03, +1.36, and 2.44 respectively at 7 yr. BMI SDS increased from -0.19, -0.92 and -2.23 at 3 y to +0.39, +0.32 and +0.17 respectively at 7 yr. Serum DHEAS levels were high ranging 1.6, x, 3.2 umol/L (NR<1.5) and SHBG was 33 to 51 nmol/L (50- 90). Serum LH, FSH, E2, and 17OHP levels were low normal. Basal cortisol level were 156, 268 and 305 nmol/L. Short Synacthen test showed normal response (peak cortisol 663, y, 982 nmol/L and normal cortisol/17OHP ratio (0.001, x, 0.017; NR<0.023). The cause of the change in CA-BA and virilisation in these girls with PWS remains unclear, with no features of Cushing's disease or adrenal tumour and having excluded non-classical congenital adrenal hyperplasia. ACTH deficiency has been reported in majority of patients with PWS hence the elevation of DHEAS is unexpected. Premature adrenarche with biochemical and clinical hyperandrogenism in the PWS patients is not associated with GH therapy. The premature adrenarche may explain the maturation of BA and be associated with the compromise final height in children with PWS not treated with GH. The increase in BMI SDS and advancement in BA together with elevated DHEAS levels was not associated with clinical or biochemical evidence of hyperinsulinism. The hyperandrogenism may be as a result of aromatisation of androgens in adipose tissue.

PO1-094 Genetics of Hormone Excess

Carney Complex – a rare cause of Cushing's syndrome

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Carney Complex (CRC, OMIM 160980; 605244) is an extremely rare cause of peripheral hypercortisolism in childhood.
CRC occurs sporadicallly as well as inherited and features a broad spectrum of symptoms including endocrinopathy, neoplasia and skin hyperpigmentation (Carney et al, 1985). In over 50% of the CRC cases a mutation of the tumour suppressor gene PRKAR1A is found. Constituting a part of cAMP-transmitted signalling cascades, PRKAR1A regulates the activity of protein kinase A (PKA). Lower intracellular levels of PRKAR1A lead to an increased cellular response towards cell proliferation and neoplastic deviation.
We report of the case of a 13 years old female patient presenting with hypertension, steatosis hepatis, increased weight gain and stunting of growth. She exhibited a cushing-like phenotype and perioral hyperpigmentation.
Laboratory findings indicated unsuppressible peripheral hypercortisolism without alteration of other steroids. After inconclusive MR/CT imaging, venous sampling was performed and proved bilateral adrenal hyperactivity. Accordingly, the patient underwent laparoscopic bilateral adrenalectomy. On histological examination, both adrenal glands showed macronodular deviations >1cm as well as pigmented micronodular areas, pointing to primary pigmented nodular adrenocortical disease (PPNAD). Genetic analysis showed a so far unreported mutation of PRKAR1A. Confirmation of the diagnosis of CRC warrants screening for typically associated neoplasms.
The girl is now on replacement therapy with hydrocortisone. Her phenotype normalized completely within the following 12 months. Ultrasound screening every 6 months and surveillance of endocrine functions and psychological support complete the patient's management.

PO1-095 Genetics of Hormone Excess

Molecular diagnosis of Mc-Cune Albright syndrome (MCAS) by thyroid fine needle aspiration biopsy

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Background: The Mc Cune-Albright syndrome (MCAS) is characterized by the triad of irregularly edged hyperpigmented macules, polyostotic fibrous dysplasia, and GnRH independent sexual precocity. Other autonomous endocrine hyperfunction can occur. The MCAS is due to somatic activating mutations in GNAS1 encoding the α subunit of the GTP-binding protein. The activating mutation is detected in 90% of cases in the affected tissue (mostly ovarian tissue, cyst liquid, and bone lesions) when it is available. Conversely, the activating mutation is found in blood samples in only 25% of the patients. Finally, the availability of affected tissue may be the main limitation for molecular diagnosis of MCAS.
Objective: to identify GNAS mutation in thyroid tissue in 1 patient with MCAS.
Patients: A 5- years- old girl was referred for vaginal bleeding during 5 days. When she was 6-month-old, she had had a right oophorosalpingectomy for adnexial torsion complicating an ovarian cyst. Her height was 110 cm (+0.2 DS, target height: +1DS), pubertal stage was B2P1. A small irregular hyperpigmented spot was noted on the abdomen. The GnRH test confirmed GnRH independent precocious puberty. No other autonomous endocrinopathy was found. Skeletal radiography was normal. Puberty regression occurred spontaneously. No GNAS mutation was found in blood samples using highly sensitive nested PCR method.
At the age of 8 years, systematic biological control showed thyroid hyperfunction: TSH 0.1mUI/L (0.66-4.1), FT4 14.1 pmol/L (11.6-21.5), T3 6.4 pmol/L (4.1-7.9). Thyroid echography showed a normal sized gland, with 2 hypoechogenic nodules (respectively 17x11mm and 5x4 mm) with micro calcifications in the left lobe. In the right lobe, 2 smaller nodular lesions were identified with similar appearance. Fine needle aspiration biopsy (FNAB) showed normal thyroid cells. Nested PCR found the arginine 201 mutation of the Gαs protein in the FNAB thyroid specimen, confirming the diagnosis of MCAS.
Conclusion: Thyroid specimen by FNAB may be easily available for molecular diagnosis of MCAS. Whether the mutation could be found when there is no apparent involvement of the thyroid gland remains to be studied.

PO1-096 Genetics of Hormone Excess

Gastro-intestinal polyps in McCune Albright syndrome – expanding the spectrum of a well known disorder

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Background: McCune Albright Syndrome (MAS), a disorder caused by somatic activating mutations in the GNAS gene, usually presents with cutaneous, skeletal and endocrine manifestations. Focal lesions involving multiple tissues have been identified in MAS but gastrointestinal polyps have not been reported. Following observation of perioral freckling in two of our patients, endoscopy was performed in five.
Methods: Upper and lower gastrointestinal endoscopy and pill camera were employed to identify possible pathologic lesions.
Results: Hamartomatous gastrointestinal polyps were observed in 4 of 5 subjects, either in stomach or upper duodenum. Lower bowel polyps were not found. Because of the similarity between perioral freckling and associated bowel polyps, investigation for possible genetic links were performed. STK 11(LKB1 mutations) as found in Peutz-Jegher (PJ) syndrome were not observed. PRKARIA mutations as seen in Carney complex were not found.
Conclusions: A putative interaction between the function of the GNAS and LKB1 genes is implicated in the pathogenesis of the two disorders, via the cyclic AMP response element binding protein (CREB), with an important role in cell growth regulation, via transcription of proto-oncogenes and cell cycle regulatory genes. Both inactivating LKB1gene mutations in PJ syndrome and constitutive increase in c'AMP kinase activation via CREB in MAS, culminate in disordered cellular growth with predisposition to tumour development.These findings indicate an overlap between MAS and PJ syndrome, highlighting the need for routine gastroenterological endoscopy in patients with MAS

PO1-097 GH and IGF Use I

A phase I, three-way crossover trial to assess the bioequivalence, pharmacokinetics (PK), safety and tolerability of a new liquid multi-dose formulation of recombinant human growth hormone (r-hGH) versus Saizen® freeze-dried (FD) formulation

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Background: Two liquid formulations of Merck Serono r-hGH have been developed: 5.83 and 8.0 mg/mL.
Aims: To assess the bioequivalence, safety and tolerability of the two liquid formulations in comparison to the FD reference formulation of Saizen® and describe the PK parameters of the liquid formulations.
Methods: An open label study in which healthy volunteers (aged 18–45 years) were given sc injections of the reconstituted FD and two liquid formulations in random sequential order, each at 4 mg/dose, with 1 week washout between doses. To suppress endogenous GH secretion, iv somatostatin (118 µg/h) was infused continuously 1 hour before to 24 hours after each dose. Blood samples for PK analysis were taken hourly up to 10 hours, then at 12, 18 and 24 hours post-dose. Vital signs, safety and tolerability were assessed before and 24–26 hours after each dose and 14±3 days after the last dose. Primary PK endpoints were area under the serum concentration time curve (AUC_{0-t}) and maximum serum concentration (C_{max}). A mixed model was fitted to each log-transformed primary endpoint with fixed effect terms for treatment, period and sequence, with subject as a random effect. For each of the two concentrations, bioequivalence was concluded if the 95% CIs for the ratios of geometric means were within the standard pre-specified acceptance range (0.80–1.25).
Results: Fifteen men and 15 women enrolled (safety population n=30; PK population n=28). Bioequivalence could be shown for AUC_{0-t} and C_{max} with no significant differences between the three treatments in half-lives, time to reach C_{max}, clearance or volume of distribution. Intra-subject variabilities were 12% for AUC_{0-t} and 16% for C_{max}. The ratios of geometric means (95% CI) were 1.046 (0.980, 1.169) for AUC_{0-t}, 0.954 (0.875, 1.040) for C_{max} for 5.83 mg/mL; 0.991 (0.929, 1.058) and 0.955 (0.876, 1.041), respectively, for 8.0 mg/mL. After injection, 26 subjects had mild pain (≤29/100 mm by visual analogue scale, mean intensity 3.52 mm), 19 had injection site reactions (all mild). There were no clinically significant abnormal vital signs, ECG or laboratory findings. There were 56 treatment-related adverse events (AEs): 49 mild, 6 moderate, 1 severe (vomiting). The pattern of AEs was as expected, with most (e.g. nausea, vomiting, headache, dizziness) related to somatostatin. All resolved by study end.
Conclusions: The liquid multi-dose formulations were bioequivalent to the FD reference formulation and were well tolerated.

PO1-098 GH and IGF Use I

Outcome of rhGH treatment in patients with achondroplasia and skeletal dysplasias

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Background and Aims: Achondroplasia (ACH) is an autosomal dominant skeletal dysplasia characterized by rhizomelic dwarfism, with an average attained adult height of 131±5.6 cm for men and 124±5.9cm for women. Previous studies have shown that the use of rhGH may result in a transient increase in the growth rate, however, there has been no clear demonstration of benefit

with respect to adult height. The aim of our study was to evaluate a cohort of patients with ACH and other skeletal dysplasias who have been treated with rhGH and evaluate their response during treatment and attainment of adult height.

Methods: We studied retrospectively 24 patients with the diagnosis of achondroplasia, who have been treated with rhGH (mean dose 30U/m^2/wk) over the last 15 years.We noted their auxological characteristics at the start of treatment, within the first three years, and at the end of treatment and attainment of final height. Height SD scores were calculated according to the published data for patients with ACH.

Results: Patients with ACH (Male: 71%, Female: 29%) started treatment at a mean age of 3.4±2.5 yrs (range 1.2-9.0), with a HtSDS of -1.14±1.5 (range -3.30 to 1.86). They received rhGH for 10.2±3.2 years (range 4-15 years). At the end of the first year of treatment their growth velocity was 7.8±1.2 cm/yr which was significantly higher compared to the growth velocity observed in the second and third years (5.5±1.5 and 4.8±0.9 cm/yr respectively, *p<0.05*). At the time of the study 75% (n=18) had completed treatment, 71% (n=17) had reached final height and seven patients (30%) had had a limb lengthening procedure. Results for males and females with ACH are shown in Table 1. Auxology at the time of puberty was available for 13/24 patients with ACH. Taking into account the height gained by a limb lengthening procedure, children with ACH gained in average of 8.8±5.5 cm during puberty, with great variability among individuals (range 0.2-21.8 cm).

	Male (n=17)	Female (n=7)
Start Age (yrs)	3.8±2.8	2.4±1.2
Ht SDS (start)	-1.4±1.3	-0.68±1.8
Duration	9.93±3.3	10.7±3.2
Ht SDS (end of treatment)	1.3±0.92	1.4±1.5
Final Ht	12 /17	5/7
Final Ht SDS	1.3±0.85	1.94±0.7
Complete Tx	11	7
Limb lengthening	5	2

Conclusions: At end of treatment, there was a change in Ht SDS from -1.14±1.5 pre-treatment to 1.4±1.2 (p<0.05). In order to interpret final height data limb lengthening procedures should also be taken into account.

PO1-099 GH and IGF Use I

Idiopathic short stature – evaluating growth results from clinical trials in the KIGS database

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Background:

The results of a randomized controlled clinical GH trial (JCEM. 2008:93(11):4342) show that short children without GHD respond to GH treatment with a dose dependent increase in adult height$_{SDS}$: mean increase 1.3 SDS, with a broad individual gain, 0-3 SDS, and with a reached target height. Another randomized clinical trial (JCEM 2009:94(2):483) show that individualized GH dosing based on responsiveness (estimated from prediction model) make the children reach a predefined target height. Thus, already before start of GH treatment it is known which short child will respond or not, and if treated which GH dose will be needed to reach target height.

Aim:

To evaluate the growth results of two randomized clinical trials in a new cohort of ISS children treated worldwide with GH and followed within the outcome research data base KIGS.

Patients:

The study group comprised of 1146 children with ISS. Excluded were children born preterm and/or SGA or with GHD, other diseases or syndromes. At GH start, all children were prepubertal, with age (yr) of 9.3 (5.5 – 12.1), height$_{SDS}$ of -3.1 (-4.2 ot -2.4) and MPH$_{SDS}$ of -1.8 (-3.1 to 0.3) and parental adjusted height$_{SDS}$ of -1.3 (-2.9 of 0.0).

Results:

374 children reached a near adult height$_{SDS}$ of -1.7 (-3.1 to -0.4), a mean gain of 1.4 SDS (9 cm) with a wide range, -0.8 to 4.5. The GH dose was 31 (21 of 44) µg/kg/day over a 7 (5-11) yr period and they reached a parental adjusted height$_{SDS}$, of -0.1 (-1.4 to 1.2).

Using the KIGS-ISS 1st yr prediction model (Horm Res 2007;68:53) the predicted growth response met the observed growth response, with a median studentitized residual (SR) of -0.14 and mean/SD: -0.08 (1.3).

2nd yr on GH, the median SR was -0.08, and for the 3rd yr the median SR was -0.28, when using the KIGS-GHD prediction model (JCEM 1999;84(4):1174). The adult height was predicted to a SR of -0.10 with use of the ISS prediction model.

Conclusion:

The ISS children followed within KIGS as a group respond to GH (mean 1.4 SDS, 9 cm) with a broad variation of 5.3 SDS that already before GH start could be estimated using prediction models. Thus, we conclude that the results obtained in the two randomized clinical GH trials also was obtained in KIGS, i.e. in clinical practise worldwide.

PO1-100 GH and IGF Use I

Prediction model for individualized GH dosing

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Background

Today, it is possible to individualize the GH treatment using a prediction model, that provides an indirect measure of the individual GH responsiveness, in order to reach a predefined goal in height outcome (JCEM 2009, 94(2):483). In short children being GHD, ISS or SGA without using any cut off values for GH$_{max}$ or size at birth. All these models have been found valid when tested on data from new cohorts of children (Ped Res 2000, 48(4):475, BMC 2007, 7:40).

Aim

With time, growth references as well as methods for GH evaluation changes, making it necessary to update existing prediction models. Swedish prediction models are modeled in children on 33 µg/kg/day, making it relevant to add a dose module over the GH dosing range (17-100 µg/kg/day).

The aim is to explore the usefulness of updated prediction models with addition of dose modules for individualizing the GH dose for children to be treated with GH.

Methods

We now updated our models a) regarding growth reference: from children born 1956 to born 1974, b) regarding GH assays from poly- to monoclonal antibodies, c) adding the published KIGS SGA dose module (JCEM 2003:88(1):125) as well as a new dose module made from the individualized GH dose trial, with GH range 17-100 µg/kg/day.

Patients

Around 500 Swedish children of both model and validation groups. A new group of 128 patients fulfilling the criteria of the 2 sets of prediction models, was used to validate the updated models.

Results

2000: For the auxological model (SDres 0.37), with addition of KIGS SGA dose module, and with the individualizing dose module, the SDres (mean Res) became 0.51 (0-17), 0.4 (-0.02), 0.39 (-0.06), respectively; for the best model (SDres 0.26), the SDres (mean Res) became 0.5 (0.18), 0.37 (-0.01), 0.36 (-0.07), respectively.

The corresponding values for 2007 was for the auxological model (SDres = 0.34), SDres 0.41 (0.05), 0.38 (-0.02), 0.37 (-0.05), respectively, and for the best model (SDres = 0.25), the SDres became 0.36 (0.06), 0.33 (0.02), 0.31 (0.003), respectively.

Conclusions

We here present prediction models, updated for the most recent growth reference and GH assay, with addition of the published KIGS SGA dose module, but also with addition of a new individualized GH dose module for doses 17-100 µg/kg/day, to be used with a narrow prediction interval in clinical practice for individualizing the GH dose.

PO1-101 GH and IGF Use I

Evaluation of the prediction model used in a randomized individualized GH dose study in short GHD and ISS children, with addition of KIGS SGA dose module

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Background:
In a randomized trial (no TRN 0198-003) we found that individualized GH dosing (17-100 µg/kg/day) to short children gave similar growth response after 2 yr in those diagnosed GHD or ISS, due to a 32 % ($p < 0.003$) reduced range in the primary outcome variable, parental adjusted $heigh_{SDS}$ (diff MPH_{SDS}). We used the predicted growth response as an estimate of each child's responsiveness for selection of the GH dose (JCEM 2009;94(2):483).
Aim:
To evaluate, and validate when appropriate, the prediction model used in the individualized GH dose setting of a randomized trial also with a standard GH dose.
Methods:
The prediction model used (Ped Res 2000;48(4):475) was constructed on data from on children on standard Swedish GH dose 33 µg/kg/day, with Model SD_{res} = 0.28, making validation possible in children selected for this dose (n=27). In order to evaluate the results in all the other children different GH doses, we added to our prediction model the published dose module with the broadest dose range (17-100 µg/kg/day) i.e. the KIGS SGA dose module (JCEM 2003:88(1):125), however adjusted in order to be able to use SDscores for heights.
Patients:
Data from the 128 prepubertal children (the Per Protocol Population) was used, who completed the 2 yr GH treatment.
Results:
The comparison of the predicted versus the observed growth response of the group of children selected for 33 µg/kg/day were found valid, SD_{res} = 0.25. Growth response data from the children on the other five GH doses were evaluated using the prediction model with addition of the KIGS SGA dose module, SD_{res} = 0.40. The dose group responsible for the increased SD_{res} result was the group treated with 17 µg/kg/d, whereas growth responses from children treated with doses above 33 µg/kg/d were correctly predicted.
Conclusion:
The prediction model used for selecting GH dose in the randomized GH dose trial was found valid, when tested with an added dose module over the GH dose range. Thus, the updated model can be used for individualizing the GH dose in children.

PO1-102 GH and IGF Use I

Alternatives to daily injectable growth hormone: clinical studies with ALTU-238, a crystalline extended-release rhGH

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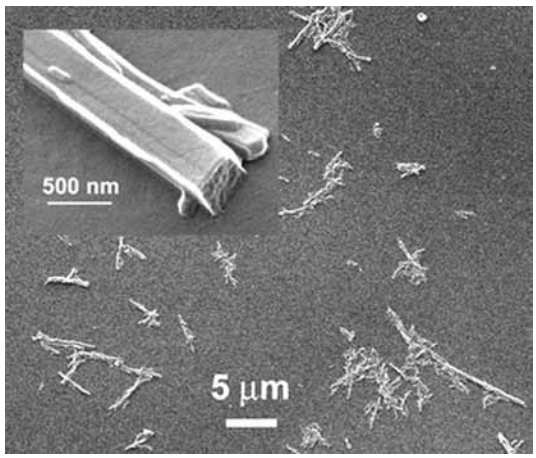
INTRODUCTION. Recombinant human growth hormone (rhGH) was one of the first proteins to be produced by rDNA technology and marketed as a therapeutic (1985). rhGH is a 191 amino acid protein, 22 kD, and has generally been administered by once daily SC injection. It has been used as a proof-of-concept test molecule for numerous alternative delivery systems, including formulations designed for other routes of administration (e.g., oral, inhaled) which have been difficult to develop due to significantly reduced bioavailability. Extended release injectable formulations have typically involved protein modification (e.g., fusion, PEGylation) or polymer encapsulation (e.g., PLGA, hyaluronate), resulting in challenges that include protein concentration, viscosity, syringability, initial "burst", injection site reactions, and immunogenicity. ALTU-238 is an extended-release, highly stable, concentrated, crystalline formulation of rhGH (Figure) requiring no protein modification or encapsulation. The resulting aqueous suspension has low viscosity, allowing administration with a very small gauge needle.



CLINICAL RESULTS. Data from three Phase 1 studies in healthy adults and one Phase 2 study in GH deficient adults demonstrate pharmacokinetic (GH) and pharmacodynamic (IGF-1) concentration-time profiles consistent with weekly dosing of ALTU-238. GH bioavailability, based on area under the curve (AUC), for a single injection of ALTU-238 was approximately 50% compared with 7 injections of daily rhGH, whereas IGF-1 AUC was >80%. GH and IGF-1 concentrations increased in a dose-dependent manner with no unexpected adverse events or laboratory abnormalities to date. The most common injection site reaction observed was erythema, with no instances of nodules or lipoatrophy.
CONCLUSIONS. ALTU-238 is an extended-release crystalline rhGH formulation with no protein modification or encapsulation that demonstrates serum GH and IGF-1 profiles consistent with weekly SC administration. Long-term efficacy and safety are currently being evaluated in pediatric patients.

PO1-103 GH and IGF Use I

Evaluation of hypothalamic-pituitary (HP) abnormalities on magnetic resonance imaging (MRI) in patients with non tumorous growth hormone (GH) deficiency (GHD) retested at the end of growth

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Background
Structural alterations of the HP region on magnetic resonance imaging (MRI), such as pituitary stalk (PS) interruption and ectopic posterior pituitary (EPP), have been associated with isolated GHD and multiple pituitary hormone deficiency (MPHD) and are considered as good markers for persistent GHD into late adolescence (the consensus cut-off being a peak GH < 5 µg/l). In contrast anterior pituitary hypoplasia (APH) has a similar prevalence in patients with normal GH secretion.
Objective
The aim of this study was to evaluate whether the presence of an HP abnormality on MRI, in patients with non tumorous GHD during childhood, provides support for this consensus cut-off value for peak GH.
Population
A retrospective analysis identified 53 patients with GHD, treated with GH during childhood, evaluated with MRI and retested at the end of growth (median age 17.0 years – range 13.2–21.3). Patients with GHD due to a tumour were excluded. Forty patients (75%) had a diagnosis of isolated GHD without defined aetiology (24 males) and 13 (25%) had septo-optic dysplasia (4 males). Seventeen patients (32%) had MPHD (3 SOD). At retesting, peak GH was <5 µg/l in 34 patients (64% - range 0.1-4.6 µg/l; group 1), between 5 and 10 µg/l in 11 patients (21% - range 5.4-9.6µg/l; group 2), and >10µg/l in 8 patients (15% - range 11.1-40µg/l; group 3).

Results
Among the patients in group 1, 16 of the 34 (47%) subjects had APH compared with 7 out of 19 (37%) subjects in groups 2 and 3.
Among the patients in group 1, 18 (53%) had an EPP ± abnormal stalk, compared with 5 (26%) in groups 2 and 3. EPP was associated with an absent or thin stalk in 15 cases (83%) in group 1 and in 4 (80%) in groups 2 and 3. In group 1, of the patients with a normal posterior pituitary (NPP) (47%), 6 (38%) had an abnormal stalk, compared with 3 out of 14 (21%) patients in groups 2 and 3.
Comparing patients with a GH peak >5µg/l, an EPP and/or PS abnormality was present in 6 (55%) patients in group 2 and in 2 cases (25%) in group 3.
Conclusions
The incidence of EPP ± an abnormal stalk decreased as the peak GH increased (Table). However EPP was present in those with peak GH of 5-10µg/L and >10µg/L. We suggest that a) the current cut-off for GHD in adolescence should be reviewed, and b) patients with a peak GH >5µg/L and a HP abnormality need to be followed up.

Group	EPP ± PS	NPP ± PS	Total abnormal	Total N	% abnormal
1	18	6	24	34	71
2	4	2	6	11	55
3	1	1	2	8	25

PO1-104 GH and IGF Use I

Is current endocrine care engaging patients with non tumour hypothalamic pituitary disease in the transition period?

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Background
Paediatric patients with hypopituitarism that is considered idiopathic or related to an MRI abnormality represent a significant cohort. Retesting GH status at final height is important to identify patients eligible for GH therapy in the transition period as recommended by the ESPE consensus statement.
Objectives
To audit the impact of consensus recommendations on initiation of GH therapy in the transition period in patients previously treated for GHD of non tumour origin in childhood.
Population
A retrospective analysis identified 42 patients who had received GH therapy until final height for non acquired GHD in childhood and were currently aged between 20 and 25 yrs. 12 patients had additional pituitary hormone deficiency, of those 6 had multiple pituitary hormone deficiencies (MPHD), ≥ 4 hormone deficiency, and these patients would be eligible for continuous GH therapy without retesting. Persistent GHD was diagnosed when both IGF-1 was <-2 SDS and peak GH during an arginine stimulation test was <5mcg/L.
Results
5 patients did not undergo retesting and were subsequently lost from the paediatric or young person clinic. 31 patients underwent retesting. 23 (including the 6 patients with MPHD who were eligible for continuous GH therapy) (62% of 37) were eligible for GH therapy in the transition period. 17 (74%) restarted GH therapy although 6 discontinued before the age of 25, 4 within a year of restarting. The mean duration of GH therapy in the remaining 11(48%) is 4.75 years. Of those eligible for GH therapy 7 (30%), including 2 with MPHD, are no longer attending clinic (paediatric, young person or adult) and are considered lost and 6 are reported to have poor compliance.
Conclusions
Although 75% of patients eligible for GH therapy in the transition period are restarting, only 48% continue. In addition 29% of patients are lost to follow up. Current endocrine care is failing to educate and encourage young people with hypothalamic pituitary disease to comply with medication and engage with endocrine services.

PO1-105 GH and IGF Use I

What is a poor growth response to rhGH therapy? A comparison of different definitions of growth response in 456 Nordic short statured children treated with rhGH

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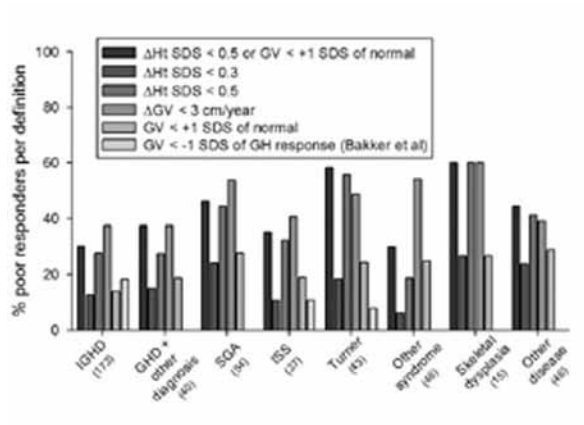
Recombinant human (rh)GH was until recently the only available therapy to promote growth in pre-pubertal children. Approval of rhIGF-I therapy in severe primary IGF deficiency has increased the focus on poor responders (PR) to rhGH. However, consensus on how to define a poor response is lacking.
We retrospectively assessed the proportion of PR using Δ height (Ht) SDS < 0.5 and/or growth velocity (GV) < +1SDS (primary endpoint) and other common definitions (figure legend). From 900 patient records in 10 centers, a heterogeneous group of 456 children (Ht SDS < -2) remaining pre-pubertal during at least one yr of rhGH were included. They were divided into 8 groups (figure; x-axis). Mean age at start of rhGH was 6.6 yrs, Ht SDS -3.31, target Ht SDS -0.93 and dose 31 mg/kgxd. First yr Δ Ht SDS was 0.69, GV SDS 2.59, and Δ GV 3.46 cm/yr. First yr Δ Ht SDS and GV were significantly lower in SGA, Turner and skeletal dysplasia compared with IGHD, GHD (incl. MPHD, malignancies) and Other syndrome (incl. PWS, Noonan). In SGA and Turner as many as 2/3 were PR. In IGHD, up to 1/3 of patients were PR. A similar proportion of PR in IGHD and ISS may suggest a similar pathology. First yr Δ Ht SDS and GV decreased with age while Δ GV and first yr GV SDS did not. In IGHD with start of rhGH < 10 yrs of age 22 % had Δ Ht SDS < 0.5 underlining the importance of early rhGH start. Basal GH and IGF-I correlated with response while rhGH dose, target Ht and birth weight correlated weakly.



This study may help to define PR to rhGH therapy. GV SDS is age-independent but the reference is old, not widely used and in many children GV SDS > +1 does not allow catch-up. Δ GV < 3 cm/yr relies on accurate basal GV and results in disproportionally many PR in SGA and Other syndromes. Bakker et al defines lower cutoffs in groups with poor mean response. A Δ Ht SDS of 0.3 is within the measuring error of a 1 yr Ht response arguing for a 0.5 cutoff. The high proportion of PR using Δ Ht SDS < 0.5 (preferred) may support a more active management of rhGH treated children considering compliance, dosing and alternative growth promoting therapy, reconsidering diagnosis or stopping therapy.

PO1-106 GH and IGF Use I

Growth hormone excess and gigantism in a 8 year old boy with Mc Cune Albright syndrome

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Mc Cune-Albright Syndrome (MAS) is defined by the trias of fibrous dysplasia of bone, café-au-lait skin pigmentation and hyperfunctioning endocrinopathies such as precocious puberty. In some children with MAS gigantism and growth hormone (GH) excess can be seen. MAS is a very rare condition with an estimated prevalence between 1/100'000 and 1/1'000'000. GH excess with or without MAS and its clinical consequences are also poorly reported in childhood and the therapeutical experience is very limited. We report of an 8-year old boy with MAS, gigantism and subsequent octreotide therapy because of proven GH excess. At the age of 4 years and 8 months, the boy was transferred to our department for the first time because of assumed precocious puberty in MAS. At this time point body height was 116.9 cm (SDS + 1.8), body weight 26.6 kg (SDS + 3.5), and no pubertal signs (Tanner I) could be confirmed in the clinical examination. Furthermore gonadotropines and testosteron were low, Greulich and Pyle bone age corresponded to 4 years and 6 months. Four years later, the boy was presented again at the age of 8 years and 9 months. Meanwhile he showed accelerated growth with body height of 153.8 cm (SDS + 3.4), body weight 55.6 kg (SDS + 3.9) and development of pubertal signs according to Tanner III. However the gonadotropines and testosteron parameters remained in the infantile range, but the prolactin (5'353 mlU/l), GH (38 ug/l), IGF-1 (81 nmol/l) and alkaline phosphatase (1038 U/l) were all clearly elevated. Repetitive measurements confirmed these findings and a glucose tolerance test was performed. GH values were not suppressable (>32 ug/l) and cranial MRI imaging showed multicystic transformations of the pituitary gland. Octreotide therapy was then started with 2 s.c. injections of 100 ug per day and growth factors markedly decreased already after 3 days (GH from 40.2 to 4.5 ug/l, IGF-1 from 84 to 63 nmol/l). Because of persistent hyperprolactinemia cabergolinum (Dostinex®) was added as therapeutical agent. The growth factors however were increasing again after only 4 weeks of therapy and octreotide therapy had to be intensified. Excess of GH in childhood remains a therapeutical challenge and has to be re-evaluated continuously.

PO1-107 GH and IGF Use I

Water deprivation test (WDT) and plasma antidiuretic hormone measurement in the differential diagnosis of polyuria and polydipsia in children: clinical and laboratory variations

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Diagnosis of Central Diabetes Insipidus (CDI) is based on clinical and laboratory studies. In the absence of a clear diagnosis, a water deprivation test (WDT) should be performed, however scarce information exists in children regarding the clinical and laboratory variations observed in patients with these disorders. To clarify these issues we studied 35 children (aged 6.98. ± 4.8 years) that underwent a WDT to rule out CDI. All patients were allowed ad libitum fluid intake until the WDT was initiated. Weight and urine output were measured hourly. Initial and final plasma and urinary osmolality (freezing point), and plasma ADH (extracted RIA) were measured. Final plasma ADH was plotted on a nomogram related to serum osmolality in a normal pediatric population. Patients were grouped according to their ability to concentrate the urine at the end of the WDT in Group A (n=24), poor concentration capacity, final U/P Osm ratio (f U/POsm) ≤ 1.5 and Group B (n=11) with a better concentrating capacity f U/POsm > 1.5.

Patients from group A had a significant decrement of weight ($p < 0.01$) and higher urine output ($p < 0.002$) than group B. Increment of plasma ADH after the test was significantly lower in group A (0.34 ± 1 pg/ml) vs. group B (2.47 ± 2.8 pg/ml), $p = 0.05$. The positive predictive value of f U/POsm ≤ 1.5 towards an inadequate final plasma ADH was 96%, and the negative predictive value of 72%, with a diagnostic efficiency of 84%.

In summary, decrements in body weight as well as the urine output during this test are useful information to define pediatric patients with a real incapacity to concentrate the urine. Moreover, the measurement of plasma ADH enabled us to establish that a cut-off f U/P Osm ratio ≤ 1.5 is likely associated with inadequate plasma ADH secretion in our population, highlighting the usefulness of the information provided by the WDT in the differential diagnosis of polyuria and polydipsia in the pediatric age.

PO1-108 GH and IGF Use I

Long-term safety and efficacy of Increlex® treatment: results from the Increlex growth forum database (IGFD) registry

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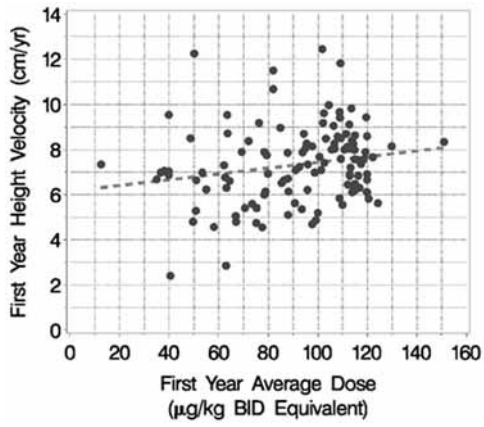
Objective: To report the long-term safety and efficacy of Increlex® (mecasermin [rDNA origin] injection) treatment in children enrolled in the Increlex Growth Forum Database (IGFD) Registry.

Methods: This is a multi-center, open-label, observational study monitoring the safety and efficacy of Increlex treatment. Children were eligible for enrollment in the IGFD Registry if they received Increlex from a qualified practitioner and their parents (or legally authorized representatives) gave informed consent. The first patients enrolled in the IGFD Registry in May 2006. Targeted adverse events (AEs) were selected AEs observed in previous Increlex or growth hormone (GH) studies.

Results: As of February 3, 2009, there were 610 patients enrolled in the IGFD Registry (mean age, 10.8 years). Of these, 506 patients had at least one follow-up visit and were included in the safety analysis. The two most frequently reported targeted AEs were hypoglycemia (6.3% of subjects) and headache (4.5%). There was no apparent relationship between dose and either AE for the range of doses studied. Three serious adverse drug reactions were reported in 3 patients: intracranial hypertension (n=2) and headache requiring cessation of therapy (n=1). The efficacy analysis included 119 prepubertal patients with height measurements and Increlex dose information available at one year. There were dose-dependent increases in first-year height velocities (HVs) (Figure; r=0.19, p=0.0342). First-year HVs were not predicted by baseline age, height SD score (SDS), baseline IGF-1 SDS or maximum stimulated GH.

Figure. First-year height velocity versus average Increlex dose.



Conclusions: IGFD Registry data suggest that Increlex treatment was generally safe and well tolerated and that there was no apparent relationship between dose and AEs for the range of doses studied. There were no new safety signals or unexpected serious adverse drug reactions. Data from prepubertal children with height and dose information at one year suggest that there were dose-dependent increases in first-year HVs such that higher Increlex doses were associated with better growth rates.

PO1-109 GH and IGF Use I

Development of multiple pituitary hormone deficiency (MPHD) in pediatric patients originally diagnosed with isolated GH deficiency (IsGHD)

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Patients originally diagnosed with IsGHD may develop additional pituitary hormone deficiencies later in life. This analysis aimed to identify factors that predict development of MPHD and to characterize the time course of specific hormone deficiencies. New-onset hormone deficiency (MPHD) during follow-up of 4833 pediatric patients with IsGHD in an observational study (GeNeSIS) was collected by check box on case report forms, by adverse event reporting or by reported initiation of replacement therapy. Limiting the time window of observation to patients with at least 3.5yr follow-up or development of MPHD within 4.5yr resulted in a total of 1058 patients, of whom 116(11%) developed at least 1 additional pituitary hormone deficiency. Baseline characteristics in those who developed MPHD vs. those who did not (mean[SD], p by ANOVA): age at diagnosis (9.2[4.4] vs. 8.2[3.4] yr, p=0.003), height SDS (-3.0[1.4] vs. -2.6[0.9], p<0.001), stimulated GH peak (median[1st,3rd quartile]) (3.2[1.1;7.0] vs. 7.3[4.7;9.8] µg/L, p<0.001) indicating more severe GHD in those who developed MPHD. Those who developed MPHD (%, p by chi-square test) had more frequently delivery complications (10.3 vs 4.8, p=0.017), breech presentation (11.2 vs 3.4, p<0.001), perinatal asphyxia (9.5 vs 2.8, p<0.001) and neonatal complications (15.5 vs 9.3, p=0.031). The proportions of patients who developed MPHD in the various diagnostic GHD sub-groups were clearly different: idiopathic (68/880, 8%), congenital (29/128, 23%), acquired (19/48, 40%), abnormal pituitary development (20/71, 28%). Deficiency of TSH was the most frequent (75%), followed by LH/FSH (16%), ADH (10%) and ACTH (9%); no PRL deficiency; 90% had 1 additional hormone deficiency and 10% had 2. In the entire population the time (yr) from diagnosis of GHD to the additional hormone deficiency (median[Q1;Q3]) was 0.9[0.4;3.2] for ADH, 1.6[0.8;3.2] for TSH, 2.0[1.4;2.7] for ACTH, and 4.6[2.5;8.6] for LH/FSH. Multiple logistic regression modeling identified the following significant (p<0.001) predictors for development of MPHD (odds ratio[95% CI]): organic cause of GHD (3.6[2.2-6.0]) and low stimulated GH peak (0.5[0.4-0.6]) for 1 unit increase in log(GH peak). **Conclusion:** In patients with IsGHD additional pituitary hormone deficiencies may develop especially in those with perinatal problems, organic cause of GHD and severe GHD. Therefore, patients with IsGHD require continuous monitoring for development of MPHD.

PO1-110 GH and IGF Use I

Prediction of final height in GH-treated children with GH deficiency (GHD)

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Various factors have been identified that influence the growth response to GH treatment in children with GHD. A number of mathematical models for prediction of short-term response have been reported, however prediction models for final height (FH) are sparse. Therefore, this study aimed to develop models for prediction of FH (cm) in GH-treated children with GHD. Patients from an observational study (GeNeSIS) were included, if they had a diagnosis of GHD with a maximum stimulated GH peak of <10µg/L, chronological age ≥2yr, and a FH measurement at the age of ≥14yr (females) or ≥16yr (males). Attainment of FH was assumed, if the last height velocity was <2cm/yr or epiphyses were closed or the last bone age was ≥14yr (females) or ≥16yr (males). A total of 253 patients with complete datasets (154 males) were randomly split into a model population (n=191) and a validation population (n=62), which did not differ with respect to the variables investigated. Linear multiple regression modeling was performed with the model population. The finally selected model with the response variable FH(cm) showed an adjusted R^2=0.78 and an error SD=4.5cm.

Table. FH prediction model: Parameter estimate of included variables, P and partial correlations.

Variable	Parameter estimate	P	Partial correlation
Intercept	47.9	<0.0001	
Baseline height (cm)	+0.459	<0.0001	0.24
Target height (cm)	+0.466	<0.0001	0.23
Gender (m=1, f=0)	+7.285	<0.0001	0.20
Baseline age (yr)	-1.879	<0.0001	0.16
GHD acquired (1), non-acqu. (0)	-6.384	<0.0001	0.11
Bone age/ chron. age at baseline	-13.520	<0.0001	0.10
Ln(max. GH peak) at baseline	-0.838	0.037	0.02

Applying this model to the validation population underestimated FH on average by 0.8cm. R^2 was 0.82 and error SD was 5.1cm. **Conclusions:** The presented model includes only easily available variables at start of GH treatment. Its precision is comparable to commonly used methods for FH prediction in GH-untreated patients with short stature (e.g. Bailey-Pinneau). This model may be helpful in predicting FH after GH therapy, which may be of interest especially in patients with 'partial GHD'.

PO1-111 GH and IGF Use I

Prediction of final height SDS gain in GH-treated children with GH deficiency (GHD)

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A number of mathematical models for prediction of short-term response to GH treatment in children with GHD have been reported. However, prediction models for final height (FH) gain due to GH treatment are lacking. Therefore, this study aimed to develop models for prediction of FH SDS gain (FH SDS – baseline height SDS). Patients from an observational study (GeNeSIS) were included, if they had a diagnosis of GHD with a maximum stimulated GH peak of <10µg/L, chronological age ≥2yr, and a FH measurement at the age of ≥14yr (females) or ≥16yr (males). Attainment of FH was assumed, if the last height velocity was <2cm/yr or epiphyses were closed or the last bone age was ≥14yr (females) or ≥16yr (males). A total of 253 patients with complete datasets (154 males) were randomly split into a model population (n=191) and a validation population (n=62), which did not differ with respect to the variables investigated. Linear multiple regression modeling was performed with the model population. The finally selected model with the response variable FH SDS gain showed an adjusted R^2=0.59 and an error SD=0.44 SDS.

Table. FH SDS gain prediction model: Parameter estimate of included variables, P and partial correlation (MPHD: multiple pituitary hormone deficiency).

Variable	Parameter estimate	P	Partial correlation
Intercept	-2.471	0.10	
GHD acquired (1), non-acqu. (0)	-1.209	<0.0001	0.18
Baseline age (yr)	+0.276	<0.0001	0.15
Target height (cm)	+0.052	<0.0001	0.15
Baseline height (cm)	-0.052	<0.0001	0.15
Bone age/ chron. age at baseline	-2.030	<0.0001	0.11
Gender (m=1, f=0)	-0.576	0.0003	0.07
Ln(max. GH peak) at baseline	-0.204	0.0006	0.06
MPHD (1) vs. isolated GHD (0)	+0.510	0.0314	0.03
GH dose at start (mg/Kg/wk)	+1.254	0.0436	0.02

Applying this model to the validation population overestimated FH SDS gain on average by 0.07 SDS; R^2 was 0.61 and error SD was 0.8 SDS. With a cutoff of 0.8 SDS for poor and good responders, the accuracy of prediction was 81%. We conclude that this model may be helpful in predicting the height gain due to GH therapy, which may be of special interest in patients with 'partial GHD'.

PO1-112 GH and IGF Use I

First year growth response to growth hormone (GH) treatment in short children born prematurely – data from KIGS

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The aim of the present study was to evaluate the 1st year growth response to GH treatment in short children born prematurely. We selected patients in the KIGS database with gestational age (GA) between 24 and 37 weeks and birth weight SDS available. They should remain prepubertal during the 1st year of GH treatment. Patients with chromosomal disorders, genetic syndromes, malformations, maternal history of alcohol or drug addiction and bone dysplasia were excluded. Values are given as median. Height measurements are expressed as standard deviation score (SDS) using Prader reference. A total of 3215 children were selected. They were grouped according to their GA as preterm (PT, GA < 37 weeks) and very preterm (VPT, GA < 33 weeks), and according to weight at birth in appropriate for gestational age (AGA, birth weight between -2 and 2 SDS) and small for gestational age (SGA, birth weight ≤ -2 SDS). Table 1 shows information at GH start.

At GH start	AGA			SGA		
	Preterm	Very Preterm	P	Preterm	Very Preterm	P
Age (years)	7.5	7.2	NS	6.7	6.0	NS
Bone Age	5.3	5.0	NS	4.2	4.8	NS
Height SDS	-3.2	-3.5	<0.001	-3.5	-3.5	NS
Weight SDS	-2.4	-3.0	<0.001	-3.1	-3.7	<0.001
Ht-MPH SDS	-1.8	-2.5	<0.001	-2.3	-2.7	0.035
HV (cm/ year)	4.7	4.7	NS	4.9	5.3	NS
IGF-I SDS	-1.9	-1.7	NS	-1.4	-1.2	NS
GH Dose (mg/kg/ week)	0.21	0.23	<0.001	0.24	0.28	<0.001

Ht-MPHt SDS= Parental Adjusted Height

Table 2 shows information at 1 year of GH treatment.

At 1 year of GH treatment	AGA			SGA		
	Preterm	Very Preterm	P	Preterm	Very Preterm	P
Height Velocity (cm/year)	8.4	8.5	NS	8.2	8.6	0.012
Change in Height SDS	0.64	0.67	NS	0.62	0.73	0.003
GH Dose (mg/kg/ week)	0.20	0.23	<0.001	0.25	0.31	<0.001

We conclude that short children born prematurely respond well to GH treatment. AGA children born PT or VPT respond similarly and SGA children born prematurely needed a higher GH dose for the same growth response of AGA children.

PO1-113 GH and IGF Use I

Safety and efficacy results of an 86-week, open-label study of PK-based, once-daily dosing of rhIGF-1 in children with primary IGF-1 deficiency

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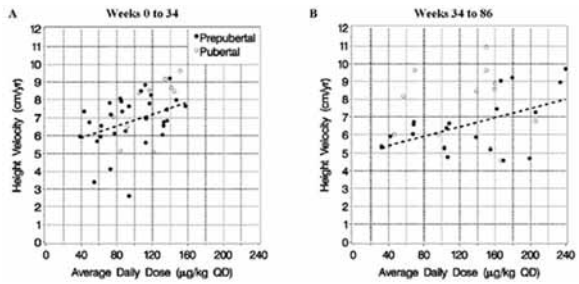
Introduction: Weight-based dosing is a traditional dosing strategy for rhIGF-1. The current study used a pharmacokinetic (PK)-based once-daily (QD) dosing strategy, with rhIGF-1 doses adjusted to serum IGF-1 targets, in children with primary IGF-1 deficiency (Primary IGFD).
Methods: This was an 86-week, open-label trial assessing QD dosing of rhIGF-1 in 45 treatment-naïve prepubertal children with Primary IGFD (height and IGF-1 SD scores [SDS] < -2, stimulated GH ≥ 7 ng/mL). The study evaluated the feasibility, safety and efficacy of rhIGF-1 when doses were adjusted at each visit to achieve serum IGF-1 targets. The initial IGF-1 SDS target was +1, but the annualized height velocities (aHVs) were only minimally increased, so the SDS target was increased to +2. The study was initially a 34-week pilot study, which was subsequently extended to 86 weeks.
Results: In the subjects who remained prepubertal, QD dosing significantly increased aHV in a dose-dependent manner during both weeks 0-34 (n = 33; r = 0.38, p = 0.031) and 34-86 (Figure; n = 19; r = 0.50, p = 0.028).
Figure.



For these prepubertal subjects, average daily dose correlated with age during weeks 0-34 (mean = 98 µg/kg; r = 0.47, p = 0.0056) and 34-86 (mean = 135 µg/kg; r = 0.48, p = 0.039). Younger children (4-7 years old) received average µg/kg doses that were approximately 70% of the other children's doses. The increase in mean dose during weeks 34-86 prevented the decline in aHV often observed during the second year of treatment with rhIGF-1. Most AEs were mild, transient, manageable and resolved without dose reduction. The most common AEs were headache (40% of subjects) and vomiting (40%), which

were concurrent in only one subject and the brevity of symptoms (~24 hours) did not suggest intracranial hypertension.
Conclusions: QD dosing of rhIGF-1 was generally well tolerated in children with Primary IGFD. There was a dose-dependent increase in aHV for prepubertal children during both weeks 0-34 and 34-86; these doses were age-dependent. PK-based dosing of rhIGF-1 resulted in younger children receiving lower doses and growing at a lower rate, and so is not recommended in young children.

PO1-114 GH and IGF Use I

Carotid artery intima-media thickness and body composition: sensitive and early markers of cardiovascular disease in growth hormone deficient young adult men

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Background: Growth hormone deficiency (GHD) in childhood may result in early onset cardiovascular disease (CVD) in adulthood. Beneficial effects of GH therapy in preventing CVD have been reported. The age of onset of the increased cardiovascular risks (CVR) in GHD adolescents/young adults is unclear. Previous studies evaluated CVR, carotid artery intima-media thickness (CIMT), cardiac structure and function in previously treated GHD adolescents who completed final height. No significant differences were noted 6-24 months after GH therapy was discontinued. Other studies have found abnormalities in body composition, lipid profile, vascular endothelial function, CIMT, cardiac morphology and performance in GHD adolescents 6-36 months after GH therapy was discontinued. Objective: To assess CVR, CIMT in 12 GHD male patients (mean age 21.6 yr) off GH therapy for at least 3 yr (mean 4.5 yr) compared with 12 age/sex matched control subjects. GHD patients had a mean peak GH of 4.75 ng/mL by GH stimulation test and had completed a mean of 3.5 years of GH therapy. Auxological data, metabolic parameters, body composition, CIMT measures were compared using t-test and Wilcoxon rank sum test. Results: Systolic and diastolic blood pressures, IGF-1, HbA1c, fasting lipid profile, glucose, and insulin levels were normal and comparable between groups. Mean weight was 11.6 kg greater in GHD group compared to healthy subjects. Mean percent body fat was significantly greater in GHD males (14%) vs. control subjects (9.3%). Statistically significant ($p<0.05$) and greater values were found in GHD patients for the following: BMI, waist circumference, hip circumference, waist/hip ratio, skin fold thickness. GHD patients exhibited a thicker mean CIMT (0.49±0.04 mm SD) compared to control subjects (0.47±0.03 mm SD). Atherosclerotic plaques are deposited in an irregular pattern, hence maximum CIMT (MaxCIMT) was determined. Mean of MaxCIMT was significantly increased in GHD patients (0.73±0.14 mm SD) vs. control subjects (0.59±0.05 mm SD) ($p<0.05$). Conclusions: Severely GHD patients treated until completion of linear growth demonstrated normal metabolic parameters. GHD patients showed increased visceral adiposity and increased CIMT as early as 4.5 years after GH therapy was completed. Changes in body composition and increased CIMT occur as independent, sensitive, cardiovascular risk factors in GHD young adult men.

PO1-115 GH and IGF Use I

Acromesomelic dysplasia, type maroteaux (AMDM) – is a growth hormone responsive skeletal dysplasia

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Introduction: Acromesomelic dysplasia, is a rare autosomal recessive cause of severe short stature caused by loss of function mutations in the Natriuretic Peptide Receptor (NPR)-B, the receptor for C-type natriuretic peptide. It has an integral role in growth plate development. The majority of skeletal dysplasias are remarkably resistant to growth hormone treatment in respect of short and long term sustained improvement in growth. In contrast, the molecular defect in AMDM entails defective NPR-B signalling, which might impair growth hormone (GH) and insulin-like growth factor-I (IGF-I) responsiveness at the growth plate. A case report (Olney et al, JCEM 2006) suggested that GH treatment (50 mcg/kg/day) was ineffective in this disorder. We report a successful trial of GH treatment in 2 siblings with AMDM at higher GH dose.
Patients & Methods: We present two siblings (AS & MS) diagnosed in early childhood with clinical and radiological signs of AMDM. Genetic studies for NPR mutations are in progress. Parents are first cousins. Mother Ht : -0.65 SDS, Father Ht: -2.08 SDS (Tanner & Whitehouse UK - TW). Assessment of their GH response to 3 hr Glucagon test showed relatively high GH levels throughout (AS 7.6 yrs, male, Height –5.6 SDS and MS 5.7 yrs, female, Height –4.5 SDS, with peak GH 65 mU/L & 36 mU/L). Serum IGF-I levels (60 and 37 mcg/L respectively) were below age/sex +/- 2SD Ref. Range (DPC Immulite). These results suggested a relative resistance to GH. GH treatment (®*Norditropin*) was started at a dose of 70 – 75 mcg/day sc. Annual height velocities increased from Pre- to 1 year on GH treatment from 3.7 to 6.9 (AS) and 4.5 to 9.0 (MS) cm/yr. Serum IGF-I levels increased to 315 and 318 mcg/L respectively. GH dose was increased to 1.5 mg/day for year 2 and 2.0 mg/day subsequently to sustain serum IGF-I levels near top of reference range for age (60 – 300 mcg/L). After 2 years of GH treatment, Ht SDS had improved to – 4.8 SDS (AM) and – 3.0 SDS (MS). No adverse effects of this relatively high GH dose were reported.
Conclusion: AMDM is a rare chondrodystrophic disorder secondary to NPR-B mutations. It differs from most skeletal dysplasias, showing a relative resistance to GH but responding to high doses of GH treatment which enhance circulating IGF-I levels and growth rate. Impact of GH treatment on final height remains to be determined.

PO1-116 GH and IGF Use I

The relationship between IGF-1 response and 1st year height velocity on two different doses of GH in idiopathic GH deficiency (IGHD): a randomized, prospective clinical study

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Background:A correlation between height gain and IGF-1 levels has been shown in some studies.However, there is not yet agreement that dose of GH should be tailored according to IGF-1 levels, except for safety issues.
Objective: To investigate the relationship between IGF-1 response and growth response after one year of treatment in children with IGHD on two different doses of GH.
Patients and methods: In this study we present the 1st year results of a 2-yr randomized, multicentre, prospective study. Forty-one patients with IGHD were randomized into 4 groups according to pubertal status and GH dose (μg/kg/day): Group 1(8prepubertal,GH dose :25), Group 2 (8pubertal, GH dose:25), Group 3 (13prepubertal,GH dose:37.5) and Group 4 (12 pubertal,GH:37.5).

Auxological measurements, IGF-1,IGFBP-3) were evaluated at months 0,3,6,12,18 and 24. Height (Ht) and IGF-1 were expressed as SDS. Results: The mean(SD) ages of the groups were 8.0(4.9), 12.9(1.1), 8.4(3.4), 13.4(1.9) years, respectively. Baseline results and the change in height ('Δ');Ht) SDS and ('Δ');IGF-1SDS of the patients are presented in Table. There was only a significant difference IGF-1SDS between Group 3 and Group 4 (p=0.04). Correlation between ('Δ');Ht SDS and ('Δ');IGF-1SDS was noted in Group 4 only(r=0.8, p=0.04).

Parameters at onset and on GH therapy, mean (SD) values are given.

	Initial Ht SDS	Basal IGF-1 SDS	Ht-SDS Δ	IGF-1 SDSΔ
Group1:	-4.0(1.6)	-2.7(2.4)	0.8(0.5)	1.5(2.7)
Group2:	-3.2(0.6)	-2.6(1.2)	0.5(0.2)	1.8(1.9)
Group3	-3.2(1.1)	-2.3(1.7)	0.8(0.7)	1.1(1.3)
Group4	-3.0(1.1)	-1.8(1.7)	0.4(0.7)	4.1(3.1)

('Δ'); Ht SDS of combined low dose groups (Group1+2) was 1.6 ±2.3 and lower than the respective value (2.2± 2.6) of high dose groups(Group3+4) (p<0.05) whereas the ('Δ');IGF-1SDS were 0.8±0.4 and 0.7±0.4 (NS), respectively. Conclusions: Increasing the dose of GH in puberty does not cause a parallel increase in height velocity. Furthermore the high values of IGF-1 SDS during puberty without a concomittant increase in height velocity raises an issue of concern for safety. There was no correlation between height gain and the change in IGF-1 SDS in either of the doses and in either of the groups. Evaluating IGF-1 for efficacy of GH therapy and tailoring the dose accordingly does not seem to be warranted. IGF-1 should be monitored and evaluated for safety of GH therapy.

PO1-117 GH and IGF Use I

Influence of the exon 3 – deleted polymorphism of the GH receptor on glucose and lipid metabolism in GH treated subjects with Prader-Willi syndrome: results of a preliminary study

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GH has contra-insulin actions and exogenous GH can reversibly reduce insulin sensitivity in patients treated with GH. It has been recently reported that the exon 3 – deleted (d3) isoform of the GH receptor (GHR) appears to be preventive for type 2 diabetes mellitus in adult subjects (GH&IGF Res 2007;17:392). Aim of this study was to investigate possible influences of the GHR-d3 isoform on glucose metabolism, lipid profile and BMI in children with Prader-Willi syndrome (PWS), a category of subjects at particular risk for glucose intolerance and type II diabetes, and in whom GH treatment is indicated. We studied 44 PWS subjects (19 male). Mean age was 25.3 (range: 3.0-42.8) years. Of the 44 subjects, 32 had been (n° 9) or were being (n° 23) treated with GH at a mean dose of 0.06 mg/kg/week, while the remaining 12 never received GH therapy, and were therefore used to analyse the sole baseline variables. Patients' genotype at GHR-exon 3 locus was determined by simple multiplex PCR. Height-SDS, BMI-SDS, fasting glucose, insulin, total and HDL-cholesterol, triglycerides, oral glucose tolerance test (OGTT), QUICKI and HOMA-R indexes were regularly evaluated during treatment. Informed written consent was obtained from the subjects and/or their parents where appropriate. The full-length (fl) GHR exon 3 isoform was found in 21 subjects in homozygosity (group fl); d3 was found in 21 subjects in heterozygosity and in 2 in homozygosity (group d3), which corresponds to the common distribution of the exon-3 isoforms in the general population. No differences in the above mentioned parameters were found comparing the two groups at treatment start, during and at the end of treatment. Furthermore, height-SDS and BMI-SDS did not differ between the two groups neither at baseline or during treatment. On the basis of these preliminary data, d3 does not seem to influence glucose and fat metabolism during GH treatment in PWS subjects.

PO1-118 GH and IGF Use I

The effect of oxcarbazepine and valproate therapy on growth in children with epilepsy

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This study aimed to evaluate the effects of monotherapy with valproate (VPA) or oxcarbazepine (OXC) on physical growth in children with idiopathic epilepsy. Seventy-six patients were initiated epilepsy medication of OXC or VPA in the study. They were evaluated at baseline and at the 3rd, 6th and 18th months of therapy for the standard deviation of height (Z-score) and body mass index (BMI). Serum ghrelin, insulin-like growth factor (IGF)-1 and insulin-like growth factor binding protein (IGFBP)-3 levels were measured. In prepubertal patients receiving OXC, height Z-scores increased at 3rd, 6th, and 18th months (p=0.009, p=0.008, p=0.001, respectively), while in pubertal patients significant elevations was observed at the 18th month of therapy compared to baseline (p=0.004). BMI values of prepubertal and pubertal patients at the 3rd, 6th, and 18th months of VPA therapy showed elevations compared to baseline (p=0.002, 0.009, 0.019; 0.001, 0.001, 0.002, respectively). In prepubertal OXC patients, serum IGF-1 and IGFBP-3 levels were significantly higher at the 18th months of therapy compared to baseline (p=0.005, p=0.004, respectively). Serum ghrelin levels, in pubertal VPA patients at the 18th month of therapy significantly decreased compared to baseline (p=0.006) while showing significant decreases at 6th and 18th months as BMI values increased or vice versa, in all VPA patients [(r=-0.361, p=0.028) (r=-0.588, p=0.001) respectively]. In epilepsy patients, OXC medication stimulated linear growth via mechanisms seemed to involve IGF-1 and IGFBP-3 release. Probably as a response, VPA cause alterations in body weight and serum ghrelin levels.

PO1-119 GH and IGF Use I

Perceptions of stature and self-esteem in ISS children from start of GH treatment to adult height

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AIMS: To investigate the perception of short stature and self-esteem from the perspective of the child and the family in idiopathic-short-stature (ISS) children treated with growth hormone (GH) from treatment start through to final height.
PATIENTS AND METHODS: One hundred and two short (below -2 SD) ISS children (mean age at start: 11.5 years) and their parents completed the Silhouette Apperception Test (SAT), a self-esteem questionnaire (I think I am) and a visual analogue scale designed for short stature children. All assessments were made at GP-GRC, Queen Silvia Children's Hospital, Gothenburg at start of treatment and thereafter yearly until adult height.
RESULTS: At adult height, a significant improvement was found in perceptions of physical characteristics (p<0.006), psychological strength (p<0.001) and body image (p<0.001) as reported by the children. Both the parents and children reported significant improvements in self-esteem (p<0.001&0.024, respectively) and the parents reported improved emotional stability (p<0.054). At start, 55% of the parents and 52% of the children indicated on the SAT a perceived height of -2SD, coinciding with their actual height. 57% of the parents and 87% of the children expected adult height would be normal or greater when asked at the start of GH treatment. When the children actually achieved their adult height, 91% of the parents and 86% of the children judged their height to be normal or above, whereas, 40% actually reached an adult height of greater than -1 SD (here defined as 'normal').
CONCLUSION: In a group of GH treated ISS children, the well-being and psychosocial functioning improved significantly from GH start to adult height. The perception of current height was overestimated by the child and the parents, both prior to GH treatment and at adult height perhaps reflecting the high level of self-esteem.

PO1-120 GH and IGF Use I

Genomic markers improve the prediction of short-term IGFBP3 response to growth hormone (GH) in girls with Turner syndrome (TS): the PREDICT study

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Background: The PREDICT Phase IV study is examining the relationships between conventional biomarkers and genomic markers after 4 weeks of GH therapy in GH treatment-naïve prepubertal children with GHD or TS.
Objective: To identify factors that best predict IGFBP3 SDS level after 4 weeks of recombinant human GH therapy in girls with TS using PREDICT data.
Methods: Akaike's Information Criteria for multivariate model selection were used to identify the factors best predicting IGFBP3 SDS level at week 4 in 149 girls with TS (intention-to-treat population). Potential covariates included birth, auxological, treatment and parental characteristics, and baseline biomarkers. The best-fit model was then applied in subgroup analyses by selected genetic markers (single nucleotide polymorphisms; SNPs) in 117 girls with TS to evaluate their impact on IGFBP3 SDS level prediction.
Results: The best-fit model identified included the following predictive factors:
• IGFBP3 SDS at baseline
• Fasting triglycerides at baseline (mmol/L)
• TSH at baseline (mIU/L)
• Age at baseline (years)
• Weight SDS at baseline
• Mean GH dose (mg/kg/day)
The coefficients for all these factors were positive, except for fasting triglycerides at baseline and age at baseline, which were negative. These factors accounted for 53% of the variability in IGFBP3 SDS level at week 4 (N=118). The absence of a SNP in PIK3CB (N=89) increased the adjusted R^2 to 60%. The absence of SNPs in both BCL2 and CDK4 (SNP B) (N=55) increased the adjusted R^2 to 64%.
Conclusions: The PREDICT study provides evidence that adding selected genomic markers to conventional predictive factors in a best-fit model improves the prediction of IGFBP3 SDS level at week 4 in girls with TS, stressing that pharmacogenomic research is key to understanding responsiveness to GH.

PO1-121 GH and IGF Use I

Genomic markers improve the prediction of short-term IGF-I response to growth hormone (GH) in GH-deficient (GHD) children: the PREDICT study

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Background: The PREDICT Phase IV study is examining the relationships between conventional biomarkers and genomic markers after 4 weeks of GH therapy in GH treatment-naïve prepubertal children with GHD or Turner syndrome.
Objective: To identify factors that best predict IGF-I SDS level after 4 weeks of recombinant human GH therapy in children with GHD using PREDICT data.
Methods: Akaike's Information Criteria for multivariate model selection were used to identify the factors best predicting IGF-I SDS level at week 4 in 169 children with GHD (intention-to-treat population). Potential covariates included birth, auxological, treatment and parental characteristics, and baseline biomarkers. The best-fit model was then applied in subgroup analyses by selected genetic markers (single nucleotide polymorphisms; SNPs) in 160 children with GHD to evaluate their impact on IGF-I SDS level prediction.
Results: The best-fit model identified included the following predictive factors:
• IGF-I SDS at baseline
• Study period adherence: received/planned (%)
• Weight SDS at baseline
• Mean GH dose (mg/kg/day)
• Age at baseline (years)
The coefficients for all these factors were positive. These factors accounted for 66% of the variability in IGF-I SDS level at week 4 (N=162). When subgroup analyses were carried out, the presence of a SNP in CDK4 (SNP A) (N=35) or in LEPR (N=32) increased the adjusted R^2 to 81% and 71%, respectively.
Conclusions: The PREDICT study provides evidence that adding selected genomic markers to conventional predictive factors in a best-fit model improves the prediction of IGF-I SDS level at week 4 in children with GHD, stressing that pharmacogenomic research is key to understanding responsiveness to GH. How these genes, not in the traditional scope of the GH-IGF-I axis, relate to GH action deserves additional research.

PO1-122 GH and IGF Use I

Second neoplasms (SN) in growth hormone (GH) treated childhood cancer (Ca) survivors (CCS): analysis from a large prospective observational study

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The Childhood Cancer Survivor Study (CCSS) has suggested that GH-treated CCS have a low, but increased, risk for SN compared to untreated CCS[1,2]. We reviewed the incidence of SN and risk factors in 442 CCS identified from 13081 GH-treated patients enrolled in the GeNeSIS observational study (Table 1).

Table 1: Characteristics of GH-treated CCS in CCSS[2] and GeNeSIS

	CCSS, N=361	GeNeSIS, N=442 unless stated)
Age (yr) at Ca diagnosis [median (range)]	3.5 (0-17.2)	5.4 (0-16.8), N=386
Male	65.7%	57.7%
Medulloblastoma survivor	19.7%	33.5%
Leukemia survivor	33%	14.0%
Age (yr) at GH therapy start [median (range)]	11 (1.0-20.8)	10.8 (1.0-18.0), N=432
Duration (yr) of GH therapy [median (range)]	4.6 (0.1-14.0)	2.8 (0-13.5), N=396

SN were reported for 14 GH-treated CCS (Table 2; 3.2%, 11 male, all previously irradiated) during 3087 person-yr of follow-up (N=376; with dates of 1st Ca diagnosis, last follow-up or SN diagnosis available), giving an estimated rate of 4.5/1000 person-yr of follow-up.

Table 2: SN in GeNeSIS

*1st Ca	SN	Yr after GH start	Yr after 1st Ca
E	Bone sarcoma	9.7	13.4
L	Bone cyst	1.6	9.1
M	Meningioma	5.0	7.3
M	Meningioma	3.0	7.4
M	Meningioma	1.9	13.3
M	ALL	1.8	8.4
M	AML	2.2	5.2
M	Myelodysplastic syndrome	2.4	6.1
M	Glioma	1.4	4.7
M	Pleomorphic xanthomatous astrocytoma	8.8	11.1
M	Granular cell tumor	3.0	6.3
M	Spinal cord tumor	1.6	10.6
N	Osteochondroma	1.6	10.4
N	Pheochromocytoma	4.4	8.6

*(E=Ependymoma, L=Leukemia, M=Medulloblastoma, N=Neuroblastoma)

Ten of the 148 medulloblastoma CCS had SN (6.8%); menigiomas were the most common SN (21.4%), all following medulloblastoma. The mean period between diagnosis of SN and start of GH therapy was 3.5 yr. The mean GeNeSIS latency period for developing SN from 1st Ca diagnosis was 8.7 yr overall and 9.3 yr for meningiomas specifically. The CCSS meningioma latency period in GH-treated CCS was significantly shorter than in untreated CCS (12.2 vs 19 yr[2]); this appears to be supported by the GeNeSIS data but may be explained, at least in part, by ascertainment bias.
The GeNeSIS SN rate of 3.2% in CCS treated with GH for a median of 2.8 yr compares to 4.2% in those treated for 4.6 yr in the CCSS[1]. Despite the differences between the CCSS and GeNeSIS cohorts, the rate of SN in GH-treated CCS from GeNeSIS is consistent with the CCSS findings.
[1]Sklar et al. 2002. JCEM 87: 3136-41.
[2]Ergun-Longmire et al. 2006. JCEM 91: 3494–98.

PO1-123 GH and IGF Use I

Ten family members with a growth hormone gene mutation and treatment with growth hormone since 1976

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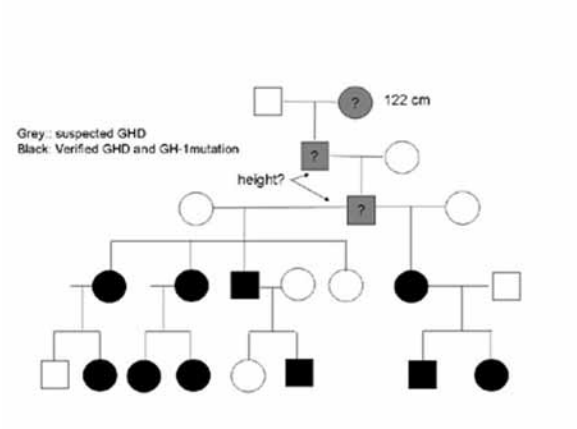
Background: Familial short stature is not always idiopathic, but may rarely be due to dominant mutations, including growth hormone 1 (GH-1) gene mutations.
Methods: Descriptive, retrospective study of a family with GH-1 mutation, the index patient treated with growth hormone since 1976.
Results: The index patient was diagnosed aged 6 years in 1976 with isolated growth hormone deficiency (GHD; height below -5SDS, bone age 4 ys retarded, no GH response to insulin test, normal pituitary by later MRI) and was treated with human pituitary-extracted growth hormone weekly, then 6 times weekly, but with poor growth response with an adult height of 144 cm. She had two children both diagnosed early (11 and 5 mo., respectively) with isolated congenital GHD. Three half siblings of the index patient also had GHD and was treated with rGH daily from late childhood. Of their 6 children, 4 were diagnosed early (-12-22 mo) with GHD.



The affected family members were born at gestational age 35-40 weeks, birth weight was normal (2120-3100 g), and average birth length was -.6 SDS only (43-50 cm). Linear growth was quickly compromised and bone age was quickly retarded. Basal p-GH and IGF-I levels were low and no response was seen on GH stimulation tests. All 5 children had a good response to low dose of recombinant growth hormone (rGH, 16-22 mcg/kg/d) with normalisation of growth.
Mutational analysis revealed a known, dominant GH-1 mutation, IVS 3+2 T>A, in the all affected family members.
Conclusion: Short or poorly growing family members to a patient with severe GHD benefits from early detection of GHD and modern treatment with rGH.

PO1-124 GH and IGF Use I

Analysis of cytosine adenine repeat polymorphism of the IGF-I promoter gene in children with idiopathic short stature

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Purpose: A polymorphism in the IGF-I gene promoter region is known to be associated with serum IGF-I levels, birth weught, and body length, suggesting that IGF-I polymorphism might influence postnatal growth. The present study aimed to investigate the role of this polymorphic cytosine-adenine(CA) repeat of the IGF-I gene in children with idioapthic short stature(ISS).
Methods: In this study,131 children(72 boysand 59 girls) diagnosed as ISS, aged 7-15 years were involved. Genomic DNA was extracted from anticoagulated peripheral whole blood. The primers were designed to cover the promoter region xcontaining the polymorphic CA repeat. Date were analyzed using GeneMapper software. The correlations between age and serum IGF-I levels were analyzed using Spearmans correlation coefficient.
Results: The CA repeat sequence ranged from 15 to 22, with 19 CA repeats the most common with an allele frequency of 40.6%. Homozygous for 19 CA repeat was 13.0%, heterozygous for 19 CA repeat was 56.5%, and 19 CA non-carrier was 30.5%. The three different genotype groups showed no significant difference in height, body weight and body mass index, and serum IGF-I levels. The serum IGF-I level and age according to the IGF-I genotypes were significantly correlated in the entire group, 19 CA repeat carrier group, and the non-carrier group. The three groups also showed no significant differences in the first year responsiveness to GH treatment.
Conclusion: There were no significant different correlations between 19 CA repeat polymorphism and serum IGF-I levels according to genotype. Our results suggest that the IGF-I 19 CA repeat gene polymorphism is not functional in chidren with ISS

PO1-125 GH and IGF Use I

The effect of four weeks of growth hormone (GH) therapy on whole blood gene expression profiles in growth hormone deficient (GHD) or Turner syndrome (TS) children: the PREDICT study

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Background: The PREDICT Phase IV study is investigating relationships between genomics and biomarkers before and during GH therapy in children with GHD or TS.

Aim: Analysis of mRNA expression in relation to changes in IGF-I SDS after 4 weeks of GH therapy in GH treatment-naive prepubertal children with GHD (n=169) or TS (n=149).

Methods: Blood samples were obtained before and after 4 weeks of GH therapy (Saizen®, Merck Serono). Serum IGF-I was measured centrally (Immulite®, Siemens), converted to standard deviation scores (SDS, Elmlinger) and change in IGF-I SDS after 4 weeks of GH therapy was categorized by quartiles. mRNA from blood was used for microarray analysis (GeneChip® HG-U133 plus 2.0, Affymetrix). Genes showing a significant change in expression ($p<0.05$), ≥1.4-fold change in absolute value and a false discovery rate (FDR) <25% were analysed in children with an IGF-I SDS change within the lower (Q1) or upper (Q3) quartiles, designated low and high IGF-I responders, respectively. If differentially-expressed transcripts were extensive, only the first 200 were listed with the FDR of the last indicated. Treatment response compared differential expression at week 4 with baseline (BL) in low and high responders. Patient response compared differential expression between low and high responders at BL and at week 4.

Results: Treatment response in GHD low responders resulted in 200 differentially-expressed transcripts (FDR≤7.9%), related to ubiquitin/proteasome, mitochondrial respiration and cell proliferation pathways. Treatment response in TS low responders resulted in 66 differentially-expressed transcripts, related to inflammation and cell proliferation pathways; 22 were common to both GHD and TS low responders. No treatment response transcripts were identified for GHD or TS high responders. Patient response for GHD identified 22 differentially-expressed transcripts at BL and 44 at week 4 and, for TS, 200 at BL (FDR≤17%) and at week 4 (FDR≤0.3%).

Conclusions: Significant changes in gene expression over 4 weeks of GH therapy were only seen in GHD and TS low IGF-I responders, in different signalling pathways in the two conditions. The absence of differentially-expressed genes in high responders may relate to greater heterogeneity between subjects. For patient response, when comparing low and high responders at BL and 4 weeks, differentially-expressed genes were identified in both TS and GHD, with larger numbers at greater significance in TS.

PO1-126 GH and IGF Use I

Correlation between genetic markers and short-term IGF-I response: the PREDICT study

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Introduction: The PREDICT Phase IV study is examining the relationships between genomic markers and conventional biomarkers after 4 weeks of GH therapy in children with GHD or Turner syndrome (TS).

Aim: To evaluate the association of single nucleotide polymorphisms (SNPs) in 98 growth- and metabolism-related genes with the change in IGF-I SDS after 4 weeks of GH therapy in GH treatment-naïve prepubertal children with GHD (n=160) or TS (n=139).

Methods: Blood was taken at baseline for DNA extraction and IGF-I measurement and after 4 weeks of GH therapy (Saizen®, Merck Serono) for repeat IGF-I level. 98 candidate genes, selected by an international advisory board, were analysed for 1536 SNPs (GoldenGate, Illumina) and IGF-I levels analysed centrally (Immulite®, Siemens) and converted to standard deviation scores (SDS, Elmlinger). The association of genotypes at a given locus with IGF-I SDS change was assessed by non-parametric tests, with adjustment for multiple testing. SNPs with a significant association underwent categorical analysis: carriage rate of a given genotype was evaluated in children classified as low (Q1), intermediate (Q2-Q3), or high (Q3) responders based on quartiles of IGF-I SDS change.

Results: The responder groups were defined by a week 4 IGF-I SDS change <0.81 for Q1 and >1.91 for Q3 in GHD, <1.15 for Q1 and >2.63 for Q3 in TS. Categorical analysis short-listed 5 markers in GHD, 3 single SNPs and 2 combinations, and 9 markers in TS, 6 single and 3 combinations, with the probability of having a low/high IGF-I SDS at week 4 shown in Tables 1 and 2.

Incidence of SNPs and probability of low IGF-I SDS response in GHD

	Incidence (%)	Low responders within SNP category (%)
CDK4 (SNP A)	22	9
CDK4 (SNP A) or LEPR or both	38	11

Incidence of SNPs and probability of high IGF-I SDS response in TS

	Incidence (%)	High responders within SNP category (%)
CDK4 (SNP A)	42	9
CDK4 (SNP B)	19	0
BCL2	19	11
SH2B2	27	13
PI3KCB	26	14
SH2B2 or BCL2 or both	41	12
PI3KCB or BCL2 or both	38	13
CDK4 or BCL2 or both	55	9

In GHD, carriers of the PTPN1 SNP (28% of the cohort) had a 16% probability of being a high responder. In TS, carriers of the ARRB1 SNP (22% of the cohort) had a 17% probability of being a low responder.

Conclusions: SNPs located in growth and metabolism genes are associated with the week 4 change in IGF-I in GHD and TS. Selected SNPs, alone or in combination, are correlated with high or low IGF-I response to GH therapy in children with GHD or TS.

PO1-127 GH and IGF Use I

Clinical, hormonal and radiological characteristics of 117 patients with short stature and ectopic posterior pituitary lobe

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Introduction: the biological criteria for the diagnosis of growth hormone deficiency (GHD) based upon provocative growth hormone (GH) stimulation tests are still difficult and controversial. Magnetic resonance imaging (MRI) of the pituitary-hypothalamic axis is of great value for the diagnosis of non-acquired GHD. Ectopic posterior pituitary lobe (EPL), a marker of pituitary stalk interruption syndrome, is found in many cases of growth hormone deficiency. **Objective:** the aim of this study was to describe the clinical, hormonal and radiological characteristics of patients with short stature and EPL. **Patients and methods:** one hundred and seventeen patients (86 males) with short stature associated with EPL were characterized clinical and biologically. Endocrine evaluation included growth hormone stimulation tests and dosage of free thyroxin, TSH levels, prolactin, cortisol, FSH, LH and testosterone or estradiol (in patients of pubertal age). Pituitary MRI scanning obtained specific pituitary cuts of 3-mm-thick slices on sagital and coronal views. **Results:** The mean ages at the beginning of treatment and at last evaluation were, respectively, 12.5 ± 5.5 (1.7- 27.1) and 18.0 ± 6.0 (5.6-32.6) years. The frequency of breech delivery was 33.3%. One patient had anophtalmia. Cryptorchidism and microphallus were seen in seven boys. Among 106 patients who underwent provocative GH stimulation tests, nine (8.5%) showed GH peaks clearly above the reference values. Combined pituitary hormonal deficiency (CPHD) was described in 74 patients (63.2%). Pituitary hypoplasia was described in 112 cases and abnormal pituitary stalk was also seen in 112 cases (80 patients with absent stalks and 32 with very thin ones). Malformations of brain structures were found in ten patients. There was no difference in the frequency of CPHD in the patients with either absent or very thin pituitary stalks, but TSH and GnRH deficiencies were more prevalent in the first group (p=0.006 for both). **Conclusion**: this study shows the clinical and hormonal heterogeneity of patients with EPL and points out the importance of the pituitary MRI especially in patients with unexplained short stature and normal GH peak levels after stimulatory tests.

PO1-128 GH and IGF Use I

The exon 3 deleted/full length growth hormone receptor polymorphism and response to GH therapy in GH deficiency and Turner syndrome: a multicenter study

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Background: The exon 3 deleted / full length (d3/fl) GH receptor polymorphism (d3/fl GHR) has been associated with responsiveness to GH therapy in some diagnostic groups.However there are still controversies on this issue.
Aim: To evaluate the effect of d3/fl GHR polymorphism on the growth in the 1st and 2nd year of GH therapy in GH deficiency (GHD) and in Turner Syndrome (TS).
Materials and methods: 199 patients with GHD (109 M / 90 F) and 44 patients with TS were included in the study. Ages of the patients are shown in Table1 and Table2. GH was given in a mean (SD) dose of 29.5(25.8) µg/kg/day in GHD and 41.3(8.4) µg/kg/day in TS. Anthropometric parameters and IGF-1, IGFBP-3 were evaluated annually. GHR isoforms were studied using simple multiplex PCR method. Height was expressed as SDS. 1st year and 2nd year height velocity(HV) was expressed in cm/year.
Results: The growth response and GHR isoforms are presented in Table1 and Table2. There were no differences between TS and GHD in distribution of GHR exon 3 isoforms. There was a significant increase in height SDS in both diagnostic groups on therapy; however there were no differences in height SDS and Δ height velocity between fl/fl; fl/d3 and d3/d3 groups. Neither was a correlation between distribution of GHR exon3 isoforms and change in IGF-1 SDS and IGFBP-3 SDS levels on GH therapy in either of the diagnostic groups.

Anthropometric findings of GHD patients in three GHR exon3 genotype groups, mean(SD) values are given.

	fl/fl (n=57)	fl/d3 (n=105)	d3/d3 (n=37)
Age (yrs) onset of therapy	9.9(4.2)	10.3(3.7)	11.2(3.2)
Height SDS- 0	-3.5(1.8)	-3.6(1.2)	-3.4(1.3)
Height SDS -1st	-2.7(2.4)	-3.1(1.1)	-2.7(2.4)
Height SDS -2nd	-2.6(1.2)	-3.4(5.2)	-2.7(1.3)
HV 1st yr (cm/yr)	9.8(3.3)	9.3(3.0)	8.8(2.5)
HV 2nd yr (cm/yr)	7.2(2.1)	7.8(1.9)	7.4(2.6)

Anthropometric findings of TS patients in three GHR exon3 genotype groups, mean(SD) values are given.

	fl/fl (n=12)	fl/d3 (n=22)	d3/d3 (n=10)
Age (yrs) onset of therapy	10.7(3.1)	9.8(3.1)	9.8(3.1)
Height SDS -0	-3.3(1.1)	-3.3(1.2)	-3.1(1.4)
Height SDS -1st yr	-0.9(7.2)	-3.4(1.6)	-3.1(1.8)
Height SDS-2nd yr	-2.1(1.1)	-3.2(1.1)	-3.1(1.9)
HV -1st yr(cm/yr)	6.1(2.8)	6.8(2.1)	7.2(1.5)
HV-2nd yr(cm/yr)	6.7(2.7)	6.4(1.9)	6.6(1.7)

Conclusions: These results suggest that responsiveness to GH therapy does not differ according to the exon 3 GHR genotypes in GHD and TS.

PO1-129 GH and IGF Use I

Isolated growth hormone deficiency (GHD) versus multiple pituitary hormone deficiency (MPHD): phenotype and response

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Background:GH therapy increases height prognosis in GHD successfully, however, adult height data are still limited.
Aim: To investigate near adult height (NAH) in patients with idiopathic GHD (GH peak < 10 µg/L, no organic pathology) grouped as isolated GHD and MPHD.
Methods: All patients were registered in KIGS database. Median (10to90th percentile) values are given and measurements are expressed as SDS. Parental adjusted height(PAH) defined as:height SDS-(fathers height SDS-mothers height SDS/1.61).
Results:GH was started at an age of 9.2(4.9to12) in isolated GHD (n= 1619, 60 % males) and at 7.7(2.8 to12.2) years in MPHD (n=554, 66 % males) (p<0.001) in similar doses (0.20mg/kg/wk). Height SDS at onset of therapy was -3.1(-4.5 to-2.1) and -3.8(-5.7 to-2.3), respectively (p<0.001). Max GH peak and IGF-ISDS were significantly lower in MPHD than in isolated GHD. Both groups showed a significant increase in height SDS at 1 year and up to the onset of puberty.PAH at start of puberty was -0.1(-1.6 to1.1) in isolated GHD and -0.4(-1.9 to1.2) in MPHD. Parental adjusted NAH SDS in isolated GHD was 0.0 (-1.5 to 1.2) and slightly but significantly higher than the NAH [-0.3(-2.1 to 1.2),p<0.001] in MPHD. In isolated GHD total Δheight SDS on

GH therapy was 1.6(0.5to3.2) and Δheight SDS in puberty was 0.1(-0.7to1). The respective values were 2.6(0.9to4.6) and 0.2(-1to1.3) in MPHD. PAH was slightly lower in girls than in boys in isolated GHD but no gender difference was observed in MPHD. Puberty was induced in 74 females at age 13.6 (11.7 to 16.8) and in 132 males at 14.4 (12.6 to 17.2) years. Spontaneous puberty occurred at age 11.8 (10.3 to 13.5) years in females(n=342) and at 12.8 (11.3 to 14.5) years in males (n=489). There were no differences in NAH between the induced and spontaneous puberty groups. Age at onset of puberty did not show correlation with NAH and correlated negatively with total pubertal growth in both sexes. Multivariate analysis showed that higher birth weight, taller parents, greater height at onset, 1st year growth response and index of responsiveness were the most important predictors of NAH in both isolated GHD and MPHD.

Conclusion:89%of isolated and 81%of MPHD children reach a NAH within their genetic potential on GH therapy. Most of the height gain occured during prepuberty. There seems to be no need to delay puberty for further height gain provided that the child is treated adequately in prepubertal period.

PO1-130 GH and IGF Use I

Traumatic brain injury (TBI) in children: a retrospective report from the Genentech national cooperative growth study (NCGS) on the use of recombinant human growth hormone (RHGH) in pediatrics

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Background: Much remains unknown about the prevalence of TBI in children or the resultant pituitary hormone disturbances. A recent report from KIGS, an international rhGH registry, revealed that 0.6% of their pediatric idiopathic growth hormone deficiency (IGHD) patients were diagnosed with TBI-induced GHD, a surprisingly low percent compared to what could be expected from epidemiologic studies of TBI in childhood[1]. Given the well-established connection between head trauma and hypothalamic pituitary abnormalities, more data must be collected to alert physicians about the endocrine consequences.

Objective: To determine how often TBI is mentioned as an etiology for GHD in NCGS and compare the clinical characteristics of the TBI patients to those with IGHD and other causes of organic (O)GHD.

Design/Methods: From 1985-2008, 143 patients in NCGS carried a diagnosis of GHD secondary to TBI, (0.6% of IGHD). 100 were rhGH-naïve and their characteristics were compared to patients diagnosed with IGHD and OGHD.

Results: Baseline characteristics of TBI and IGHD groups differed only in the use of antidiuretic hormone and corticosteroids. Compared to the OGHD group, the TBI group had fewer females, were older at enrollment, had higher mean maximum stimulated GH, and a smaller percentage of glucocorticoid use.

Table 1: Baseline characteristics (mean)

	TBI (n=100)	OGHD (n=6840)	IGHD (n=23,535)
Age (years)	10.8	9.1*	10.6
Female (%)	22.0	37.5*	24.8
Prior Growth Rate (cm/yr)	4.4 (66)	4.2 (3844)	4.5 (11,115)
Ht SDS	-2.5	-2.4	-2.5
Pubertal (%)	32.3	22.0*	27.1
Max Stimulated GH (ng/mL)	6.4 (83)	3.5* (4761)	5.7 (17, 854)
Concomitant meds ADH (%), Glucocorticoids (%)	9.0, 8.0	9.7, 17.4*	0.2*, 0.6*

(n) for those with complete information. Comparisons made to TBI group * p-value <0.05

Conclusions: Our data are similar to those previously reported with presumably relatively low numbers of TBI patients. While the NCGS does not capture the age of the TBI event, the similar enrollment age and ht compared to IGHD suggests it is only when growth failure becomes severe, rather than TBI history and initial growth failure, that there is evaluation and ascertainment. More education of pediatricians regarding the association of TBI and pituitary dysfunction may result in earlier suspicion and diagnosis.

[1] McDonald A et al (2008). Traumatic brain injury is a rarely reported cause of growth hormone deficiency. *J Pediatr*; 152: 590-3

PO1-131 GH and IGF Use I

Different thresholds of metabolic GH effects in prepubertal children

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In addition to growth hormone (GH) effects to promote linear growth in children, GH also has substantial effects on insulin sensitivity, lipolysis, lipids, and body composition. A dissociation between anabolic and lipolytic GH effects has been suggested.

Objective: The objective of the present study was to further investigate dissociated GH effects by calculating the GH doses to attain half of a given metabolic effect, the effective dose 50% (ED50%).

Hypothesis: The hypothesis was that there are dose-dependent thresholds in different variables reflecting metabolism.

Design: A randomized, prospective, multicentre trial was performed for a 2 years period, with two treatment regimens in short prepubertal GHD and ISS children a) individualized GH dose with six different dose groups ranging 17-100 µg/kg/day (n=87) and b) fixed GH dose of 43 µg/kg/day (n=41).

Results: Contrary to changes in fat mass, leptin, lipids and skinfold measurements, there was evidence for different thresholds in metabolic variables for a given GH dose when performing ANOVA, $p<0.001$. Δ was calculated as the difference between 2 years and start of GH treatment. Besides Δ height (SDS), growth related variables like weight SDS and Δ BMI (kg/m²); measures of body composition such as Δ fat-free mass (FFM) (kg), Δ FFM index (FFMI) (kg/m²), Δ waist (cm), Δ hip (cm); as well as biochemical markers like Δ IGF-1 (SDS), Δ IGFBP-3 (SDS); Δ insulin (mU/L) showed dose-dependency with different ED50% levels (Table 1).

Table 1

GH dose (µg/kg/d)	Δ FFMI (kg/m²)	Δ height SDS	Δ insulin (mU/L)	Δ weight (SDS)	Δ IGF-1 (SDS)	Δ Hip % (cm)
17	-2.2	0.8	3.7	0.6	1.8	11
33	0.4	0.9	4.8	0.6	2.0	11
40	0.1	1.5	5.0	1.0	2.3	12
50	0.8	1.4	6.2	1.1	2.6	13
66	0.8	1.7	8.8	1.5	3.5	16
100	1.7	2.0	8.8	2.0	4.3	21
50% Δ effect	-0.3	1.4	6.2	1.3	3.0	16
ED 50%	*37.0*	*52.5*	*52.5*	*58.6*	*59.3*	*65.7*
ANOVA (p-value)	*<0.001*	*<0.0001*	*<0.05*	*<0.0001*	*<0.0001*	*<0.0001*

Mean group changes (Δ) of metabolic variables between 2 years of GH treatment and start in the six different GH dose groups are listed. Bold values are closest to half of the mean GH dose effect within each dose group [50% Δ effect]. Ranges of GH doses closest to [50% Δ effect] are marked categorically with grey boxes. Effective GH dose at [50% Δ effect] is calculated according to regression of Δ group means [ED 50%]. P-values for mean differences between the GH doses for a linear trend are specified (one-way ANOVA). Δ FFMI (Δ fat-free mass index); Δ hip % (Δ hip circumference at 2 years in % of hip circumference at start).

Conclusions: Differences of the ED50% on metabolic variables were seen in-between different GH dose spans. Thus we propose that there are different thresholds in GH effects on variables reflecting different metabolic aspects, suggesting muscle tissue being more sensitive than linear growth, GH induced insulin resistance, the rise in IGF-1 and the increase in hip circumference as a measure of 3-dimensional body growth.

PO1-132 GH and IGF Use I

Pseudoexon (6 Ψ) mutation in three siblings with growth hormone insensitivity: response to rIGF-I therapy

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Background: Growth Hormone insensitivity (GHI) may be caused by a range of mutations in genes coding for proteins with specific roles in the GH-IGF-1 axis. The majority of molecular defects have been identified in the extracellular domain of the GH receptor (GHR). GHI due to an intronic mutation in the *GHR* gene has been recently described and may result in the activation of a pseudoexon and the incorporation of additional amino acids in the receptor extracellular domain. We now report the molecular, endocrine, and phenotypic characteristics of three additional GHI patients with pseudoexon (6 Ψ) mutation, and describe their response to rIGF-I therapy.
Patients: Three siblings from consanguineous parents of Pakistani ethnicity, were referred for poor growth. All three patients presented with typical GHI facial features, had severe post-natal growth failure, normal to high GH levels, and abnormally low serum IGF-I levels that failed to increase in an IGF-I Generation Test (table 1), consistent with a diagnosis of GHI.

Clinical and hormonal data

Patient, Sex & Age (yr)	Ht (SDS)	HV (cm/yr)	IGF-I (ng/mL)	Post-GH* IGF-I (ng/mL)	BP-3 (mg/dL)	GH (ng/mL)	Stimulated GH** (ng/mL)
1,M 7	-4.5	4.0	28	28	1.3	3.7	32.3
2,M 5	-3.9	4.8	37	37	1.4	4.8	10.6
3,F 2	-4.3	8.0	< 25	---	< 0.5	40.0	58.8

*GH: 0.050 mg/Kg/d 7 days; **L-dopa; Reference values: IGF-I; age, 2-6 yr: 80-234 ng/mL; 7 yr: 152-310 ng/mL; BP-3; 1-3 yr: 0.8-4.1 mg/dL; 3-5 yr: 1.0-4.9 mg/dL; >5-7 yr: 1.2-5.8 mg/dL.

Results: Sequence analysis of the *GHR* gene revealed the presence of the previously published mutation A_{-1}®G_{-1} at the 5' pseudoexon 6 Ψ splice site in the three siblings in a homozygous state. Sequencing of parental DNA revealed that both parents, who were of normal stature, were heterozygous for the mutation, as was the normal statured oldest sister. The two brothers have been treated with rIGF-I for 1 yr, and HV has increased from 4.0 cm/y (Patient 1) and 4.8 cm/y (Patient 2) to 10.4 cm/y and 9.3 cm/y, respectively.
Conclusion: We have identified 3 siblings whith GHI due to a 6 Ψ mutation in the *GHR* gene. The normal stature of the parents and oldest sister, heterozygous for this mutation, indicated that the mutation is autosomal recessive. The heights and variable biochemical characteristics of our patients are consistent with published cases. The positive response to one year of rhIGF-I therapy indicate that such patients can be successfully treated for short stature with IGF-I.

PO1-133 GH and IGF Use I

Effects of recombinant human growth hormone (hGH) on red blood cell indices in children with idiopathic growth hormone deficiency (GHD)

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There are many lines of evidence suggesting that the GH/IGF-I axis is involved in the regulation of the red blood system. For instance, blood hemoglobin is positively correlated to serum IGF-I levels in healthy children, and in children with Laron syndrome, IGF-I has a strong stimulatory effect on erythropoiesis.
Objective: To study the impact of hGH replacement on blood cell indices in children with GHD at start of hGH therapy and thereafter in 12 months intervals. **Patients and Methods:** We studied 139 children with idiopathic isolated GHD (85 males, 54 females). At start of hGH therapy, the mean chronological age was 8.81 ± 3.33 (SD) yrs.; 118 children were prepubertal. The mean GH dose (mg/kg/wk) at start of therapy was 0.20 ± 0.07 (SD), and at the end of the observation period 0.25 ± 0.05. The mean duration of GH therapy was 4.4 ± 2.2 yrs. Evaluation of absolute values of red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was performed at baseline and thereafter in 12 months intervals of GH treatment. Effectiveness of GH therapy (start until 5 yrs.) was documented by a significant increase in median height SDS from -3.2 to -1.3, height velocity from -1.6 to 0.47, and serum IGF-I levels from -1.6 to 0.40. **Results:** Age and sex at start of GH therapy had no impact on red blood cell indices. At start of GH, mean Hb concentration was 12.8 g/dl, Hct 38.6 %; RBC 4.68 mill/µl; MCV 82.8 fl; MCH 27.4 pg, and MCHC 33.3 g/dl. Overall, blood Hb concentrations increased on average by 0.17 g/dl per treatment year; Hct by 0.28, MCV by 0.42, MCH by 0.41, and MCHC by 0.28. The used median GH dose (< 0.24 vs ≥ 0.24 mg/kg/wk) had an effect on Hb concentration, Hct and MCV. The mean increases of Hb (0.12), Hct (0.01), and of MCV (0.15) per year were significantly lower ($p<0.05$) in GHD children treated with the lower GH dose than in those treated with the higher dose (Hb 0.27; Hct 0.54; MCV 0.66), whereas this effect was not found in RBC, MCH and MCHC. Positive correlations were observed between blood Hb and IGF-I SDS levels ($r = 0.19$, $p = 0.05$), and Hct and IGF-I SDS levels ($r = 0.24$, $p < 0.01$) only after 3 yrs on GH. **Conclusions:** Our results are in accord with earlier observations and show that hGH therapy at physiological doses has an effect on erythropoiesis in children with GHD.

PO1-134 GH and IGF Use I

Growth hormone supplementation increased latency to tumorigenesis in A*tm*-deficient mice

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The growth hormone and insulin-like growth factor-1 (GH/IGF-1) axis is important for cell growth and differentation. GH has multiple effects on lymphoid tissue and may promote blast cell proliferation and cancer development. Ataxia teleangiectasia (AT) is a devasting human recessive disorder that is characterized by progressive cerebellar ataxia, immunodeficiency, chromosomal instability, radiosensivity and cancer susceptibility. In addition, endocrinological abnormalities such as insulin resistance, glucose intolerance and growth retardation are described. Since AT patients show altered serum IGF-1 levels the question has been raised as to wether GH therapy could be beneficial and/or increase the cancer risk in AT. Therefore we studied the role of GH on longevity and tumor formation in A*tm*-deficient mice, an established model of the human cancer prone syndrome AT. A*tm*-deficient mice were supplemented with GH (1.5 mg/kg/d) to test its effects on tumorigenesis, wheight gain, T-cell immunity and locomotoric behavior.
We found that supplementation with GH did not result in higher weight gain in A*tm*-deficient mice, but treatment significantly increased longevity of A*tm*-deficient. Remarkably, GH seems to ameliorate general condition of the mice and improve T-cell immunity. Treatment with GH did not increase the risk of cancer and seems to have positive effects on the general conditions of A*tm*-deficient mice.

PO1-135 GH and IGF Use I

Prevalence of insulin-like growth factor-I deficiency in prepubertal children with isolated short stature

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Background/Aims: Primary Insulin-like Growth Factor-I Deficiency (IGFD) is defined by low levels of IGF-I without a concomitant impairment in GH

secretion in the absence of secondary cause. The aims of this study were to evaluate the prevalence of non-GH deficient IGFD in prepubertal children with isolated short stature (SS) and to describe this population.
Methods: This retrospective study included all children with isolated SS seen in our Paediatric Endocrinology Unit from January 2005 to December 2007. Children were included based on the following criteria: (i) short stature with current height SDS below or equal to -2.5, (ii) age ≥ 2 years and (iii) prepubertal status. Exclusion criteria were: (i) identified cause of short stature and (ii) current or past therapy with rhGH. IGF-I-deficient children were defined as children without GHD and with IGF-I levels below or equal to -2 SDS.
Results: Among 65 children with isolated SS, 13 (20%) had low IGF-I levels, consistent with a diagnosis of primary IGFD, 4 of which were born small for gestational age (SGA) and 9 were born appropriate for gestational age (AGA). When compared with non-IGFD children, IGFD children had higher birth weight (-0.7 vs. -1 SDS, p=0.02) and birth height (-1.7 vs. -2 SDS, p=0.04) and more delayed bone age (2.6 vs. 1.7 years, p=0.03).
Conclusion: The prevalence of primary IGFD was 20% in children with isolated short stature. Concerning the pathophysiology, our study emphasizes that IGFD in some children may be secondary to nutritional deficiency or to maturational delay.

PO1-136 GH and IGF Use I

rhIGF-I therapy in combination with IGFBP-3 or alone in severe primary IGF-I deficiency (PIGFD) due to a GHR mutation (Laron syndrome): effects on longitudinal growth, body composition and insulin sensitivity

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We have previously reported short time metabolic and growth effects of rhIGF-I/IGFBP-3 treatment (~ 200 µg/kg/d of IGF-I) in two siblings with severe PIGFD (compound heterozygous for two missense mutations in exon 7 in the GHR gene). After 1.5 yrs the rhIGF-I/IGFBP-3 was stopped. rhIGF-I (100 ug/kg BID) was started and longitudinal growth, body composition and insulin sensitivity were studied for an additional yr.
After an overnight fast with 30 min blood sampling, hyperinsulinemic euglycemic clamps were performed during 4 h (M-value based on 2 h steady state). This procedure was repeated on 5 occasions. DEXA was also performed.
Both patients had been on rhIGF-I since infancy. Due to lack of drug, they were studied after 1 yr off therapy. OA was 14.5 yrs of age and CA was 12.2 yrs of age, both were pre-pubertal and growth velocity (GV) was 0.8 and 1.6 cm/yr, respectively. The first yr GV on rhIGF-I/IGFBP-3 was 3.5 and 2.8 cm/yr. Pubertal development accelerated in OA and GnRH agonist was started after 6 w on rhIGF-I/IGFBP-3. The first year GV on rhIGF-I alone was 5.3 and 5.5 cm/yr. CA started GnRH agonists directly after start of rhIGF-I. Total abdominal fat mass by DEXA increased by 15.2 % and 15.5 % during the yr off therapy. After 1 yr on rhIGF-I/IGFBP-3 a 22% reduction was observed in CA, while OA slightly increased adiposity (+ 3.7 %; GnRH agonist effect?). After 1 yr on rhIGF-I, adiposity was reduced in both (-10.3% and -5.5 %; GnRH agonist started in CA). Clamp M-values demonstrated that OA was insulin resistant with no short or long term changes after start of rhIGF-I/IGFBP-3 (and GnRH agonist) but improved insulin sensitivity after 6 w on rhIGF-I. CA was insulin sensitive, displayed a slight long term improvement on rhIGF-I/IGFBP-3 but after start of rhIGF-I (and GnRH agonist) he returned to the level off therapy. Overnight AUC insulin decreased and AUC IGFBP-1 increased after 6 w on rhIGF-I/IGFBP-3 with further changes in the same direction on rhIGF-I suggesting an improved hepatic insulin sensitivity
Comparison of GV in these children with severe PIGFD (Laron syndrome) using the same dose of rhIGF-I (200 ug/kg/d) suggests that delivery alone is superior to that in combination with rhIGFBP-3. The effects on body composition and insulin sensitivity may be affected by rapid induction of puberty and start of GnRH agonist treatment. rhIGF-I appears to reduce adiposity and increase insulin sensitivity while GnRH agonist counteracts these effects.

PO1-137 GH Physiology

GH level, bone age, and height in children with growth hormone deficiency

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Introduction
Growth hormone (GH) is a potent stimulator of longitudinal bone growth. It acts directly by a local action on the growth plate and indirectly by stimulation of IGF-I production in the liver and locally at the growth plate.
Objective:
Bone age (BA) is determined routinely in children with short stature and, in those with GH deficiency (GHD), is known to be delayed. However: How much is this delay? What is the relationship between GH levels and BA? What is the relationship between height and BA?
The aim of our study is to try to answer these questions which have not been previously addressed in the medical literature.
Method and Patients:
The medical records of 266 children who were referred to the GH Committee of the Jordanian Ministry of Health as having GHD were reviewed. 177 of them met the study inclusion criteria which included 2 GH stimulation tests with a maximal response <10 ng/mL. Those who were previously treated with GH or who had any other chronic (including endocrine) disease were excluded. Glucagon, *l*-dopa, clonidine, insulin, and exercise were used as stimulators of GH.
Sex, chronological age (CA), BA, BA SD, height, height SD, and peak serum GH after stimulation were recorded and analyzed by using SPSS 11 statistical software.
Results:
While the mean CA of these patients was 122 mo, the mean BA was 88 mo or -3.23 SD. The mean peak GH response was 3.9 ng/mL. The correlation between the maximum GH level and BA SD was very small (r=0.045) and non-significant (p=0.562), while the correlation between GH level and height SD was greater (r =0.216) and significant (p=0.05), and the same is true for the correlation between height SD and BA SD (r=*.325 and* p=0.00).
Conclusions:
Although the peak stimulated GH level and skeletal maturation are subnormal in children with GHD, this study showed no significant relationship between them.
On the other hand, there was a significant correlation between peak GH level and height SD and between BA and height SD.
This suggests that skeletal maturation is related more to height rather than to GH level, and this may indicate a weak effect of GH on skeletal maturation or simply inaccuracy of GH stimulation tests in detecting GHD, inaccuracy of bone age estimation, or both.

PO1-138 GH Physiology

Isolated growth hormone deficiency type II is caused by ER stress responses triggered by unfolded and mutant GH accumulated in ER

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[Background] Isolated growth hormone deficiency typeII (IGHDII) is typically caused by a splice site mutation at the donor site of IVS3 in the GH1 gene. A mutant 17.5kDa GH is lacking in exon 3 corresponding to 32-71 amino acids, and several studies have shown that 17.5kDa GH from a mutant allele had a dominant negative effect on secretion of wild 22kDa GH from a wild allele, leading to GH deficiency. However, a molecular mechanism of the dominant negative effect is unclear. Recently, Kannenberg et al reported that large dense-cored vesicular structures were found in GH4C1 cells in which both wild and mutant GH were stably expressed. The mutant GH lacks two disulfide bonds, considered to be crucial for normal folding of GH. Thus, we hypothesized that mutant and unfolded GH was accumulated in ER and ER stress responses could be evoked.
[Aim] The aim of this study is to validate the dominant negative effect of

mutant GH on synthesis and secretion of wild GH, and investigate a possibility of ER stress responses.
[Materials and methods] We identified a heterozygous single base g>a substitution at IVS3 +34 in the GH1 gene of a 3-year-old boy. The substitution was located within an XGGG repeat, previously reported that a spliceosome enhancer was associated, and we confirmed a skipping of exon 3 by RT-PCR. To investigate the dominant negative effect, constructs containing the wild and mutant GH1 gene (both genome and cDNA) were transiently transfected into GH4C1 cells, and synthesis and secretion of wild and mutant GH were analyzed by immunoblotting. To evaluate ER stress responses, we established GH4C1 stable cell lines which produced both of wild and mutant GH and investigated signal transductions.
[Results] In transient transfection, the dominant negative effect was not comfirmed. In addition, we are going to show some results suggesting that ER stress responses have an important role in developing GH deficiency in IGHDII patients.
[Discussion] A GH deficiency in IGHDII patients seems not to be due to the dominant negative effect by mutant GH in a short time course which can be demonstrated in a transient transfection assay, but more likely due to ER stress responses by accumulated mutant GH.
[Conclusion] ER stress responses by mutant GH may be a major cause of developing GH deficiency in IGHDII patients.

PO1-139 GH Physiology

Sleep architecture in children with growth hormone deficiency

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Bi-directional interactions between sleep and somatotropic system are documented. An association between slow wave sleep (SWS) and nocturnal GH secretion has been described. A reduction in REM sleep, partially normalized after GH substitution has been reported in children with GH deficiency (GHD). Sleep microstructure, by means of Cyclic Alternating Pattern (CAP) analysis, has never been studied. CAP is an EEG expression of the arousal level instability during non REM (NREM) sleep. It represents a periodic EEG activity, characterized by repeated spontaneous sequences of transient events (phase A), recurring at intervals up to 2 minutes long. CAP phases are classified into A1, A2 and A3 subtypes and measured as the number of a specific subtype per hour of NREM sleep (index).
We analysed, by video-polysomnography, the sleep architecture of 3 GHD children (mean age: 6.2 ± 1.4 yrs), compared to 12 healthy controls matched for age and gender (mean age: 7.0 ± 1.2 yrs). No respiratory disturbances, sleep related EEG abnormalities, periodic limb movements or ECG abnormalities were observed. GHD patients showed a decrease of REM sleep period time (REM spt%: 13.42 ± 3.85 vs 24.65 ± 5.56 - p: 0.004). Wakefulness after sleep onset (WASO%), that is the percentage of time spent awake after sleep onset, and the awakenings per hour (AWN/h) were increased (WASO%: 10.70 ± 4.17 vs 2.00 ± 2.72 - p: 0.0009; AWN/h: 3.72 ± 1.68 vs 0.49 ± 0.64 - p: 0.0007). The study of sleep microstructure showed that GHD patients had a decreased percentage of total NREM sleep time occupied by CAP sequences (CAP rate%) in S1 and S2 sleep stages (p: 0,03 and p: 0.02 respectively). CAP rate% in SWS was also reduced, but the difference did not reach statistical significance. The number of phases A1 per hour of NREM sleep (A1 index) was not different in cases and controls in S1, S2 and SWS. A2 index was significantly reduced in GHD children in S2 and SWS (p: 0.01 and 0.02 respectively); A3 index was reduced in S1 and S2 (p: 0.03 and p: 0.02 respectively). Our data indicate that the quality of sleep is significantly impaired in untreated GHD children. We confirm the previously described reduction in REM sleep time in GH deficient patients. In addition, the sleep microstructure seems to be severely disrupted, with a significantly reduced CAP rate during NREM sleep. Other studies are required to explore if GH substitutive therapy is able to affect sleep architecture and in particular to modify CAP.

PO1-140 GH Physiology

Hypopituitarism is rare after structural traumatic brain injury in early childhood

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Introduction: Post traumatic hypopituitarism (PTHP) is reported to occur in 10-61% of children following traumatic brain injury (TBI). Assessment was by Glasgow Coma Scale (GCS) a crude functional assessment of structural brain injury severity. Little is known about the risk in young children with structural head injuries, who may be at higher risk of hypopituitarism.
Aim: To assess the risk of PTHP amongst a defined group of young children with radiological evidence of CNS trauma following TBI.
Methods: Children <11 years were recruited who sustained structural TBI (skull fracture and/or intracranial haemorrhage) in early childhood (<3 years of age) and had CNS radiology performed who were assessed for evidence of PTHP >1 year after TBI. Growth hormone stimulation (arginine and clonidine 150 mgm/m^2) and low dose synacthen tests (0.1 mgm) were performed with normal peak responses defined as ≥7 mcg/l and >500 mmol/l respectively. TSH, Free T4, prolactin and fasting serum osmolality were measured at baseline. Males <9 yrs and females <8 yrs with evidence of puberty had a GnRH stimulation test performed. Subjects with abnormal results were followed up for 6-12 months.Values expressed as mean±SD.
Results: 70 children were studied aged 7.2±2.0 years, which was 6.0±1.8 years after head injury (56% were male, 53% European and 47% Maori or Pacific Island). Mechanisms of injury were falls 61%, motor vehicle accident 21% and other 18%. Injury severity by initial hospital GCS was mild (12-15) in 65%, moderate (9-12) in 20% and severe (<9) in 18%. CNS radiology revealed 92% with a skull fracture, 52% intracranial haemorrhage and 42% with cortical injury. Four subjects had low GH levels (peak GH 3.4 to 5.8 mcg/l) with normal serum IGF-I values and at follow-up all had a height velocity >25th percentile over >6 months. Three of the 4 subjects were obese. Low cortisol levels (peak cortisol 400-474 mmol/l) were found in 2 further subjects. At follow-up both had a repeat stimulated cortisol >500 nmol/l. One subejct developed central precocious puberty within 12 months of the intial head injury.Overall there was only a 1.4% prevalence of PTHP following initial evaluation and follow-up.
Conclusions: Young children with structural TBI are at low risk of PTHP following rigorous assessment of pituitary function and follow-up. Evaluation of the specific risk factors for PTHP following TBI is needed to guide which selected children require assessment of pituitary function.

PO1-141 GH Physiology

Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) resistance in a child with an IκBα mutation

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Introduction:
NF-κB is a family of transcription factors involved in cell proliferation, differentiation, and apoptosis. Humans with defects of the NF-κB pathway have severe immune defects. In addition, they exhibit growth failure, which suggests that NF-κB may also be involved in skeletal growth. We have recently demonstrated that NF-κB is expressed in the growth plate and it mediates the growth-promoting effects of IGF-1 on chondrogenesis and longitudinal bone growth. Here we describe a patient with short stature harboring a mutation of IκBα, an essential component of the NF-κB pathway.
Case:
This is a male infant born to nonconsanguineous parents. In the first 6 months of life, he suffered from several severe infections, including meningitis and pneumonia, which eventually subsided after starting IV IgG supplementation and antibiotic prophylaxis.
A heterozygous mutation at serine 32 in IκBα was eventually found.
While his length was normal at birth (-1 SDS), it gradually declined (-2 SDS at 1 year and – 4.1 SDS at 2.4 yrs). At age 2, he was found with low IGF-1 and

IGFBP-3, and a GH stimulation test ruled out GH deficiency. To determine whether he was GH resistant, a IGF-1 generation test was performed: the administration of GH for 7 consecutive days did not elicit any significant IGF-1 response.
To assess whether GH resistance was due to the IκBα mutation, we first cultured the patient's skin fibroblasts with GH (0-100 ng/ml) and studied cell proliferation (assessed by thymidine incorporation) and NF-κB activity (measured by an enzyme-linked immunosorbent assay). While 100 ng/ml GH induced cell proliferation ($p<0.05$) and NF-κB activity ($p<0.05$) in control fibroblasts, it had no effect on the patient's fibroblasts. In addition, GH stimulated Stat5b phosphorylation (by Western) and IGF-1 expression (by real-time PCR, $p<0.05$) in control, but not in the patient's fibroblasts. Control fibroblasts treated with 100 ng/ml IGF-1 exhibited induced cell proliferation ($p<0.01$), NF-κB activity ($p<0.01$), and increased mRNA (by real-time PCR, $p<0.01$) and protein (by Western) expression of TDAG51, a gene specifically induced by IGF-1. In contrast, none of these effects was elicited by IGF-1 in mutant fibroblasts.
Conclusions:
Our findings suggest that our patient's growth failure may have been due to both GH and IGF-1 resistance. Such dual resistance is likely due to the impaired NF-κB activity.

PO1-142 GH Physiology

Hormonal determinants of bone mass changes after stopping GH therapy at final height in adolescents with childhood onset GH deficiency

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Discontinuation of GH therapy at near-final height in adolescents with childhood onset GH deficiency (GHD) has been associated with either a further increase or a stabilization of bone mass. We hypothesized that beside the severity of GHD, changes in body fat (either by a direct or indirect mechanism) might explain this variability in bone accretion after stopping GH.
In this study, we therefore analyzed the changes both in bone mass and body fat as well as in circulating IGF-1, estradiol, insulin and leptin concentrations in male GHD adolescents at the moment of discontinuation of GH and subsequently 1 and 2 years later.
In 12 male patients with documented GHD, aged between 15.9 and 18.8 yr, presenting 3 months after stopping GH therapy (0.025 mg/kg.day) with a peak GH value at an insulin stimulation test < 5 ng/L, whole body (WB)and lumbar spine(LS) bone mass(BMC) were studied by DXA (Hologic QDR 4500) at discontinuation of GH at near final height (height increase < 1 cm last 12 months)and subsequently after 1 and 2 yr. At these moments and additionally at 3 months, fasting serum samples were taken for centralized hormonal measurements.
The WB and LS BMC increased respectively with 12.5 and 6.7 % above baseline after two years, independently of peak GH concentration and IGF-1 level at 3 months. IGF-1 declined between baseline and 3 months ($p<0.001$). No statistically significant changes in IGF-1 thereafter and in the other studied hormonal parameters occurred. Mean(SE)absolute increases in WB BMC (281(79)g)and LS BMC (3.6(1.1)g) correlated significantly with WB body fat increase (6079(1368)g).The WB BMC increase correlated positively with the basal leptin at 3 months and its increase between 3 and 24 months, while LS BMC change correlated negatively with basal oestradiol and its increase in between 3 and 24 months.
In conclusion, in this 2-year prospective study in male patients with a persisting GHD a prolonged bone acquisition was observed in accordance with a further body fat accumulation when GH therapy was stopped. We suspect that changes in bone mass after final height in untreated GHD adolescents depend rather on leptin production (by stimulation of radial bone growth and cortical thickening) than on estradiol secretion. More prolonged observation is needed to detect whether a sufficient bone formation will occur in the following years, allowing the achievement of a normal peak bone mass.

PO1-143 GH Physiology

GH response to GHRH+arginine stimulation in children is influenced by pubertal stage

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Discrepant data on the effects of pubertal development on the stimulated GH secretion have been reported. While an increase with puberty in the peak GH response to conventional stimuli was described by some AAs, others failed to detect significant differences in stimulated GH secretion between prepubertal and pubertal subjects. To assess the effect of pubertal stage on the GH response to maximal stimulation by GHRH+arginine, 92 short-normal boys were studied. The study group was subdivided into 3 subgroups according to pubertal development defined by Tanner stage (group A [n = 42]: Tanner stage I; group B [n = 16]: Tanner stage II-III; group C [n = 34]: Tanner stage IV-V). Mean age, bone age, and BMI were significantly different among the 3 groups, as expected (age: 10.41 ± 0.47 vs 13.97 ± 0.32 vs 21.05 ± 0.77 yr, ANOVA $p< 0.0001$ mean ± SEM; bone age: 8.99 ± 0.51 vs 12.43 ± 0.39 vs 17.89 ± 0.10 yr, $p< 0.0001$; BMI 16.26 ± 0.35 vs 19.69 ± 0.56 vs 22.50 ± 0.33 kg/m^2, $p< 0.0001$). No significant differences in height SDS, and height velocity SDS for bone age were detected among the groups (height SDS: -2.02 ± 0.14 vs -2.10 ± 0.23 vs -2.05 ± 0.12; height velocity SDS for bone age: -0.14 ± 0.64 group A vs -0.90 ± 0.61 group B).
The GH response to GHRH + arginine was significantly greater in group A than in group B or C and in group C compared to group B (group A: 65.28 ± 5.6 µg/l; group B: 35.11 ± 3.39 µg/l; group C: 56.46 ± 4.93 µg/l; ANOVA $p < 0.002$). The same differences were obtained after correction of the data for BMI. A positive correlation ($r = 0.47$) between height velocity SDS for bone age of the whole group and GH response to GHRH + arginine was detected. The IGF-I SDS levels corrected for BMI were similar in the 3 groups.
In conclusion, the results of the present study indicate that the GH response to maximal stimulation in short-normal male subjects varies with pubertal development, being maximal in prepuberty, decreasing in mid-puberty, and increasing again at completion of puberty. The reduced stimulated GH secretion detected in mid-puberty might reflect the deflection in height velocity that physiologically occurs at this age, and explain the false positive responses for GH deficiency among children in this age group.

PO1-144 GH Physiology

The functional to total IGFBP-3 ratio as an index of binding protein fragmentation in children with chronic kidney disease before and after treatment, compared to healthy children, children with growth hormone deficiency, and children born small for gestational age

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Introduction: In chronic kidney disease (CKD), IGFBP-3 levels are considerably elevated. This is thought to be due to the accumulation of low-molecular forms. There is an inverse correlation between the glomerular filtration rate and IGFBP concentrations. The functional role of circulating IGFBP-3 variants

for IGF-I binding in CKD is unclear. To study this, we measured total and functional IGFBP-3 in children with CKD and compared the results to healthy children, children with growth hormone deficiency (GHD) and children born small for gestational age (SGA). **Patients and Methods:** 253 serum samples of 29 healthy children, 42 children with GHD, 34 children with SGA, and 148 children with CKD were obtained, the latter group including children with end-stage renal disease (ESRD, n=33), children undergoing peritoneal dialysis (PD, n=17) or hemodialysis (HD, n=9), and children after renal transplantation (RTx, n=89). None of the patients received growth hormone (GH) treatment. Samples were analysed for total (t) and functional (f) IGFBP-3, using commercial assays (Mediagnost, Reutlingen, Germany). The functional assay quantifies only those IGFBP-3 molecules capable of binding labelled IGF-I. Data were analysed by ANOVA, followed by Tukey's test. **Results:** Mean tIGFBP-3 SDS differed between controls and patient groups ($P < 0.0001$), being elevated in ESRD and the two dialysis groups, with a mean of +3.9 (PD), +3.2 (HD), and +2.1 (ESRD). It was lowest in GHD (-1.5). Intermediate values were obtained for SGA (-0.5), controls (+0.2), and RTx (+1.7), with the means of PD and HD being higher than the means of all other groups ($P< 0.005$). Mean fIGFBP-3 also differed between groups ($P< 0.0001$). In HD (2.6 mg/l), it was significantly ($P< 0.005$) elevated over the means of the other groups except RTx (controls 1.3 mg/l, ESRD 1.5 mg/l, PD 1.5 mg/l, GHD 1.2 mg/l, SGA 1.3 mg/l). The functional to total ratio differed between groups ($P < 0.0001$). It was high in controls, GHD, SGA, HD and NTx (0,35-0.45) and low in ESRD (0,29) and PD (0,22). **Conclusions:** Our data indicate that in ESRD and PD elevation of tIGFBP-3, as measured by conventional assays, can be caused by the accumulation of inactive fragments. The low functional to total ratio in the two groups is compatible with this notion. FIGFBP-3 concentrations in ESRD and PD that are not different from controls would argue against a concept of growth failure caused by excessive IGFBP-3 blocking IGF-I action in these conditions.

PO1-145 GH Physiology

Nocturnal secretion of renin and its relationship to the secretion of GH, DHEA, MLT and sleep quality in children with short stature

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Introduction
Circadian monitoring of renin in adults has shown that renin secretion peaks during sleep and correlates with the NREM sleep phase. Sleep deprivation increases its secretion in NREM sleep phase (Brandenberger et al., 1994, Murck et. al., 2005).
In recent studies we analyzed the dynamics of nocturnal secretion of GH, DHEA, LH, FSH and MLT in children and adolescents and also its relation to sleep.
The goal of this study was to find if the described correlation between Renin secretion and sleep in adults exists in childhood too and which relationships exist to the secretion of GH, DHEA, LH, FSH and MLT.
Subjects, Material and Methods
We studied in 23 children aged 6 to 21 years affected by short stature with (18) and without (5) growth hormone deficiency the nocturnal blood secretion of Renin (IRMA, DSL), GH (ELISA), DSL) and MLT (RIA, DSL) over 12 hours by sampling every 30 minutes using a Conflow Pump and in parallel performing polysomnography. The programs Pulsar and AnCoPuls (Merriam and Wachter, 1982, Albers, 1992) were used for secretion analysis, SPSS for statistical analysis, ALICE for automatic analysis of polysomnography. The sleep staging and efficiency were determined according to Rechtschaffen and Kales, 1968. The nocturnal EEG-profiles were visually analyzed to synchronize results from polysomnography and blood parameter measurements.
Results
The blood concentration of renin did not alter during the night and pulsations could not be matched to any sleep phases. In contrast, pulsatility of GH, DHEA and MLT could be found in all children. Furthermore the renin profiles were not significantly different between children with short stature without and children with GHD, showing a similar average concentration around 19 pg/mL and an average cumulative secretion of around 200 pg/mL. The reference value for adults ranges between 1.4 and 17 pg/mL.
Conclusion
A correlation between renin blood concentration or overall secretion and sleep quality could not be found, in contrast to GH, DHEA and MLT.

PO1-146 GH Physiology

Effects of growth hormone on bone modeling and remodeling in hypophysectomized female rats

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Background
Our study investigated the mechanism of action of GH on bone growth, bone formation, modeling and remodeling in the hypophysectomized (HX) female rats.
Methods
Thirty female Sprague-Dawley rats at age of 2-month were divided in groups with 10 rats each; group (C), age matched control; group (X), hypophysectomized; group (G), SC administration of 3 mg/kg daily of recombinant human GH for 4 weeks. When sacrificed, undecalcified proximal tibial metaphysis (PTM) and tibial shaft were embedded and pairs of serial 6-um sections were cut. Villanueva stained sections were used for calcein fluorochrome and polarized light analysis, Toluidine Blue-stained sections were used to view cement lines and wall thickness and Goldner's trichrome-stained sections were used to measure tissue area, bone area, osteoid area, osteoclast surface, and bone surface.
Results
Hypophysectomy resulted in cessation of bone growth in length and circumferential area. Periosteal bone formation decreased and bone turnover rate of endocortical and trabecular surfaces increased compared to group C. GH administration for 4 weeks restored weight gain, bone growth and mitigated the decrease in bone density after hypophysectomy. The femoral, tibial area and cortical % of the group G increased to levels that did not differ from that of group C. However, trabecular bone mass in the PTM was significantly lower in group G than in group C. In the PTM, 0-1mm (Z1) and 1-4mm (Z2) zones beneath the growth plate were examined. Dynamic histomorphometric analysis showed that both periosteal bone formation and growth plate elongation of Z1 were significantly higher in group G than in group X. New bone formed in the Z1 area beneath the growth plate was predominantly woven bone in the group C and group G. In endocortical and Z2 area of the PTM, where bone remodeling predominated, GH administration enhanced both bone formation rate and resorption activities and both modeling and modeling-remodeling mixed modes were also significantly higher than that of the group X.
Conclusion
GH administration to HX rats reactivated modeling activities in modeling predominant sites. In remodeling predominant sites it activated both modeling and remodeling activities giving limited net gain in bone mass. The fact that limited net gain in bone mass in trabecular bone was proven requires further investigation using higher dose of recombinant human GH or combination of anti-resorptive agents.

PO1-147 GH Physiology

Interactions of IGFBP-3 with nuclear receptors and co-activators regulates adipocyte function

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IGFBP-3 is the most abundant circulating IGF binding protein and is expressed in many tissues. IGFBP-3 not only regulates IGF bioavailability and action,

but also mediates IGF independent actions on cell survival and apoptosis. We have recently shown that IGFBP-3 leads to the induction of insulin resistance *in vitro* in 3T3-L1 adipocytes and *in vivo* in rats. Adipocytes are emerging as an important endocrine source of adipocytokines as well as a target tissue for various cytokines that modulates insulin sensitivity. We carried out a series of experiments to elucidate the effects of IGFBP-3 on adipocytokine expression. We treated maturing 3T3-L1 adipocytes with IGFBP-3 (1 mg/ml) and measured the mRNA levels of adipokines by RT-PCR and the concentration of adipokines in culture medium by ELISA. To elucidate the possible mechanism of the effects of IGFBP-3 we studied its *in vitro* binding with PPAR-γ and other nuclear receptors

IGFBP-3 inhibited both the mRNA expression of adiponectin, leptin and resistin and their secreted peptide levels. No effect on IL-6 expression was observed. Importantly, we found that IGFBP-3 bound directly to PPAR-γ. We therefore assessed the effects of IGFBP-3 on the interaction between nuclear receptor co-activators (TIP-2, SRC-1, p300) and PPAR-γ and demonstrated enhanced interactions.

We conclude that the effects of IGFBP-3 on adipocytes are related to the regulation of several adipokines that modulates insulin sensitivity. This may occur via a direct interaction with PPAR-γ and/or modulation of PPAR-γ binding with other nuclear receptors.

PO1-148 GH Physiology

The effect of corrective surgery on serum IGF-1, IGFBP-3 levels and growth in children with congenital heart disease

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OBJECTIVE. To evaluate growth monitoring, IGF-1, IGFBP-3 levels in children with congenital heart disease before and one year period after the corrective surgery and to determine if these parameters have any relationship to the type of congenital heart disease, and nutritional status.

METHODS. This prospective study conducted in 40 patients with congenital heart disease (8.61±6.15 (0.1-22) months, 20 cyanotic, 20 acyanotic) and 32 healthy children. We also categorised patients as pulmoner hypertensive or not. Measured antropometric values, serum levels of IGF-1 and IGFBP-3 compared with healthy controls.

RESULTS. Acyanotic patients and pulmonary hypertensive patients presented significantly more growth failure and malnutrition prevalence. Malnutrition prevalence was 61.1 % for cyanotic, 94.7 % for acyanotic group.

Lowest serum IGF-1 levels before surgery were revealed in acyanotic group (32.85±7.87 ng/ml) than cyanotic and control groups (43.14±25.9, 44.71±20.81 ng/ml, respectively) (p>0.05). Samely, serum IGFBP-3 levels were significantly lower in acyanotic group than the control and cyanotic groups (1.80±0.95, 2.82±0.83, 2.03±1.17 ug/ml), respectively. No relationship was detected between IGF-1, IGFBP-3 levels and pulmonary hypertension or hypoxia. Three months after surgery malnutrition prevalence, serum levels of IGF-1, IGFBP-3 were similar between acyanotic and cyanotic groups. One year after surgery, degrees of malnutrition were not different between two groups. But height sds was lower in acyanotic group (p<0,05).

The progress of IGF-1 and IGFBP-3 levels in a year demonstrated that the values in the third month and first year were higher than the preoperative values (p<0,05).

CONCLUSIONS. We suggest that IGF-1, IGFBP-3 levels may be more useful for academic purposes. Still, antropometric measurements are cheaper, more practical and non-invasive methods for the evaluation of nutrition. However, timing for corrective surgery especially before chronic malnutrition or pulmonary hypertension developed is the most important issue to maintain a normal growth for children with congenital heart disease.

PO1-149 GH Physiology

Nonoperative octoreotide-long acting release (LAR) treatment for a 12-year-old boy with pituitary gigantism

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INTRODUCTION: Growth hormone (GH)-producing pituitary adenoma in childhood is very rare. In such cases, transsphenoidal surgery is most acceptable therapy and is followed by somatostatin analogue as an adjuvant therapy. Recent study has shown an efficacy of single agent therapy with long-acting somatostatin analogue, which could shrink pituitary tumor in adult cases. But there is no report in children. We described a clinical course of a 12-year-old boy with pituitary gigantism who underwent octreotide-LAR alone and showed remarkable shrinkage of the tumor.

CASE: A 12-year-old boy visited our clinic because of petechiae and prolonged fever. He was diagnosed acute lymphocytic leukemia (ALL). At that time, he was noticed tall stature (+2.81 S.D. in Japanese reference). The height velocity began to increase around six-year-old of age. Bone survey did not show fibrous dysplasia. Serum GH and IGF-1 level were remarkably elevated (60.3 ng/ml and 570 ng/ml, respectively). Serum GH level was not suppressed by 75g OGTT and showed paradoxical response to TRH. Pituitary MRI revealed a cystic macroadenoma (19 mm x 23 mm x 20 mm). We put priority on the treatment for ALL. During chemotherapy, high blood sugar level continued. First, short-acting somatostatin analogue of octreotide was administrated for his gigantism. However, it was difficult to maintain fair blood glucose levels (maximum insulin infusion required above 3.0 Unit/kg/day). Subcutaneously octreotide-LAR was started at 20 mg in dose, follwing 10 days. With this therapy, he could be free from insulin infusion. Serum IGF-1 level was decreased to 70 ng/ml after 4 weeks treatment. In MRI finding, pituitary tumor size dramatically had shrunk. (6 mm x 12 mm x 14 mm of cystic tumor after 2 months, following no cystic tumor after 8 months later).

DISCUSSION: In adult cases, it has been reported that long-acting somatstatin analogue could be more effective than short-acting one for tumor shrinkage. Our case revealed diminished tumor size with octreotide-LAR. There are few reports concerning long time treatment of octreotide-LAR. This single therapy could be potential option for nonoperative cases, because it could be less invasive and might keep other anterior pituitary hormone intact.

PO1-150 GH Physiology

CHARGE syndrome: height and bome mineral density outcomes in adults

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Background : CHARGE Syndrome describes a combination of congenital malformations (coloboma, heart defects, choanal atresia, delayed development, genital hypoplasia, ear abnormalities, and/or hearing loss) occurring in about 1/12.000 births worldwide.

Aim: An observational study of CHARGE syndrome after transition.

Subjects and Methods: Clinical notes were reviewed in 8 subjects, 4 males and 4 females, attending endocrine clinics at a University Hospital in London. Age ranged between 20-28 years. Outcomes of height and bone density were compared with age matched subjects with primary and secondary hypogonadism.

Results: All had Hypogonadotrophic Hypogonadism and were on sex steroid replacement. None had attempted fertility. 2 were GHD and 3 received GH. All subjects had low BMD, 5/8 (62.5%) had osteopaenia and 3/8 (37.5%) had osteoporosis. Vit D was measured in 6 subjects and was low in 1. Differences in height, BMI and Bone Mineral Density within the three groups are displayed in table 1 and graphs 1&2. Subjects with CHARGE were shorter and with low BMD when compared to reference groups.

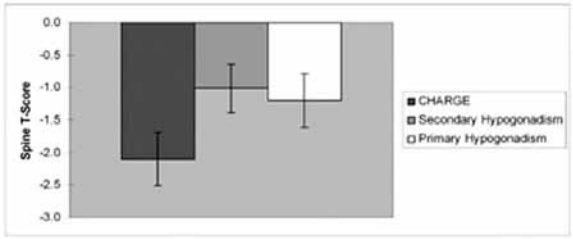
Conclusion: Height outcomes were suboptimal. Reduced spine BMD was observed even after correction for height.Given that patients with Hypogonadism were exhibiting better outcomes on height and BMD, we believe that other factors such as reduced nutrition and mobility, an impaired GH-IGF1 axis or bone dysplasia may account for low BMD in CHARGE. We conclude that low

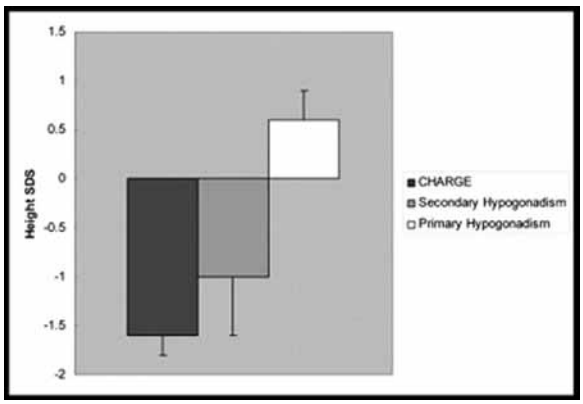
BMD and short stature may require more intensive management in pediatrics than was received by this group.

Height, BMI and BMD outcomes in age-matched subjects with CHARGE and Hypogonadism

	CHARGE	Hypo/Hypo	Klinefelter/POF
Age	21.5	22	21.7
Height	158.6*	165.4	172.5
Height SD	-1.6	-1	-0.3
BMI	21.5	25.4	22.9
Spine T-Score	-2.4	-1	-1.1
Hip T-Score	-0.6	-0.2	-0.3

* P<0.05





PO1-151 GH Physiology

Preliminary results of pituitary function evaluation in children with suspected pituitary abnormalities after traumatic brain injury (TBI)

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Study aim: Pituitary function evaluation in children with suspected endocrine disorders after Traumatic Brain Injury (TBI). **Subjects**: 12 children, 2 girls and 10 boys (age 0,3 15,2 years) recovered from mild, to severe TBI. Prospective follow up has been done for all subjects. Clinical and biological assessment were performed 12 months after TBI. **Methods**: General clinical examination and specific neurological status and endocrine function evaluations were performed on the basis of a standardized investigation protocol including determination of FT4, TSH, PRL, IGF-I, Testosterone/E2, FSH and LH. Corticotroph and Somatotroph axis were evaluated using pharmacological tests : Betaxolol - Glucagon test or GHRH-Arginin for investigation.**Results** : The preliminary result on our limited series of patients confirmed the presence of two isolated hormone deficiencies: one patient with GH deficiency (GHD) and one patient with thyrotrope deficiency. Associated deficiencies were not observed among our patients.**Conclusion**: These preliminary results from our cohort confirmed the high risk of pituitary insufficiency following TBI. the recommendation for evaluation of pituitary function in patients with mild to severe TBI.Growth velocity and as to be assed and survey for all children with pituitary insufficiency after TBI. Pituitary function evaluation has to be systematically performed when clinical symptoms are present.

PO1-152 GH Physiology

POU1F1 involved in a familial form of isolated GH deficiency

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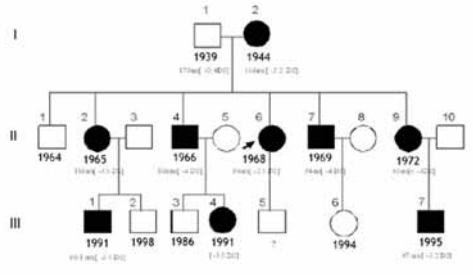
POU1F1 is a pituitary-specific nuclear transcription factor playing a key role in pituitary development and expression of the GH, PRL and TSH genes. Mutations in the POU1F1 gene are usually associated with combined pituitary hormone deficiency (CPHD). Autosomal dominant familial forms of CPHD due to POU1F1 mutations are rare.

We report on a large three-generation family with a new POUI1F1 mutation.



Clinical and biological data are summarized in Table 1.

Case	Height at	Peak GH ng/ml		TSH	fT4	Prolactin ng/ml	
	Diagnosis	Test 1	Test 2	mUI/l	pmol/l	Basal	Peak
I2	-3,2	ND	ND	ND	ND	ND	ND
II2	-3,5	2,6	2,3	4	13,4	3,3	19,9
II4	-4	2,2	2,2	4	16,2	ND	ND
II6	-2,5	1,5	2	3,8	13,3	1,4	4,6
II7	-4	2	2,5		12,5	ND	ND
II9	-3	2	3	1,02	10,7	ND	ND
III1	-3,5	3,1	8,6 (GRF)	1,9	12,3	3,7	12,5
III4	-3,5	1,9	2,4	0,67	15,2	5,1	16,7
III7	-3,2	3,4	5,2	1,09	11,9	0,9	4,6

Magnetic resonance imaging (MRI) of the pituitary region showed normal or pituitary hypoplasia.

Molecular analyses revealed a novel heterozygous POU1F1 mutation (c.227C>T, p.Pro76Leu) in the transactivation domain of the protein. The mutation segregates with the pituitary phenotype. The functional consequences of this mutation are under investigation.

The phenotype associated with this POU1F1 mutation is variable within this family. Most importantly, in contrast with previous reported cases, the majority phenotype is characterized by isolated GH deficiency. Of the 6 adults, two have been lost for follow-up; of the others four only one has a combined full GH and partial TSH deficiency. Two patients are still under 18 years,: one has partial TSH deficiency with T4 below normal at the age of 6 years (III7) Basal prolactin levels were found to be slightly decreased in two patients, but with normal response to TRH.

This familial observation, which shows some intrafamilial variability in the expression of the POU1F1-associated phenotype, points to a so-far-unsuspected complexity of POU1F1-dependent mechanisms involved in pituitary function. From a clinical viewpoint, this observation reveals that POU1F1 mutations may be associated with isolated GH deficiency.

PO1-153 GH Physiology

Two cases of GH secreting pituitary adenoma with slipped capital femoral epiphysis

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[Introduction] GH secreting pituitary adenoma is often diagnosed by acromegaly over the age of adolescence. In the present report, two cases of GH secreting pituitary adenoma with gigantism were able to diagnose before adolescence due to their slipped capital femoral epiphysis.
[Case1] 12 years old boy visited an orthopedist with left coxalgia. He was diagnosed as slipped capital femoral epiphysis. His body height was 190 cm (+4.9 SD) and body weight was 135 kg. His pubic hair was Tanner 2 and testis volume was 4ml. Basal GH was 12.3 ng/ml and IGF-1 was 1170 ng/ml. GH level in blood was not suppressed by an oral glucose tolerance test (OGTT). GH level was markedly increased (from 7.87 to 100 ng/ml) in response to GRF, TRH, LHRH and CRH, while TSH, PRL, LH, FSH, ACTH and cortisol levels were within the normal response. Heel pad thickness was 24 mm. Cranial MRI scan show an intrasellar tumor of 1cm diameter.
[Case2] 14 years old girl visited an orthopedist with left coxalgia. She was diagnosed as slipped capital femoral epiphysis. After the operation, she was pointed out a gigantism by the orthopedist and was introduced to our department. Her body height was 173.3 cm (+3.3 SD) and body weight was 69.3 kg. Her breasts were Tanner 2 and pubic hair was Tanner 1. Basal GH was 450 ng/ml, IGF-1 was 1280 ng/ml, LH 0.31 mIU/ml, FSH 1.79 mIU/ml and E2 <10 pg/ml. GH level in blood was not suppressed by OGTT.GH level was markedly increased (from 405 to 5910 ng/ml) in response to GRF, TRH, LHRH and CRH, while TSH, PRL, LH, FSH, ACTH and cortisol levels were within the normal response. Heel pad thickness was 24 mm. T1-weighted image of MRI showed an isointense mass involving the sella and suprasellar space.
[Discussion] These patients were pointed out a high statue starting with their slipped capital femoral epiphysis, and were able to diagnose as the GH secreting pituitary adenoma. The continuous high level of GH cause the loss of stability of their proximal femoral growth plates and the slipped capital femoral epiphysis occurred by their heavy weight. We need to pay attention to the diagnosis of GH secreting pituitary adenoma when we examine a case of slipped capital femoral epiphysis with high statue.

PO1-154 GH Physiology

Growth hormone concentration in short statured children with normal pituitary function

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Short stature is caused by growth hormone (GH) deficiency only in 8% of all cases. In the remaining group of short children the GH secretion is normal. In most non GH deficient patients the IGF-1 concentration is within normal range. The coexistence of normal GH concentration and low IGF-1 concentration can suggest a pathology in GH signal transduction due to a mutation in GH receptor (GHR) gene or a mutation in genes coding factors involved in GH signal transmission inside the cell. These all cases are called insensitivity to GH. The abnormal response for GH in peripheral tissues can be connected with increased levels of growth hormone. In the literature there is no defined cut-off for abnormal high GH levels. The aim of the study is to estimate GH concentration in short children with excluded GH deficiency. The calculation of mean and standard deviation for GH concentration can allow to define abnormal high GH levels, which can be an indication for further molecular diagnostics.
The study group consisted of 102 children aged 4,5-16,5 years with short stature and excluded GH deficiency. In all children the GH sleep test and IGF-1 measurement were performed. The results: the mean maximum GH concentration and standard deviation (SD) in whole analyzed group were as follows 44,56 mIU/ml +/- 17,25. In 13 children (12,7%) the IGF-1 was equal or below 5th centile according to age ranges. In these patients mean maximum GH concentration was 48,6 mIU/ml +/- 11,59. In children with IGF-1 above 5th centile the mean peak GH concentration was 44,13 mIU/ml +/- 17,93. There was no statistical significant correlation between maximum GH concentrations and IGF-1 concentrations (p=0,44, R=0,08). There was no statistical significant difference between maximum GH concentrations in children with IGF-1 above 5th centile and children with IGF-1 equal or below 5th centile (p= 0,18). Analyzing the data, from a clinical point of view the GH value 45,0 mIU/ml could be used as a cut-off level. For this value the specificity, sensitivity, accuracy, negative predictive value were as follows: 59, 76, 61, 95 %. The peak GH concentration in sleep test above this cut–off is connected with a higher risk of low IGF-1 concentration in laboratory tests. In a coexistence of high GH and low IGF-1 concentrations the clinician should consider to perform IGF-1 generation test and molecular analysis. The results show also how the diagnostic process of short stature in children is difficult.

PO1-155 GH Physiology

Decreased IGF-I and GHBP circulating levels in Babinga pygmy children correlate with their stunted growth

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African Pygmies' dwarfism has been an enigma for centuries. The study of their growth and hormonal levels is important for better understanding the mechanisms regulating and determining human stature.
In this study we investigated height and hormonal levels in Pygmy children of different ages to evaluate if a defect in GH/IGF-I axis is already present in childhood.
We enrolled 15 Babinga Pygmy children (8 F and 9 M, age from 1 to 12 years), living in South-East Cameroon on the edge of the forest. We obtained their informed consent relying on local interpreters-nurses. Height and BMI were expressed as standard deviation score (SDS), using the standards by Tanner and Whitehouse (Arch Dis Child 1976). Serum GH and IGF-I concentrations were measured by an automatic chemiluminescent assay and the reference values reported by Elminger et al. (Clin Chem Lab Med 2004) were used. Circulating levels of GHBP were measured by a commercially available ELISA kit.
No signs of malnutrition are observed among Pygmy children following standard clinical examination, confirmed by normal BMI.

Anthropometrical and biochemical data of Pygmy children

Subjects	Age (years)	Height (SDS)	BMI (SDS)	IGF-I (SDS)	GHBP (pmol/ml)
1	1	-	-	-2.32	205
2	2	-5.21	0.62	-2.86	210
3	3	-3.69	-0.22	-3.96	100
4	4	-1.05	0.53	-2.13	240
5	4	-4.23	-1.03	-1.90	220
6	5	-2.61	0.08	-0.64	275
7	5	-5.29	1.2	-0.93	305
8	6	-3.55	-0.25	-2.85	300
9	7	-5.06	0.14	-2.85	240
10	9	-3.51	0.61	-1.43	340
11	9	-3.67	0.66	-3.19	245
12	10	-2.08	-1.0	-2.9	225
13	10	-4.97	0.72	-3.3	235
14	12	-4.20	-0.23	-2.32	315
15	12	-3.64	-1.28	-2.15	310

The heights of Babinga Pygmy children of different ages are almost all closely below the 3rd percentile of the European population with a mean height <2 SDS (mean±standard deviation: -3.77±1.23 SDS).
Hormonal findings show that Pygmy children have decreased levels of IGF-I compared to sex and age-matched European standards, with concentrations generally <1 SD below the mean. Furthermore, GHBP levels are below the normal range for ages (0-5 years: 773±159 pmol/ml; 5-10 years: 993±216 pmol/ml; 10-20 years: 1100±240 pmol/ml).
Our results indicate that growth failure in Pygmies occurs already in childhood in according to reduced circulating IGF-I and GHBP levels.

PO1-156 GH Physiology

Extrapituitary congenital malformations are frequent in growth hormone (GH) deficient children with and without pituitary stalk interruption (PSI)

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BACKGROUND: High frequency of extrapituitary defects are reported in children with GH deficiency (GHD) associated with PSI suggesting a role of common development factors. The question of the frequency of such malformations in children with GHD without PSI can be raised as it may appears as a functional rather than developmental process.

AIM: The objective of this study is to describe clinical phenotypes and associated extrapituitary malformations of children with GHD and to compare the prevalence of these malformations depending on the presence or not of PSI.

PATIENTS: Ninety eight children with a diagnosis of GHD were evaluated retrospectively. All had cerebral magnetic resonance imaging (MRI) including the pituitary region.

RESULTS: In the whole cohort, 23.5% (23/98) had normal MRI, 27.5% (27/98) had isolated pituitary hypoplasia and 47,5% (47/98) had PSI. One had two posterior pituitary signals with a hypoplasic anterior pituitary. Extrapituitary abnormalities were found in 51,4% (51/98) patients. Among them, 16% had a well defined syndrome; the others 35% had cerebral, ophtalmic or craniofacial malformations, concerning midline structures (23/35), but also had digestive and renal ones. The most frequent abnormalities involve the brain and the eyes like cerebral midline structure interruption or agenesia, anophtalmia or coloboma. A major result was the absence of significative difference in the prevalence of associated extrapituitary malformations between children with and without PSI. Morevover, there was no significative difference between patients with and without PSI for the frequency of ophtalmic malformations (38.3% vs 28.8%; p=0.44) nor craniofacial or ORL (otorhinolaryngologic) abnormalities.

Comparison of the patients depending on the presence of PSI.

	PSI + (n=47)	**PSI -** (n=51)	*p*
Malformations (M)	51,4%	51,9%	0,91
Ophtalmologic M	38,3%	28,8%	0,44
CPHD	55,3%	32,7%	0,04

CPHD: combined pituitary hormone deficiency

CONCLUSIONS: We report high prevalence of extrapituitary malformations in GHD patients, even if there is no pituitary stalk interruption. This supports a developmental origin of the pituitary deficiency without PSI. We suggest systematic ophtalmic and craniofacial examinations in children with proven GHD. Precise description of clinical phenotype will help to define candidate genes involved in GH secretion.

PO1-157 GH Physiology

Inhibition of the protein expression of CIS ubiquitin ligase overcomes the impairment in the GH signalling pathway in growth hormone transduction defect (GHTD)

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Introduction: We have previously described 4 GHTD children with severe growth delay, impaired phosphorylation of the transcription factor STAT3 and increased expression of the cell cycle inhibitor p21 who responded to hGH therapy. These children also showed over-expression of the negative regulator CIS and increased proteasome activity probably mediated by the over-expression of CIS protein acting as a ubiquitin ligase. **Objective:** To examine the association of CIS over-expression as a possible cause of defective GH signalling in GHTD. **Methods:** In fibroblasts from gingival biopsies of 1 control and 1 GHTD patient we inhibited CIS protein expression with silence RNA CIS (siCIS). The protein expression and cellular localization of CIS, the GH receptor (GHR), pTyrSTAT3 and p21 were studied by immunofluorescent microscopy (IF) at a basal level and after induction with 200ng/ml GH with and without inhibition of CIS by siCIS. **Results: 1)** After siCIS treatment, CIS expression was decreased by 70% in GHTD fibroblasts (GF) and C fibroblasts (C). **2)** Before siCIS, GF had mainly cytoplasmic localization of the GHR near the nucleus wheras C had both membrane and cytoplasmic localization of the GHR. After siCIS, GF had both membrane and cytoplasmic localization of the GHR as observed in C whereas C had only cytoplasmic accumulation of the GHR. **3)** Before siCIS, in GF, induction with 200ng/ml hGH did not cause any changes in the localization of the GHR, whereas after siCIS, induction with 200ng/ml hGH caused increased membrane localization of the GHR in both GF and C. **4)** Normal phosphorylation of STAT3 after induction with 200 ng/ml GH was observed in GF after -siCIS. **5)** Before siCIS, nuclear overexpression of p21 was observed in GF in comparison to C. In GF, after-siCIS the p21 over-expression was decreased and p21was localized in the cytoplasm as was observed in C.**Conclusions**: In GHTD patients, it appears that the over-expression of CIS protein reduces the availability of the GH receptor at the membrane, reduces the activation of STAT3 and promotes the overexpression of the cell cycle inhibitor p21, and may be one of the main causes of pathological GH signalling in these children.

PO1-158 GH Physiology

Overnight growth hormone (GH) concentrations are supressed by testosterone (T) infusion in eumenorrheic and polycystic ovary syndrome (PCOS) adolescents

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Chronic exposure to gonadal steroids increases GH secretion. It has been demonstrated that lean PCOS patients have increased GH secretion. This study was aimed to explore acute effects of T on the characteristics of overnight GH profile in eumenorrheic and PCOS adolescents with normal BMI. Seven non obese PCOS adolescents and 8 matched controls (C) were studied in the early follicular phase of 3 consecutive menstrual cycles or in 3 consecutive months. Overnight GH profiles (1900 to 0700 h) were determined during saline (B) and constant testosterone (T) infusions, low dose (T-LD): 0.75 and high dose (T-HD): 2.5 mg/12 h iv. Blood samples were withdrawn every 20 minutes. GH (ICMA) and T (ECLIA) were determined during all 3 infusions. Pulsatile GH characteristics were analyzed by Cluster program. **Results:** On T-LD, T levels increased 2-3 folds from B in control group (1.4±0.25 to 4.1±0.46 nmol/L) and in PCOS (from 2.4±0.30 to 4.6±0.54 nmol/L), whereas on T-HD values rose to 8.5 ± 0.96 and to 10.4±0.67 nmol/L in C and in PCOS, respectively. None of the characteristics of GH release was modified by T-LD infusion in either C or PCOS. However, T-HD blunted mean GH concentrations (Control B: 2.0±0.41 vs T-HD: 1.4±0.26 µg/L; PCOS: 2.7±0.54 vs 1.5±0.28 µg/L), maximum GH peak height (Control B: 6.7±1.4 vs T-HD: 4.1±0.76 µg/L; PCOS: 8.4±1.5 vs 5.2±0.81 µg/L), and increment peak amplitude (Control B: 6.4±1.4 vs T-HD: 3.9±0.75 µg/L; PCOS: 8.1±1.4 vs 5.0±0.80 µg/L) (all parameters $p<0.05$ vs B). GH frequency did not change. **Conclusions:** Similar responses to acute T infusion in both groups suggest that the effect of T in GH system is maintained in PCOS. The selective decrease in GH pulse amplitude by T-HD in both groups of adolescents may reflect inhibition by nonestrogenic metabolites of T.

PO1-159 GH Physiology

Treatment with growth hormone decreases small dense LDL particles and high sensitive CRP

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Objectives: To investigate the antiatherogenic properties of growth hormone, we analysed lipoproteins, LDL subclasses, and hsCRP before and after 1 year of treatment. 57 patients aged 6 to 16 years with short stature and an indication for the treatment with growth hormone were included. Among them, 30 had SGA related short stature, 6 had a Turner syndrome, and 21 suffered from growth hormone deficiency.
Methods and Results: LDL subclasses were determined by ultracentrifugation and hsCRP on the Roche Modular PP analyser using Roche CRP (Latex) HS assay. Total LDL (p=0.004) and LDL small dense subclasses 5 and 6 (p<0.004), and hsCRP (p=0.006) decreased significantly in the total group. In SGA patients, total LDL decreased from 83.2 to 77.0 mg/l, p<0.03 and LDL-5 from 10.05 to 8 mg/l, p<0.01. Moreover, hsCRP decreased from 0.6 to 0.2 mg/l, p<0.03. In Turner patients, exclusively hsCRP decreased significantly (p<0.03), whereas in patients with growth hormone deficiency, LDL-5 decreased from 9.4 to 8.7 mg/dl, p=0.003, and LDL-6 decreased from 10.3 to 9.6 mg/dl, p<0.03.
Conclusion: Treatment with growth hormone shows distinct antiatherogenic effects. Based on these preliminary results, especially patients with SGA seem to benefit with respect to prevention of premature atherosclerosis.

PO1-160 GH Physiology

The glucagon test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years

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Among the various pharmacological GH stimulation tests, few studies have addressed the diagnostic value of glucagon in children with suspected GHD.
Objective: To investigate the diagnostic value of glucagon test as an alternative test to insulin tolerance test (ITT) and arginine in children with GHD younger than 6 years.
Design and Setting: This study was conducted in two Pediatric Endocrinology Centers.
Patients and Methods: Forty-eight children (median age 4.2 years, median height -3.0 SDS) with GHD confirmed by a peak GH to ITT and arginine < 10 µg/L (median 4.7 µg/L and 3.4 µg/L, respectively, P=NS), underwent a glucagon stimulation test. MRI showed normal hypothalamic-pituitary anatomy in 24 children with isolated GHD (IGHD), isolated anterior pituitary hypoplasia (APH) in 7 (6 IGHD and 1 with multiple pituitary hormone deficiencies, MPHD) and structural hypothalamic-pituitary abnormalities in 17 (2 IGHD, 15 MPHD).
Results: Median peak GH response to glucagon (13.5 µg/L) was significantly higher than ITT and arginine (P<0.0001).

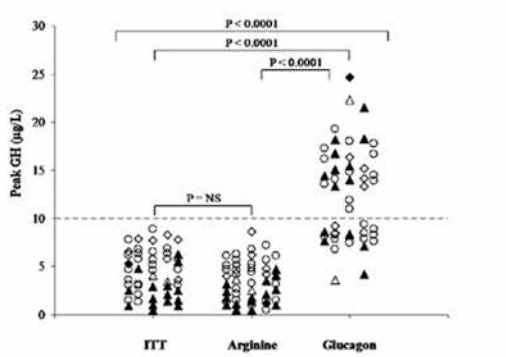

Figure 1.

Distribution of peak GH response to ITT, arginine and glucagon in 48 subjects affected by:

○ normal MRI and IGHD

△ anterior pituitary hypoplasia and IGHD

▲ anterior pituitary hypoplasia and MPHD

◇ structural hypothalamic-pituitary abnormalities and IGHD

◆ structural hypothalamic-pituitary abnormalities and MPHD

(ITT, Insulin tolerance test; IGHD, isolated GH deficiency; MPHD, multiple pituitary hormone deficiencies).

Peak GH to glucagon was <10 µg/L in 20 (group 1) and >10 µg/L in 28 subjects (group 2), without significant clinical or biochemical differences between the two groups. Median peak GH to glucagon was not statistically different neither between those with MPHD or isolated GHD nor between subjects with and without structural HP abnormalities, while median IGF-I SDS (-3.3 SDS vs. -1.9 SDS, P<0.0001 and -3.3 SDS vs. -1.9 SDS, P=0.0003, respectively) and median peak GH to both ITT (2.3 µg/L vs. 5.8 µg/L, P=0.0002 and 2.5 µg/L vs. 5.8 µg/L, P<0.0001) and arginine (1.8 µg/L vs. 4.5 µg/L, P=0.0004 and 2.1 µg/L vs. 4.6 µg/L, P=0.0014) were lower in the former. Peak GH to glucagon was negatively related to age at diagnosis (ρ=-0.636, P<0.0001).
Conclusions: We have shown that the glucagon test is a potent test for GH secretion in young children with GHD. Normative data for this test in young children needs to be established before its use in clinical practice

PO1-161 GH Physiology

Posterior pituitary function in patients carrying structural hypothalamic-pituitary abnormalities

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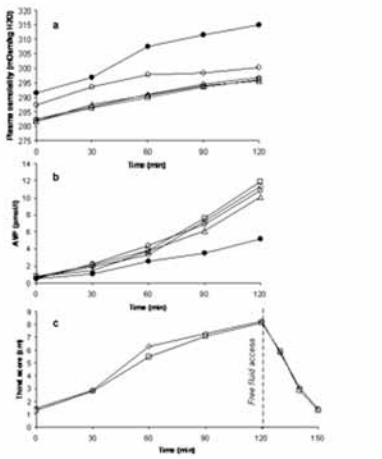
Background We performed an i.v. osmotic challenge to evaluate posterior pituitary function in subjects with anterior pituitary defects and structural hypothalamic-pituitary (HP) abnormalities at MRI.
Subjects 42 subjects (19 F, 22 M; mean age 17.1±1.0 years) with anterior pituitary dysfunction were divided into 5 groups according to their endocrine-MRI features: 8 subjects had isolated GH deficiency (IGHD) with normal MRI (group 1); 30 with an ectopic posterior pituitary (EPP), of whom 15 with isolated EPP [8 IGHD, 7 multiple pituitary hormone deficiencies, (MPHD) of whom 1 with central diabetes insipidus; group 2], 7 with septo-optic dysplasia (SOD) and corpus callosum or septum pellucidum anomalies (3 IGHD, 4 MPHD; group 3) and 8 with SOD, MPHD and other complex HP abnormalities including EPP (group 4). Four subjects (1 IGHD, 2 MPHD; group 5) showed SOD and other HP anomalies with normal posterior pituitary location.
Methods After an overnight fast, a peripheral venous line was placed and plasma osmolality (Posm), arginine-vasopressin (AVP) and a thirst score (based on a 10-cm long visual analog scale) were measured at time 0 and after 30,60,90 and 120 minutes of hypertonic (855 mmol/liter) saline i.v. infusion (rate 0.05 ml/kg/min). At the end free access to oral fluids was allowed and thirst score evaluated after 10,20 and 30 minutes in groups 1 and 2.
Results All patients showed significant increase of Posm and AVP after saline infusion (P<0.0001).



Patterns of plasma osmolality (a), arginine-vasopressin (AVP) concentration (b) and thirst score (c) during 2 hours of hypertonic saline i.v. infusion and subsequent 30 minutes of free oral fluid intake in five groups (NB: figure 1c: only groups 1 and 2):
□ Group 1: normal MRI
◊ Group 2: Isolated ectopic posterior pituitary without other hypothalamic-pituitary anomalies
▽ Group 3: Ectopic posterior pituitary, septo-optic dysplasia, corpus callosum and septum pellucidum abnormalities
● Group 4: ectopic posterior pituitary, septo-optic dysplasia, corpus callosum and septum pellucidum abnormalities, other complex hypothalamic-pituitary abnormalities
△ Group 5: normal posterior pituitary, septo-optic dysplasia corpus callosum and septum pellucidum abnormalities

Mean Posm at baseline and during infusion was significantly higher in group 4 than in the others (P<0.0001). While no significant differences were observed at baseline in mean AVP concentrations between the 5 groups, AVP reached the lowest levels in group 4 as compared to the other groups after saline infusion (P<0.0001 at all times). Thirst score evaluation was not different between groups 1 and 2.

Conclusion Posterior pituitary function is not dissimilar between subjects with normal posterior pituitary location or EPP. Subjects with SOD, structural HP abnormalities and EPP showed a major impairment of hypothalamic-posterior pituitary function as compared to subjects with milder phenotypes.

PO1-162 GH Physiology

Infant fed soy formula have normal growth, puberty and final height

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An increased rate of male infertility, abnormalities in reproductive organs and the high rate of precocious puberty, increased the awareness to the potential hormonal effects of endocrine disruptors as evidenced from experimental animals and wildlife. phytoestrogens (PE) and in particular the isoflavones(IF) have structural homology to estradiol. These IF are the major PE in soy formulas (SF). Several studies in animal models and human adults have demonstrated estrogenic effects of IF Similar studies in infants are lacking, The IF in infant formulas may cause to100-200 times higher IF than in breastfed (BF) infants. Population: Since1977 longitudinal data were available on 999 healthy girls who reached final height at the time of analysis were included in the study. 312 of these children were BF for 1 year and 111 for 6 months. 125 were fed on SF for 5-8 months and 451 were on milk based protein formula. *Anthropometry* Weight and length/height from birth to 18 years of age was measured in addition, parental and grand parent height were obtained. Puberty: Breast bud and menarche were recorded. Clinic visiting intervals were 1 to 2 months for the first 6 months of life, and every 3 months during the following 18 months, and yearly thereafter, until final height. At each visit, anthropometric measurements and the occurrence of pubertal signs were obtained and recorded. Results: similar to our previous report (J Pediatr Gastroenterol Nutr. 2008;46:191), a small increase in the occurrence of breast bud in the second year of life in soy fed girls was noted. There was no difference in the timing of appearance of breast bud or menarche between soy fed or formula and breast fed babies. Final height was similar in all groups a similar secular trend was noted in all groups. Final comments: Our results in a longitudinal study do not show any estrogenic adverse effect on puberty or growth in soy fed infants during the first year of life. Additional studies are needed for older age groups although these results on infants make the possibility of adverse effect of soy, unlikely.

Height and puberty markers by nutrition				
nutrition	Breast fed 12m	breast fed 6 m	soy formula	milk formula
number	312	111	125	451
final height (cm)	165.4±6.3	165.2±6.4	165.5±6.6	166.0±6.7
mother height (cm)	163.5±6.7	163.1±6.6	163.6±6.9	163.2±6.8
breastbud % at 7 yrs	1.9	1.8	2.3	2.2
breast bud % at 8 yrs	3.8	4.5	4.8	5.3
age at menarche (yrs)	11-13.6	11.1-13.7	11.3-13.5	11-13.8

PO1-163 Gonads and Puberty I

Hypothalamic-pituitary-gonad function of 88 patients treated for medulloblastoma or ependymoma during childhood

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Background: Posterior fossa tumours are treated by surgical resection, cranial irradiation (CR) plus spinal irradiation and/or chemotherapy. CR may induce early puberty, or no puberty due to gonadotropin deficiency. The spinal irradiation and chemotherapy may alter gonad function. The association of gonadotropin deficiency and gonad lesions, which increase gonadotropin secretion, makes it difficult to evaluate the hypothalamic-pituitary (HP) gonad (HPG) axis.

Objective: To analyse the HPG axis and/or gonad lesions; factors influencing its alteration and frequency of LH/FSH deficiencies, compared to the other deficiencies.**Patients and methods:** The 88 patients were treated for medulloblastoma (n=73) or ependymoma (n=15) at 6.8 (0.6-16) years by surgical resection and CR [median 29 (5-49) Gy], associated with spinal irradiation in 68 and with chemotherapy in 69. They were evaluated at age 14.2 (5.4-24.2) years, among them 75 (45M/30F) of pubertal age. **Results:** Early puberty occurred in 26 (13M/13F). At evaluation, 57 (37M/20F), including the two girls who underwent ovarian transposition, had normal clinical-biological puberty, 13 (6M/7F) had peripheral gonad insufficiency and 5 (2M/3F) had absence or no progression of puberty. The basal plasma FSH concentrations were elevated in 13 for FSH (> 9 IU/l, 10M/3F) and in 12 for LH (> 5 IU/l, 5M/7F), and low in 8 for inhibin B (3M/5F). Most of them were given spinal irradiation and chemotherapy. None had low LH/FSH. In males, the inhibin B (233±25 pg/ml) correlated with FSH (r=-0.58, p=0.0006) and LH (r=-0.52, p=0.002), but not with age at irradiation, testicular volume or testosterone. In females, the inhibin B (53±12 pg/ml) correlated with estradiol (r=0.42, p=0.05) and age at irradiation (r=0.50, p= 0.01), but not with FSH or LH. In the whole group, inhibin B was not influenced by the type of irradiation (cranial or craniospinal), however it was much lower in patients given chemotherapy (137±19 pg/ml) than in those who were not (256±50 pg/ml, p = 0.012).The other deficiencies were: 76% GH; 29% abnormal thyroid function (peripheral hypothyroidism in 26, hyperthyroidism in 2); moderate hyperprolactinemia in one.

Conclusions: In the patients treated for posterior fossa tumours, the HPG axis lesion expresses as increased FSH/LH and decreased inhibin B plasma concentrations due to the spinal irradiation and/or chemotherapy. This probably masks the partial gonadotropin deficiency induced by CR.

PO1-164 Gonads and Puberty I

Age at pubertal growth spurt in girls and boys in relation to body mass index during the emerging obesity epidemic

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Background Recent studies suggest that girls may be entering puberty at younger ages than previously, and it has been hypothesized that the increasing prevalence of childhood obesity is contributing to this secular trend. The purpose of this study was to analyse the association between prepubertal BMI and pubertal timing as assessed by age at onset of pubertal growth spurt (OGS) and at peak height velocity (PHV) in a large cohort of Copenhagen school children to determine if the reported obesity epidemic has any impact on the secular trends in the timing of puberty.

Methods and materials Annual measurements of height were available in all children born from 1930 to 1969 who attended primary school in the Copenhagen Municipality. 156,835 children fulfilled the criteria for determining age at OGS and PHV. The effect of prepubertal BMI at age 7 years on these markers of pubertal development was analysed.

Results Age at OGS and PHV was significantly negatively influenced by prepubertal BMI. Dividing the children into 5 categories of BMI at 7 years of age we found a significant relation between BMI and age at OGS and at PHV. The secular trend towards earlier maturation was evident in all BMI categories. The five categories were completely separated with no overlapping confidence intervals in girls, e.g. the heaviest girls at 7 years of age entered puberty significantly earlier than any of the lighter categories, and the lightest girls entered puberty significantly later than any other category. In boys a similar trend was found in the cohorts born after 1945. The decline in age at OGS and PHV during the study period was most pronounced in the girls with the highest prepubertal BMI.

Conclusion We found that the heaviest girls at age 7 entered puberty significantly earlier than any of the leaner girls, and the same tendency was evident in boys, although less strong. There was a downward trend in the age at attaining puberty in all BMI categories in both boys and girls, suggesting that the obesity epidemic is not solely responsible.

PO1-165 Gonads and Puberty I

Physiological estrogen replacement therapy during mid-puberty in girls

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Background

In girls, we have previously shown that it is possible to mimic the spontaneous levels as well as the diurnal pattern of serum estradiol in early puberty, by cutting a transdermal estradiol matrix patch and attaching a part of it, corresponding to 0.08-0.12 microgram estradiol/kg body weight, to the skin nocturnally (JCEM 2001:86;3039-44). However, the optimal dose for mimicking mid-pubertal estradiol levels is not defined. During mid-puberty, nocturnally application resulted in pretreatment serum estradiol levels which are not physiological. In this study we show a physiological way to administer estrogen replacement therapy during mid-puberty.

Study patients

29 hypogonadal girls with induced puberty. Their ages ranged 12,8-18,9 years, weight ranged 38-77 kg and Tanner breast stages ranged 2-5, all before menarche.

Methods

A transdermal matrix patch of 17beta-estradiol (Evorel ® (=Systen ®), Janssen-Cilag, Belgium; 25 microgram/24 hours) was cut into 6.25-18,75 microgram/24 hours) or kept as 25 microgram/24 hours. The patch was thereafter divided and attached to the skin overnight. In the morning, one of the parts was removed and the other remained attached during day. The day-patch was removed the following evening and replaced by a new pair. In 6 girls, samples were collected every 2 hours during 24 hours, in 4 girls in the morning and evening and in the other 19 girls sampling were done only in the morning with patches in situ. Serum estradiol concentrations were determined by extraction radioimmunoassay (detection limit: 4.0 pmol/L). The results were compared to the estradiol concentrations seen in girls during spontaneous mid-puberty.

Results

Doses of 0,17-0,51 microgram Evorel/kg girl mimicked the morning serum estradiol levels (75-131 pmol/L), 0,09-0,26 microgram Evorel/kg mimicked the nadir estradiol levels (20-43 pmol/L) seen in healthy mid-pubertal girls as well as the diurnal variation seen during spontaneous mid-puberty.

Conclusion

By keeping half the estradiol night-dose during day, it is possible to mimic the estradiol levels as well as the diurnal rhythm seen in girls during spontaneous mid-puberty.

PO1-166 Gonads and Puberty I

The role of fibrillin in polycystic ovary syndrome in mice

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Objective: Transmission/disequilibrium testing and genetic analysis has linked polycystic ovary syndrome (PCOS) to fibrillin-3 [1,2]. Fibrillins interact with various members of the transforming growth factor (TGF)-β superfamily [3-9]. Different members of the TGF-β superfamily have been found to be either abnormally high or low in PCOS ovaries [10-13]. The purpose of this study is to determine whether mice deficient in the fibrillin gene develop PCOS or show aberrant levels of different members of the TGF-β superfamily within the ovary.

Methods: Heterozygous fibrillin-1 deficient and fibrillin-2 null mice on regular and high fat diets were evaluated by nuclear magnetic resonance, dual energy X-ray absorptiometry, quantitative real-time polymerase chain reaction, immunohistochemistry, and enzyme immunoassay hormonal blood testing.

Results: Heterozygous fibrillin-1 deficient mice are larger than wild-type with higher amounts of fat. Homozygous fibrillin-2 null mice are smaller than heterozygous mice which are smaller than wild-type mice. Blood sugars do not correlate with body size. Anti-Müllerian hormone levels are elevated in fibrillin-2 null mouse ovaries. Bone morphogenetic protein (BMP)-4, BMP-7, and growth differentiation factor-9 are decreased in heterozygous fibrillin-1 deficient and fibrillin-2 null mouse ovaries.

Conclusions: There are no mouse models for PCOS. Heterozygous fibrillin-1 deficient and fibrillin-2 null mouse ovaries have aberrant TGF-β superfamily signaling, as is also seen in human PCOS ovaries. Fibrillin deficient mouse models may help to determine the mechanisms of abnormal growth factor signaling, which may lead to new treatments for the most common cause of infertility.

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PO1-167 Gonads and Puberty I

Growth in bone marrow transplanted children: does conditioning regimen matter?

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It is known that growth problems after bone marrow transplantation (BMT) are dependent upon the preparative regimen received that may include alkylating agents as cyclophosphamide (CY) with or without busulphan (BU) and total body irradiation (TBI). To evaluate the real impact of BMT on growth we studied 59 patients (pts), 34 males, without history of cranial radiotherapy or cranial tumour, transplanted at a median age of 9 years (yrs) for malignant (34), not malignant (9) haematological disease or solid tumours (16), after pre-BMT conditioning with high dose chemotherapy (CT) with (n.12, group 1) or without TBI (n.47, group 2). 32/59 underwent allogeneic BMT. 41/59 pts were prepubertal at BMT and 28/59 reached final height. The median follow-up is 5,6 yrs (range 1-11 yrs). Auxological data are expressed in standard deviation (SD).

Group 1: height at BMT (H0) is -0,60±1; the difference between height from BMT to 1 yr after(H1-H0) is -0,3±0,3. At last control (follow-up 6,1±3 yrs), H-H0 is -0,35±1. 9/12 reached final height (FH -0,82±0,9 SD) and FH-H0 is -0,51±1; FH-Target height (FH-TH) is -0,50±0,9.

8/12 pts were prepubertal at BMT: H1-H0 is -0,4±0,2; height at start puberty(P) was measured and HP-H0 is -0,79±0,4. 6 pts reached final height (FH) and FH-H0 is -0,8±1;FH-HP -0,26±1; FH-TH is -0,54±1.

7 pts was tested for GH deficiency and 2 started treatment.

Group 2: H0 is -0,21±0,9; H1-H0 is -0,23±0,5 and H-H0 -0,30 ±0,8 (follow-up (5,5±3 yrs). 19/47 pts reached FH and FH-H0 -0,25±0,7; FH-TH is -0,37±0,9.

33/47 pts were prepubertal at BMT, H0 is -0,3±1; H1-H0 is -0,27±0,5; H-H0 is -0,29±0,9 (follow-up 6,2±3 yrs).20 pts started puberty and HP-H0 is -0,26±0,7; The 33 prepubertal pts were subdivided in 2 groups: with (A) or without (B) BU based pre-treatment regimens.

Group A (23/33): H1-H0 is -0,09±0,5; H-H0 is -0,22±1,1; in 13/24 HP-H0 is -0,2±0,7. *Group B* (10/33): H1-H0 is -0,74±0,2; H-H0 is -0,43±0,6; in 7/10 HP-H0 is -0,4±0,7. 16 pts were treated for subclinical hypothyroidism and 11 pts for hypogonadism. Our pts did not show severe growth failure, regardless pre-BMT conditioning. In all pts growth impairment occurred in the first years after BMT and before puberty. It continued until first yr post BMT in group 2 and until puberty in group 1: only group B pts showed a partial catch up growth in the prepubertal period. Regular monitoring of growth and development post BMT is mandatory to detect early growth impairment and treat hormone deficiencies.

PO1-168 Gonads and Puberty I

Comparative follow up study of body mass index (BMI) in girls with idiopathic central precocious puberty treated with GNRH analogs (GNRHa) and nontreated

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Objectives: Evaluate the body mass index (BMI) of girls with idiophatic central precocious puberty (ICPP), and compare one year data of those treated with GNRH analogs (GNRHa) with non-treated controls.

Methods: We performed a review of 115 charts of patients with ICPP followed at our unit from december 2000-2008. We identified 18 girls (15,7%) treated with GNRHa, and compared with 30 non treated girls, after one year of follow up. Variables analyzed were age at beginning of treatment or follow up, weight, height, BMI, BMI SDS and nutritional classification.

Results: Chronological age was lower in the treatment group 5,35±2,69 vs 6,66 ± 2,15 in the control group (p=0,06) at initial evaluation. BMI SDS was not different between treated and non- treatment groups at initial evaluation or after one year follow and did not change significantly within groups during treatment with GNRHa (before=1,75±1,63 vs after=1,97 ±2,12) or during follow up in the non-treated group (before=1,31 ±1,51 vs 1 year =0,82 ± 1,14). Nutritional classification was done according to WHO criteria and the proportion of overweight or obese children (BMI>+1 SDS) significantly decreased in the follow up group (53,3% vs 30% p =0,006) and was lower although not significant (72,2% vs 53% p= 0,49) in the treated girls.

Conclusion: In our population BMI was similar in girls with ICPP indicated for treatment and those followed up for pubertal progression. BMI SDS did not change during treatment with GNRHas. The number of overweight children decreased during follow up in both groups although it did not reach statistical significance in the treated girls.

PO1-169 Gonads and Puberty I

Final height in girls with idiopathic precocious puberty treated with leuprolide: dose-titration approach

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The impact of treatment of girls with idiopathic central precocious puberty (ICPP) with GnRHagonists on height remains controversial, due to limited data regarding final height. Furthermore, GnRHagonist dose used varies between Europe and North America, higher doses are used in the latter. We report final height data of 16 girls with idiopathic central precocius puberty (ICPP) treated with Leuprolide acetate for average duration of 27±8 months with a dose-titration approach between 2000-2009. Control group consisted of 16 girls with ICPP presented within the same period but did not receive any treatment because of older age at presentation or refusal of treatment. Exclusion criteriae were organic etiology and patients with history of IUGR.

Treatment was started with Leuprolide acetate 3.75 mg/q4week in all patients and the dose was increased only if there is inadequate suppression of LH and/or undue bone age advancement. In six patients, the dose of 3.75 mg/q4week was sufficient throughout treatment duration. The dose had to be increased to 3.75 mg/q3week in six patients, 7.5 mg/q4week in three patients and 11.25 mg/q8week in one patient.

In treated patients, Mean±SD predicted adult height (PAH) at the onset of treatment was 161.5 ± 6.8 whereas target height was 159.4± 5.0 cm. Final height reached was 161.5 ± 6.0 cm.

In untreated patients, PAH at diagnosis was 157.7 ± 6.6 using advanced table and 153,4 ± 5.8 using average table of Bayley-Pinneau. Target height was 156.5± 5.2 cm. Final height reached was 154.5± 7.2 cm.

Only seven patients in the treatment group remained below PAH whereas twelve patients in the untreated group remained below PAH.

BMI SDS at the onset, at completion of treatment and at final height were 1.0± 0.7, 0.9± 0.8 and 0.4 ± 1.0 respectively. In untreated patients BMI SDS at diagnosis and at final height were 0.9 ± 0.7 and 0.9 ±1.1 respectively.

In conclusion, low dose-titration approach is effective in delaying menarche and preserving height in girls with ICPP. An initial dose of Leuprolide 3.75 mg/q4wk is efficient in most children with ICPP. Treatment is not associated with increase in BMI SDS. Bayley-Pinneau method overpredicts height in untreated girls with ICPP when advanced table is used. Using average table predicts adult height in untreated patients with ICPP with better precision.

PO1-170 Gonads and Puberty I

Gonadal function in a large pedigree of patients with the IMAGe syndrome

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The IMAGe Syndrome has recently been described in a few patients. Although genital anomalies have been reported, little information is available about

clinical variability and gonadal function. We report clinical evaluation and gonadal function in 8 patients (5 boys) with the IMAGe Syndrome from a large Argentinian pedigree.
Clinical data were obtained from all the patients; whereas gonadal function was available from 5 boys and one girl (age range 21 days – 17 yr). Serum LH and FSH were measured by IFMA; Inhibin B, Pro-αC and AMH were measured by ELISA.
In males 3/5 had micropenis, 3/5 cryptorchidism and 3/5 microorchidism (testicular volume < 1 ml). No genital ambiguity was observed. Serum Inhibin B, Pro-αC and AMH were low or in the lowest 25th centile of reference values. Serum FSH, LH and testosterone were normal in all prepubertal patients. One patient started puberty at a normal age; however, at Tanner genital stage 3, his testicular volume was small (2-3 ml each) and only reached 8 ml at 17 yr (Tanner genital stage 5). Inhibin B was undetectable and FSH was 20.5 IU/l.
The three girls had normal external genitalia. Gonadal function in the only girl studied showed normal serum gonadotropins and inhibins at 21 days of age.
In summary, boys with the IMAGe Syndrome had at least one clinical genital anomaly indicative of primary hypogonadism of late onset in fetal life. Testicular function showed a primary Sertoli cell dysfunction and apparently normal interstitial function (at least in early infancy and in one patient evaluated at puberty). In girls, external genitalia were normal; however, hypogonadism at a prepubertal age cannot be ruled out. More patients will need to be studied throughout puberty in order to: a) further characterize the anomalies of testicular function to evaluate up to what extent their fertility can be affected and b) determine whether ovarian function is impaired.

PO1-171 Gonads and Puberty I

Pubertal development in children and adolescents with type 1 diabetes: normal timing and progression of puberty in well controlled type 1 diabetes

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Objective: To investigate the effect of type 1 diabetes on pubertal onset and development in children and adolescents with type 1 diabetes. In previous studies delayed pubertal onset has been reported in children with mediocre diabetes control.
Patients and methods: In 102 patients with type 1 diabetes (53 females, 49 males) stages of pubertal development were examined every three months. Start of puberty was defined as Tanner stage B2 in girls and as testicular volume of three milliliters in boys. Girls were asked for the age at menarche and auxological data was recorded. For comparison pubertal development data form Largo and Prader were used (Largo RH, Prader A 1983 Pubertal development in Swiss girls/boys. Helv Paediatr Acta 38: 229-243/211-228).
Results: At manifestation of type 1 diabetes patients had a mean age of 8.1±3.8 years (girls 7.9±3.8 years, boys 8.3±3.7 years). Mean diabetes duration until onset of pubertal development was 3.8±3.1 years (girls 3.5±3.1 years, boys 4.2±3.1 years). Diabetes control until the onset of pubertal development was rather good with a mean HbA1c of 7.1±0.7% (girls 7.1±0.7%, boys 7.2±0.6%). Tanner stage B2 was achieved at a mean age of 10.8±1.1 years (vs. Largo & Prader at 10.9±1.2 years), pubic hair Tanner stage two in girls was documented at 10.9±1.2 years (vs. Largo & Prader at 10.4±1.2 years) and mean age at menarche was 12.9±1.0 years (vs. Largo & Prader at 13.4±1.0 years). The period between onset of puberty and Tanner stage five was 2.5±0.8 years in girls. At start of puberty BMI-SDS was already above average with 0.3±1.0 and height-SDS was 0.9±1.2 SDS.
In boys testicular volume of three milliliters was achieved at 12.2±1.3 years of age (vs. Largo & Prader at 11.8±0.9 years), pubic hair Tanner stage two in boys was documented at 12.5±1.3 years (vs. Largo & Prader at 12.2±1.5 years). The period between onset of puberty and Tanner stage five was 2.5±0.7 years in boys. At start of puberty BMI-SDS was already above average with 0.6±0.9 and height-SDS was 0.3±1.0 SDS.
Conclusion: Pubertal timing and development are not delayed in children and adolescents with well controlled type 1 diabetes. At onset of puberty weight is already above average in children with type 1 diabetes.

PO1-172 Gonads and Puberty I

Gonadal function of 100 patients after bone marrow transplantation (BMT) during childhood

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Background: Conditioning for BMT may alter the viability of germ cells and production of gonad hormones. **Objective:** To analyze the risk factors for gonad failure and evaluate the plasma inhibin B concentrations as marker of the gonad function. **Patients and methods:** The gonad function of 100 patients given BMT was evaluated. None had factors that might interfere with their gonad function other than conditioning for BMT. They were classified as: normal gonad function (spontaneous pubertal development and normal FSH and LH plasma concentrations), or gonad failure (elevated FSH and/or LH); this group was subdivided into partial (spontaneous pubertal development and normal plasma testosterone/estradiol) or complete (no pubertal development) failures. We analyzed the roles of the initial disease (malignant or not), the conditioning protocol and age at BMT for each group. The conditioning protocol was: total body irradiation (TBI) fractionated 12 Gy or single 8-10 Gy; total lymphoid irradiation (TLI), 5 or 6 Gy; or chemotherapy alone. We measured, retrospectively, the plasma inhibin B of 78 patients (43 boys). **Results:**

TESTICULAR FUNCTION			
	Normal	Failure	p
N=53	26.5%	73.5% (47%, 21%, 5,5%)*	
Disease			
Malignant/ Non malignant	20% / 62.5%	80% / 37.5%	
Conditioning			
TBI / TLI / Chemo	17.5% / 57% / 50%	82.5% / 43% / 50%	
Age BMT, yr (M±SD)	8.4±3.5	8.3±3.6	ns
inhibin B pg/mL (M±SD)	210±238	47±53 (62/18/15)*	0.001

* (tubular failure, tubular and partial leyding failure, tubular and leydig failure)

OVARIAN FUNCTION			
	Normal	Failure	p
N=47	19%	81% (29%,52%)**	
Disease			
Malignant/ Non malignant	16% / 33%	84% / 67%	
Conditioning			
TBI / TLI / Chemo	13% / 50% / 25%	87% / 50% / 75%	
Age at BMT, yr (M±SD)	6.3±2.5	7.7±3.9	ns
Inhibin B pg/ml (M±SD)	78.5±55	21.2±26.7 (26.5,11.7)**	0.0003

** (partial ovarian failure, complete ovarian failure)

Inhibin B correlated negatively with FSH ($p<0.0001$ in boys, <0.004 in girls), but not with testicular volume ($p=0.058$).**Conclusion:** Gonad function was abnormal in 73.5% of boys and 81% of girls. The increased frequency of gonad failure in patients treated for malignant disease is probably due to the chemotherapy given before BMT. TBI seems to be the major risk factor of gonad failure, while age at BMT is not. Inhibin B seems to accurately reflect the severity of gonad damage after BMT.

PO1-173 Gonads and Puberty I

Factors predicting the adult height and age at first menstruation in 138 girls with idiopathic central precocious puberty

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Background: It is difficult to decide whether to give a girl with idiopathic central precocious puberty (CPP) gonadotropin hormone releasing hormone (GnRH) analog: as it may be hard to distinguish between CPP and premature thelarche, as between classical and slowly progressing forms. Because of this variability, randomized prospective trial comparing treated to untreated girls seems to be inappropriate.

Objective: To predict at initial evaluation the difference between adult height (AH) and target height (TH), and (for untreated girls) the age at first menstruation (M1).

Patients and methods: Of the 138 girls with idiopathic CPP who reached their AH, 76 were given GnRH analog (7.9±1.5 to 10.7±0.9 yr) because their predicted AH was below 155 cm (n=28), their LH/FSH peaks ratio over 0.66 (n=42) and/or their estradiol over 20 pg/ml (n=40). The 62 others, whose predicted AH was greater than 155 cm (n=56) and/or they had a very low estrogenic activity, were followed without treatment. The intervals between the onset of CPP and evaluation were similar for the treated and untreated girls (0.9 yr).

Data analysis: Multiple linear regression was performed on several subsets of variables. The models selected were those that gave the best trade-off between quality and simplicity. They used the height (SD) at initial evaluation (H1).

Results:

Treated: AH–TH (cm) = 3.53 (H1–TH) – 1.88 (H1–predicted AH) – 3.50 with R2 = 0.78. The actual AH of 11 (17%) was lower than this prediction by more than 3 cm; 4 of them were obese.

Untreated: AH–TH (cm) = 2.54 (H1–TH) – 2.78 LH/FSH peaks ratio – 3.40 with R2 = 0.78. The actual AH of 8 (14%) was lower than this prediction by more than 3 cm; 6 of them had a bone age advance greater than 2 years at initial evaluation. Age M1 – age CPP (yr) = 12 – 1.07 age CPP – 0.35 (H1–TH) with R2 = 0.74.

Conclusions: The difference between AH and TH of treated and untreated girls can be predicted by two formulas using the height at initial evaluation, TH, predicted AH (calculated by the Bayley and Pinneau method) and the LH/FSH peaks ratio. Height (SD) greater than the TH at initial evaluation in both treated and untreated girls, and a lower LH/FSH peaks ratio in untreated girls, suggest greater AH. These formulas may help when deciding whether to treat a girl when it is difficult to distinguish between classical and a slowly progressing forms.

PO1-174 Gonads and Puberty I

Pharmacodynamics of leuprolide acetate stimulation testing in diagnosing central precocious puberty

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Introduction: GnRH agonists are available in short- and long-acting preparations and are used for both treatment and diagnosis of central precocious puberty (CPP). Because GnRH is unavailable in the U.S., aqueous leuprolide acetate (LA) has become the standard stimulating agent for diagnostic testing. Initial studies measured LH and FSH at 3-24h, but subsequent data have shown an earlier LH rise. Still, there is confusion regarding the expected timing of the LH or FSH peak.

Methods: To assess the pharmacodynamics of Gn secretion following LA (20 mcg/kg sq), serum was obtained at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min for LH and FSH (Esoterix ICMA) in 11 girls with PP. The subjects were grouped based on clinical features: likely CPP, CPP on depot lupron with suboptimal control (Rx), likely premature thelarche (PT), and a variant with androgenization before feminization (Var).

Results: At 30, 90, and 180 min after LA injection, serum LH reached a mean 74, 83, and 93%, respectively, of the maximum achieved at any time point. Mean LH reached its peak earliest in girls with CPP, with 93% of maximum at 30 min (table) followed by plateau. In contrast, the low LH levels in PT and variant groups kept rising slowly (55-63% of peak at 30 min). FSH rose throughout the time course in all groups without discernible peak. Because LH rose more quickly than FSH in all groups, sampling at 30 min exaggerated the peak LH/peak FSH ratio.

Gn response after LA				
Means (total n=11)	**CPP (3)**	**Rx (3)**	**PT (2)**	**Var (3)**
LH at 30 min (IU/L)	25.3	4.1	3.3	1.2
Peak LH	27.3	5.5	4.9	1.9
% of peak LH at 30 min	93	81	63	55
FSH at 30 min (IU/L)	10.9	5.6	9.6	4.2
Peak FSH	18.0	10.2	24.5	10.8
% of peak FSH at 30 min	60	62	42	43
LH/FSH at 30 min	2.70	0.73	0.33	0.26
Peak LH/Peak FSH	1.53	0.55	0.22	0.20
% of peak LH/FSH at 30 min	166	134	147	142

Conclusion: LH after LA is near maximum by 30 min in girls with CPP or treated CPP, but not in those without CPP. This is consistent with prompt secretion of Gn stores in true puberty, as seen after GnRH. Gn response is prolonged following aqueous (or depot) LA, suggesting extended production of new LH and particularly of FSH when compared with GnRH effects. Gradual LH rise is more evident in those without CPP because of the minimal early peak, whereas in CPP the robust immediate release of LH after LA permits a single 30+ min sample for diagnosis and monitoring.

PO1-175 Gonads and Puberty I

Precocious puberty audit

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BACKGROUND and AIM

Nowadays, national media makes precocious puberty (PP) a current issue and we noticed the increasing number of PP patients admitted to our pediatric endocrinology clinic. Therefore, we aimed to evaluate these cases retrospectively.

METHODS

A total of 98 cases with pubertal problems (girls aged 0.6-10.8 years) were assessed. They were classified as premature thelarche (PT), premature adrenarche (PA), thelarche variant (TV), central precocious puberty (CPP), peripheral precocious puberty (PPP) and early puberty (EP). All cases were subdivided into 3 groups according to their ages: group 1 (<6), group 2 (6-8) and group 3 (>8).

RESULTS

The mean age was 6.5 ± 2.2, median 7.2 years. Frequency of diagnosis and age distribution is shown in table 1. The most common symptom was breast development (80%). Of those, 5% was at Tanner stage IV/V and all had CPP. Only 6% presented with vaginal bleeding (4 had CPP, 1 PPP and 1 PT). The most common diagnosis was PT in group 1 (52%), CPP in group 2 (40%) and group 3 (44%). The number of admissions due to pubertal problems increased in the last 2 years. Moreover, the ratio of idiopathic CPP increased almost twice (table 2). Four of the cases (aged<6.5) with CPP had organic lesions in cranial MRI; hydrocephalus in 2, hamartoma in 1 and pineal cyst in 1.

CONCLUSION

Our study has shown that not only the number of pubertal problems but also diagnosis of idiopathic CPP increased in the last 2 years in our region. Noticeability of this raise needs to be confirmed with population based studies. Idiopathic CPP cases should also be assessed as progressive or non-progressive.

Table 1. Frequency of diagnosis and age distribution

Diagnosis	n	%	Mean age ± SD	Median	Range
PT	23	23.5	4.4 ± 2.4	5.5	1.2-8.1
PA	14	14.3	7.1 ± 2.5	7.6	0.6-10.8
TV	17	17.3	7.3 ±1.0	7.3	4.4-8.8
CPP	38	38.8	7.1 ± 1.7	7.5	3.4-10.1
PPP	1	1	5.5		
EP	5	5.1	8.5 ± 0.3	8.4	8.2-8.9

PT: Premature thelarche, PA: Premature adrenarche, TV: Thelarche variant,

CPP: Central precocious puberty, PPP: Peripheral precocious puberty, EP: Early puberty

Table 2. Diagnosis of pubertal problems across the years

Diagnosis	2005	2006	2007	2008
	n (%)	n (%)	n (%)	n (%)
PT	3 (27.3)	7 (43.8)	3 (11.5)	4 (16.7)
PA	2 (18.2)	1 (6.3)	3 (11.5)	2 (8.3)
TV	2 (18.2)	3 (18.3)	4 (15.4)	5 (20.8)
CPP	3 (27.3)	4 (25.0)	15 (57.7)	12 (50.0)
EP	1 (9.1)	1 (6.3)	1 (3.8)	1 (4.2)

PO1-176 Gonads and Puberty I

Evaluation of the interest of inhibin B measurement for differentiating boys with hypogonadotropic hypogonadism from those with constitutional delay of puberty

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Introduction. Delayed puberty in boys is one of the commonest causes for referral to a pediatric endocrinologist. The differential diagnosis lies between constitutional delay of puberty (CDP) and hypogonadotropic hypogonadism (HH). Physiological and stimulation tests did not allow for differentiating between the two conditions with 100% sensitivity and specificity. More recently, the ability to measure circulating inhibin B (INHB) produced by Sertoli cells could offer a simple way to discriminate between CDP and HH as INHB concentrations are detectable during prepuberty and show significant changes during normal puberty.
Aim. To assess the interest of INHB measurement for differentiating HH and CDP in 72 boys with pubertal delay, 21 with HH and 51 with CDP.
Subjects and Methods. 72 boys who presented with delayed puberty between the ages of 14 to 18 years to the Pediatric Endocrinology Departments in Angers University Hospital and Saint Vincent de Paul Hospital in Paris between January 2000 and January 2005 were included. Fifty-one subjects had subsequently a complete progression of pubertal development (Tanner stage V), and received a diagnosis of CDP. Twenty-one subjects did not undergo complete spontaneous pubertal development and received a diagnosis of HH, including seven with combined pituitary hormone deficiency, 7 with Kallmann syndrome, and 7 with isolated HH. Inhibin B was measured by means of a solid-phase sandwich assay (Serotec, Oxford, UK). Intraassay precision was 7.4 and 4.2% at levels of 44 and 225 pg/ml, respectively. RIA were used to measure plasma concentrations of testosterone, LH and FSH were measured by immunoradiometric assays.
Results. Age was 16.0 ± 1.5 yrs, with no difference between groups. INB was 28 ± 41 vs. 117 ± 70 pg/mL ($p < 0.001$, HH vs. CDP), testosterone was 0.74 ± 1.6 vs. 1.5 ± 1.0 nmol/L ($p < 0.01$, HH vs. CDP), LH was 0.7 ± 1.1 vs. 1.3 ± 1.1 IU/L (NS, HH vs. CDP), and FSH was 1.1 ± 1.2 vs. 2.2 ± 1.5 IU/L (($p < 0.01$, HH vs. CDP). The area under the ROC was 0.90 ± 0.05 for INHB, 0.74 ± 0.07 for testosterone, 0.76 ± 0.07 for FSH, and 0.72 ± 0.09 for LH. INHB measurement (cut-off 55 pg/mL) gave a sensitivity and specificity of 85%.
Conclusion. Our data suggest that INB measurement may help in differentiating HH from CDP.

PO1-177 Gonads and Puberty I

No evidence of germline variants of the transcription factors genes (*TTF-1 and EAP1*) controlling GnRH secretion in a large cohort of patients with central pubertal disorders

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Background: Gonadotropin releasing hormone (GnRH) secretion is under a complex regulatory system that includes excitatory and inhibitory transsynaptic, as well as glial inputs and its pulsatile discharge is essential for the pubertal development. Thyroid transcription factor 1 (*TTF-1*) and enhanced at puberty (*EAP1*), genes encoding trascription factors, are consistently more expressed at the time of puberty in the hypothalamic areas implicated with puberty onset in primates. Both genes were proved to transactivate GnRH promoter and repress preproenkephalin promoter. In addition, TTF-1 directly enhances KiSS1 gene expression through specific consensus motif at KiSS1 promoter. Mice with hypothalamic knockout of *TTF-1* and with silencing of *EAP1* have delayed puberty, reduced reproductive capacity and short reproductive span. To date no molecular analysis of TTF-1 or EAP1 genes were performed in patients with central pubertal disorders.
Aim: To identify molecular defects in TTF-1 and EAP1 genes in a selected multicentric cohort of patients with central pubertal disorders: central precocious puberty (CPP) – idiopathic form and due to hypothalamic hamartoma, and idiopathic isolated hypogonadotropic hypogonadism (IHH).
Patients/Methods: Sixty-two patients with idiopathic CPP (59 girls); 14 patients (7girls) with CPP due to hypothalamic hamartoma and 47 patients (13 females) with IHH were selected. CPP was diagnosed when pubertal signs appeared before 8 yr in girls and 9 yr in boys, associated to pubertal LH response and normal magnetic resonance in "idiopathic" form or presence of hamartoma. IHH was documented based on clinical signs of hypogonadism, prepubertal or low testosterone or estradiol levels for age, low or inappropriately normal gonadotropin levels, normal basal and stimulated levels of the other anterior pituitary hormones, absence of anosmia and normal hypothalamic-pituitary imaging. Genomic DNA was extracted followed by amplification and automatic sequencing of all three exons of *TTF-1*, as well as its putative promoter region and *EAP1*, which is an intronless gene.
Results: No mutations or polymorphisms in *TTF-1 and EAP1* coding regions and *TTF-1* promoter region were found.
Conclusion: Although both genes seem to be highly conserved, germline *TTF-1 and EAP1* mutations are probably not implicated in pubertal disorders. However, we cannot exclude somatic defects and/or TTF-1 and EAP1 expression abnormalities.
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PO1-178 Gonads and Puberty I

Prepubertal gynecomastia and advanced skeletal maturation due to topical absorption of coumestrol

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Introduction: We report a 5yo boy with severe gynecomastia and skeletal age advancement due to topical absorption of the phytoestrogen coumestrol.
Clinical Case: A 5 2/12yo male presented with bilateral breast tissue and growth acceleration. PE revealed height 120cm (97%ile), weight 26.3kg (97%ile), bilateral Tanner III breast tissue with areolar pigmentation, Tanner II pubic hair, prepubertal phallus and 3ml firm testes. Lab studies revealed detectable serum and urine estrone, 24-hour urine total estrogens of 19µg/gCr (normal adult male 4-23µg/gCr), random LH 0.1 mIU/L, FSH 0.7 mIU/L. Prolactin and adrenal steroids were normal and β-HCG was undetectable. Testicular ultrasound was normal. Bone age (Greulich and Pyle) was 5years advanced, with a predicted final adult height of 160.6cm (<3%ile and 12cm less than MPH).
A visit to the patient's farm-based home revealed that this child regularly

fed calves fresh alfalfa mixed with Rumensin, a lipophilic chelating agent commonly used as a feed additive in the beef industry. The skin of both arms and hands had been exposed several times daily to the feed mixture over the preceding year during hand-mixing. Analysis of the alfalfa revealed significant quantities of the phytoestrogen coumestrol. Cessation of exposure resulted in gradual reduction in breast size and slowing of growth rate and skeletal maturation. However, precocious central puberty subsequently occurred, necessitating the use of leuprolide acetate treatment. He had completion of puberty and skeletal maturation by 12 years of age and reached an adult height of 167cm. **Clinical Lessons:** Endocrine disruptors are receiving increasing attention as potential causes of adverse health and reproductive outcomes in animals and humans. The phytoestrogen coumestrol (first identified in alfalfa but also found in soybeans and other plants) is known to have peripheral estrogenic actions. We propose that our patient experienced a significant exposure to coumestrol. Chelation with Rumensin likely enhanced absorbtion of the lipophylic complex through the skin, with subcutaneous fat acting as a reservoir. This case is an extreme example of the effects of a widely-dispersed environmental estrogen causing peripheral estrogen effects - gynecomastia, growth acceleration and skeletal maturation, secondary central precocious puberty, and compromised adult height - in a prepubertal boy. It highlights the importance of a careful history regarding exposure to such substances.

PO1-179 Gonads and Puberty I

Peripheral precocious puberty in girls with McCune-Albright syndrome: preliminary results of treatment with ketoconazole

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Introduction: Classic McCune-Albright syndrome (MAS) refers to the at least 2 features of the triad of polyostotic fibrous bone dysplasia, (italic) *cafe au lait* (/italic) skin pigmentation, and precocious puberty (with or without additional endocrinopaties). Precocious puberty, the most common endocrine feature of MAS, is a result of gonadotropin independent autonomous ovarian or testicular function. In MAS, an activating missense mutation in the gene for the alpha subunit of G_s, results in uncontrolled activation of adenylate cyclase leading to cAMP formation and resultant hyperfunction of endocrine cells and other tissues. Some drugs have been tried to treat MAS, such as steroid synthesis inhibitors (ketoconazole), an estrogen-receptor blocker (tamoxifen) and aromatase inhibitors (testolactone and anastrozole). The objective of this study is to present the results of treatment of the peripheral precocious puberty (PPP) with ketoconazole in girls with the diagnosis of MAS. Methods: Retrospective study, which included five girls with MAS and PPP, in treatment with ketoconazole at Joana de Gusmão Children's Hospital – Pediatric Endocrine Unit, Florianópolis, Brazil. Data included clinical and laboratorial findings from diagnosis to the end of the treatment and the presence of side effects. Results: The average age at the diagnosis was 5,1 yr (2,9 - 7,7) and the average of bone age was 7,1 yr (4,5 - 11). They all presented bone dysplasia and PPP. One patient did not present advanced bone age and (italic) *cafe au lait* (/italic) pigmentation; three of them presented with vaginal bleeding and two with hyperthyroidism. Prior treatment with gonadotropin-releasing hormone analogs (GnRHa) and/or progesterone were used in three patients, without regression of the PPP. The luteinizing hormone releasing hormone agonists (LHRHa) stimulation test, excluded precocious central puberty. The dose of ketoconazole was 400-600mg/day, with no side effects. There was regression of the puberal stage followed by withdrawal bleeding. One patient finished the treatment with 11 yo, menarche at 12 yo, reaching final height above the genetic target. Conclusion: In this study, the regression of puberal charactheres, absence of vaginal bleeding and side effects, demonstrated that ketoconazole was efficient and safe for the treatment of PPP in the MAS.

PO1-180 Gonads and Puberty I

Final height in girls with idiopathic central precocious puberty

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Introduction: Central precocious puberty (CPP) is mainly due to the precocious activation of hypothalamic-pituitary-gonadal axis. The early linear growth acceleration and bone maturation leads to short adult height. The majority of girls presenting with precocious puberty have the central form, with the underlying neurological abnormalities being unusual. Gonadotropin-releasing hormone analogs (GnRHa) are used to supress pituitary-gonadal activity in children with CPP. The stimulus for accelerated skeletal maturity is removed once sex esteroid levels are supressed. Bone age x-rays with concomitant heights can be used to estimate growth potencial and hence predict adult height. Changes in height prediction during therapy are directly related to skeletal age at onset of therapy. Objective: Identifying final height (FH) in girls with idiopathic CPP treated at Joana de Gusmão Children´s Hospital – Pediatric Endocrine Unit, Florianópolis, Brazil. Methods:The study evaluated the registration of girls that had reached the FH. Data included chronological age (CA), bone age (BA), age at diagnosis, age at FH, duration of treatment, duration of follow-up from the beginning of the treatment to FH, treatment used, height at the beginning and at the end of the treatment, predicted height by Bayley- Pinneau (BP), target height (TH) and FH. Database was done using Microsoft ® Excel 2002 and were analysed through the EpInfo ® 6.04. Results: The 54 appraised girls were treated with intramuscular leuprolide acetate or triptorelin 3,75 mg each 28 days. The average period of treatment was 3,1 years. The BA, CA and height were valued in accordance to the table:

Baseline and final characteristics

Treatment	CA (years)	BA (years)	Difference between BA and CA (years)	Height (cm)	Height predicted by BP (cm)
Beginning	7,6 (1,6 SD)	9,6 (4,6 SD)	1,9 (1,2 SD)	129,8 (11,5 SD)	155,2 (10 SD)
End	10,9 (0,6 SD)	11,9 (0,6 SD)	1,1 (0,7 SD)	147,5 (6,3 SD)	160,5 (3,7 SD)

The average TH was 158,7 cm. The difference between height predicted by BP in the end and in the beginning of the treatment was 5,6 cm. The z score of FH was 0,04 SD of average. The difference between FH and height in the end of the treatment was the average of 13,2 cm and the difference between FH and TH was the average of 2,4 cm. FH average was 160,7 cm (5,6 SD) and was reached at 14,2 years. Conclusion: In this study, girls with CPP treated with GnRHa reached their TH.

PO1-181 Gonads and Puberty I

Pelvic ultrasound in girls with precocious puberty is a useful adjunct in diagnosis and therapy monitoring

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Background: Gonadotropin-releasing hormone analogs (GnRHa) are known to be efficacious in the treatment of central precocious puberty (CPP). However, there are no clear-cut criteria for initiation of therapy or the means for monitoring suppression during treatment. We have previously shown that pelvic ultrasound can be used to differentiate CPP from premature thelarche (PT). The contribution of pelvic ultrasonogrphy to the decision on treatment initiation and to treatment monitoring has not been extensively studied.

Aim: To prospectively assess the use of pelvic ultrasound in documenting progression of precocious puberty before GnRHa therapy and in monitoring suppression during therapy.
Patients and methods: Girls referred because of appearance of breast buds before age 8 years were recruited consecutively. All underwent general and endocrine evaluation. The diagnosis of CPP (n=25) was based on the clinical judgment of an experienced clinician after 6 months of follow up. Transabdominal pelvic ultrasound was performed with a 5-MHz real-time sector scanner on referral, 3 and 6 months later, and every 6 months thereafter.
Results: Before treatment a significant increase in height –SDS ($p=0.003$), uterine volume ($p<0.01$), fundus diameter ($p<0.05$) and endometrial thickness ($p<0.001$) was observed after 3 months of follow-up in girls with CPP but not in controls (girls with PT). Three months after beginning therapy there was a significant decrease in uterine length (mean 4.2±0.6 vs 3.8±0.7 cm, $p=0.01$), uterine volume (4.4±2.2 vs 3.0±1.0 ml, $p<0.003$) and ovarian volume (3.2±2.3 vs 1.9±1.0, $p<0.01$) but no significant change in height-SDS or bone age to chronological age ratio. No further changes in either height-SDS or ultrasound measurements were documented during 2 years of treatment.
Conclusions:
The increase in uterine measurements indicating progression of puberty may be used as an adjunct when considering GnRHa treatment. The significant decrease in both uterine and ovarian measurements as soon as 3 months after therapy initiation suggest that ultrasound may be an early useful means for monitoring suppression.

PO1-182 Gonads and Puberty I

Partial normalization of puberty timing in female mice with DSS colitis treated with anti-TNF-a antibody: further argument for effect of inflammation on puberty

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Background: Inflammatory bowel disease (IBD) and resultant colitis occurring prior to puberty are frequently associated with a delay in puberty and losses of growth and bone mineralization. Some of this delay in puberty may be due to colonic inflammation and associated systemic inflammation. To date no treatments for IBD have been shown to normalize the timing of puberty.
Objective: Determine whether there is a normalization of the timing of puberty during treatment of colitis using monoclonal antibodies to TNFα (TNFα-ab).
Methods: We induced colitis in 23 day-old C57Bl6 female mice using dextran sodium sulfate (DSS), producing a chemical colitis. We treated animals with 3% DSS for 7 days, followed by removal of DSS for an additional 3 days, resulting in 10 days of worsening colitis. DSS mice were evenly divided by weight into two groups: one which received injections of neutralizing-TNFα-ab 0.1 mg IP on days 4 and 8 of DSS treatment and the other receiving treatment with non-specific control antibody (C-ab). These mice were compared to Control (C) mice that received injections of TNFα-ab or C-ab. All groups were followed for the timing of vaginal opening until 33 d.o., when they were euthanized and their serum was collected and their colons prepared for histologic examination.
Results: DSS-treated mice treated with TNFα-ab had lower levels of systemic IL-6 and a partial normalization of timing of vaginal opening compared to mice that received C-ab (see Table). There were no differences in weight gain or growth between the TNFα-ab or C-ab groups over the course of the experiment.

	Control+TNFα-ab	DSS+TNFα-ab	DSS+C-ab
Weight gain, d.o.l. 23-33	45.1% ±2.6 n=17	13.7% ±3.1 n=21***	19.6% ±3.4 n=19***
Growth, d.o.l. 23-33 (% initial)	19.8% ±0.9 n=17	17.3 ±1.8 n=21	18.5 ±1.6 n=19
Chronic inflammatory index (colon)	0 ±0 n=17	2.44 ±0.12 n=20***	2.29 ±0.12 n=17***
IL-6 (pg/mL)	13.1 ±3.4 n=16	37.1 ±3.5 n=21*,#	60.9 ±11.7 n=14***
Age of Vaginal opening (days)	30.2 ±0.6 n=17	31.2 ±0.27 n=21#	32.3 ±0.4 n=19**

Significance vs. control+TNF-α-ab: * $p<0.05$; *** $p<0.001$. Significance vs. DSS+C-ab: # $p<0.05$.

Conclusions: DSS colitis causes delay in puberty in sexually-immature mice that is partially normalized via treatment with anti-TNFα-ab treatment despite a lack of improvement in weight gain. This adds support to the hypothesis that systemic inflammation contributes to delayed puberty in the setting of colitis.

PO1-183 Gonads and Puberty I

Ovarian cysts and torsion

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Background: Importance of ovarian pathology like cysts/torsion in infants & pre/peripubertal girls with acute abdominal pain and/or mass is inadequately suspected and documented.
Data & methods: 46 girls, median age 6.7yrs (2mths-16yrs) were referred for above complaints, with/without signs of early puberty (EP) & retarded growth (PCOS excluded). Clinical evaluation, hormonal profile, USG and doppler & CT/MRI (as indicated) were done.
Results: After collating data patients were categorized as **GroupA: Simple Ovarian Cysts** n=30(65%) subdivided into(i)with torsion(11),(ii)without torsion(19); **GroupB: Ovarian Cysts with Hypothyroidism** n=12(27%) & **GroupC: Neoplasm** n=4. GroupA girls presented with abdominal mass(67%), pain(50%) & EP (47%). Antenatal cyst was detected in 10% of GroupA. In GroupA(i),11 had mean cyst diameter 5.4±3.6cm, ovarian volume 33cm^3 except 1 with large torsed ovary but no cyst. 6/11 had oophorectomy inclusive of 1 diagnosed antenatally; detorsion in 3/11 & oophoropexy in 2/11. In GroupA (ii), of the19 patients, 2 of 3 diagnosed antenatally had surgical intervention(cyst aspiration, bilateral cystectomy), 1 resolved on follow up. Recurrent ovarian cysts occurred in 4 & spontaneous resolution in 11. In GroupA, mean E2-42 pg/ml, LH, FSH suppressed. In GroupB all 12(mean age 8.2yrs) noted to be hypothyroid (meanTSH-152.3μIU/ml, T4-1.72μg/dL) presented for abdominal complaints & 60% with EP & retarded growth(mean Ht.SDS-2). 2 had partial torsion, 1 referred after oophorectomy for acute abdomen. Mean cyst diameter 5.2±1.2cm, ovarian vol. 48 cm^3.Thyroxin resolved symptoms in <1week, cysts regressed by 8 wks without recurrences. Normal puberty occurred at appropriate age. In neoplasia USG showed complex mass, diameter >10cm, treated surgically.
Conclusion: Ovarian cysts/torsion should be suspected in girls with acute abdomen and/or mass & EP. Hypothyroidism is a clinical possibility. Simple idiopathic ovarian cysts were seen in 65%, of which 36% had torsion. Cyst diameter>3.5cm and ovarian vol. >30cm^3 predisposed to torsion except in hypothyroidism. Diagnostic delay led to oophorectomy for gangrenous changes in 50%. Early diagnosis, detorsion, bivalving and oophoropexy can salvage ovary. Early cyst aspiration if diameter >5cm may prevent torsion. Antenatally detected cysts need follow up in infancy. Ovarian cysts in hypothyroidism resolve with Thyroxin. Complex ovarian mass suggests neoplasia. USG was most important tool in diagnosis.

PO1-184 Gonads and Puberty I

A population-based anthropometric study of U.S. male puberty in a primary care research network: progress report from the American academy of pediatrics (AAP) PROS (pediatric research in office settings) study of secondary sexual characteristics in boys

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Periodic assessment of secular trends in age of pubertal onset is important from clinical, public health and environmental perspectives. While longitudinal studies are ideal, cost, duration, and patient retention make them impractical. We describe an ongoing population-based, cross-sectional study of male pubertal development being conducted by primary care physicians (PCPs) within a pediatric practice-based research network (PBRN). **Methods:** Study data are being collected by PCPs in the AAP's PROS (Pediatric Research in Office Settings) network. PCPs are trained to perform Tanner staging and testicular volume measurement using a modified Prader orchidometer on consecutive boys 6-16 years old being seen for well child care. To enhance detection of potential ethnic/racial differences, recruitment strategies have been designed to over sample minorities by targeting enrollment of 2200 boys each among Non-Hispanic Whites (NHW), African-Americans (AA), and Hispanics (H). Historical data on characteristics and factors relevant to male genital and pubertal development, such as congenital genital anomalies and chronic diseases/medications, is collected on each subject. **Results:** (1) an inter-rater reliability study using paired PCPs in 10 practices validated the PCP sample's post-training ability to reliably stage pubertal markers, with intraclass correlations all significant at p<.001 and kappas for initiation of puberty ranging from .49 to .79; (2) although the 2200-subject goal will be met for NHW boys, enrollment of AA and H boys has lagged; (3) preliminary data on reported histories of cryptorchidism (1.3%) and hypospadias (0.6%) indicate prevalences similar to those previously reported; (4) 23.1% of subjects had histories of chronic medications prescribed, with stimulants (7.9%) most common. **Conclusions:** (1) PCPs can be successfully trained to assess male pubertal development; (2) although under representation of practices serving racial/ethnic minority youth in the PROS network has presented recruitment challenges, this is being addressed by collaboration with other PBRNs serving high-minority populations; (3) the congenital genital anomalies data support the representativeness of the PROS sample; (4) recent trends in treatment of U.S. male children with stimulants and other psychotropic medications that can impact growth and pubertal maturation are important to consider in the interpretation of data on temporal trends in male pubertal development.

PO1-185 Gonads and Puberty I

A distinct case of Mc Cune-Albright syndrome. Effect of anastrozole treatment

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Background. Mc Cune-Albright syndrome (MAS) is a rare disorder resulting from a somatic mutation of the GNSA1 gene which occurs in a mosaic distribution, early in embryonic life. Clinically, MAS usually presents with the triad of cafe au lait spots, polyostotic fibrous dysplasia and gonadotrophin independent precocious puberty (GIPP).Two of the three criteria are necessary for the diagnosis, however, atypical cases have been reported.
Case report.A 3 year old female patient presented in our Endocrine unit with a two month history of thelarche. She had height and weight at +2SDs, breast and pubic hair Tanner stage 2,and bone age 3 years.LH, FSH and Oestradiol levels as well as the ovarian volume on ultrasound were prepubertal.She was lost to follow-up and returned at the age of 4,5 years due to increased vaginal secretions. Clinically she was prepubertal.However,a month later, she presented with vaginal bleeding lasting for 4-5 days and breast development Tanner stage 3 with nipple hyperpigmentation. The ovarian volume on ultrasound was then 4-5 ml bilaterally, E2 was 633 pg/ml, and the GnRH test was completely blunted, compatible with GIPP.Subsequently, she was presenting with cyclic breast development, increased ovarian volume and vaginal bleeding every 2-3 months. At the phase of cyclic oestrogen excess, she additionally had increased basal GH and IGF-1 values .She had neither cafe au lait spots nor polyostotic fibrous dysplasia in bone scan.DNA analysis was not confirmatory of MAS. Her growth rate was increased with rapidly progressing bone age which prompted treatment. She was started on anastrozole at a dose of 0.5 mg once daily with no effect.Nine months later it was increased to 1 mg once daily, which led to bleeding episodes arrest, decrease in growth rate and deceleration of bone age 15 months after initiation of treatment.
Conclusions. The cyclic bleeding episodes due to oestrogen excess concomitant with GH hypersecretion in our patient lead to the diagnosis of atypical MAS. Anastrozole seems to be effective in decreasing bleeding episodes and decelerating bone age advancement at a dose f 1mg,once daily.

PO1-186 Gonads and Puberty I

Longitudinal measurement of follicle stimulating hormone and luteinizing hormone during childhood in a boy with anorchia

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Objective
Regulation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) involve stimulatory effects of gonadotropin releasing hormone and inhibitory effects of sex steroids and inhibin B. In boys with anorchia the inhibitory effects from testes are absent, and high FSH and LH values would be expected the first months of life and at the age of puberty. FSH and LH values during childhood in boys with anorchia are not well described.
We present longitudinal measurements of FSH and LH including gonadotropin releasing hormone tests in a child with anorchia.
Case story
The child was the first boy of unrelated Caucasian parents born after an uneventful pregnancy at term. The birth weight was 3290 g and the birth length 54 cm. At birth normal male genitalia was (mentioned), but with an empty scrotum. The child had a normal male chromosomal karyotype, 46,XY. The boy was referred 18 months old for further investigations. He had cryptorchidism, but a normal penis, and otherwise a normal somatic examination. Thyroid parameters were normal. No increase in testosterone was seen in human chorionic gonadotropin stimulation test. Serum inhibin B was less than the detection limit (< 20 pg / ml). FSH and LH were 75 and 19, 5 IU / L, respectively. Laparoscopic examination showed normal male internal genitalia, but no intraabdominal testes. The following years the boy had a normal growth along the 50 percentile. FSH and LH were examined at the age of 26, 34, 60 and 70 months; at the age of 34 and 70 months he also had a gonadotropin releasing hormone test (**Table).**

Longituditional FSH and LH measurements							
Age (months)	18	26	34	34*	60	70	70*
FSH IU/L	75	79	58	137	40	37	75
LH IU/L	19.5	6.9	3.4	59	1.1	1.1	24

*= stimulated values

Discussion
The pituitary - gonadal axis is interrupted in anorchia with no inhibin B formation and no central inhibition of FSH formation. In our patient a continuing high level of FSH was shown up to the age of 70 months, with a small decline in basal and stimulated FSH from the age of 18 months to the age of 70 months. In order to switch of the secretion of FSH a minimal amount of inhibin B may be necessary. LH had decreased to a normal basal value at 70 months may be due to inhibition by adrenal sex steroids

PO1-187 Gonads and Puberty I

Long term treatment with aromatase inhibitors in a father and son with aromatase excess syndrome

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Background: Aromatase excess syndrome is a rare autosomal dominant condition resulting in gynecomastia and accelerated growth in childhood, and hypogonadism and short stature in adulthood.
Case History: A 5.5 year old male presented with prepubertal gynecomastia, tall stature, and a bone age of 13 y with predicted adult height (PAH) of 152 cm. His father had a history of prepubertal gynecomastia of unknown etiology; review of his medical chart revealed a right adrenalectomy to rule out an estrogen producing tumor, and cessation of growth before 10 y. At adulthood, he had a height of 157 cm, high-pitched voice, paucity of facial hair, decreased libido, and a history of bilateral mastectomies. Genetic analysis in both revealed a heterozygous inversion in chromosome 15 causing over-expression of

aromatase. (NEJM 2003; 348:1855)
Results: The son underwent a 2-month trial of testolactone with no benefit, followed by prednisone for 1.7 y, which was successful in suppressing adrenal precursors and estrogen, but caused excessive growth reduction. He was then placed on anastrozole (A) for 5.5 y and switched to letrozole (L) for more potent suppression as gynecomastia worsened in puberty. He entered puberty at 11.5 y and virilized normally. At last measurement at 17.4 y, his height was 172.6 cm. DEXA scans have revealed normal bone mineral density throughout therapy.

Therapy for Aromatase Excess (Son)

	Baseline	Testolactone (24mg/kg-d)	Prednisone (2.5mg bid)	Anastrozole (2mg qd)	Letrozole (2.5mg qd)
Age at start of therapy (y)	5.4	5.5	5.7	7.4	13
Bone Age (y)	13	-	13.5	14	14
Growth Velocity (cm/y)	9.2	12.6	1.6	5	7®3
Androstenedione (ng/dL)	16	483	<3	28	113
Estrone (ng/dL)	8.3	17	1.1	2.4	0.59
Testosterone (ng/dL)	<3	7.9	<3	4.9	591
Estradiol (ng/dL)	1	0.9	<0.5	0.5	1.6
LH (IU/L)	0.06	-	-	0.41	6.7
FSH (IU/L)	0.36	-	-	0.64	3.8

The father was started on A and transitioned to L. On A, testosterone increased from 248 ng/dL (NL > 350) to 1300; estradiol suppressed from 23 ng/dL (NL 0.8-3.5) to 2.8. Two unaffected sons were born in the first 3 y of therapy.
Conclusion: We describe aromatase inhibitor (AI) treatment (sequential A and L) in a father and son with aromatase excess. Both have used AI therapy for over 10 years with no side effects or clinically significant decreases in bone density. Earlier identification of the son and directed treatment resulted in a final height 20 cm above initial PAH and 15 cm above his father's height.

PO1-188 Gonads and Puberty I

A randomized trial of 1- and 3-month depot leuprolide doses in the treatment of central precocious puberty

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Background: Depot leuprolide (DL) is available in monthly and multi-monthly formulations for treatment of central precocious puberty (CPP). In the U.S., considerable variation exists in DL dosing, with many providers switching from the approved monthly (1m) DL to off-label use of three month (3m) preparations for ease of compliance. We previously reported in a sequential dosing study that peak gonadotropin (Gn) levels were better suppressed on 7.5mg-1m DL compared to low-dose 3.75mg-1m and 11.25mg-3m DL. It was unclear if higher dose 3m DL might provide Gn suppression similar to monthly dosing.
Methods: Naive patients with clinical characteristics of CPP and basal LH >0.3IU/L or >5IU/L after aqueous leuprolide acetate (LA) were randomized to 7.5mg-1m, 11.25mg-3m or 22.5mg-3m DL. Pubertal stage, height, and hormonal suppression were assessed every 3 m and bone age (BA) yearly. Serum Gn (Esoterix ICMA) and sex steroids (MS/MS) were obtained 40 min after AL or DL injection (alternating visits).
Results: 50 patients (45F) were enrolled (mean age 7.9±1.5y (F) at first DL). Mean peak LH and FSH were higher in the 11.25mg-3m group compared to the 7.5mg-1m and 22.5mg-3m groups; however, FSH was higher in this group at baseline. There were no differences between the 7.5mg-1m and 22.5mg-3m groups.

Mean LH and Estradiol during therapy

		Time 0	3 M	6 M	9 M	12 M
LH mIU/ml	7.5mg-1m	20.3±21.9	1.7±1.1	1.4±0.8	1.7±1.0	1.4±0.8
	11.25mg-3m	25.2±21.4	2.6±1.3* †	2.5±1.5* †	2.8±1.4* †	2.4±1.1*
	22.5mg-3m	15.4±11.4	1.6±0.8	1.4±0.7	1.5±0.7	1.6±0.8
E2 ng/dl	7.5mg-1m	2.38±1.61	0.20±0.14	0.12±0.03	0.22±0.13	0.17±0.08
	11.25mg-3m	1.73±1.15	0.17±0.08	0.21±0.15	0.18±0.18	0.17±0.08
	22.5mg-3m	2.42±3.17	0.18±0.08	0.22±0.22	0.16±0.10	0.22±0.11

p<0.05 t-test 11.25mg-3m vs.*7.5mg-1m and †22.5mg-3m

Mean growth velocity during the first year was 6.6±2.0 cm/y and BA change was 0.96±1.1 y, with no differences between groups.
Conclusions: Mean peak LH, whether measured after LA or DL injection, was higher in the 11.25mg-3m DL arm compared with the higher-dose arms. In contrast, estradiol, growth rate, and change in BA were identical among all groups. While the lower 3m DL dose is associated with a higher peak LH, it remains unclear if this has any clinical consequences.

PO1-189 Gonads and Puberty I

Molecular investigation of *XbaI and PvuII* polymorphisms of the estrogen Treceptor α gene in girls with precocious puberty

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Backround. Polymorphisms (PLMs) of the estrogen receptor (ER)α gene influence the pubertal development and age of menarche in girls. The aim of this study was to investigate the single nucleotide polymorhisms (SNPs) XbaI and PvuII of the ER α gene in girls with precocious puberty (PP).*Patients and methods.* Fifty girls (6-12 yrs) having PP or early menarche and 30 girls matched for age without signs/history of PP were investigated. Genotyping for XbaI and Pvu1II using PCR-RFLP was performed *Results.* PpXx haploptype was found 59.37% in PP and 55.55 % in controls. Only 2% of all individuals have the ppxx haplotype, exclusively the PP group. Girls having XX present adrenarche 0,5 and 0,7 yrs later than genotypes Xx and xx .Genotype PP present adrenarche 1,4 and 2 yrs later than genotypes Pp and pp. PPXX presents adrenarche 0,38 and 1 yrs later than PpXx and ppxx respectively.*Conclusion*. Girls presenting the restriction fragment sites for theXbaI and PvuII have earlier puberty than girls without them. This is indicative for possible implication of these PLMs in the timing of puberty of girls.

PO1-190 Gonads and Puberty I

Prepubertal gynecomastia in Peutz-Jeghers syndrome in two monozygotic twins: one-year treatment with anastrozole and genetic study

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Peutz-Jeghers syndrome (PJS) is a rare autosomal-dominant disorder characterized by multiple gastrointestinal hamartomatous polyps, mucocutaneous pigmentation and increased predisposition to neoplasms. Endocrine manifestations in PJS include gynecomastia due to estrogen production by large-cell calcifying Sertoli cell tumors(LSCT).
We present two 9 yr old male monozygotic twins, both with PJS and bilateral

progressive prepubertal gynecomastia. Their bone age was advanced of 2,5 years. In both ultrasound showed testicular bilateral multifocal calcification, suggesting a diagnosis of LSCT. Father had PJS but no history of gynecomastia or testicular calcification.
To reduce gynecomastia and delay skeletal maturation, both started treatment with an aromatase inhibitor, anastrozole, 1 mg orally, once daily. Growth velocity decreased and gynecomastia diminished in diameter.After one year, anastrozole is still currently used at age 10 with no side effects.
We decided not to perform orchidectomy, as the low risk of malignancy, also to allow sperm preservation in future.
As to genetics, our study describes for the first time monozygotic twins with prepubertal gynecomastia and LSCT in PJS. It has been described an incomplete penetrance of this complication and the fact that both twins unlike their father had the same manifestation suggests a strong role of a genetic determinant.
First, we did not find any mutation in the tumor suppressor gene *LKB1/STK11,* responsible for about 59% of PJS.
Second, we assessed whether father's genotype for aromatase cytocrome P45019, a key enzyme in estrogen biosynthesis, were different from his children.
In particular we genotyped the tetranucleotide (TTTA)n polymorphism and TCT I/D polymorphism in intron 4 and a 3'-UTR C/T polymorphism in exon 10. Father and children had the same genotype: (TTTA)7 and TCT deletion in intron 4 and e heterozygousity for 3'-UTR T allele.
Only about 30 cases with prepubertal gynecomastia and PJS have been described until now, and most of them have been treated by orhidectomy.
Anastrozole is an efficacious medical treatment, alternative to orchidectomy, to control the effects of estrogens excess.
Aromatase gene seems not to be implicated in determination of endocrine complications of PJS, but it would be interesting to study other genes, such as estrogen receptor. Finally a remarkable genetic heterogeneity exists, as the *LKB1/STK11*mutation is not essential for the occurrence of PJS related endocrine complications.

PO1-191 Gonads and Puberty I

Pubertal development in a large longitudal cohort study: the new normal?

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Background: Cross sectional studies have reported the onset of pubertal changes at earlier ages than in the past; however, there are few longitudinal investigations. The CYGNET Study (Cohort study of Young Girls' Nutrition, Environment and Transitions) is a longitudinal project of the Breast Cancer and the Environment Research Centers (BCERCs), and is examining environmental and other predictors of early puberty in girls recruited from Kaiser Permenante Northern California.
Objective: The BCERCs are funded by NIEHS & NCI to study the impact of early environmental exposures that may lead to earlier pubertal development, and thus increase the risk of breast cancer. This consortium of basic scientists, epidemiologists, and community advocates is investigating mammary gland development and evolution over the lifespan. CYGNET is the San Francisco Bay Area epidemiologic project of these centers.
Design/Methods: Longitudinal study of 444 girls recruited at age 6-7, with anticipated follow-up for at least 5 years. Annual visits included physical examination, biospecimen collection (urine and blood), and questionaires. Research staff were trained in sexual maturity ratings by one master trainer. Dietary Assessment was conducted with 4 24 hr recalls spaced 3 months apart. We are concluding year 4.
Results: Overall ethnic breakdown was: 25.9% Hispanic, 20.4% Black, 11.3% Asian, 41.7% White non-Hispanic. Most girls were age 6-7 y at baseline visit. Of these, 7.5% had Breast Tanner 2 (BT2) or higher at baseline, with 9.4% at Pubic Hair Stage 2 (PH2) or higher. At Year (Yr) 2 visit (age 7-8), 16.2% participants were at BT2 or higher and 18.4% were at least PH2. At Yr3 (age 8-9), 37.2% were at BHT2 or more and 28.9% were at PH2 or more. At Yr4 (age 9-10), 71.4% were BHT2 or more and 53% were at least PH2. Analyses of associations with BMI, ethnicity, and environmental biomarkers are ongoing.
Conclusions: Preliminary results from this large longitudinal study demonstrate that in girls, sexual maturity is occurring at younger ages than seen in previous cross-sectional investigations. This study provides an opportunity to determine associations with possible environmental factors and endocrine disrupters.

PO1-192 Gonads and Puberty I

Boys with precocious puberty from transdermal transmission of testosterone gel

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Because precocious puberty occurs less commonly in boys than girls, pediatricians are often trained to more aggressively seek out a pathologic cause in boys. We present two boys with precocious puberty resulting from the transdermal transmission of testosterone gel used by family members. In one case, a ten month-old boy presented with a 7 cm phallus and Tanner II pubic hair. His grandfather used testosterone gel as part of post-oncologic medical care, and the patient was exposed by crawling on his grandfather's shirtless abdomen throughout the summer. In another case, a four year-old boy presented with an 8 cm phallus and Tanner III pubic hair. His father used testosterone gel for bodybuilding and the boy shared his dad's towel. On diagnostic evaluation, both patients had elevated serum testosterone levels (346 ng/dL and 89 ng/dL, respectively) despite prepubertal testicular volumes. Pediatricians should be alerted to this relatively new cause of precocious puberty in boys, so they can elicit the appropriate history when working up their differential diagnoses (will be reviewed). With the increasing prevalence of illicit testosterone use, such history may not be offered readily.

PO1-193 Gonads and Puberty I

Short-term changes of lumbar bone mineral density during GnRHa treatment with or without rhGH in precocious girls

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Objective In precocious girls, estrogen deprivation by using gonadotropin releasing hormone analogue (GnRHa) might cause a significant decrease in bone mineral density (BMD). We investigated the short-term changes of BMD during GnRHa treatment and further the effect of rhGH co-treatment.
Method Forty girls with idiopathic central precocity or early puberty were included. Twenty six girls received GnRHa alone (Group I), and the others received GnRHa with rhGH (Group II). BMD was measured by dual energy X-ray absorptiometry (DEXA).
Results For total precocious girls, lumbar BMDs before and after treatment were in normal range according to chronologic age (CA), but significantly lower according to bone age (BA), Total patient showed no significant changes in BMD-SDS during treatment according to CA, significantly different according to BA. In group I, there was no significant change in BMD-SDS according to BA during treatment. But, in group II, BMD-SDS according to BA was significantly increased after treatment.

Changes of BMD-SDS before and after treatment				
		Total (n=40)	Group I (n=26)	Group II (n=14)
Before Tx	CA (yr)	9.0±1.5	8.6±1.2	9.9±1.5
	BA (yr)	11.6±1.3	11.2±1.3	12.4±1.0
	BMD-SDS vs CA	0.06±1.05	0.20±0.95	-0.20±1.20
	BMD-SDS vs BA	-1.36±0.93 *	-1.19±1.01	-1.67±0.68 †
After Tx	CA (yr)	10.2±1.5	9.7±1.1	11.1±1.5
	BA (yr)	12.2±1.2	11.8±1.1	12.8±0.9
	BMD-SDS vs CA	0.04±0.99	0.13±0.91	-0.13±1.15
	BMD-SDS vs BA	-1.12±0.95 *	-1.08±1.04	-1.19±0.81 †
Duration of Tx (yr)		1.62±0.7	1.6±0.9	1.7±0.6

* $P < 0.05$ compared to normal range, and $P < 0.05$ changes in BMD-SDS during Tx, † $P < 0.01$ changes in BMD-SDS in Group II during Tx

Conclusion At initial presentation of precocious puberty, lumbar BMD was proper chronologically, but lower if compared to bone age, that is, bone mineralization catch-up was incomplete. Significant increase in BMD according to BA was attributed to co-treatment group. Therefore rhGH might be considered for improvement of BMD during GnRHa treatment, and BMD should be followed even after discontinuation of therapy, especially for GnRHa only group.

PO1-194 Gonads and Puberty I

As clinical and laboratory differentiation between precocious puberty and premature thelarche

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Objective: The aim of the present study was to reveal clinical, biochemical and ultrasonographic parameters in children who referred to endocrin department to found differences between central precocious puberty (CPP) and premature thelarche (PT).

Material and methods: Our study group was consisted of children whose breast budding was observed before 8 years old (n=114). The differentiation between CPP (n=61, 53.5%) and PT (n=53, 46.5%) was made according to clinical evaluation. The date of signs related with CPP and PT were noted. Birth weight, age at the first singns and at referral, height SD, bone age, FSH, LH, estradiol, pelvic ultrasonography (size of ovary and uterus), peak LH and FST in GnRH test, peak LH/FSH ratio in GnRH stimulation test results and adenohypophysis length with magnetic resonance imaging were compared between CPP and PT.

Results: Bone age (9.5±8.8 vs 8.4±1.6, p:0.001), basal (luteinizing hormone) LH (1.0±2.0 mIU/ml vs 0.4±0.7mIU/ml, p:0.026) and (foliculostimulating hormone) FSH (3.5±2.0 mIU/ml vs 2.3±1.6 mIU/ml, p:0.001), peak LH (9.5±8.8 mIU/ml vs 4.6±4.9mIU/ml, p:0.025) and peak LH/FSH ratio (0.8±0.6 vs 0.4±0.4, p:0.006) in Gn RH test, transverse uterine diameter (17.8±5.7mm vs 14.7±5.6 mm, p:0.022) were found to be significantly different.

Conclusion: We recommend that in differentiating CPP and PT the following parameters; bone age, basal LH, FSH, transverse uterine diameter, peak FSH/LH levels may be used.

PO1-195 New Technologies

Enhancement of endothelial function in healthy children with increase of estradiol and DHEAS during puberty

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Purpose;- To study the relationship between Estrogen levels and the development of endothelial function in healthy children during puberty. To measure the endothelial function by reliable novel technique Peripheral Arterial Tonometry (PAT).

Methods:- We studied 100 healthy school children. Data on height, weight, waist circumference, & percent (%) of body fat, BMI, BMI z-score blood pressure (BP) was collected.

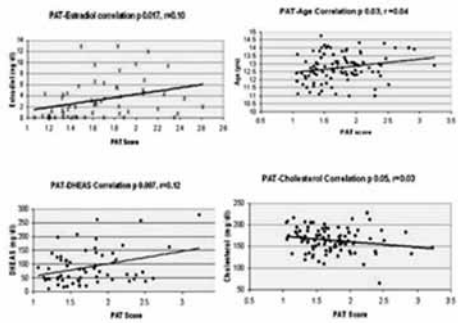
	Average	Females	Males	p value
Number=100		53	47	
Age yrs	12.7	13.0	12.8	ns
SDS	0.8	0.8	0.9	
BMI kg/m^2	20.0	20.0	19.6	ns
SDS	3.2	3.2	3.2	
BMI Z score	0.3	0.1	0.4	ns
SDS	1.0	1.0	1.0	
Body Fat %	22.9	23.5	20.7	0.001
SDS	6.5	5.7	6.7	
Waist circum (cm)	69.9	69.1	69.0	ns
SDS	9.6	8.3	10.9	

ns=not significant

Estradiol, DHEAS, Estrone, Estrone sulfate, fasting lipid profile, insulin & glucose levels were measured. Children with diabetes, hypertension, chronic illness, dyslipidemia, elevated fasting insulin or glucose levels and BMI z score <-2 or >2 were excluded from analysis.

All children underwent evaluation of endothelial function using the Endo-PAT 2000 device, which measures arterial pulse amplitude changes at the fingertips. Phase 1 is 5 minutes of baseline recording, Phase 2 is 5 minutes of blood flow occlusion to one arm, Phase 3 is 5 minutes of recording post occlusion. The PAT score is a corrected ratio between the corrected post to pre- occlusion average signals. The better the endothelial function the greater is the PAT score.

Results:-The PAT score increased and positively correlated with increase in age, increase of Estradiol & DHEAS levels. It negatively correlated with total cholesterol levels.



There was no significant correlation of PAT score with Estrone, Estrone sulfate, fasting glucose, insulin, BMI, BMI z-score, waist circumference, percent body fat, BP, LDL, HDL, triglyceride levels.

Conclusion:-The increment in age, estradiol and DHEAS levels during puberty

play a positive role in the improvement of the endothelial function as measured by PAT. This phenomenon might have a physiological role in the maturation of endothelial function during puberty.

PO1-196 New Technologies

The use of continuous glucose monitoring sensors (I-PRO) to aid in the diagnosis of post-prandial hypoglycemia in two children

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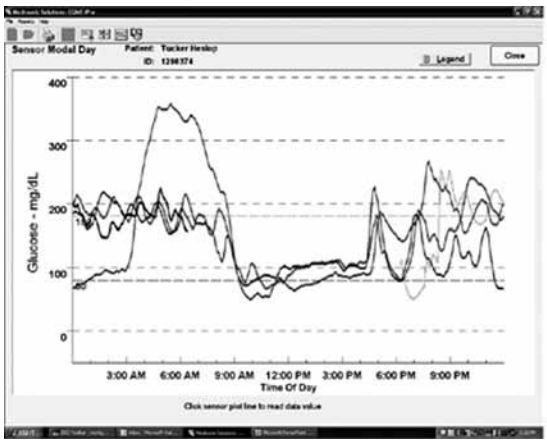
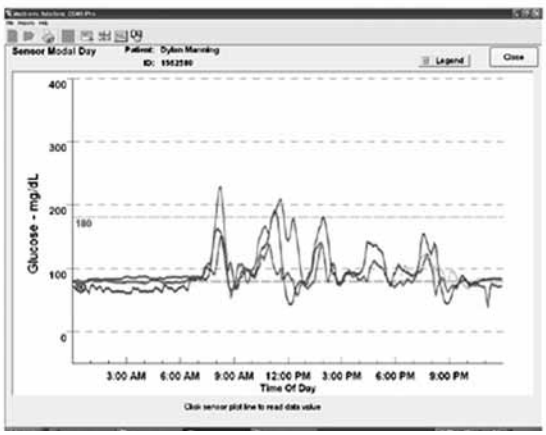
The diagnosis of dumping syndrome & post-prandial hypoglycemia can be challenging in the pediatric population.We describe 2 patients who underwent 72 hour continuous glucose monitoring (I-PRO) in which post-prandial hypoglycemia was demonstrated.
Case 1:A 5 yr male w/ Hirshsprungs Disease was evaluated for erratic blood glucoses of 57-298 mg/dL. Patient was receiving continuous overnight feeds via G-J tube.An I-PRO measured interstitial glucose every 5 min for 72 hrs.



I-PRO readings were calibrated with 4 fingerstick glucose readings/day.Normal I-PRO glucose values were noted during the day. Glucoses of 180-353 mg/dL during overnight feeds were seen.Glucose values abruptly dropped after 8am (when continuous feeds were stopped) to 54-82 mg/dL.Based on these findings, acarbose was initiated in an attempt to slow glucose absorption.
Case 2:A 22 mo male w/ Trisomy 21 fed via g-tube was evaluated for suspected hypoglycemic seizures s/p Nissen fundaplication.An OGTT demonstrated hyperinsulinism(insulin=52) but not hypoglycemia by definition (glucose=59mg/dL).An I-PRO sensor was placed for 72 hrs.The patient received 5 bolus feeds/day and hypoglycemia occurred with glucose values of 40-50mg/dL 60-90 min after bolus feeds.



Acarbose was initiated w/ subsequent resolution of hypoglycemia & seizures. A 72 hr I-PRO will be repeated 3 months after the initiation of Acarbose in both cases to compare trends.
We hypothesize in both cases that post-prandial hypoglycemia is caused by hyperinsulinism.Case 1 also shows hyperglycemia during overnight feeds. This could be due to insulin secretion defect & damage to beta cells caused by prolonged hyperinsulinism & additional risk factors (prograf; steroids).
Conclusion: I-PRO can be a useful tool in the diagnosis of post-prandial hypoglycemia in children receiving g-tube feeds.

PO1-197 New Technologies

Evaluation of a difficult case of recurrent hypoglycemia in an infant using unique resources

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An 8 week old male infant was admitted with nonketotic hypoglycemia. During the course of the hospitalization a number of routine diagnostic studies including cortisol, growth hormone, ammonia, amino acids, organic acids, and acylcarnitine profile were normal. Insulin and C-peptide levels were obtained during hypoglycemia on 3 different occasions. These 3 specimens yielded contradictory results. The first had a high insulin level and low C-peptide, concerning for exogenous insulin administration. However, 2 subsequent specimens had low insulin and C-peptide levels. The inconsistent results led us to explore the ability of the our commercial lab's assay to detect analog insulins. Our investigation revealed that the initial insulin levels were determined using a two site electrochemiluminescent immunoassay which published data indicated had little cross reactivity with commonly prescribed analog insulin preparations (lispro, aspart, glargine). We therefore made use of two alternative assays which published data suggested would have cross reactivity with analog insulins. We compared the insulin immunoreactivity in each of the two low insulin samples using the three immunoassays. Each of the alternate assays detected appreciable insulin levels, inappropriate for hypoglycemia and discordant with the low C-peptide in both samples. The results provided strong evidence for the presence of analog insulin in the patient's blood. In an effort to obtain more definitive evidence of exogenous insulin administration we turned to a liquid chromatography-tandem mass spectrometry technique developed for the purpose of detecting insulin doping by athletes. Blinded samples from 1) patient when hypoglycemic, 2) patient when euglycemic, 3) two individuals with type 1 diabetes using analog insulin, and 4) individual without diabetes were submitted for analysis to the German Sport University in Cologne. Lispro was detected in a sample from the patient's hypoglycemic episode but not during euglycemia. This technique also correctly identified human and analog insulin in the other blinded specimens. This confirmed that hypoglycemia in this child was due to Munchausen syndrome by proxy. Our experience highlights 1) the importance of understanding the properties of standard insulin assays when evaluating hypoglycemia, especially when exogenous insulin administration is a consideration and 2) the value of looking for and utilizing unique resources to explore difficult clinical cases.

PO1-198 New Technologies

A novel stable isotope dilution / benchtop gas chromatography – mass spectrometry (ID/GC-MS) assay for profiling estrogens, their biologically active metabolites and testosterone in human urine

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Estrogens, such as estrone (E1), 17β-estradiol (E2), estriol (E3) and their biologically active metabolites 2-methoxyestrone (2MeOE1), 16-ketoestradiol (16-OE2), 16-epiestriol (16-epiE3), 2-hydroxyestradiol (2-OHE2) and testos-

terone (T) play an important role in physiological and pathological developmental processes. GC-MS allows for highest specificity in steroid analysis. We therefore aimed at developing an assay, based on stable isotope dilution/benchtop GC-MS for these analytes. The method consisted of equilibration of urine (5 ml) with stable isotope labeled internal standards (d_2-estrone, d_4-17β-estradiol, d_3-estriol, d_3-testosterone, d_4-2-methoxyestrone, d_5-16-ketoestradiol, d_2-16-epiestriol and d_5-2-hydroxyestradiol), solid phase extraction, enzymatic hydrolysis, re-extraction, purification by anion exchange chromatography and derivatisation (trimethylsilyl-ethers). The samples were analyzed by GC-MS (Agilent 6890N/5975; fused silica capillary column 25m x 0.2mm i.d., 0.10µm). Calibration plots were linear and showed very good reproducibility with coefficients of determination (r^2) between 0.999 to 1.000. Intra-assay coefficients of variation (CV) ranged between 1.00% (for 16-epiestriol) and 1.92% (for estrone). The inter-assay CVs were between 0.65% (for testosterone) and 2.21% (for estriol). Sensitivity was highest for 2-hydroxyestradiol (0.25pg absolute injection) and lowest for 16-epiestriol (5pg absolute injection). Accuracy – determined in a two level spike experiment – showed relative errors between 0.15% (16-ketoestradiol) and 11.63% (2-hydroxyestradiol). Chromatography showed clear peak shapes for all components analyzed.
In summary, we describe a novel sensitive, convenient and specific assay based on ID/GC-MS to measure estrone, 17β-estradiol, estriol and their biologically active metabolites 2-methoxyestrone, 16-ketoestradiol, 16-epiestriol and 2-hydroxyestradiol and testosterone in human urine.

PO1-199 New Technologies

GH-releasing peptide (GHRP) 2 test as a safe, potent and simple test to evaluate GH secretion in children

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[Background] The diagnosis of GH deficiency (GHD) should be currently made upon GH stimulation tests. Insulin tolerance test (ITT) is recognized as the first-line test both in childhood- and adult-GHD, however, it is sometimes risky and tough especially for children due to hypoglycemia. GHRP2 is a potent peptide that stimulates GH secretion from pituitary mainly via hypothalamic GRF. In adult GHD, GHRP2 test is reported as a comparable diagnostic tool to ITT. [Aims] To evaluate the clinical usefulness of GHRP2 test in the diagnosis of GHD in children, and determine the cut-off levels. [Methods] 154 children (63 GHD (37 severe GHD and 26 moderate GHD) and 91 short stature without GHD), aged from 1 to 19 years old were studied. The diagnosis of GHD was determined by two of following tests; Insulin, Arginine, Glucagon, Clonidine tolerance tests (ITT, ATT, GTT, CTT). GHRP2 administered intravenously at a dose of 2 µg/kg BW (max. 100 µg) in the morning after overnight fast. The blood was withdrawn at 0, 15, 30, (45), 60 min after injection. Serum GH was measured by RIA. Correlation of peak GH (pGH) in GHRP2 and other stimulation tests were analyzed by linear regression analysis. Relationships between pGH after GHRP2 and serum IGF-I levels, height SDS, % overweight, chronological age, pubertal stage were also analyzed by t-test and ANOVA. [Results] No adverse event was observed. Among 91 non-GHD subjects, pGH after GHRP2 showed no significant correlation with height SDS or with % overweight. When compared between Tanner stage groups, pGH in Tanner 2 was significantly higher than in Tanner 1 ($P<0.05$). In all subjects, Serum IGF-I significantly correlated with pGH of GHRP2 ($r=0.22$, $P=0.02$). pGH in GHRP2 highly correlated with pGH in ITT ($r=0.57$, $P<0.0001$). pGH in ATT and CTT significantly correlated with pGH in GHRP2, while pGH in GTT and GRF test showed no significant correlation. The cut-off levels for GHD was determined by plotting sensitivity and specificity. For severe GHD, the sensitivity and the specificity were 0.83 at the pGH of 10.5 ng/ml. For moderate GHD, the value was 0.75 at pGH of 16 ng/ml.
[Conclusion] GHRP2 test is a safe and simple test for the evaluation of GHD in children. It is comparable to the established tests including ITT.

PO1-200 New Technologies

Evaluation of sensitive detection methods for somatic mosaicism of GNAS1 mutation in patients with McCune-Albright syndrome

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Background: Activating mutations of arginine (Arg) 201 in the guanine-nucleotide-binding protein α subunit 1 (*GNAS1*) gene, is responsible for McCune-Albright syndrome (MAS). Gene analysis using sample from affected tissue is essential for the diagnosis because of nature of somatic mosaicism in this disorder. However, this may be harmful and more invasive for the patients. Therefore non-invasive method for diagnosis should be developed. Recently two highly sensitive detection methods of nested PCR method and PNA clamping method using peripheral blood for detection of Arg 201 mutation. We evaluated their usability as a diagnostic tool for MAS.
Methods: Ten clinically diagnosed MAS patients were included in this study. Eight patients presented with three characteristic phenotypes of MAS, and other two patients presented only with two phenotypes. DNA samples extracted from peripheral blood leukocytes were analyzed by Nested PCR and PNA clamping method as reported previously.
Results: Arg 201 mutation of *GNAS1* was detected in eight samples. In two patients presenting only with two phenotypes, the mutation was not detected. Nested PCR method could not detect one mutation although PNA clamping method detected. Nested PCR method required more time than PNA clamping method.
Conclusions: These two methods could detect less mutant alleles due to somatic mosaicism for a mutation of *GNAS1* using peripheral blood. In patients with triad of MAS, especially, these methods are more useful because the detection rate is much higher. We considered that PNA clamping method was superior to nested PCR method for usability.

PO1-201 New Technologies

Demirjian's system in the skeletal and dental assessments in Northeastern Brazilian children

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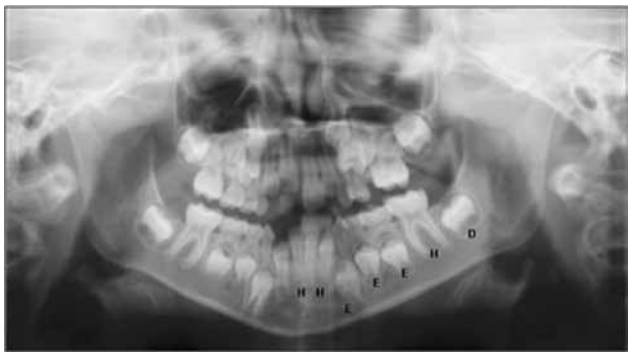
This study aimed at verifying the interrelationships among chronological, dental and skeletal ages (CA, SA and DA) of northeastern Brazilian children. The sample consisted of 325 subjects (134 boys and 191 girls), between 7 and 16 years, of whom were reviewed a carpal and a panoramic radiographs each, both been taken at a same date, in a private dental image clinic of Fortaleza-Ceara, Brazil. CA was determined from date of birth. DA assessment was done by Demirjian et al. system (figure 1) and skeletal assessment applied Greulich-Pyle standards.



The sample was divided into 3 age groups (<10, 10-13 and >13 years). The results showed a difference between means of DA and CA (1.74 ± 0.03, in boys and 1.68 ± 0.09, in girls). In both sexes, mean values of CA and DA in all age groups significantly differed ($P< 0.01$). Correlations between CA and DA were significant in the <10 and 10-13 groups, respectively 0.64, 0.53 ($P<0.01$), for boys and 0.77, 0.58 ($P<0.01$), for girls. CA and SA mean values in boys were similar in all age groups. In girls, they were statistically different ($P< 0.01$) in <10 and 10-13 years. Correlations between CA and SA in boys were significant only in the 10-13 and > 13 groups (0.47 and 0.59; $P< 0.01$). In girls, the correlations between CA and SA were significant in all age groups (0.66, 0.66 and 0.41; $P< 0.01$). We conclude that Demirjian system is not accurate to be applied in northeastern Brazilians and Greulich-Pyle standards are more suitable for boys, especially >10 and girls > 13 years.

PO1-202 New Technologies

Prediction of adult height based on automated determination of bone age

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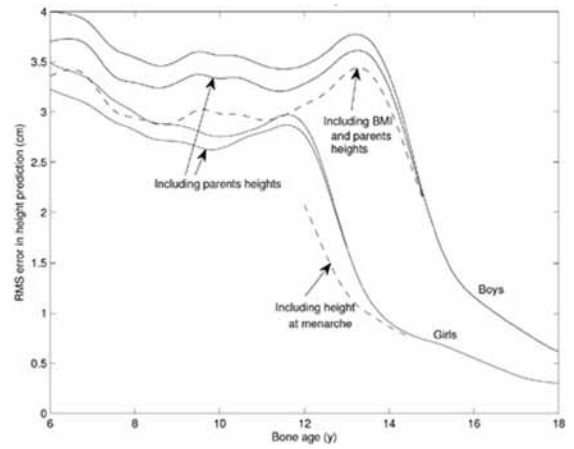
Adult height prediction is a common procedure in pediatric endocrinology. However, it is associated with a considerable operator variability and bias from the bone age (BA) rating.

Objective: A new method for adult height prediction is presented, based on automated bone age determination and improved mathematical modelling

Method: The method predicts the fraction of height left to grow from current height, age and BA. The BA is determined using the automated BoneXpert method, which provides a BA value on the Greulich-Pyle scale. The prediction is refined by dragging it towards the population mean, or alternatively towards the adult height predicted from the parents' heights. BMI can optionally be included as a predictor for boys, and height at menarche for girls. The uncertainty of the prediction is an integral part of the model.

Participants: We included 231 healthy children from the first Zurich Longitudinal Study, followed from 5 to 20 years with annual height measurements and X-rays of the left hand. This study was previously used to derive the Tanner-Whitehouse 3 (TW3) model for adult height prediction based on manual TW3 BA.

Results: The root mean square (RMS) error (compared to the TW3 method) was 3.3 cm (3.5 cm) for boys, 10-15 years, and 2.7 cm (3.1 cm; $p<0.005$) for girls, 8-13 years. High BMI before puberty affected adult height of boys negatively, independently of bone age, and inclusion of BMI lowered the prediction error for boys (10-15 years) to 3.1 cm. The figure shows the RMS errors versus BA of various variants of the new model including for instance the parents' heights or menarche information.



Conclusions: With the new method, adult height prediction has become evidence-based, since the dependence on manual bone age rating is eliminated. This could lead to a radically improved basis for accurate diagnosis of idiopathic short or tall stature. The method is also well-suited to analyse retrospective studies and build a consistent body of evidence of the relation between maturation, body mass and growth across populations, conditions and ethnicities

PO1-203 New Technologies

Bone age assessment in pediatric age: comparison between radiographic and sonographic methods

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Introduction.

Determining skeletal maturity in children takes great importance, not only in the evaluation of growth disorders, but also in the emerging need to attribute an age to children without identity documents. In clinical practice, the most used methods (Greulich & Pyle, Tanner-Whitehouse and Fels) require a radiograph of the left hand and wrist. In the last years several ultrasound-based techniques have been developed for the estimation of skeletal age without radiation.

The aim of this study was to compare the performance of a new ultrasound method (BonAge, Sunlight Medical Ltd., Tel Aviv, Israel) with the three most widely means of estimating bone age.

Methods.

From June to October 2008, 84 subjects (69 patients with proven or suspected growth disorders and 15 patients with slight traumas) who had undergone left hand and wrist radiography, were also examined sonographically in the non-dominant hand.

Children with previous or actual fractures of distal ulna or radio were excluded. Two paediatric radiologists evaluated all hand radiographs using TW2, Greulich & Pyle and Fels methods. Interpretation of the radiographs was performed blindly without knowledge of the age and height of the patient. Each patient performed BoneAge test afterwards in the same day or in the immediate following days.

The Pearson correlation coefficient (r) between BoneAge and each radiographic method was performed.

Results

A good correlation between the BoneAge measurement and the G&P (r=0,94) and TW2 (r=0,94 for RUS and r=0,95 for XX) methods was observed. Similar results were observed with Fels method (r=0,93)

Then we branched our population in subgroups on the basis of gender and pubertal status and we observed a similar correlation for all clusters, even when we controlled our findings for age, gender, pubertal status, BMI values, weight, height.

In a regression model BoneAge is predicted by height, age and BMI (r=0,85 β=0,86; 0,43; 0,23 respectively) as we observed in all radiographic methods.

Conclusions

The BoneAge results are highly correlated with skeletal age evaluated conventionally using the radiographic methods. Moreover, it is easily reproducible because of its lack of ionizing radiation and its objectivity.

PO1-204 New Technologies

The European paediatric regulation and its impact on drug development in paediatric endocrinology

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Since July 2008, pharmaceutical companies who want to obtain marketing authorization for a new medicinal product in the European Union (EU) must provide results of paediatric studies (done in accordance with an investigation plan agreed with the EMEA); alternatively, the applicant and the EMEA need to agree on a waiver from such obligation, or a deferral to provide these data until a later stage. In addition, from January 2009 this obligation is extended to existing patented medicinal products, when a new indication or formulation or route of administration is requested. These requirements will certainly translate into an increased number of industry-sponsored clinical trials in the paediatric population. The limited availability of paediatric patients may pose recruitment issues; this is particularly true for conditions that are highly prevalent in the adult population but not in children/adolescents (for example, type 2 diabetes or hypertension), and for which many medicinal products are under development. The role of paediatric networks is central for the success of clinical trials in children and adolescents, and therefore the EMEA is coordinating a network of existing European paediatric networks.

The Paediatric Committee (PDCO) at the EMEA meets monthly since July 2007. By April 2, 2009 the PDCO had received a total of 432 applications, of which 110 are requests for total waivers (no studies to be conducted in paediatrics), and 322 included a proposed paediatric investigation plan with one or more studies. The three therapeutic areas with the highest number of applications were endocrinology/metabolism/gynaecology-reproduction, cardiovascular diseases, and oncology, with the first area representing 17.5% of the total applications. For example, 27 applications for medicinal products intended to treat type I and/or type 2 diabetes have been submitted (including 6 fixed dose combination products). Seven applications for contraception-related products have also been submitted.

The EU paediatric regulation provides unprecedented opportunities for the assessment of the safety and the efficacy of medicinal products in all paeadiatric populations, from premature infants to adolescents until 18 years of age. Academic centres, health services, industry and regulatory agencies need to cooperate to maximize the benefits and fulfil the spirit of the legislation.

PO1-205 Obesity, Fat I

Serum chitotriosidase activity: is a new inflammatory marker in obese children?

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Background: Chitotriosidase (ChT) was the most promising inflamatory marker and received great attention for the last decade. Serum ChT activity was reported to be significantly higher in individuals with atherosclerosis. Obesity is a systemic low-grade inflammation and causes atherosclerosis. In this study, we aimed to investigate serum ChT activity in obese subjects and to determine the relation with insulin resistance and high-sensitivity C-reactive protein (hsCRP).

Subjects-Methods: A total of 80 obese subjects (10.37 ± 2.75 years of age, 48 male patients) and 44 age and gender matched healthy lean subjects (11.62 ± 0.42 years of age, 17 male patients) were included in this study. Obesity was defined as the body mass index (BMI) being over 97% percentile of the same gender and age. Fasting serum glucose, insulin, hsCRP, ChT and lipid profile were measured.

Results: There was no statistically significant increase in serum ChT activity of obese subjects, while there was statistically significant difference in serum hsCRP levels when compared to healthy lean subjects (27.43±18.72, 22,56 ±17.54 p=0.154, 2.26±3.03, 0.71±1.16, p=0.001 respectively). Obese subjects had significantly higher BMI-SDS, HOMA-IR and trigliseride levels when we compared with the control subjects (2.30±0.32 & 0.03±0.78, 3.60±2.26 & 1.68±1.52, 123.63±66.74 & 90.5±46.26, p<0.05 respectively). There was no significant correlation between ChT activity and hsCRP, HOMA-IR and BMI-SDS (r=0.031, p=0.774, r=0.114, p=2.95, r=-0.065, p=0.552) while, hsCRP only significantly correlated with BMI-SDS (r=0.282, p=0.008).

Conclusion: These results suggest that serum ChT concentrations may not be a useful marker for monitoring systemic low-grade inflammation in obese subjects when compared to hsCRP.

PO1-206 Obesity, Fat I

Evaluation of visfatin and adiponectine levels in obese children and relation with insulin resistance

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Background: Visfatin is a new cytokine that act as an insulin analogue on the insulin receptor and may link obesity and insulin resistance. In this study we aimed to investigate the relationships between plasma visfatin, adiponectine, insulin sensitivity, lipid profile and anthropometric parameters in obese children.

Material and methods: A total of 40 obese subjects (mean age; 10.91 ±2.65 year, 20 girls) and 40 lean subjects (mean age; 11.02±3 year, 20 girls) were included in this study. Obesity was defined as the body mass index (BMI) being over 97% percentile of the same gender and age. Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR).

Results: Obese and healthy lean subjects showed no significant difference in terms of age, total cholesterol and LDL-cholesterol. Subjects in the obese group had significantly higher BMI-SDS, HOMA-IR, HDL-C, triglycerides levels than control subjects (p<0.05). Plasma visfatin levels (31.3 ± 11.1 vs. 18.5±10.7, p<0.001) were significantly elevated while, plasma adiponectine levels were significantly lower (2.01 ± 1.02 vs. 12.5 ± 6.2, p<0.001) in obese subjects. Insulin-resistant group had higher visfatin (36 ±9.7 vs. 22.9 ±7.6, p<0.001) and lower adiponectin levels (1.7 ± 1.05 vs. 2.5 ±0.77, p=0.016). Correlation analysis showed that visfatin was positive correlated with BMI-SDS and HOMA-IR (r=0.61, p<0.001 and r= 0.63, p<0.001) while, adiponectin was negative correlated with BMI-SDS and HOMA-IR (r= -0.46, p=0.002 and r=-0.44, p= 0.004).

Conclusion: These results suggest that plasma visfatin and adiponectine level is a specific marker for visceral fat accumulation and significantly associated with BMI and insulin resistance in obese children.

PO1-207 Obesity, Fat I

Subepicardial adipose tissue thickness and relation with anthropometric and clinical parameters in pubertal obese children

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Aim: To determine the relation of echocardiographic subepicardial adipose tissue (SAT) thickness with anthropometric and clinical parameters in pubertal obese children.
Subjects-Methods: A total of 52 pubertal obese subjects (13.1 ± 1.56 years of age, 27 male patients) and 39 age and gender matched pubertal lean subjects (13.0 ± 1.28 years of age, 16 male patients) were included in this study. The criterion for diagnosing obesity was defined according to the World Health Organization classification as the body mass index (BMI) SDS being over +2 SD of the same gender and age. Serum glucose, lipid profile, and insulin levels were measured during the fasting state. Each subject underwent a transthoracic echocardiogram and the SAT thickness was measured during end-diastole from the parasternal long-axis views.
Results: The obese pubertal subjects had significantly higher SAT and triceps skin fold (TSF) thickness (mm), waist, mid-arm muscle circumference (MAC) (cm) values compared with the pubertal subjects in the control group ($p<0.05$). Simple linear regression analysis showed significant correlation between SAT, age (year), BMI (kg/m^2), SDS-BMI, hip circumference (cm), MAC, TSF, and insulin resistance (HOMA-IR) (r=0.293, r=0.475, r=0.347, r=0.449, r=0.301, r=401, r=305, $p<0.05$, respectively), whereas there was no significant correlation between SAT, waist (cm) and waist-hip ratio (r=0.228, r=0.074, $p<0.05$, respectively). As an optimal cut off point, a SAT thickness of 5.25 mm determined insulin resistance with 91.3% sensitivity and 60.9% specificity.
Conclusions: Our study showed that the SAT was significantly correlated with age, BMI, SDS-BMI, waist, MAC, TSF, and insulin resistance but not with hip circumference and waist-hip ratio. This is the first study in obese pubertal children to demonstrate the relation of SAT thickness and insulin resistance and anthropometric parameters. In addition to this relation, our study suggest in pubertal obese subject that a 5.25 mm cut-off of SAT thickness might be used as a simple and non-invasive screening method because of its ability to predict insulin resistance with high sensitivity in obese pubertal children.

PO1-208 Obesity, Fat I

Obesity and *cardiovascular* risk factors in adolescents

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Background: Obesity is associated with comorbidities in childhood as well as increased risk of chronic disease and decrease life expectancy in adult life.
The aim of this study was to find the impact of obesity on carotid –intima media thickness and Lt ventricular mass and function.
Patients and methods: A case control study was carried out on 52 adolescents with obesity aged 10-18 years as well as 52 age and sex matched controls recruited consecutively from the out patient clinic of **Suez Canal University Hospital**. All were subjected to thorough medical history& clinical examination and laboratory investigations including: fasting blood sugar, serum triglyceride, HDL-cholesterol, LDL-cholesterol and carotid artery scanning by echocardiography using a 7.5-MHz linear array transducer to detect the impact of obesity on cardiovascular risk factors.
Results: BMI of obese adolescents was 39.98 ±39.36 kg/m2 and mean fat % 26.2% versus 17.90± 1.01 kg/m2& 7.99% for controls respectively. Significant increase in diastolic blood pressure, total cholesterol, triglyceride, HDL- cholesterol, LDL- cholesterol& fasting blood sugar in obese compared to control. (p=0.001& p= 0.008& p= 0.007& p= 0.005& p= 0.009& p= 0.017 respectively)
Right& Left carotid intima media thickness (CIM)(cm) were significantly increased in obese compared to control (0.51± 0.1& 0.50± 0.02 vs 0.40± 0.02& 0.40± 0.02) (p=0.001).Sigificant increase in Left ventricularl mass& posterior wall thickness and interventricular septum was found in obese adolescents compared to control.
Conclusion: Obese adolescents have an increased cardiovascular risk factors (dyslipidemia & greater IMT& Left ventricular hypertrophy and hypertension).

PO1-209 Obesity, Fat I

Risk factors for the early prediction of obesity among childhood survivors of suprasellar brain tumours

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Background: Excessive weight gain leading to obesity is a well recognised complication in children surviving suprasellar brain tumours. However, factors that predict obesity in these children have not been explored.
Aims: To assess changes in body mass index (BMI) from diagnosis to last follow up in survivors of childhood suprasellar brain tumours, and to determine the factors that could predict which of these children are at risk of developing obesity.
Methods: Retrospective review of 46 children (16 boys) aged 7.6±4.6yrs at diagnosis of tumour, and followed up for 4.9±3.5yrs. Height and weight at diagnosis and follow up were ascertained from clinical records. BMI standard deviation scores (SDS) and changes in BMI SDS (ΔBMI SDS) during follow up were calculated. Survival analyses were used to explore risks of developing obesity (International Obesity Taskforce defined) during follow up.
Results: There were no significant differences between boys and girls in age at diagnosis of tumour (9.0±4.3yrs vs 6.8±4.6yrs; p=0.1), duration of follow up (4.5±2.8yrs vs 5.1±3.8yrs; p=0.6); tumour types (glioma [total n=21], craniopharyngioma [13], prolactinoma [4], other suprasellar tumours [8]); endocrinopathies [39]; and treatment modalities (surgery [29], radiotherapy [23], chemotherapy [14], none [10]). Baseline BMI SDS was similar in boys (0.64±1.51) and girls (0.70±1.33); p=0.9. ΔBMI SDS/year was significantly greater in girls (0.52± 0.81) than in boys (0.09±0.34); p=0.02 – especially in the first year after diagnosis (1.24±1.30 in girls vs 0.19±0.89 in boys; p=0.002). Three of the 46 children (6%) – 1 boy and 2 girls – were obese at diagnosis of tumour, increasing to 20 (43%) – 3 boys (19%) and 17 girls (57%) – by last follow up (p=0.04); hazard ratio for obesity in girls was 5.0 (95%-confidence interval (CI) 1.2–21.7) compared to boys. Eighteen of the 34 children (53%) whose baseline BMI SDS > 0, developed obesity by last follow up, compared to only 2 of the other 12 children (17%) whose baseline BMI SDS ≤ 0 (p=0.02); hazard ratio was 4.7 (95%-CI 1.1–20.8).
Conclusions: In our cohort of childhood survivors of suprasellar brain tumours, female gender and baseline BMI SDS > 0 were risk factors enabling early prediction of subsequent obesity. Significantly greater BMI increases in girls occurred during the first year after diagnosis, highlighting the importance of early targeted interventions in the high-risk patients to minimise weight gain and associated morbidities.

PO1-210 Obesity, Fat I

Correlation between ambulatory blood pressure and anthropometric and metabolic parameters in obese children

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Introduction: Childhood obesity is associated with features of insulin resistance, including elevated blood pressure. Ambulatory blood pressure (ABP) monitoring is superior to clinic (office) blood pressure (CBP) monitoring in predicting cardiovascular morbidity. It also allows the diagnosis of Masked Hypertension (elevated ABP in face of normal CBP), which is associated with increased cardiovascular risk.
Objectives: To evaluate the relationship between ABP, BMI, and components of the metabolic syndrome in obese children.
Methodology: 44 children (7 – 17 years old) were recruited. Inclusion criteria were: BMI SDS > 2 and normal CBP. ABP monitoring, OGTT, lipids, urine microalbumin, height, weight, and waist circumference were obtained. HOMA and BMI were calculated.
Results: 15 (34.1%) of the children had at least one abnormal ABP parameter: 6 (13.6%) had an elevated (≥ 95th percentile) 24-hour systolic blood pressure (SBP) (4 of whom had also an elevated wake SBP); 9 (20.5 %) had an elevated sleep SBP (2 of them, or 4.5%, had an elevated sleep diastolic blood pressure,

DBP). None of the subjects exhibited elevated 24-hour or wake DBP. When we analyzed the results according to the subjects' BMI SDS (2-2.5 SDS vs. > 2.5 SDS), those with more severe obesity had higher 24-hour ($p<0.05$) and sleep SBP ($p<0.01$). No differences were found between the two groups regarding the DBP parameters.
Subjects with more severe insulin resistance (highest quartile vs. lowest quartile HOMA) had a higher triglyceride ($p<0.05$) and wake SBP ($p<0.05$) than those with milder insulin resistance.
When we grouped the subjects according to the SBP parameters (≥ 95th percentile vs. < 95th percentile), those with elevated sleep SBP had greater BMI SDS ($p<0.01$) and waist circumference ($p<0.05$), while those with elevated 24-hour SBP exhibited a greater BMI SDS only ($p<0.05$). Subjects with elevated wake SBP had a higher 120-min glucose ($p<0.05$). No difference was found between groups with respect to HOMA, fasting glucose and insulin, urine microalbumin, and lipid levels.
Subjects with elevated DBP did not differ from those with normal DBP for any of the parameters considered.
Conclusions: Our findings suggest a high prevalence of masked hypertension in obese children. Elevated ambulatory blood pressure (especially systolic) is associated with more severe obesity and abdominal adiposity, thus prompting the need for ambulatory blood pressure monitoring in selected high-risk children.

PO1-211 Obesity, Fat I

Metabolic syndrome prevalence in obese children and adolescents aged 10-16 years according to new IDF criteria

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AIM: Childhood obesity and related disorders are growing worldwide to become the most prevalent chronic disease of the youth population. The metabolic syndrome (MS) is a clustering of cardiovascular disease and type 2 diabetes mellitus risk factors, which includes central obesity, hypertension, dyslipidemia and glucose intolerance. In contrast to the definition of MS in adults, no standard definition for use in pediatric population exists. Recently, the International Diabetes Federation (IDF) has proposed a new set of criteria to define MS in children and adolescents with central obesity as the core of the definition.
In this study, we aimed to explore the MS prevalence in our obese children and adolescent patients aged 10-16 years according to new IDF criteria.
METHODS: A total of 148 obese children and adolescents (79 male and 69 female aged 10-16) referred to the Department of Pediatric Endocrinology, Erciyes University in Kayseri, Turkey between April 2008 and December 2008 were included in this study. Exclusion criteria were the presence of major disease and/or genetic and endocrinological disease causing secondary obesity and use of any medication. The IDF criteria for MS in adolescents aged 10-16 years include abdominal or central obesity (90th percentile of waist circumference or adult cutoff if lower) plus at least two of other clinical features of the following: fasting plasma TG ≥150mg/dl; HDL-C ≤40mg/dl; systolic BP ≥130mmHg and/or diastolic BP ≥85mmHg; fasting plasma glucose >100mg/dl or known type 2 diabetes mellitus.
RESULTS: Metabolic syndrome was found in 35.1 % of the subjects, with a rate among males 35% and females 34%. The prevalence of two components in addition to abdominal obesity was 27%, three components 6.8%, and four components 1.4%.
CONCLUSION: MS prevalence in our obese children and adolescents aged 10-16 was found as 35.1 % according to new IDF criteria.

PO1-212 Obesity, Fat I

The melanocortin system and insulin resistance: insights from a patient with complete POMC deficiency and type 1 diabetes mellitus

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POMC (proopiomelanocortin) is the precursor to five biologically active proteins made in the anterior pituitary or hypothalamus and skin. POMC plays an important role in the leptin-melanocortin system in that POMC-expressing neurons are targets of leptin signaling, and α-melanocyte-stimulating hormone (α-MSH) is the POMC cleavage product that activates melanocortin-4 receptor. The classic triad of POMC deficiency (adrenal insufficiency, severe obesity and red hair) is due to the functional absence of ACTH from the anterior pituitary, and α-MSH in the hypothalamus and skin. Complete POMC deficiency is rare in that only seven human cases have been reported. Interestingly, POMC-null mice are hypersensitive to the adverse metabolic effects of glycocorticoids and develop diabetes mellitus 12 weeks after glucocorticoid treatment.
Objective: To report the eighth case of complete POMC deficiency, to demonstrate that the patient's mutation abolishes the production of POMC protein, and to describe the clinical phenotype of our patient which includes diabetes mellitus.
Clinical Case: A 9 year-old male with isolated ACTH deficiency, severe early-onset obesity, red hair and type 1 diabetes mellitus was referred to our institution. The patient had a history of hypoglycemia during the newborn period. He was diagnosed with isolated ACTH deficiency at 3 months of age and was started on hydrocortisone. At 5.5 years he presented with diabetic ketoacidosis with positive ICA-512 autoantibodies. His insulin requirements ranged from 0.7 to 1 unit/kg/day. His HbA1C ranged from 7.1 to 7.5. The parents are first cousins of Scottish and German descent. The patient's BMI is 35kg/m2 (>99.99 percentile for age).
Results: Direct sequencing of the patient's POMC gene showed that he was homozygous for the mutation C3804A. A homozygous C to A change in the 5' untranslated region that creates a novel ATG codon 11 basepairs upstream of the actual translational start site. This frameshift mutation results in complete absence of the POMC transcript.
Conclusion: This is the first report of a patient with a homozygous POMC mutation and type 1 diabetes mellitus. Our study demonstrates that the patient's mutation results in complete absence of the POMC protein. The POMC deficiency in our patient did not increase his insulin requirements compared to patients of the same age and BMI, indicating that absence of a functional melanocortin system in humans does not promote insulin resistance.

PO1-213 Obesity, Fat I

Hyperleptinaemia with possible leptin-resistance in obese boys with delayed puberty

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Background: The adipocyte-derived hormone, leptin, is supposed to play a permissive role in initiating puberty. **Objective:** The study aim at measurement of serum leptin level in obese boys with delayed puberty (DP) and its relation to some other puberty-relevant hormones and somatic and sexual anthropometric indices. **Subjects and Methods:** Delayed puberty was defined as absence of signs of puberty at upper limit of the chronological age for onset of puberty (Mean ±SD) equal 13.8 years. Twenty-five obese boys with DP (group1) their BMI ≥95th percentile for age and twenty normal age-matched control subjects with normal onset of puberty (group 2) were included. The age in obese and control was(14.3±0.4) and (13.8±15.8) respectively. For all subjects, heights and body weights were measured; body mass index, fat percentage and fat mass were calculated. Sex Maturity Rating stages were identified. The levels of leptin, FSH, LH, GH, T_4 and free testosterone were determined. Bone age was determined for group1 only. **Results:** Serum level of leptin in obese and control was (25.2±17.1) and (6.16±2.9) $P<0.001$ respectively. All other variables showed significant differences between the two groups except serum levels of GH and T4.

Clinical and hormonal profile among obese boys with delayed puberty and control subjects

	Obese	Control	P
Fat %	21.4±3.6 (15.4-30.3)	9.1±3 (3.9-14.5)	0.001
Fat mass	15.3±4.8 (9.5-28.5)	6.4±1.8 (1.9-9.2)	0.001
Testicular vol. (ml)	2±0.6 (1.2-3)	10.3±2.4 (7.5-14.5)	0.001
FSH (mIU/ml)	1.56±0.9 (0.45-3.15)	4.76±1.8 (2.6-8.5)	0.001
LH (mIU/ml)	0.52±0.5 (0.1-1.6)	3.06±1.1 (0.8-5.2	0.001
GH (ng/l)	6.3±1.5 (4.3-8.9)	6.33±1.2 (4.5-8.7)	0.93
T4 (n mol/dl)	8.83±1.52 (6.2-12.3)	8.60±1.85 (5.9-12.1)	0.65
Total testost. (ng/ml)	0.18±0.25 (0.02-1.1)	1.63±0.84 (0.2-3.74)	0.001
Leptin (ng/ml)	25.2±17.1 (4.8-70.4)	6.16±2.9 (2.1-12.6)	0.001

In Obese children there were significantly positive correlations between serum leptin and body weight, BMI, fat mass and fat percentage. Obese boys with DP exhibited a subtle retardation of bone age by 6 % of their chronological age.
Conclusion: Obese boys with DP are hyperleptinaemic. Presence of defective expression of leptin receptors might be responsible for this distinctive association of obesity and delayed puberty.

PO1-214 Obesity, Fat I

Succesful outcome in an adolescent with obesity and mental delay treated with pharmacological therapy

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A 18- year-old adolescent diagnosed with Sotos syndrome and morbid obesity is presented. He started to gain weight and height rapidly since the age of 4 years and had failed to respond to dietetic therapy. He was the only child born from the first gestation from non-consaguineous parents. Normal delivery. Birth weight: 3.600g. Birth length: 54 cm. Psycomotor delayed. Normal Kariotype. 2 hip epyphisiolisis. Both parents are moderately obese. **Physical examination:** weight: 227 kg. Height: 190 cm. BMI: 62,8 Kg/m2. Waist/Hip circumference: 165/165 cm. Blood pressure: 129/56 mmHg. Morbid obesity. Prognatism. Achantosis nigricans. Estriae over abdomen and lower extremities. Tanner stage: 4. Investigations: hyperleptinemia**,** hypertransaminasemia, oral glucose tolerance test showed: hyperinsulinsm with carbohydrate intolerance. Abdominal ultrasound showed liver esteatosis. Genetic test were negative for: Sotos, X-fragile and Prader-Willi. Outcome: He started Metformin and Orlistat therapy with very good tolerance. After 30 months of therapy he lost 90 kg and all the biochemical alterations returned to normal values.

Summary of the case report

	Day1	2m	5m	9m	13m	24m	30m
Weight (kg)	227	217	195	178.2	163	151.6	137
BMI (Kg/m2)	62.9	60	54	49.3	45	41.5	37.5
Insulin (mcU/mL)	41.9	25.6		23.1	15	16.9	12.4
Leptin (mcg/mL)	101		39.5		39.6	29.7	19
AST (UI/L)	45	68	47	25	16	12	13
ALT (UI/L)	68	117	86	47	30	16	11
GGTP (UI/L)	63	56	46				
Metformin 850 mg	1-0-1	1-1-1	1-1-1	1-1-1	1-1-1	1-1-1	1-1-1
Orlistat 120 mg	0-1-1	1-1-1	1-1-1	1-1-1	1-0-1	0-1-0	Stop

m: months

Conclusion: the very succesful respond to pharmacological therapy in this patient with morbid obesity and mental delay is an incentive to encourage the treatment of obesity in these type of patients.

PO1-215 Obesity, Fat I

Effect of orlistat on cardio-vascular risk factors in obese adolescents

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Aim: to study efficacy and safety of orlistat-120 on cardio-vascular risk factors in obese adolescents.
Materials and methods: we examined 28 adolescents aged from 12 to 17 with obesity of various severities. 20 healthy adolescents of matching sex and age were included into the control group. The examinees were divided into two groups: 11 persons in the 1st group (O) on hypocaloric diet (1,500 ccal a day only) and with increase in physical activity received orlistat in the dose of 1 drop thrice a day at meal, 17 obese persons on hypocaloric diet and with increase in physical activity (life style change, LSC group) in the 2nd experimental group. Duration of treatment and follow-up was 6 months. Anthropometric parameters, arterial pressure, MAU, carbohydrate and lipid profile, IRI with HOMA index were measured.
Results: after 6 months of treatment confident decrease in body mass by 13.9% (P<0.05) WC by 11.9% and BMI by 13.8% (P<0.05) was observed in the O group, in LSC group only BMI decreased confidently by 8.4% (P<0.05). Triglycerides were found to decrease by 19.2% and HDP to increase twice (P<0.01) in persons receiving Orlistat. In the 2nd group there was non-confident decrease in all parameters of lipid metabolism. Arterial systolic and diastolic pressure in the O group confidently decreased by 13.2 mm Hg (11.3%) and 7.32 mm Hg (10.7%), respectively (P<0.05). In these persons MAU was 36,4%, in the LSC group being 35.3%. MAU in persons receiving Orlistat for 6 months decreased by 37.1% having confident difference (P<0.05) with the initial one, in the LSC group decrease in MAU was non-confident (16%). Initially HOMA index was increased in 70% of persons in the 1st group IR confidently decreasing while on Orlistat (P<0.05), in 43% of them IR normalization being observed and 57% and significant decrease without normalization being found. In the LSC group HOMA index was increased in 50% of patients with no normalization within 6 month of follow-up.
Orlistat was well tolerated by more than 82% of obese adolescents. Side effects in the form of fatty stool and meteorism were registered in 2 (18%), after correction of dose no withdrawal being necessary.
Conclusions: 6-month therapy with Orlistat in the dose of 240-360 mg/d in 12-17- year old adolescents with exogenous-constitutional obesity showed high safety and confident efficacy in decrease of cardio-vascular risk as compared with life style change only.

PO1-216 Obesity, Fat I

Incidence of arterial hypertension and microalbuminuria in insulin- and non insulin resistant adolescents with exogenous-constitutional obesity

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Aim: to study incidence of hemodynamic parameters in insulin-resistant and non insulin resistant adolescents with and without exogenous –constitutional obesity (ECO).
Materials and methods: we examined 54 adolescents aged from 10 to 16 with ECO (girls 53.7%, boys 46.3%). 56 healthy adolescents of matching age and sex were included into the control group. In dependence of HOMA index (normal <1.8) they were divided into groups: the 1st one consisted of 34 insulin resistant adolescents with ECO and the 2nd one - of 20 non insulin resistant persons with ECO. BMI, waist circumference, systolic and diastolic arterial pressure were measured in the morning and in the evening in compliance with 2 - 18-year age norms recommended by IDF (2008). Microalbuminuria (MAU) was assessed by means of commercial RIA kit (Immunotech, Czech Republic) and Micral-test (Roche, France).
Results and discussion: the findings showed that morbid obesity (BMI > 35 kg/m2) in the 1st group was observed more frequently (26.4% n=9), than in the 2nd one (5%, n=1). In the 1st and 2nd groups WC ≥ 90 percentile was 88.2% and 80%, respectively. Increase in systolic arterial pressure > 90 percentile in the 1st

group was observed in 8.8% (n=3), in the 2nd one being detected in 10% (n=2). MAU parameters upon ECO in both groups (20 ± 2.4;19.5 ± 2.7 mg/ml) had no confident difference, but were confidently increased as compared with those in the control group (7.1±1.2) mg/ml. (Ð<0.001). Upon insulin resistance (IR) MAU was observed more frequently in 30% of cases (n=9), than in persons without IR in 15% (n=3). The value of MAU >20 mg/ml is the evidence for starting dysfunction of endothelium as well as for early risk of atherosclerotic affection of renal and cardiac vessels even in adolescents under 16 years.
Conclusions: 1. There is higher incidence of morbid obesity (26.4%), arterial hypertension (8.8%) and increased WC ≥ 90 percentile (88.2%) in insulin resistant adolescents with exogenous-constitutional obesity as compared with those having exogenous-constitutional obesity without insulin resistance.
2. Higher incidence of microalbuminuria can be seen upon insulin resistance in adolescents with exogenous-constitutional obesity.

PO1-217 Obesity, Fat I

Evaluation of FETUIN-A as a serum marker for childhood obesity comorbidity: no association with HOMA or fatty liver disease

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Background: The liver derived protein FETUIN-A (also known as "alpha2-Heremans-Schmid Glycoprotein) has been suggested as a risk factor for type2 diabetes and increased liver content in adult obese patients (Stefan et al. 2006,2008). In a recent study based on a small cohort of childhood obese patients (n=36) FETUIN-A was shown to be associated with fatty liver disease and HOMA (Reinher et al 2008). To validate the relevance of FETUIN-A as a serum marker for childhood obesity we measured FETUIN-A serum levels in a large cohort of normal weight and obese children.
Methods: FETUIN-A was measured with an ELISA kit (Bio Vendor Lab, Czech Republic). Cohorts consisted of 98 normal weight children (BMI-SDS<2), 139 obese children ((BMI-SDS>2), 79 obese children with impaired glucose tolerance (HOMA>95P.) and 24 obese children with NASH (non-alcoholic-steato-hepatitis). FETUIN-A levels were compared to measurements of the established obesity markers leptin and adiponectin.
Results: We found the expected association of leptin with BMI-SDS, pubertal stage and HOMA. However, FETUIN-A was only weakly associated with BMI-SDS in boys but did not revealed any association with BMI-SDS in girls, with pubertal stage, HOMA or NASH in both genders.
Conclusion: Although findings in FETUIN-A knockout mice were suggestive for a role of FETUIN-A as a marker of obesity phenotypes, studies in humans so far revealed only weak correlations with obesity comorbidity. Our results in a large childhood obese cohort including children with impaired glucose tolerance and fatty liver disease did NOT confirm a relevant role of FETUIN-A as a marker for obesity comorbidity phenotypes in childhood.

PO1-218 Obesity, Fat I

Lung function impairment in obese children

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In adults, pulmonary function abnormalities are well reported complications of obesity; the most frequently reported abnormalities are reductions in lung volumes and expiratory flow rates. Data from similar studies in the pediatric population are however limited and conflicting.
The aim of this study was to determine the predominant pulmonary function abnormality in a group of obese children, and the to assess the correlation between the severity of lung function impairment and respectively the body mass index (BMI) and waist circumference (WC).
Seventy–four (10±1.8 years) children with BMI for age and sex more than the 95th percentile, and a group of sixty normal weight and age-matched reference subjects, underwent pulmonary function study.
Results are expressed as mean±SD, and P<0.05 was accepted as the level of significance. Statistical analysis was performed using Student's t-test to compare differences in mean values between groups, and linear regression analysis to study the correlation between functional indices and single variables.
Adjusted values expressed as SD score of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1), and the forced expiratory flow 25–75 (FEF25–75) were found to be significantly lower in obese children than in children with normal weight (-0.1±1.0 vs 0.3±1.0 p<0.05; 0.0±0.9 vs 0.4±0.9 p<0.05; -0.2±0.8 vs 0.2± 0.9 p<0.01; respectively).
Total lung capacity (TLC) and residual volume/TLC ratio (RV/TLC ratio) were significantly higher in the group of obese children than in controls (0.4±1.2 vs. 0.0± 1.1 p<0.05; 24±8 vs 21±7 p<0.05, respectively).
There was a significant relationship between pulmonary function indices and BMI. The effect of increased BMI was negatively associated with FVC, FEV1 and FEF25–75, and positively associated with RV/TLC ratio but not with TLC. WC but not BMI was negatively associated with a decline in FEV1/FVC.
In conclusion this study supports the view that the lung is functionally involved in obese children. There was a significant relationship between lung function impairment and measures of obesity (BMI and WC). The underlying mechanisms remain to be clarified.

PO1-219 Obesity, Fat I

Deletion of lipopolysaccharide receptor toll-like receptors confers protection against lipid induced insulin resistance in murine model

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Obesity and diabetes characterized by inflammation and insulin resistance. Toll-like receptors (TLR) are suspected in providing metabolic crosstalk between both inflammatory and insulin signaling pathways. TLR are found in innate immune system and in insulin sensitive cells, including muscle, liver, and adipose, and bind LPS and fatty acid moieties. We investigated whether mice lacking TLR were protected from insulin resistance caused by a high fat diet (40.5% fat).
Age matched TLR knockout (TLR KO) mice and wild type C57/B10 were fed either a standard chow or high fat diet (HFD) for nine weeks. HFD led to similar weight gain and adiposity (measured by DXA) in both groups. However, HOMA values at 6 weeks HFD were lower for TLR KO (WT HF: 40.45 +/- 6.34 vs KO HF: 12.73 +/- 1.80; p<0.05) and liver triglyceride levels were lower for TLR KO (WT HF: 31.28 +/- 3.38 ug/mg tissue vs KO HF: 11.5 ug/mg tissue +/- 0.53; p<0.05). In addition, insulin sensitivity as measured by intraperitoneal insulin tolerance testing and intraperitoneal glucose tolerance testing were improved in TLR KO on HFD (WT HF vs KO HF; p<0.05). These results appear to indicate that absence of TLR may confer protection against high fat induced insulin resistance and type 2 diabetes.

PO1-220 Obesity, Fat I

Acylated, unacylated ghrelin and obestatin regulation during oral glucose tolerance test in obese prepubertal and pubertal children

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Introduction. Three peptides, acylated ghrelin (AG), unacylated ghrelin (UAG) and obestatin are derived from a common prohormone, preproghrelin by posttranslational processing, originating from endocrine cells in the stomach. Circulating ghrelin levels are decreased in obesity and in metabolic syndrome (MS) in adulthood and increased by fasting and anorexia nervosa, but the physiological role of the three peptides is poorly understood in particular in childhood.
Aim. In order to understand the biological implications of these three peptide

in obesity and MS, we measured AG, UAG, obestatin, at fasting and every 60 min during an oral glucose tolerance test (OGTT) in 60 prepubertal (PP-OB; 32) and pubertal (P-OB; 28) children. Auxological parameters, glucose and insulin during OGTT, lipid profile and blood pressure were also evaluated to divide patients according to paediatric IDF 2007 criteria for MS.
Results. In PP-OB group, 20 (62.5%) children had MS and 4 of them had glucose intolerance (impaired fasting glucose or impaired glucose tolerance). In P-OB group, 28 (100%) children had MS and 5 of them had glucose intolerance. UAG ($p<0.004$), obestatin ($p<0.05$) and nearly to significance AG ($p=0.07$) were lower in OB-P when compared to OB-PP. AG levels were higher in both prepubertal and pubertal males when compared to females ($p<0.04$). During OGTT: 1) AG levels decreased at 60 min ($p<0.04$) and returned to basal levels at 120 min in both PP-OB and P-OB; 2) UAG levels decreased for all the testing session ($p<0.0001$) in both groups and more significantly in PP-OB when compared to P-OB ($p<0.02$); 3) obestatin levels decreased at 120 min ($p<0.04$) in both groups. Fasting UAG ($p<0.01$) and during OGTT obestatin levels ($p<0.01$) were lower in children with MS when compared to those without MS. Fasting UAG ($p<0.05$) and obestatin ($p<0.02$) were also lower in PP-OB children with glucose intolerance when compared to euglycemic PP-OB. The levels of three peptides were positively correlated each others ($p<0.004$). The AG decrease was correlated with glucose nadir during OGTT (r: 0.357; $p<0.02$). The UAG decrease was associated with fasting insulin, HOMA index and insulin nadir (r: 0.372; $p<0.02$) during OGTT. The obestatin decrease was associated with fasting HDL-cholesterol (r: -0.497; $p<0.002$).
Conclusions. AG, UAG and obestatin levels were differently inhibited during OGTT in OB children. MS influences the glucose-induced regulation of ghrelin system also in childhood.

PO1-221 Obesity, Fat I

Evaluation of birth weight and length among overweight and obese children and adolescents

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Introduction: Overweight and obesity are multifactorial conditions, determined by complex interactions among biological, genetics and environmental factors. They represent a global worry epidemy affecting all ages, including the pediatric group. Recognition of risk factors or risk groups could contribute to increase the efficacy of the preventive multidisciplinary healthy systems works attempting to revert this situation.
Objective: To evaluate if there is correlation between overweight and obesity in childhood and adolescence with weight or length at birth.
Patients and methods: This retrospective study was comprised of 116 overweigh or obese patients, aged 2 to 18 years, followed at a specialized ambulatory care. Patients were classified according to weight/length for gestational age in small (SGA), appropriate (AGA) and large (LGA), using regional charts. In the first visit, they were classified in overweight or obese according to the percentiles they were in on the body mass index (BMI) chart (*CDC/NCHS, 2000*). Data were presented and analyzed in terms of *z-score* of the body mass index (*z-BMI): overweight- z*-BMI from 1 to 1.9sd; obesity- *z*-BMI ≥ 2.0sd. *Spearman* and *Student tests* were used for statistical analysis.
Outcomes: There were 59 girls (50.9%); the whole group mean age at the beginning of follow-up was 9.3±2.9 years; mean *z*-BMI was 2.0±0.5sd. Overweight was diagnosed in 43.1% of patients and 56.9% were obese. The girls' mean *z*-BMI (1.9±0.4sd) was statistically lower than the boys' (2.1±0.5sd), $p=0.024$. It was observed an inverse correlation between age and *z*-BMI ($p = 0.0008$; $r = -0.306$). It was also found that the patients born LGA presented a statistically significant higher *z*-BMI in the first visit than the ones born SGA: LGA*weight x SGAweight* ($p=0.02$); LGA*length x SGAlength* ($p=0.036$). There was no statistical difference when comparing the *z*-BMI of patients born AGA to the LGA and SGA groups and neither when comparing the *z*-BMI of patients born full-term to the pre-term ones ($p=0.433$).
Conclusion: In this pediatric group, it was not observed correlation between obesity in childhood and adolescence and weight nor length at birth, but it was found that children and adolescents born LGA presented a statistically significant higher *z*-BMI than the ones born SGA. It was also seen that younger ages and male sex were variables correlated to a statistically higher *z*-BMI in the beginning of follow-up.

PO1-222 Obesity, Fat I

What is the Bogy mass index threshold value for children that metabolic syndrome develops?

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Objectives: Recently obesity and metabolic syndrome(MetS) have become a growing problem in pediatric age group.Although Body Mass Index(BMI) is a criteria of MetS, it does not show the body's fat distribution.Abdominal perimeter and Bioelectrical Impedance Analysis(BIA) are promising methods that can be used in children to measure the increase in body's fat balance.It is not clear why all obese children don't have MetS what is the risky percentile of BMI for MetS.The aim of this study is to determine at which BMI percentile that MetS risk appears first and if there is a relationship between fat distrubion of the body and developing MetS.
Methods: In this study total 191 cases (7-18years old) were evaluated.Besides anthropometric measurements and pubertal evaluation, all of them were weighted with Tanita BC 418.The families of the children were ask to fill out a public survey for MetS' point of view and nutritional status, physical activity states.Patients were divided in to three groups according to their BMI percentile.Cases were evaluated according to MetS criteria(Cook S et al.)
Results:1. In this study MetS frequency in whole group was found 33.5% (n:191).

The frequency of patients with MetS according to their BMI percentile			
	BMI:85-90%(n:21)	BMI:90-95%(n:47)	BMI:over 95%(n:123)
Frequency of MetS (%)	14.3%(n:3)	23.4%(n:11)	40.7%(n:50)

2. According to BMI percentiles the frequency of the patients who had increased body fat proportion was given at table 2.

The distribution of the patients with increased body fat proportions according to their BMI precentile.			
	BMI:85-90%(n:21)	BMI:90-95%(n:47)	BMI:over 95%(n:123)
Frequency of the patients with increased body fat proportion (%)	19%(n:4)	38.3%(n:18)	74%(n:91)

3. Body mass index being over 95% increases the MetS risk 4.93 times (p:0.016)
4. According to Turkish Standards, in 28.7% of the cases who had a waist perimeter over 90% had also MetS.
5. The existence of acantosis nigrikans, history of unbalanced eating habits and inadequate physical activity are found to be risks for MetS.
Conclusion: We suggested that BMI measurements combined with BIA is useful method to determine MetS risk not only for obese but also overweight children. If BMI is used in the diagnosis of MetS without BIA, it will be more convenient to use 85% as a cut-off value, instead of above 95%.
Acknowledgment: We are thankfull to Prof.Dr.G.Öcal and Prof. Dr.M.Berberoglu for their necessary help during this research.

PO1-223 Obesity, Fat I

Generalizability of a family-based behavioral treatment of obesity: a pilot study

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Introduction: Family-based behavioral treatment (FBBT), developed by Epstein and colleagues, has been shown efficacious over a 25-year period[i]. Generalization across settings and populations is required for the treatment to be considered an applicable intervention in general settings.

Methods: Participants were 16 obese children and a parent participating with each child. The families were randomly assigned to receive 11 sessions of FBBT delivered over 16 weeks at two different times. Starting treatment right away or having a delayed treatment onset of 11 months during which the participants received standard care (a pediatric endocrinologist and a nutritionist consultation). Among the measures collected was height and weight at baseline, 16 weeks, 10 months and 15 months and BMI and BMI-SDS were calculated. Acceptability interviews and questionnaires were administered post treatment. Both groups included 8 obese children (BMI > 2.4 SDS), 2 boys and 6 girls and a participating parent with each child (7 mothers and 1 father in both groups). The groups both included a child with a diagnosis of ADHD, a child with a low IQ (75 and 76 according to WISC-III) and two children in each group who had emotional difficulties (peer problems, depression and anxiety). Children's mean age at treatment onset was 10.5 years.
Results: BMI-SDS remained constant for the eight children in the delayed treatment onset group from baseline until start of treatment at 11 months (mean BMI-SDS = 3.24 at baseline and 3.22 at 11 months, p = 0.88). Thirteen families completed 16 weeks of treatment during which the children lost an average of 2.9 kg from pre to post treatment (p < 0.001), and lowered their BMI-SDS by 0.33 points (p < 0.001). The children who received treatment first (n = 7) maintained their new BMI-SDS from post treatment to 1-year follow-up (2.95 vs. 2.92, respectively).
Conclusions: FBBT required only minor changes for implementation with a diverse group of children in a clinical setting and was well liked by participants and program staff. Results indicate that significant changes in BMI-SDS can be achieved with FBBT. Further testing of treatment effectiveness with two-year follow-up is underway in a larger sample of obese Icelandic children.
[i] Epstein, L. H et al. *Health Psychology, 26* (4), 381-391.

PO1-224 Obesity, Fat I

Prediabetes in Italian obese children and youngsters

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Childhood obesity is epidemic in certain European countries and is accompanied by an increase in prevalence of intermediate glucose metabolism alterations.
Objective Aim of our study was to define the metabolic abnormalities underlying prediabetic status of isolated impaired glucose tolerance (IGT), isolated impaired fasting glucose (IFG), and combined IFG/IGT in Italian obese children and youngsters.
Research Design and Methods We evaluated OGTT results (glucose and insulin) of obese subjects of 8 (girls) or 9 (boys) to 20 years of age (mean age and BMI-SDS 12.7±2.5 years and 2.2±0.3, respectively) and divided them in four groups according to the results: normal glucose tolerance (NGT), IGT, IFG and IFG/IGT. Subjects of the 4 groups were then matched for age, gender, BMI-SDS. HOMA-IR, hepatic insulin resistance, insulin sensitivity and insulinogenic index were calculated from OGTT measurements. Disposition index was defined as the product of insulinogenic and insulin sensitivity index (tab.1).

	NGT (n=178)	IGT (n=78)	IFG (n=11)	IFG/IGT (n=6)	P values (One-way ANOVA)
HOMA-IR (Matthew 1999)	3.3±2.6	4.2±2.3	6.3±4.2	6.2±3.0	<0.001
Hepatic insulin resistance (Abdul-Ghani 2007)	6.3±4.3	7.4±4.7	7.5±3.3	10.20±6.0	0.090
Muscle insulin sensitivity (Abdul-Ghani 2007)	0.7±0.9	0.4±0.5	0.6±0.4	0.3±0.3	<0.001
Insulinogenic index	2.0±1.3	2.0±2.2	2.6±2.0	1.7±1.6	0.331
Disposition index (Kahn 1993)	6.7±5.7	4.1±5.2	5.3±5.0	2.7±1.5	<0.001

Results Subjects with IFG and IFG/IGT showed the highest values of HOMA-IR, IFG/IGT subjects the highest hepatic insulin resistance index. Muscle insulin sensitivity was reduced in IGT and IFG/IGT. Insulinogenic index was similar among groups; however, disposition index of IFG/IGT showed the lowest values (tab.1).
Conclusions IGT in obese children and youngsters is linked to reduced muscle insulin sensitivity. Otherwise the IFG group appears to be affected by a more severe degree of hepatic insulin resistance. IFG/IGT is hallmarked by both reduced muscle insulin sensitivity and increased hepatic insulin resistance and by an additional defect in disposition index. Thus, combined IFG/IGT seems to produce the severest alterations in glucose handling and likely is the metabolic category preceding type 2 diabetes in young Italian obese individuals.

PO1-225 Obesity, Fat I

Obese children with low birth weight demonstrate increased prevalence of metabolic syndrome compared to obese children with high birth weight

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Objective Epidemiological studies have shown a U-shaped association among birth weight and future risk of metabolic syndrome (MS), with individuals born either small or large at increased risk. We sought to investigate the influence of birth weight on the development of MS in obese children.
Research design and Methods Children were enrolled in the present investigation if they were obese or overweight, Italians, born at a gestational week ≥36th. Exclusion criterion was maternal diabetes. Two hundred and twenty-three obese children aged 11.6±2.3 years were divided into 3 groups according to birth weight percentile: 36 were small (SGA) (<10th percentile), 143 appropriate (AGA) (25th-90th percentile) and 44 large (LGA) (>90th percentile) for gestational age. Participants underwent oral glucose tolerance test, lipids and blood pressure measurements. MS was defined as having 3 or more of following risk factors: obesity, impaired glucose tolerance, high blood pressure, low HDL-cholesterol, high triglycerides. One-way ANOVA and $\chi 2$ test were used to compare the 3 groups.
Results Mean birth weight, as expected, was significantly different between groups (SGA, AGA, LGA= 2.5±0.3, 3.4±0.3, 4.1±0.3 Kg respectively, P<0.001). BMI, BMI-SDS, corrected height for target height were not different (mean values in the whole group 30.1±4.4, 2.2±0.3, -0.7±1.0 respectively). HDL cholesterol, triglyceride, 2-h post load glucose, systolic and diastolic blood pressure were not different between groups (P= 0.36, 0.41, 0.06, 0.31, 0.87 respectively). When these cardiovascular risk factors were considered

together, higher prevalence of MS was observed in SGA, with LGA having the lowest percentage (MS prevalence in SGA, AGA and LGA= 25, 10, 7% respectively, P=0.021).
Conclusions Low birth weight negatively affects metabolic outcomes since childhood. Otherwise, high birth weight seems to protect children from MS development and to confer them a metabolically healthy obesity. Thus, any effort to avoid excessive weight gain of low birth individuals, since infancy, should be undertaken.

PO1-226 Obesity, Fat I

Double increase in the prevalence of obesity among children and adolescents in Banja Luka (Bosnia and Herzegovina) from 2004 to 2009

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Objective: Obesity has been on increase at an alarming rate in recent years, and it is considered to be a worldwide public health problem. The aim of this study was to compare the changes of the prevalence of overweight and obesity among children and adolescents in a city area in Bosnia. **Research design and methods:** Two comparable samples of, in total, 2168 children and adolescents (female 53.7 %), aged 6-17 years (mean 10.8, SD 3.0) were examined in 2004 and 2009. The international age- and gender-specific child BMI cut-points were used to define overweight (BMI corresponding to adult value of 25.0-29.9 kg/m²) and obesity (BMI ≥30kg/m²). Other measures taken recorded behaviors concerning TV, computer and physical activity. **Results:** The total prevalence of overweight and obesity was significantly higher (p<.01) in our subjects in 2009 where 19.7% were classified as overweight and 6.6% as obese compared with the 2004 generation where respective results were 12.8% and 3.3%. In the younger group (6-10yrs) the prevalence of overweight and obesity were 22.5%, and 9.7% while for adolescents (11-17yrs) they were 17.2% and 3.7%, respectively. There were no significant differences with regard to the gender. **Conclusion:** The prevalence of obesity and overweight increased significantly in the last five years, especially among the younger group (6-10yrs). The results suggest that more preventional activity is needed in the near future to combat the trend.

PO1-227 Obesity, Fat I

Cardiovascular risk factors in overweight children with family history (FH) for non transmissible chronic diseases

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In overweight Chilean children, the metabolic syndrome (MetS) affects to 25%, the hypertriglyceridemia to 33% and fasting hyperglycemia have a low prevalence (4%). **Objective**: To study the prevalence of MetS and cardiovascular risk factors (CVRF) in overweight children with a family background of non transmissible chronic diseases (NTCD) and to analyze its association with the number of relatives with NTCD. **Material and Methods**. In 185 children aged 7 to 14 years (87 males) with BMI ≥ 85 and a FH of NTCD, we assessed the BMI z (CDC / NCHS), waist perimeter, blood pressure and baseline levels of HDL cholesterol, triglycerides, glucose and insulin. The MetS and the CVRF were diagnosed using the Cook phenotype and the insulin resistance (IR) through the HOMA-IR according to a national reference. Chi^2 was used to look associations, ANOVA and Bonferroni to compare means. **Results:** The frequency of FH for DM2, hypertension and dyslipidemia were 80.5%, 87.0%, and 70.8% respectively. The 11.9% of children were slightly overweight (BMI z > 1.0 < 2.0) and only 19.5% were severely obese (BMI z ≥ 4.0); 41.4% children showed IR and 48.0% had the MetS. The hypertriglyceridemia was a high prevalence (54.6%), while fasting hyperglycemia affected 31.4% of the sample. There was no association between the number of relatives with NTCD and the cardiovascular risk profile. **Conclusion:** In overweight children with FH of NTCD, the prevalence of MetS, dyslipidemia and fasting hyperglycemia are significantly higher, than those observed in the general population of obese children. The importance of FH in the targeting of children with higher biological risk is discussed.

PO1-228 Obesity, Fat I

Healthy Buddies™: a peer-led, comprehensive health promotion program for elementary school students

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We designed Healthy Buddies (www.healthybuddies.ca, HB) as a comprehensive, school-based health education and health promotion program to empower school children to live healthier lives. The program focuses on promoting healthy attitudes and behaviours towards nutrition, physical activity, body image and key aspects of mental health from Kindergarten (K) to Grade (Gr) 7. It is based on a peer-led structure in which older buddies (Gr4-Gr7 students) help teach a younger student (K-Gr3). A randomized pilot study (2 schools using **study** teachers) demonstrated that HB improved knowledge in both older and younger buddies and decreased BMI and weight velocity in older students (S Stock, Pediatrics 2007;120:e1059). Hypothesis: large scale implementation of our program by **staff** teachers will confirm the results of the pilot study and demonstrate sustainability. Objective: To implement HB in elementary schools across British Columbia. Methods: 45 Schools were provided with all materials necessary to teach the 21-week Program (1 school year, Sep-Jun): The classroom and fitness loop bins contained lesson plans, DVD presentations, yoga resources, posters, overheads, student handouts and a variety of games. Program implementation was performed by regular school staff. Evaluation (n=1622 students) was performed in 6 schools at the beginning and end of the school year. For questionnaires only, 2 additional schools were used as controls (C). Measures: weight, BMI; questionnaires (knowledge, behaviour, habits and attitudes pertaining to health); survey (teacher and student satisfaction). Results: BMI was unchanged over 1 school-year in both K-Gr3 and Gr4-Gr7 students. For K-Gr 3 students, scores of knowledge, behaviour and healthy habits questionnaires improved (P<0.001) in HB schools but only behaviour changed in the C school (P=0.02). For Gr4-Gr7 students, scores of knowledge and healthy habits questionnaires improved in both HB and C schools, while behaviour improved in C schools only. In surveys, teachers (>76%), K-Gr3 (>76%) and Gr4-Gr7 students (>62%) had a positive opinion of the program's structure and impact on student health. Conclusions: We show that HB can be easily and successfully implemented by **in-serviced, staff** elementary school teachers, demonstrating sustainability. K-Gr3 buddies in particular showed marked benefit from the program, demonstrating the positive effects of peer-led teaching. Funded by a grant from the BC Provincial Health Services Authority.

PO1-229 Obesity, Fat I

The lipogenic effect of GHRP-6 is insulin dependent in streptozotocin-induced diabetic rats

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Poorly controlled diabetes results in a state of hypercatabolism due to the lack of insulin that results in weight loss, including loss of adipose tissue and muscle mass. In wasting diseases, growth hormone secretagogues, which exert their effects through ghrelin receptors, have been shown to stimulate food intake, improving weight gain, promoting adiposity and preventing skeletal muscle loss. Thus, our aim was to analyze the effects of GHRP-6 treatment on weight gain and the metabolic state of diabetic rats and to compare this with insulin treatment. Streptozotocin (70 mg/kg) was injected to adult male Wistar rats that were considered diabetic the day glycemia reached 300 mg/dl or high-

er. Diabetic rats were divided into four groups: those receiving saline, GHRP-6 (150 μg/kg/day), insulin (1-8 U/day) or insulin plus GHRP-6. Treatments were injected s.c. once daily for eight weeks. Control rats received saline for all treatments. Weight gain and glycemia were controlled daily and food intake weekly. Diabetic rats receiving saline or GHRP-6 alone suffered hyperglycemia, hyperphagia, polydipsia and weight loss throughout the study ($p<0.001$), whereas insulin significantly improved these parameters ($p<0.001$). Serum insulin, adiponectin and IGF-I levels were decreased in diabetic rats receiving saline or GHRP-6 ($p<0.001$) and insulin significantly increased these hormones ($p<0.001$). Diabetic rats treated with insulin plus GHRP-6 gained more weight than control rats and had increased epididymal fat and serum leptin levels ($p<0.001$). To understand the alterations occurring in adipose tissue, mRNA levels of factors involved in lipogenesis and lipolysis were measured by real time PCR in epididymal fat. Diabetes down-regulated fatty acid synthase (FAS) and up-regulated hormone sensitive lipase (HSL) and proliferator-activated receptor-γ (PPAR-γ) mRNA levels ($p<0.001$, $p<0.001$ and $p<0.01$, consecutively) and insulin returned all three to control levels. GHRP-6 alone had no significant effect compared to diabetic rats treated with saline. However, treatment with GHRP-6 potentiated insulin's effect on FAS ($P<0.001$) and HSL. In conclusion, GHRP-6 treatment to diabetic rats does not prevent diabetes-induced body weight loss, but in combination with insulin treatment promotes adiposity through increased lipogenesis of visceral fat mass.

PO1-230 Obesity, Fat I

Obesity accentuates the age-associated increase in arterial stiffness during adolescence

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Obesity and increased large artery stiffness are risk factors for cardiovascular disease. In adults, arterial stiffness increases with age and obesity. Limited studies have explored these associations in adolescence.
Aims: To determine the relationship between age and arterial stiffness in obese and control adolescents (12-18yrs). The influence of blood cardiovascular risk markers was assessed.
Methods: 20 obese (14F) and 36 normal-weight (21F) adolescents were recruited. Large artery stiffness was determined by carotid-dorsalis pedis pulse wave velocity (PWV) using a SphygmoCor Vx PWV System. Fasting assessment included insulin resistance (HOMA-IR), lipids, free fatty acids, inflammatory markers (interleukin [IL]-6), tumour necrosis factor-α, c-reactive protein [CRP]), plasma free 8-isoprostane (marker of oxidative stress) and superoxide dismutase (SOD), an erythrocyte antioxidant enzyme. Brachial mean arterial blood pressure (MAP) was determined by an automated monitor (3 readings).
Results: There were no significant differences in age, height or pubertal stage between the groups. PWV was significantly higher (increased arterial stiffness) in the obese compared to the control group (mean, 95%CI; 6.6, 6.3-7.0 and 6.2, 6.0-6.4m/s). Age was significantly associated with PWV in the obese group ($r=0.53, P=0.017$), but not the controls ($r=0.16, P=0.34$) and there was a significant difference between *r values* ($z=2.11, P=0.045$). The obese group had significantly higher heart rate (HR), MAP, HOMA-IR, total cholesterol, triglycerides (Tg), IL-6, CRP and SOD, with significantly lower HDL ($P<0.05$ for all). In the obese group, there was a trend for a positive association between PWV and Tg ($r=0.43, P=0.054$), but not in controls ($r=0.025, P=0.89$). In the obese group, SOD was significantly correlated with PWV ($r=-0.46, P=0.04$), but this was not observed in controls ($r=0.18, P=0.28$). In multivariate analysis (all subjects), age, MAP and HR were independent predictors of PWV ($R^2=0.50$), whereas BMI z-score, gender and blood biochemistry were not independently associated.
Conclusions: Obesity in adolescence is associated with increased arterial stiffness that is accelerated with age compared to normal-weight controls. The association between SOD and PWV in the obese adolescents suggests that the superoxide radical may be involved in the mechanism. These associations need further assessment in larger cohorts of obese adolescents.

PO1-231 Obesity, Fat I

Sedentary behaviour is associated with increased metabolic risk in young children

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Aims: Paralleling the increasing prevalence of childhood obesity, there has been a significant increase in the incidence of type 2 diabetes in children and adolescents in Australia. Given the increasing opportunities for children to be sedentary in their leisure time, the purpose of this study is to examine the influence of sedentary activity, as represented by screen-based behaviours, on risk factors for Type 2 diabetes in young Australian children.
Methods: The sample included 75 normal weight children, 49 overweight children and 18 obese children who are participants in a cohort study of Australian school children (n=142, 9.7±1.8 yrs). Children had a medical assessment which included a physical examination, fasting insulin and glucose levels. An oral glucose tolerance test (OGTT) was performed in a subgroup of study participants (n=82). BMI was determined, overweight and obesity were defined by international cut-offs (Cole et al), and BMI z-scores calculated. Children's physical activity and screen-based behaviours were assessed using a validated parent-proxy report questionnaire.
Results: Time spent in physical and sedentary activity was not associated with child BMI z-score. Screen-based behaviours were positively associated with levels of fasting insulin ($p<0.05$), C-peptide ($p<0.05$), and insulin resistance (HOMA-IR; $p<0.05$), independent of physical activity levels, waist circumference and BMI z-score. In a series of univariate models, increasing time spent in screen-based behaviours was predictive of higher fasting insulin concentration and greater insulin resistance (both $p<0.01$). **Conclusions:** Our findings highlight the associations between sedentary behaviour and risk factors for Type 2 diabetes, even in young children. The relationship between sedentary activity and metabolic risk was present independent of both adiposity and physical activity levels. These findings support recent research in adults and suggest that even in young children, prolonged sedentary time appears to deleteriously influence health and may be a potent contributor to diabetes development. Activity guidelines for children should not only highlight the importance of regular physical activity, but should also emphasise the importance of limiting time spent in sedentary activities.

PO1-232 Obesity, Fat I

The association of family history and BMI Z-score with obesity related complications in primary school aged children

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In Australia overweight/obesity during childhood is increasing. Obesity related complications are present in treatment seeking primary school aged children. Less is known about the prevalence of these complications in community non-treatment seeking children. By using body mass index (BMI) z-score as a measure of relative weight, we have calculated the prevalence of obesity complications in primary school children. The associations between parental obesity related diseases and child's BMI z-score has also been examined.
This study is part of a larger, prospective cohort Growth and Development Study. Children were recruited through randomly selected primary schools. A medical history including a detailed family history was taken. Anthropometric measurements and a physical examination were performed. Investigations included oral glucose tolerance test (OGTT), fasting lipid profile, liver function tests and body composition by DXA. CDC BMI z-scores were calculated for each child.
251 children, aged 6-12 yrs, were recruited: 19 obese, 77 overweight and 155 healthy, age and sex matched controls. The reported prevalence of obesity

related disease symptoms were high in the obese children. With 33% reporting symptoms of obstructive sleep apnoea 28% musculoskeletal pain and 33% described being either bullied or teased. Components of the metabolic were present in the obese children, fasting insulin was elevated in 50%, 46% had abnormal lipid profiles and 10% were hypertensive.
An increasing BMI z-score was significantly related to higher fasting triglycerides, ALT and insulin during OGTT and a lower HDL cholesterol level.
Increasing maternal BMI was associated with increasing BMI z-score in the children studied. Independent of the child's BMI z-score liver enzyme levels in children of mothers who reported having metabolic syndrome were significantly higher. Children of mothers who reported having hypertension or high cholesterol had higher total cholesterol and LDL cholesterol levels
In conclusion obesity complications are seen in obese community primary school aged children. Methods to identify community children at risk of complications need to be improved. Increasing BMI z-score is associated with an increasing risk of obesity co-morbidities. Parental medical history is an essential part of the initial and ongoing medical history when assessing a child's risk of disease.

PO1-233 Obesity, Fat I

Role of es-RAGE, s-RAGE and insulin resistance in liver steatosis in obese pre-pubertal children

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The AGE-RAGE pathway has been recently implicated in the pathogenesis of several pathological conditions, including insulin resistance (IR) and liver injury. Recently, the endogenous soluble-RAGE (es-RAGE) and soluble-RAGE (s-RAGE) have been shown in human plasma and have emerged as reliable biomarkers in a number of RAGE-mediated disorders. The aim of the study was to evaluate both es-RAGE and s-RAGE levels in obese pre-pubertal children with and without liver steatosis.
A large group of 100 obese pre-pubertal children was recruited. Anthropometric measurements, an oral glucose tolerance test (OGTT), es-RAGE and s-RAGE levels, transaminase values and a hepatic ultrasound scan were performed in all subjects. HOMA-IR and WBISI were used as indexes of IR. According to the ultrasound presence or not of liver steatosis the children were divided into group 1 (52 subjects; mean age 8.95±1.69 years) and group 2 (48 subjects; mean age 8.09±2.01 years), respectively.
s-RAGE (1013.43±274.60 vs 1361.52±553.60 pg/ml; p=0.02) and es-RAGE levels (0.75±0.46 vs 1.09±0.62 ng/ml; p=0.03) were significantly lower in group 1 than in group 2. HOMA-IR was significantly higher (4.01±2.91 vs 2.26±1.20; p=0.0001) while WBISI (3.72±1.97 vs 6.95±3.84; p=0.0004) was significantly lower in group 1 compared to group 2. Furthermore, in a multiple linear regression analysis, es-RAGE and HOMA-IR were independently related to liver steatosis (β=-2.667, p=0.01 and β=0.369, p=0.003, respectively).
Decreased s-RAGE and es-RAGE levels have been shown in obese pre-pubertal children affected by liver steatosis. The relationship between s-RAGE and liver steatosis suggests an independent role of AGE-RAGE pathway in the development of liver injury, already in pre-pubertal obese children.

PO1-234 Obesity, Fat I

Do young overweight type 1 diabetics show statistically significant difference in serum lipid levels as compared to their non-overweight type 1 diabetic counterparts?

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Background and Significance
Bogalusa Heart analyses have demonstrated that abnormal serum lipid levels have an additive correlational with atherosclerotic lesion severity. Elevated BMI (Body Mass Index) has also been identified as independently increasing atherosclerotic lesion severity in young people. There is currently an increased prevalence and incidence in pediatric obesity. We are interested in the effect of elevated BMI on serum lipid levels in children with Type 1 Diabetes Mellitus (T1DM).
Research Design
We hypothesized that overweight subject with T1DM have statistically significant increased serum lipid levels as compared to their non-overweight counterparts. We compared fasting serum LDL, HDL, and triglycerides in T1DM age 2-18 with BMI 85% or more for sex and age compared to T1DM age 2-18 with less than 85%. Subgroup analyses are also conducted by age (<10 and ≥ 10) and gender (male and female). We excluded any child on steroid therapy, retinoid therapy, protease inhibitor therapy, with a diagnosis of Cushing's syndrome, with familial hypercholesterolemia syndrome.
Results
Overall, data was entered to the database for 53 type 1 diabetes patients with 21 boys and 32 girls. Average overall age was 12.7 years.

Lipid profile by BMI percentile

	<85% BMI	≥85% BMI	Overall	p-value
	n=28	n=25	n=53	
Age (years)	12.8 (4.0)	12.6 (4.0)	12.7 (4.0)	0.868
Trig (mg/dL)	72.9 (31.2)	100.9 (54.5)	85.7 (45.3)	0.031
HDL (mg/dL)	56.0 (12.6)	55.0 (12.6)	55.5 (12.5)	0.785
LDL (mg/dL)	83.8 (27.5)	106.4 (36.1)	94.4 (33.5)	0.015
HbA1c %	9.3 (1.7)	9.2 (2.0)	9.2 (2.0)	0.829

Trig= triglycerides

Table 1 demonstrates mean values for fasting lipid profile and Hba1c at the time of the profile. There was a statistically significant difference in triglycerides and LDL in subjects with BMI 85% and more copmpared with those with BMI less than the 85%. Subgroup analysis demonstrated significant statistical difference of mean triglycerides, HDL, LDL in girls aged 10 years and over only. Indeed, there was no difference in lipid profiles in boys or girls less than 10 years of age.
Conclusion
BMI 85% and above does reveal significantly higher fasting serum lipid levels in girls age 10 years and over with T1DM. Sample size maybe too small to reveal a difference in boys. Further investigation including other cardiovascular risk factors is warranted in this population.

PO1-235 Obesity, Fat I

Quality of life (QOL) in survivors of bone marrow transplantation (BMT) with total body irradiation (TBI) in childhood treated with growth hormone (GH): relationship with body composition and fitness

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Introduction
BMT survivors can have growth hormone deficiency (GHD), adiposity and fatigue. GHD and cancer survivorship both affect QOL. We aimed to look at QOL using two generic (SF-36 and PEDSQL) and one specific (MMQLYF29) questionnaire and relate the findings to body composition and physical fitness. These are baseline data of an exercise intervention study.
Subjects 25 BMT survivors (14 male) enrolled. All had GHD treated with GH for at least 6 months prior to study. Mean(standard deviation) age and time since BMT were 16.8(4.1)yrs and 8.4(3.8)yrs respectively. 3 subjects were pre-pubertal, 8 pubertal, and 14 post pubertal.
Methods MMQLYF29 was completed by the young person if less than 21 years. PEDSQL child and parent versions were completed for under 18s. All subjects completed the SF36 with parental help if necessary. Body composition was measured by DEXA scan, and aerobic fitness by VO2 peak on inclined treadmill test.
Results The SF-36 showed similar scores to adult normal data. There was a trend for the physical score to be lower but this was not significant (86 (13) vs95(17)). Age was negatively (-vely) correlated with physical, energy, emotional, social and total scores, all p<0.05. The MMQLYF29 (N=19) showed scores similar to reported normal data except for the physical domain, which again showed a trend to lower values in the survivors (70(18)vs82(12)). The

physical, emotional and appearance scores all correlated -vely with fatness and thinness, and % body fat, p<0.05. The physical scores correlated -vely with BMISDS, and fitness, all p<0.05. The PEDSQL (N=13) child reports showed normal scores. The parent reports (N=11) showed a trend to score lower than children in all domains as reported in the literature. Social scores correlated -vely with time from BMT, p=0.023

Discussion QOL scores in BMT survivors were generally high as reported previously in cancer survivors. Although QOL scores may be increased by repressive coping mechanisms, survivorship may improve outlook as it leads to a higher appreciation of being alive. A number of domains became more impaired with increasing age. Older survivors may have more self-knowledge and struggle to meet increasing physical and emotional demands.The MMQLYF29 is the most appropriate questionnaire for this group as it includes appearance. These results imply impaired QOL related to appearance and physical domains (associated with reduced fitness and abnormal body composition) despite GH treatment.

PO1-236 Obesity, Fat I

Impairment of adiponectin system in children and adolescents with metabolic syndrome

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Subjects with the metabolic syndrome (MS) have a dysfunctional adipose tissue microenvironment that overproduces IL-6 and underproduces adiponectin – a cytokine that is secreted exclusively by adipocytes. Several studies analyzed the rule of adiponectin system in the MS phenotype, with non univocal conclusions. The majority of studies dealt with heterogeneous cohorts, in terms of age (adolescents and/or adults) and, specially, ethnicity.

To investigate the adiponectin pattern in a cohort of Caucasian obese children and adolescents we studied 85 obese subjects with (n = 31) and without (n = 51) MS. 15 non obese age-matched subjects were also studied.

Anthropometric and metabolic characteristics are resumed in table 1.

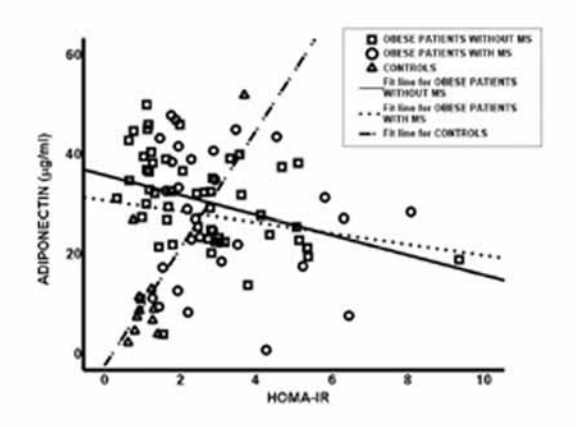
Anthropometric and metabolic characteristics of the entire cohort

	Obese children with MS	Obese children wihtout MS	Controls
Male/Female	15/16	28/23	12/8
Mean age (years)	9.8 (9.1-10.6)	9.4 (8.8-10.1)	9.6 (8.9-10.2)
Prepubertal/pubertal	20/11	35/16	13/7
z-score BMI	4.37 (4.03-4.71)a	4.20 (3.84-4.57)a	1.03 (0.56-1.51)
HOMA-IR	3.06 (2.43-3.69)a	2.51 (2.02-2.98)a	1.39 (0.79-1.99)
Adiponectin µg/ml	25.6 (22.6-28.6)ab	30.8 (28.1-33.5)a	12.6 (6.1-19.1)

a: significantly different compared to normal weight children and adolescents.
b: significantly different compared to obese children and adolescents without metabolic syndrome

As expected, in non-obese subjects, adiponectin shows a positive correlation with insulin-resistance, identified by HOMA-index (p = 0.002), confirming a possible protective action against the damages induced by insulin-resistance in MS.

An inverse correlation is observed in obese patients without metabolic syndrome (p = 0.01), but not in patients with metabolic syndrome (p = n.s.), evidencing a significant upset of the expected pattern.



Our data confirm that youths with metabolic syndrome harbour lower circulating adiponectin levels than subjects without metabolic syndrome and controls. Adiponectin could be considered as a potential screening tool for adverse health outcomes in metabolic syndrome.

PO1-237 Obesity, Fat I

Decreased level of soluble receptor for advanced glycation end-products (s-RAGE) is an independent risk factor for cIMT in obese pre-pubertal children

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Advanced glycation end-products (AGEs) and the receptor for AGEs system (es-RAGE and s-RAGE) plays an important role in the onset and progression of atherosclerosis in adult subjects.

AGE engagement of RAGE results in cellular signaling including activation of nuclear factor-B, increased expression of cytokines and adhesion molecules, and induction of oxidative stress. Both reduced es-RAGE and s-RAGE are tightly related to the risk of carotid atherosclerosis (cIMT). Therefore, we tested whether impaired es-RAGE and s-RAGE concentrations are related to increased cIMT in obese pre-pubertal children.

In 44 obese pre-pubertal children (20M/24F, mean age 7.9±1.5 yrs), anthropometric measurements, inflammatory markers (hs-CRP and PGF-2α), es-RAGE and s-RAGE, were evaluated and compared with 41 healthy gender, age and pubertal stage matched subjects (21M/20F, mean age 7±2 yrs). OGTT was performed and insulin resistance (IR) indexes (HOMA-IR, WBISI) were calculated in all patients. High resolution ultrasound techniques was used to evaluate cIMT.

Obese children had lower levels of es-RAGE and s-RAGE compared to healthy subjects (p=0.009 and p=0.001). Fasting insulin levels and HOMA-IR were higher (p=0.003 and p=0.001) while WBISI lower (p=0.006) in obese children than controls. Furthermore, compared to healthy subjects obese children showed increased levels of PGF-2α and hs-CRP (p=0.001 and p=0.007).

In addition, obese children had an increased cIMT (p=0.001). A significant correlations between cIMT and PGF-2α (β=0.341, p=0.003), between cIMT and s-RAGE (β= -0.230, p=0.030), between cIMT and HOMA-IR (β= 0.206, p=0.048) were detected by multiple stepwise linear regression analysis.

In conclusion, the receptor for AGEs system markers are reduced in obese pre-pubertal children and represent an independent risk factor of cIMT, already during pre-puberty.

PO1-238 Obesity, Fat I

Periodontal disease in obese children

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INTRODUCTION
Recently it has been suggested that the fat distribution pattern could play a crucial role in periodontal disease. Cytokines and hormones producted by fat tissue may modulate periodontitis.
The aim of this study was to evaluate the relationship between obesity, abdominal adiposity in particular, and periodontitis.
METHODS
Body mass index (BMI) and waist circumference (WC) were measured for each patient. All patients underwent a periodontal examination based on recording both soft debris and mineralized deposits on the following teeth: the first and second molars and two incisors, upper right and lower left central incisors. Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) was probed for each subject. Oral hygiene was assessed by self-reported questionnaire.
All subjects completed a multidimensional self concept scale questionnaire, which assesses global self-esteem and six specific domains of self-esteem (social, competence, affect, academic, family and physical).
RESULTS
We enrolled 64 italian healthy subjects, 34 females and 30 males, aged 10 to 18 years, 32 obese/overweight subjects and 32 normal weight controls. There were no differences in mean age and sex distribution betweeen the two groups. In obese subjects, 10 males and 10 females, mean BMI was 30.59. Fifteen of them (75%) have abdominal obesity, with a mean BMI 31,60.
Obese subjects had significantly higher plaque index (1.09±0.31 vs 0.79±0.48) and gingival index (1.26±0.40 vs 0.76±0.50) than normal weight ones (p 0.005; p 0.001); no differences were found between abdominal obesity and obesity. Gingival index in abdominal obese subjects was significantly higher (1.25±0.42 vs 0.85±0.48; p=0.005) than controls.
Oral hygiene didn't differ among groups.
Obese children had significantly lower self-esteem in the competence (p=0.039), affect (p=0.002), academic (p=0.000), family (p=0.000) and physical (p=0.000) domain than normal weight ones. Abdominal obese subjects had lower self-esteem in the physical domain than obese ones (p=0.05). Adjustment for confounding factors, including sex and age, confirmed such results.
CONCLUSION
These observations suggest a potential interaction between obesity and periodontitis. Obesity impacts also the self-perception of children entering adolescence, placing some children at increased risk of having lower self-perceptions in some, but not all domains.

PO1-239 Obesity, Fat I

Influence of Gln223Arg polymorphism of the leptin receptor gene on obesity in Turkish children

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Obesity is a growing public health problem all over the world. Several genes play a role in obese phenotypes. Among these genes, the leptin receptor (LEPR) gene, encoding leptin receptor was extensively studied for variants that could explain the obese phenotype. Polymorphisms of LEPR gene may contribute to a common form of obesity and, as a consequence, obesity-related diseases. The aim of our study was to investigate the association between LEPR Gln223Arg polymorphism and obesity in Turkish children

Material and methods: In this study, 92 obese patients (44 males, 48 females, body mass index, BMI,>95%) were evaluated, together with 99 lean or normal healthy individuals (57 males, 42 females, BMI <85%). In each child after 10 hour overnight fast, glucose, insulin, leptin and lipids: triglycerides (Tg), cholesterol total, cholesterol HDL (HDL-C), cholesterol LDL (LDL-C) were measured. The LEPR Gln223Arg polymorphism was analyzed by restriction fragment length polymorphism-PCR.
Results: There was a significant difference between the groups by the anthropometric parameters, glucose, insulin, Tg, LDL-C, HDL-C and leptin levels ($p<0.05$). Serum leptin levels were significantly higher in obese children than in the control group (15.0±6.7 ng/mL vs 2.7±2.0 ng/mL). No association was found between LEPR Gln223Arg polymorphism and serum leptin, insulin and lipid levels. There was no difference in the genotype frequencies for Gln223Arg polymorphisms between obese and non-obese subjects ($p>0.05$).
Conclusion: Gln223Arg polymorphism of the LEPR gene does not appear to be associated with obesity in Turkish children.

PO1-240 Obesity, Fat I

Subclinical hypothyroidism in obese children and adolescents: innocent bystander or early risk marker of dyslipidemia?

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Background: Subclinical hypothyroidism (SCH) is defined as elevated thyrotropin (TSH) concentrations with normal circulating levels of triiodothyronine (T3), and thyroxine (T4). Elevated thyrotropin levels are a frequent finding in obese children. Recent epidemiologic evidence suggests an association of SCH with dyslipidemia and cardiovascular disease in adult populations.
Objectives: To investigate the association of SCH with dyslipidemia in a large cohort of obese children.
Methods: Cross-sectional study in 462 obese children (239 girls, mean age 13.9 ±2.0 years, mean BMI z-score 2.6 ±0.5) with normal or elevated TSH (mean 2.76 ±1.4 mIU/l, range 0.6-11.5) and normal T3 (mean 1.66 ±0.34 nmol/l, range 0.9-3.3) and T4 (mean 8.14 ±1.34 µg/dl, range 5.1-11.48), who underwent cardiovascular risk factor profiling.
Results: 392 children (84.9%) were classified as euthyroid having normal T3 and T4 levels and a TSH below 4.0 mIU/l, 70 subjects (15.1%) were identified with SCH. 56.8% of the study participants were affected by dyslipidemia, comprising hypercholesterolemia (total cholesterol >95th percentile, 37.9%), elevated LDL cholesterol (>130 mg/dl, 39.1%), low HDL cholesterol (<35 mg/dl, 9.9%), or hypertriglyceridemia (>130 mg/dl, 25.7%). After regrouping the study population in quartiles for TSH specific for age, gender and BMI z-score, ANCOVA analysis (adjusted for HOMA-IR and pubertal stage) revealed significantly higher levels of total cholesterol (182.9 ±32.5 mg/dl vs. 197.2 ±38.8 mg/dl, p 0.001) and triglycerides (102.4 ±60.7 mg/dl vs. 119.0 ±55.3 mg/dl, p= 0.0154) in the highest TSH quartile compared to the lowest TSH quartile. There was also a trend for higher LDL cholesterol (117.6 ±27.0 mg/dl vs. 126.0 mg/dl, p= 0.051) in the highest TSH quartile. Logistic regression analysis adjusted for age, gender, BMI z-score, pubertal stage and HOMA-IR, demonstrated that a one unit increase in TSH yielded a 22% increase in risk for having hypercholesterolemia (odds ratio OR 1.224, p= 0.0064), a 24% risk increase for hypertriglyceridemia (OR 1.239, p= 0.01), and overall increased risk for any form of dyslipidemia of 18% (OR 1.182, p= 0.032).
Conclusions: SCH in obese children is associated with an increased risk for hypercholesterolemia and elevated triglycerides independent of age, gender, stage of pubertal development, BMI z-score and degree of insulin resistance. Notably, this association is present over the whole range from normal to elevated TSH levels.

PO1-241 Obesity, Fat I

Long-term effects of a non-intensive weight management program in obese children's features of the metabolic syndrome

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Introduction: Since obesity and insulin resistance are associated with an increased risk for type 2 diabetes and cardiovascular diseases, great efforts have been made to prevent/treat these conditions since childhood. While a number of short-term studies have demonstrated the positive effects of intensive weight-loss programs, little is known on the long-term effects of a non-intensive, conventional, weight management program on obese children.
Methods: Chart review of obese (BMI > 95th percentile) non-diabetic children followed at the Weight Management Center of the Section of Endocrinology and Diabetes at St. Christopher's Hospital for Children. Children were evaluated 3-4 times/year. During the visits, age-appropriate caloric intake and regular physical activity were recommended. Fasting glucose and insulin (or an OGTT) and lipid panel at the first and last visits of the follow-up period were obtained.
Results: 54 children (21 males) were included in the study. At the initial visit, their age was 10.7 ± 2.9 yrs (mean ± SD) and their BMI z-score 2.5 ± 0.5. 4 children had impaired glucose tolerance (IGT). At the end of the follow-up (43.8 ± 17.3 months), 3 of the 4 children's OGTT normalized (none of the 3 was treated with metformin), while the 4th child had persistent IGT. In addition, the population sample's BMI Z-score (last vs. first visit, $p<0.05$), 120- min glucose (by OGTT, $p<0.01$), peak insulin ($p<0.01$), and LDL cholesterol ($p<0.05$) were all decreased. In contrast, there was no change in fasting glucose, HOMA, triglycerides or HDL-cholesterol. Among children who experienced a decrease in HOMA (initial HOMA > last HOMA, $p<0.001$), we found reduced fasting ($p<0.05$) and 120-min glucose ($p<0.001$), fasting ($p<0.001$) and peak insulin ($p<0.001$), triglycerides ($p<0.05$), and total cholesterol ($p<0.05$), with no change in HDL- or LDL-cholesterol. Although this subgroup's BMI z score was lower (last vs. first visit, 2.15 vs 2.41) the difference did not reach statistical significance ($p=0.051$). In the subgroup of children who experienced a decrease in BMI z score (initial BMI Z score > last BMI Z score, $p<0.01$), the last-visit 120 min glucose ($p<0.01$), and peak insulin ($p<0.001$) were lower than the corresponding initial visit's values.
Conclusions: Our findings indicate that a non-intensive weight-management strategy may have long-term, beneficial effects on children's features of the metabolic syndrome, not necessarily related to a significant weight loss.

PO1-242 Obesity, Fat I

Insulin resistance is a better predictor of the presence of non-alcoholic fatty liver disease in obese children than parameters of body composition (DXA, waist-to-hip-ratio)

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Background: Non-alcoholic fatty liver disease (NAFLD) is a severe complication of childhood obesity because it has the potential to progress to steatohepatitis, fibrosis and even cirrhosis. Only few data are available on this topic in children.
Aim: To investigate the relationship between NAFLD and fat / muscle mass (DXA), waist-to-hip-ratio (WHR), insulin sensitivity, liver enzymes and fasting lipids.
Methods: The study-group consisted of 96 obese children (48 female) presenting consecutively to our obesity outpatient clinic. All patients were investigated by a standardized program. NAFLD was diagnosed by ultrasound examination (hyperechoic liver). Fat and muscle mass were assessed using DXA (LPX-LUNAR, GE-medical Corp.). Insulin resistance was defined as HOMA-IR>P95. Results [mean±SD]: Age [years] (12.6 ± 3.1), BMI [kg/m2] (31.4 ± 5.3), and pubertal stage did not differ between patients with (n = 23, 24 %) and without (n = 73, 76 %) NAFLD. Patients with NAFLD had higher values of HOMA-IR-SDS (5.3 ± 4.3 vs. 3.0 ± 2.6, p = 0.03), ALT [U/L] (73.6 ± 48.3 vs. 30.2 ± 15.1, p < 0.01), and gGT [U/L] (33.3 ± 20.3 vs. 18.7 ± 8.8, p = 0.003) and similar values of AST, triglycerides, total cholesterol, HDL and LDL cholesterol ($p \geq 0.05$). There was no difference in mean fat mass [%]-SDS (NAFLD: 2.4 ± 0.6 vs. without NAFLD: 2.3 ± 0.6, p = 0.75) and in mean muscle mass-SDS (NAFLD: 0.4 ± 1.3 vs. without NAFLD: 0.5 ± 1.60, p = 0.88). The mean WHR-SDS of patients with NAFLD (3.2 ± 1.6) did not differ significantly from the WHR of patients without NAFLD (3.5 ± 2.5, p = 0.3).
Conclusions: Our findings show that insulin resistance is a better predictor of the presence of NAFLD in obese children than lipid values or the absolute amount of fat / muscle mass (DXA) or WHR. This suggests that genetic factors play a major role in the pathogenesis of NAFLD.

PO1-243 Obesity, Fat I

Doppler waveform in hepatic vein and B-mode ultrasonography identify hepatic steatosis in healthy pubertal obese children; differences in metabolic findings and adiponectin levels

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Aims: The aims of our study were: (1) to determine the prevalence of asymptomatic hepatic steatosis with both Doppler and B-mode ultrasound (US) in pubertal obese children; (2) to investigate differences among degree of obesity, lipid profile and serum adiponectin levels in identifying hepatosteatosis of pubertal obese children by two different methods.
Subjects and Methods: Fifty-nine pubertal obese children with a mean age of 11.7 ± 3.3 years and 41 non-obese healthy children as aged matched control were included in this study. B-mode and right Hepatic vein (HV) Doppler ultrasonography (US) were performed to all children. Anthropometric indices, lipid profiles, and adiponectin levels were measured in all children. Obesity was defined as ≥ 85th percentile of Body Mass Index (BMI) for age and gender.
Results: We detected hepatosteatosis 50.85% of pubertal obese children by B- mode US, and 35,6 % of those by HV Doppler US. BMI was significantly higher in obese children with fatty liver (FL) detected by both two methods compared to obese children without FL ($p<0.001$). Adiponectin levels were not different between obese children with FL and those without FL by two methods ($p<0.05$). HDL-chol levels were lower in obese children with fatty liver (FL) detected by HV Doppler US compare to children without FL ($p<0.05$). HDL-chol levels were inversely correlated moderately with both B-mode and HV Doppler US (r=-0.285, p=0.004 and r=-0.328, p=0.001 respectively) and adiponectin levels was only correlated with detection of fat deposition in liver by B-mode US (r=-0,263, p=0.008). In multiple regression analysis of factor associated with FL, only BMI in all children by both two methods (B:0.0310, t: 4.290, $p<0.001$ and B:0.0582, t: 9.14, $p<0.001$). HDL-chol were the most significant factor affecting hepatosteatosis identified HV Doppler US in obese children (B:-0.0125, t: -2.27, p=0.027).
Conclusion: When compared with right HV Doppler, frequency of detection of fatty liver is higher in B-mode US. High BMI increases possibility of detecting fat deposition using B-mode US in all children. High BMI and low HDL-chol are associated with increased probability of detecting hepatosteatosis using HV Doppler US in all children. Low HDL-chol levels in obese children is an important indicator for presence of fatty liver shown by HV doppler US.

PO1-244 Obesity, Fat I

Analysis of 23bp insertion in the endothelian protein C receptor (EPCR) gene in subjects with Prader-Willi syndrome and nonsyndromic obesity

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Endothelian Protein C Receptor (EPCR) is a type I transmembrane protein, highly expressed on the endothelium of large vessels, required for efficient activation of protein C by thrombin and for multiple protective actions of activated protein C (APC) as well as anti-inflammatory and anti-thrombotic activities. The 23 bp insertion in the sequence of EPCR gene leads to the formation of a truncated receptor lacking the extracellular and transmembrane domains and, thus, unable to sustain protein C activation. This mutation has been previously reported in patients with venous thromboembolism and myocardial stroke, but to date, no conclusive evidence exist as to its role as risk factor for either disease.
High levels of C-reactive protein (CRP) have been found in subjects with Prader-Willi syndrome (PWS) and with nonsyndromic obesity. Increased levels of CPR are associated with cardiovascular disease suggesting that subjects with PWS as well as obese are at a similar increased risk.
The aim of our study was to analyze the 23bp insertion of the EPCR gene in a cohort of PWS and obese subjects to evaluate the prevalence of this mutation in subjects with syndromic and non-syndromic obesity, and to evaluate the predisposition to development of thrombotic events.
After informed consent, we obtained DNA from 133 subjects, 93 PWS (aged > 16 yrs) and 40 obese children (mean age 10.5 $\pm$ 2.9 years). Obesity was defined as BMI exceeding the 95th percentile for sex and age according to reference values, and the deviation from the mean reference value was evaluated by calculating the standard deviation scores (z-scores).
The 23bp insertion in the EPCR gene was analyzed by both PCR and Electrophoretic Analysis.
Our results showed that 1/40 (2.5 %) of obese subjects was heterozygous for the 23bp insertion.
This mutation was never found in the group of PWS subjects.
We conclude that the distribution of 23bp EPCR insertion is rather low in nonsyndromic obese subjects and is absent in PWS subjects.In conclusion, this study demonstrate the absence of the EPCR insertion in our group of PWS subjects which not show a predisposition to the development of thrombotic events.

PO1-245 Obesity, Fat I

IGF2 gene variants and risk of hypertension in obese children and adolescents

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Obese children have a greater risk of high systolic and diastolic blood pressure than non-obese children and hypertension in childhood represents the major risk factor for cardiovascular morbidity in adults. The Insulin-like Growth Factor type II (IGF-II) has a role in regulating glucose homeostasis, cardiovascular functions and lipid metabolism. *IGF2* gene variants have shown a strong association with body weight, body mass index (BMI) and metabolic profile in adult men.
We performed the molecular screening of two *IGF2* single nucleotide polymorphisms (SNPs), the 6815 A/T in the P1 promoter region and the 820 G/A (ApaI) in the untranslated region (3'UTR) of exon 9, in a population of two hundred and twenty seven obese children and adolescents in order to evaluate the potential association between *IGF2* gene variants with either obesity or high blood pressure (assessed with a 24-h holter system) or both.
We observed a significant association between the 6815 A/T *IGF2* gene variant and high systolic blood pressure, assessed with a 24-h holter system, in obese children and adolescents. In particular, obese subjects homozygotes for the T6815 allele showed, even in 24-h measurements, a higher risk to develop hypertension than those carrying the A6815 allele (OR=3.7, 95% CI: 1.59- 8.66). By contrast, we did not observe any statistically significant difference in terms of BMI between the genotype groups.
Our results suggest that *IGF2* gene variants are involved in the blood pressure regulation in obese children and adolescents.

PO1-246 Obesity, Fat I

Are ethnic differences in body composition related to participation in physical activity?

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Introduction: Lean, healthy South Asian, (SA) adolescents have previously been shown to have increased levels of body fat, (BF), more adverse lipid profiles and more insulin resistance than their white UK peers (1). In addition cardiovascular fitness has declined in lean SA children over the past 10-15 years (2). However ethnic differences in body composition and physical activity in obese children have not been investigated. Our hypothesis was that there would be no ethnic differences in these factors in obese children. We therefore aimed to objectively assess physical activity in a sample of overweight and obese young people, and to explore how physical activity variables relate to body composition.
Methods: 36 overweight and obese children, (22 females), aged 13.5yrs, underwent measurement of body composition, (Tanita BIA, UK). Ethnicity was self-reported as SA or white UK. Actiheart, (AHR) combined heart rate and activity monitors (CamNtech, UK), were worn for 5 days to record physical activity.
Results: 23 white, (age: 13.4yrs, 13f, weight: 91.1kg, BMI SDS: 2.24, BF %: 39.58) and 13 SA, (age: 13.8yrs, 9f, weight: 87.6kg, BMI SDS: 2.06, BF%: 41.9) children were assessed and no significant differences in body composition were found between groups. For any given BMI SDS in the overweight or obese range, SA had higher BF, ($p<0.001$, using ANCOVA, with BMI SDS as a covariate). Total activity counts were significantly lower in South Asians, (28,617 vs 42,540, $p<0.05$). When analysis was restricted to females, total activity counts remained significantly lower for the SA, ($p<0.05$), in addition to an apparently altered relationship between physical activity energy expenditure and BF.

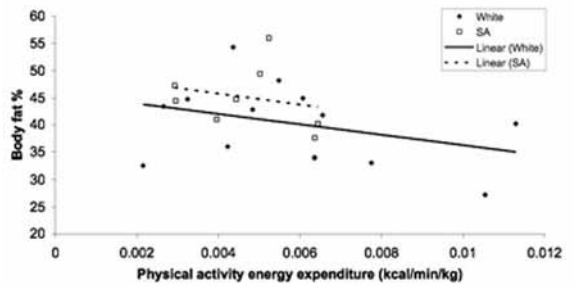

Fig. 1. The relationship between physical activity energy expenditure and body fat

For any given energy expenditure, (adjusted for body weight), SA show a trend towards a higher BF although this did not reach significance.
Conclusions: Overweight and obese SA children have higher percentage total body fat than their white UK peers; and lower physical activity. The normal relationship between physical activity and lower body fat appears to be re-set in SA children towards higher body fat at any level of physical activity.

PO1-247 Obesity, Fat I

Early impact of obesity on phenotype and cardio-metabolic profile of adolescent patients with polycystic ovary syndrome

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Background: it is well known that obesity negatively impact upon severity of metabolic and phenotypic features of adult polycystic ovary syndrome (PCOS) patients, but these aspects are poorly characterized in adolescent PCOS patients.
Aim: to determine the early consequences of obesity in adolescent patients with PCOS.
Subjects were 40 post-menarchal adolescent with PCOS (20 lean patients, group A and 20 overweight and obese patients, group B) aged 13-18 years selected from our PCOS database. **Methods**: clinical and paraclinical parameters were reviewed from medical records. Metabolic syndrome (MetS) was defined by adolescent Cook criteria. The diagnosis of PCOS was established in the presence of clinical and/or paraclinical hyperandrogenism in association with menstrual irregularity.
Results: the two groups were similar in terms of age (16,95±1,5 group A vs 17±1,8 yrs group B). We found that patients in group B had more severe hyperandrogenism as shown by higher Ferriman-Gallwey score ($p< 0,005$) and free androgen index ($p<0,001$), although total testosterone was not different between groups. Group B patients had also greater metabolic abnormalities when compared with lean subjects, as follow: higher 2h glycemia during oral glucose tolerance test ($p<0,05$), higher fasting ($p<0,005$) and 2h insulinemia ($p<0,005$), higher HOMA index ($p<0,01$). Both systolic ($p<0,001$) and diastolic blood pressure ($p<0,001$), but also triglycerides levels ($p<0,005$) were significantly higher in group B suggesting an early alteration in cardiovascular risk factors. MetS was significantly more prevalent among group B patients (43,8% vs 0%, $p<0,005$).
Conclusions: the presence of obesity in young PCOS patients contributes to a more severe clinical phenotype in terms of hyperandrogenism, metabolic alterations, but also cardiovascular risk profile.

PO1-248 Obesity, Fat I

Obesity influence on bone at puberty

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It is well known that obesity has an impact on Bone Mineral Density (BMD) in adults. In particular obese subjects show a higher BMD compared to normal lean subjects. It is still debated the influence of obesity and body composition on BMD in children and adolescents. Aim of our study was to investigate whether BMI and body composition can affect bone mass in a population of healthy obese (BMI SD>2.5) and control (cnt; BMI-SD<2.5) pubertal (Tanner T3-T4 and T5) subjects. We used DXA scan to study (lumbar) L-aBMD (areal), L-vBMD (volumetric), L-Z score, wbBMD (whole body), height normalized wbBMD (n-wbBMD) and lean mass (g) and lean/fat ratio of 151 subjects (82 females; 69 males; mean age 14.5±2.4 yrs). Testosterone and estradiol (t and E2) levels were also considered in both groups. Eighty one were obese (41 males; age 14.3±2.3 yrs; SD-BMI 4.7±1.3) and 70 were control (28 males; age 14.8±2.5 yrs; SD-BMI 0.5±1.6). males and females obese subjects had higher SD-BMI and lean mass ($p<0.001$) and lower lean/fat ratio ($p<0.001$) compared to control. No differences were found in T and E2 levels between obese and cnt sbjects. As a whole group, obese boys did not show any significant difference in bone parameters vs controls, while obese girls showed statistically higher L-aBMD, L-vBMD, L-Z score, wbBMD, and n-wbBMD ($p<0.05$).When we divided subjects according to Tanner stage we found that in males all bone parameters considered were higher in obese at T3-4 ($p<0.01$ vs cnt T3-4), and become higher in cnt at T5 ($p<0.01$ vs obese). In females subjects we did not found any statistically difference in all bone parameters at T3-T4 but at T5 L-vBMD, n-wbBMD and Z score become higher in obese compared to cnt. Furthermore we found a positive correlation in cnt such as in obese males between all bone parameters and lean mass and testosterone levels. Lean/fat ratio was positive correlated with lumbar bone parameters in cnt males and wb bone parameters in obese males. Bone parameters were positively correlate to lean mass also in females subjects, cnt and obese, but neither lean/fat ratio nor estradiol showed any correlation with bone density levels in females. In conclusion our data seem to confirm some previous reported studies about the possible negative influence of obesity on bone density in boys, during developmental age probably due to unfavourable body composition. On the other hand obesity seems not to affect negatively bone development in adolescents girls.

PO1-249 Obesity, Fat I

A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness

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Leptin is a 16-kD protein secreted from adipocytes that plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure. Furthermore, leptin influences a variety of other functions, including immune and inflammatory responses, pubertal development and fertility. Obesity in humans is not generally associated with leptin deficiency. However, two homozygous mutations in the leptin gene are known, leading to a phenotype of extreme obesity associated with marked hyperphagia, which improves under leptin therapy. Despite extensive research, only 12 affected individuals have been found worldwide so far.
We describe here a 14 year old girl with a body mass index of 31.5 kg/m² (2.46 SDS) and undetectable leptin serum levels. Sequencing of the leptin gene revealed a hitherto unknown homozygous transition (TTA to TCA) in exon 3 of the LEP gene resulting in a L72S replacement in the leptin protein. RT-PCR, Western blot and immunohistochemical analysis indicated that the mutant leptin was expressed in the patient's adipose tissue, but retained within the cell. Using a heterologous cell system we confirm this finding and demonstrate that the side chain of L72 is crucial for intracellular leptin trafficking.
In accordance to published patients, our patient showed signs of a hypogonadotropic hypogonadism. In contrast, however, our patient showed only mild obesity without hyperphagia and a normal T cell responsiveness.
In conclusion, our findings shed a new light on the consequences of leptin deficiency.

PO1-250 Obesity, Fat I

A diagnostic pitfall: new mutations in leptin receptor unravelled by late menarche in severely obese dizygote twin girls

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Homozygote leptin receptor (LEPR) mutations lead to severe early-onset obesity associated with hyperphagia, delayed pubertal development and increased plasma leptin (LEP) levels.
We report the clinical and endocrine profiles, together with the genetic analysis

of a dizygote twin pair, AF (BMI 50.6 kg/m²) and LF (BMI 49.6 kg/m²), aged 16, born from lean non consanguineous parents of French origin. In both sisters severe early obesity has developed similar weight and height gain patterns. Growth acceleration from mean to + 1SD took place between 4 and 11 years. LEP and soluble LEPR plasma concentrations were repeatedly within expected range for obesity degree. OGTT showed markedly elevated peak insulin (AF 285 UI/ml, LF 307 UI/ml). TSH, fT4 and IGF1 levels were normal as well as adrenal parameters. Both girls had delayed puberty characterized by low (LF) and normal (AF) gonadotropins levels, low plasma levels of ovarian androgens and ovaries of normal size at the pelvic ultrasound. Menarche occurred at age15 in AF and 15 7/12 in LF.
We decided to sequence the coding region of the *LEPR* gene based on these extreme obesity form associated with delayed puberty. We identified in both sisters the same two heterozygous mutations: a missense mutation in exon 9 and a base pair insertion leading to a frame shift and premature stop codon in exon 15. Only one case of *LEPR* compound heterozygous mutations has been reported so far.
The association of severe obesity with delayed puberty led to the identification of two novel mutations in the LEPR gene in a likely compound heterozygous sib pair in spite of LEPR circulating levels appropriate to the BMIs and leptin levels.

PO1-251 Obesity, Fat I

RBP4 (retinol binding protein 4) is a marker of adipose tissue mass in children – evidence from clinical, experimental and genetic studies

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Retinol binding protein 4 (RBP4) was proposed as novel adipokine that potentially links obesity with insulin resistance and cardiovascular sequelae. In this study, we applied experimental, clinical and genetic approaches to evaluate the role of RBP4 in obesity and metabolic and cardiovascular complications in children.
For the clinical study, RBP4 was quantified in 61 obese children and 68 lean controls by immunoassay.
Potential association of RBP4 with physical development in children was assessed in the healthy lean control group. There were no differences according to sex but a significant increase of RBP4 with pubertal development and age. Obese children had significantly higher RBP4 levels compared to lean controls (30.5±1.4 vs. 26.3±1.1 mg/L, P=0.015) and there was a clear association of RBP4 with BMI SDS (r=0.33, P<0.0001) and other anthropometric parameters independent of age. RBP4 levels also correlated significantly with dyslipidemia and parameters of glucose and insulin metabolism. However, when correlation analyses were corrected for BMI SDS, only C-peptide and LDL-cholesterol remained significant. Similar to the metabolic traits, cardiovascular parameters of blood pressure and endothelial function correlated with RBP4 levels, but again lost significance after correction for BMI SDS except for 24h systolic blood pressure. Multiple regression analyses confirmed the strong association of RBP4 with BMI SDS and age, while the association with metabolic and cardiovascular parameters was marginal.
Hypothesizing from the clinical studies that RBP4 is a marker of adipose tissue mass, we evaluated RBP4 mRNA expression and protein secretion during adipocyte differentiation in the human SGBS cell line. In preadipocytes, RBP4 mRNA expression was nearly undetectable but increased during differentiation to 1624±506fold, P=0.0196. Likewise, secretion of RBP4 was restricted to mature adipocytes, further indicating that RBP4 is strongly related to differentiation of adipocytes.
To finally assess the effect of genetic polymorphisms in the RBP4 gene with obesity, we genotyped the -803G>A polymorphism in our cohort. The minor A-allele was associated with lower obesity risk, lower BMI SDS and RBP4 levels, but not with metabolic or cardiovascular parameters.
In summary, our data provide evidence that RBP4 is a strong marker of obesity in children and that associations with metabolic and cardiovascular parameters are mainly attributed to this underlying relation to BMI.

PO1-252 Obesity, Fat I

Obese children demonstrate alterations in the expression of lipoproteins and coagulation factors in plasma proteomic analysis

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Background: Obesity in childhood and adolescence represents one of the most challenging health problems of our century and is associated with significant morbidity and mortality in adult life. Complications of obesity include impaired glucose tolerance, insulin resistance, diabetes mellitus type 2, hypertension, dyslipidemia, endothelial dysfunction and atherosclerotic cardiovascular disease. Proteomics is a large-scale, high-throughput analysis of proteins that provides information on protein expression levels, post-translational modifications, subcellular localization and interactions.
Aim: The aim of our study was to investigate whether obesity in childhood is associated with alterations in plasma protein expression profiles, and to determine potential biomarkers for complications of the condition in adult life.
Patients and Methods: We studied 10 obese (age: 10.75±0.16yr; BMI: 27.50±0.69kg/m^2), 10 overweight (age: 10.54±0.1yr; BMI: 21.88±0.28kg/m^2) and 10 normal weight (age: 10.89±0.19yr; BMI: 18.34±0.42kg/m^2) prepubertal boys. Plasma samples were subjected to protein fractionation and analyzed by two-dimensional electrophoresis (2-DE), followed by protein identification using matrix-assisted laser desorption-time of flight-mass spectrometry (MALDI-TOF-MS). Image analysis was conducted using the PD-Quest software.
Results: Preliminary results indicate prominent changes related to lipoproteins and coagulation factors in obese children. The expression of apolipoprotein A-I (ApoA-I) was significantly lower in obese children than in children of normal weight. In addition, apolipoprotein A-IV (ApoA-IV), angiotensinogen, antithrombin-III and fibrinogen-beta chain exhibited differential expression in obese children. Our 2-DE findings are currently being confirmed on a larger number of specimens and are being correlated with endocrine and metabolic parameters determined in these children.
Conclusions: Obese children demonstrated prominent alterations in the expression of plasma lipoproteins and coagulation factors compared to their normal weight counterparts. Decreased circulating concentrations of ApoA-I and increased concentrations of fibrinogen are associated with an increased risk of atherosclerotic cardiovascular disease. Low ApoA-I plasma expression levels in obese children might be used as a biomarker for disease severity and might predict the development of atherosclerotic cardiovascular disease in adulthood.
*AV and EC contributed equally to this project

PO1-253 Obesity, Fat I

Inflammatory markers associated with anthropometric and metabolic variables in pre-pubertal children

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Background: Low-grade chronic inflammation with elevated inflammatory markers - TNF-alpha, IL-6 and C-reactive protein (CRP) and low levels of the anti-inflammatory factor adiponectin are characteristically found in obese patients, especially with visceral adiposity. All these parameters are insufficiently investigated in children. Aim: To evaluate the relationship between

anthropometric measurements and serum levels of several inflammatory/ anti-inflammatory markers and metabolic risk factors in pre-pubertal Bulgarian children. Design and research methods: A cross-sectional study of 168 pre-pubertal urban children (78 males; mean age 8.1±1.3 years) was conducted in 2007/2008. Body weight, height and waist circumference (WC) were measured as well as blood pressure (BP); BMI was calculated. Fasting serum levels of blood glucose, insulin, lipids (triglycerides, total cholesterol, HDL- cholesterol, LDL-cholesterol), TNF-alpha, IL-6, hsCRP and adiponectin were measured. Insulin resistance was defined as HOMA-IR index. For the analysis, children were divided into 3 groups according to their BMI and WC: 1st group (BMI≤85th P and WC≤75th P, N=53); 2nd group (BMI between the 85th and 95th P and WC≤90th P, N=46); 3rd group (BMI≥95th P and WC≥90th P, N=69). For the analysis SPSS 15.0 for Windows was used, applying descriptive statistics, ANOVA and partial correlation analysis. Results: With increasing total obesity and WC by group, serum levels of hsCRP and IL-6 rose significantly (p<0.0001 and p=0.016, respectively), girls displaying higher values of IL-6 (2.12±2.03 vs. 1.56±1.51 pg/ml in boys, p=0.048). Adiponectin levels decreased with adiposity (p=0.045). No significant difference was found in TNF-alpha according to BMI and WC, while hsCRP correlated positively with BP, WC, BMI, insulin, HOMA and IL-6 levels in both sexes (p<0.0001), and negatively with HDL-cholesterol in boys (r=-0.281, p=0.017). After controlling for sex and age, IL-6 correlated with WC, BMI, systolic BP and TNF-alpha (p<0.05). Adiponectin levels were inversely related to BMI (r=-0.21, p=0.01) and WC (r=-0.195, p=0.017) regardless of sex. Conclusion: The derangements in the investigated biomarkers found in young obese Bulgarian children may suggest a pathophysiological role of inflammation in the development of the metabolic syndrome even before puberty.

PO1-254 Obesity, Fat I

Inflammatory gene polymorphisms – relation to obesity, metabolic and pro-inflammatory markers in Bulgarian pre-pubertal children

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Background: Various single nucleotide polymorphisms (SNPs) of adipokine genes (IL-6, TNF-alpha, Adiponectin) have been associated with abdominal obesity and its related metabolic abnormalities. All these factors are insufficiently investigated in children. Aim: To evaluate the associations between Adiponectin, IL-6 and TNF-alpha gene polymorphisms and abdominal obesity, metabolic and pro-inflammatory markers in pre-pubertal Bulgarian children. Design and research methods: A cross-sectional study of 168 pre-pubertal urban children (78 males; mean age 8.1±1.3 years) was conducted in 2007/2008. Body weight, height and waist circumference (WC) were measured as well as blood pressure (BP); BMI was calculated. Fasting serum levels of blood glucose, insulin, lipids, TNF-alpha, IL-6, hsCRP and Adiponectin were measured. Insulin resistance was defined as HOMA-IR index. Four SNPs located in IL-6 promoter (rs1800795), TNF-alpha (rs1800629) and Adiponectin (rs2241766, rs1501299) genes were genotyped, using the gold standard method - allelic discrimination with fluorogenic probes. Results: The genotypes were consistent with Hardy-Weinberg equilibrium proportions. Male subjects carrying IL-6-174C allele showed higher serum hsCRP concentrations, WC and BP than homozygotes for the G allele (p<0.05). Boys homozygous for the Adiponectin+276T allele were less insulin resistant with lower insulin levels and HOMA index (p=0.031 and p=0.039, respectively) compared to G allele carriers. Adiponectin+276T/G genotype was associated with family history of hypertension in boys (p=0.016), G allele conferring stronger predisposition. Again in boys, Adiponectin+45T/G genotype was associated with serum Adiponectin - 45T/T homozygotes had lower levels compared to G allele carriers (9.8±4.9 vs. 12.2±4.7 ng/ml, p=0.014). Adiponectin+276T/T genotype was associated with higher diastolic BP in both sexes, compared to G allele carriers (70.4±13.2 vs. 65.7±8.9 mmHg, p=0.05). TNFalpha-308A allele carriers had significantly higher serum levels of hsCRP, IL-6 and TNF compared to non-carriers (p<0.05). Girls with TNF-308AA genotype had the highest values of WC, systolic and diastolic BP, HOMA index and total cholesterol compared to those with AG/GG genotype (p<0.05). Conclusion: These associations suggest that adipokine gene polymorphisms may be related to metabolic syndrome features in pre-pubertal children.

PO1-255 Obesity, Fat I

Xanthogranuloma of the Sellar Region – results of a European multicenter prospective study on diagnostics, therapy and prognosis in children and adolescents

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In **KRANIOPHARYNGEOM 2000** 117 patients with newly diagnosed childhood craniopharyngioma (CRA) from Germany, Austria and Switzerland were recruited between 2001 and 2007. Additionally, 14 patients with childhood xanthogranuloma (XTO) were included in the observational part of our trial **KRANIOPHARYNGEOM 2000**. All patients were prospectively analyzed for clinical manifestations, treatment and risk factors for relapses. Histological diagnoses were assessed by a reference panel in all cases.

Differences between the cohorts of XTO and CRA patients were not detectable for gender, age at diagnosis, severe endocrine deficits, functional capacity at last evaluation and height SDS at the time of diagnosis and at last evaluation. CRA patients presented with higher BMI-SDS at the time of diagnosis (median BMI-SDS at dgx: +1.0 SD [-2.5-9.9]; p=0.019) and at last evaluation (BMI-SDS: +2.9 SD [-1.8-14.6] p=0.001) when compared with XTO patients (BMI-SDS at diagnosis: -0.6 SD [-2.1-1.7]; BMI-SDS at last evaluation: +1.3 SD [-2.3-4.1]). We observed a 3-year-OS and 3-year-EFS of 1.00 in patents with XTO in comparison with CRA patients (3-yrs-EFS: 0.63±0.09 after complete resection and 0.31±0.07 after incomplete resection). The localization of the sellar / parasellar mass was intrasellar in 8%/3%, extrasellar in 0%/23% and combined intra-+extrasellar in 92%/76% and hypothalamic involvement 46%/68% for patients with XTO and CRA, respectively. The median duration of history was 18 months (1-96) in XTO and 5 months (0.3-84) in CRA. A complete resection was achieved in 100% of XTO and 41% of CRA. Irradiation was performed in 27% of CRA patients and in none of XTO patients. Median tumour volume: 2.8 cm^3 (0.3-9.2) in XTO and 13.5 cm^3 (0.9-2.4) in CRA. Visual disturbances were less frequent observed in XTO patients (18%) when compared with CRA (60%). Hydrocephalus was observed none of the XTO patients (35% CRA).

We conclude that the overall prognosis in terms of EFS rate, visual disturbances, hydrocephalus and degree of obesity was better in XTO in comparison with CRA. XTO patients presented with smaller tumors and longer history. In contrast to the literature suprasellar extension and hypothalamic involvement in XTO was observed frequently. Surgical resection seems to be the treatment of choice in XTO.

Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO1-256 Obesity, Fat I

Randomized multicenter trial on patients with childhood craniopharyngioma (KRANIOPHARYNGEOM 2007) – update after 18 months of recruitment

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Despite high overall survival rates (92%) in patients with childhood craniopharyngioma (CP), health-related quality of life (QoL) is frequently impaired due to sequelae resulting from hypothalamic involvement. Based on the results of **KRANIOPHARYNGEOM 2000** radical surgery is no appropriate treatment strategy in patients with hypothalamic involvement of CP. Furthermore, tumor progression and relapses are frequent and early events in CP patients. The analysis of event-free survival rates (EFS) in 117 prospectively evaluated patients with CP showed a high rate of early events in terms of tumor progression after incomplete resection (EFS: 0.31±0.07) and relapses after complete resection (EFS: 0.63±0.09) during the first three years of follow-up. Therefore, innovative treatment strategies are warranted for patients with hypothalamic involvement of CP after incomplete resection.

Accordingly, in **KRANIOPHARYNGEOM 2007** QoL, and survival rates in CP pts (≥5 years of age at diagnosis) are analyzed after randomization of the time point of irradiation (XRT) after incomplete resection (immediate XRT versus XRT at progression of residual tumor). Up to now (03/09) 36 pts with CP were recruited in KRANIOPHARYNGEOM 2007 (22 pts in the randomization arm; 9 pts in the surveillance arm; 5 pts in the process of review of imaging). 9 of 22 pts were randomized. 13 pts could not be randomized due to parental decision (4 pts), insufficient organization (5 pts) and due to decision of the physician (4 pts).

In conclusion, KRANIOPHARYNGEOM 2007 represents the first randomized trial in patients with CP. Aim of the study is to analyze the appropriate time point of XRT after incomplete resection in order to improve QoL in patients with hypothalamic involvement. The recruitment rate is high. However, the compliance to randomization has to be improved. Problems in the randomization process have been improved during the first year of recruitment. International recruitment of CP patients in **KRANIOPHARYNGEOM 2007** will start in 2009.

Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO1-257 Obesity, Fat I

Serum resistin and insulin resistance in obese children

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There is a debate among previous studies, concerning the link between the level of serum Resistin, an adipocyte secreting factor, on one hand and increased fat mass and insulin resistance, on the other hand, in obese children. We aimed to assess in this cross sectional study the body mass index (BMI), body fat percentage, blood pressure, insulin resistance and level of serum resistin in obese non diabetic children. Insulin resistance was evaluated using HOMA and serum resistin level was measured in forty five obese non diabetic children, as well as thirty age and sex matched healthy controls. Calculation of the percentage of body fat was done using the Auxology calculator, while fasting serum insulin and serum resistin were assessed by ELISA technique.

All cases were exceeding 95th percentile as regards both waist circumference and body fat percentage. Insulin resistance was prevalent in 77.8% of cases. Elevated systolic and diastolic blood pressure was present in four obese children (8.8%). Obese non diabetic children had high fasting insulin level (66.6%); which was significantly related to body fat percentage. High fasting insulin, high HOMA as well as high serum resistin levels were significantly more prevalent in cases than controls (p = 0.000)

Twenty cases had high HOMA and low resistin level, among them, 8 had Acanthosis Nigricans (AN). Six cases had high HOMA and high resistin level, among them, 2 had AN. AN was significantly related to BMI and waist circumference (p = 0.01). In cases, BMI was positively correlated to each of waist circumference (r = 0.5, p = 0.01)and systolic blood pressure (r=0.6, p=0.01), while serum resistin level was positively correlated to each of fasting insulin (r=0.7, p = 0.001)and HOMA (r =0.6, p =0.001).

The role of resistin in the pathophysiology of obesity associated insulin resistance in humans is still controversial and needs more research.

PO1-258 Obesity, Fat I

Subclinical hypothyroidism and myocardial function in obese children

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Recently several studies have reported thyroid function derangement in obese subjects and there is concern about negative effect on metabolism and myocardial function of subclinical hypothyroidism in such patients.

The goal of our study is to determine if there is any association between subclinical hypothyroidism and impairment in myocardial function in a sample of Italian obese children.

We examined 21 obese children (11 males, BMI z-score 2.8±0.5, age 9.3±1.5 yrs) with subclinical hypothyroidism and 81 control obese children matched for age and sex. Serum glucose, insulin, triglyceride, HDL cholesterol, TSH, fT3, fT4, thyroid anti-bodies, blood pressure and waist circumference were determined. Each patient underwent complete baseline echocardiographics examination (GE System Seven) with a 3.5-MHz phased-array probe.

Subclinical hypothyroidism was diagnosed when TSH was higher than 4.5 μUI/ml, with normal fT3 and fT4 and no signs or symptoms of hypothyroidism; no patient had positive thyroid antibodies.

Children with TSH≥4.5 μUI/ml did not differ significantly in any of the evaluated metabolic parameters from those with normal TSH.

As to echocardiographic parameters, left ventricular function and mass, left atrial dimension, systolic and diastolic functions were similar in the two groups.

	TSH <4.5 micrUI/ml	TSH ≥4.5 micrUI/ml	P value
n	81	21	
LVM/H (g/m)	41,8±15	42,5±13	0,8
LVM/BSA(g/m2)	87±31	80±14	0,3
LA (mm)	31,8±5,5	33,3±4,2	0,3
EF(%)	67±6	68±7	0,4
E-DT(ms)	172±45	178±30	0,7

LVM/H: left ventricular mass corrected for height; LVM/BSA: left ventricular mass corrected for body surface area; LA: left atrium; EF: ejection fraction; E-DT: peak early diastolic velocity deceleration time

It is recognized that thyroid metabolism exerts profound effect on the cardiovascular system, but it is not so clear if subclinical hypothyroidism in children influence negatively cardiac function.

Obese children arouse particular interest because they have both higher cardiovascular risk and an increased incidence of subclinical hypothyroidism. Our study show, for the first time, the absence of differences in myocardial function

in obese children with subclinical hypothyroidism compared to those with normal TSH. We suggest they should not be treated with L-thyroxine because they are not at higher metabolic and cardiovascular risk and because, as reported previously, hyperthyroitropinemia is reversible after weight reduction.

PO1-259 Obesity, Fat I

Metabolic syndrome in children and adolescents with Prader-Willi syndrome: comparison between obese and non obese subjects

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Prader-Willi syndrome (PWS) is the most frequent cause of syndromic obesity. PWS adults are prone to premature death from cardiovascular disease (CVD) and disorders associated with type 2 diabetes mellitus (DM2). The metabolic syndrome (MS) is a strong risk factor for atherosclerotic CVD and DM2, and it is not possible to rule out the hypothesis that MS may be one of the mechanisms responsible for excessive mortality in PWS. Because there is evidence that MS development has its origin in children an adolescents, we estimated the prevalence of MS in a large group of children and adolescents with PWS. In addition, we compared the metabolic profile of obese PWS subjects to what observed both in non obese PWS and in a group of patients with simple obesity. One hundred and four subjects with genetically confirmed PWS, 54 males, aged 2-18 yr, were studied. Forty-seven PWS were obese and 57 non obese. As control group, a sample of 96 children and adolescents (50 males), matched to obese PWS for age (within 1 year), BMI (within 1 kg/m^2) and gender, was considered. Height and weight were measured by using standardized equipment. The published CDC standards for age- and gender-specific weight, height and BMI percentiles were used for calculating Z-score. Blood pressure (BP) was measured in each subject at least 3 times at 5-min intervals, and the mean values of 3 tests were used in analyses. Biochemical testing included measurements of fasting glucose, triglycerides (TG), and HDL cholesterol levels. According to Weiss et al. (N Engl J Med 2004), we define MS as having at least 3 of the following: BMI>2.0 Z-score, high systolic or diastolic BP, high TG, low HDL and altered glucose tolerance. Altogether, the number of PWS patients with the MS was 2.9%. However, our population of PWS included both obese and non obese subjects. In this respect, obese PWS showed a similar MS prevalence than obese controls (6 vs 12%), while none of the non obese PWS had MS. Non obese PWS showed lower TG (66±35 mg/dl), higher HDL (59±17 mg/dl), lower frequency of hypertension (12.3%) and glucose homeostasis alterations (1.7%) when compared to both obese PWS (85±53 mg/dl, 49±14 mg/dl, 31.9%, 6.4%) and obese controls (84±51 mg/dl, 46±15 mg/dl, 35.4%, 5.2%). No difference was detected between obese PWS and obese controls for all these variables. Overall, our findings seem to confirm that improvement in weight control remains the most important component of any PWS treatment program.

PO1-260 Obesity, Fat I

Effects of an educational intervention program on weight control in overweight and obese children

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Purpose: Pediatric obesity is increasing all over the word. Obese children are at risk of serious complications. Early prevention and treatment of childhood obesity is therefore very important. The aim of this preliminary study was to evaluate the effects of a short-term (2 months) nutrition and physical activity intervention program on weight control addressing overweight and obese school children aged 6-11 years. The program was planned to be integrated to the national primary school program and if successful, to become a model for a nationwide intervention program.
Methods: This study was conducted in four primary schools in Istanbul with the approval of the Local National Education Board and permission from the schools. First an informative meeting was held with the parents and their written consent was obtained. Parents filled out a questionnaire regarding their children's nutritional and physical activity habits, including a three day dietary history form. Physical examination and anthropometric measurements were conducted on 1169 children. The measurements were taken with standard methods, fat ratios were measured with bioelectiric impedance. Out of 1169 children, 224 participated in an interactive program under the supervision of a nutritionist, endocrinologist and a physical education teacher for 2 hours a week, 8 weeks in total. Four months later repeat anthropometric measurements were taken in 130 children (45 F, 85 M). The SPSS program was used in the analysis of the results.
Results: Of a total of 1169 children, 24.2% were overweight and 9.7% were obese. Overweight ratio was 22% among girls and 26% among boys. Obesity ratio was 5.5% among girls and 13.3% among boys. Four months after the initiation of the intervention program, weight SDS, BMI SDS, fat ratio and fat mass values decreased significantly in the intervention group ($p<0.001$). Waist circumference also showed a significant reduction in obese children. No significant changes were noted in the measurements of children who did not join the intervention program.
Conclusion: This study showed that an educational intervention program on diet and physical activity addressing obese and overweight children aged 6-11 years was effective. Considering the rapid increase in the prevalence of obesity, it was concluded that the integration of a similar educational program to the national primary school education programs would be an effective way to prevent obesity, particularly in urban settings.

PO1-261 Obesity, Fat I

Can IGFBP-1 level predict hepatic steatosis?

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Background: IGFBP-1 (Insulin like growth factor binding protein-1) transcription in hepatocytes is inhibited by portal insulin, therefore serving as a liver-specific marker of insulin sensitivity. Additionally, IGFBP-1 levels were recently shown to be inversely correlated with liver fat content and were obesity-independent. Also leptin and TG(triglyceride) levels are presumed to correlate with the visceral adiposity and insulin resistance but their role is not established in terms of hepatic steatosis.
Hypothesis: IGFBP-1 can be used as a marker not only for insulin sensitivity but also for hepatic steatosis
Aim: To investigate the difference in IGFBP-1 level in obese boys with normal liver enzymes (LE) and in obese boys with abnormal LE suggestive of hepatic steatosis
Methodology: 21 obese boys were divided into 2 groups according to their LE and fasting insulin. Different causes of abnormal LE like hepatitis and metabolic liver disease were excluded by blood tests. Insulin resistance indices were calculated from fasting glucose and insulin and OGTT test. Along with IGFBP-1, Leptin and lipid profile were also compared.
Results: Group 1 with normal LE (n=13, age 12.6 ± 2.2, 60 % Hispanic, 20 % AA, 20% others, ALT 22.6 ± 5.3, AST 22.1 ± 3.9), Group 2 with abnormal

LE (n=8, age 12.13 ± 4.9, 100% Hispanic, ALT 136 ± 118, AST 90.2 ± 62.2). There were no differences between the groups BMI, lipid profile and insulin resistance indices HOMA, IRG and Quicki. IGFBP-1 was significantly lower in group 2: 2.3 ± 1.1 than in Group 1: 8.8 ± 3.9, $p < 0.01$. Leptin was lower in Group 2: 17.9 ± 7.9, Group 1: 24.1 ± 4.9, but didn't reach statistical significance p=0.1.HDL was lower and TG was higher in Group 2 but didn't reach statistical significance.

Conclusions:

In addition to being a indicator of insulin resistance, IGFBP-1 may be marker for hepatic steatohepititis. However, further investigation is necessary to confirm whether elevated IGFBP-1 levels can predict hepatic steatosis. Further larger, prospective studies are required to establish definitely the role of IGFBP-1 in predicting hepatic steatosis and to evaluate its progression.

PO1-262 Obesity, Fat I

Is the natural history of hepatic steatosis in children progressive?

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Background : Steatohepatitis in adults is considered a progressive disorder. But not much is known about this condition in children.

Hypothesis: The severity of hepatic steatosis should increase with time in children

Aim: Aim of the present study was to study the natural history of progression of hepatic steatosis in children and any relation of steatosis to insulin resistance or lipid profile.

Methodology: 8 obese children were selected who also had abnormal LE (liver enzymes) suggestive of hepatic steatosis after ruling out other causes of hepatitis or metabolic liver disease by blood tests. They were followed up for 4.5±1.5 years. Insulin resistance indices HOMA and quicki were calculated from fasting glucose and insulin. Also their lipid profile and LE were measured before and after the study period. Student T-test was applied on the mean values of the results before and after the study period. 5 out of 8 children also underwent liver biopsy to find the disease severity in them.

Results: All 8 children had improvement in their liver enzymes over the study period. Their BMI, lipid profile and insulin resistance indices did not change over time. There was no correlation between their lipid profile or insulin resistance parameters and the severity of their liver enzyme derangements. 2 children on liver biopsy had simple steatosis, 2 had steatohepatitis and 1 child had normal biopsy report. One child with steatohepatitis had complete resolution of abnormal liver enzymes whereas other child with steatohepatitis still has abnormal liver enzymes.

Conclusion: In the present study, there was spontaneous improvement in liver function in obese children. But other studies suggest that steatohepatitis is progressive in nature specially in adults. So, it is still not clear which patients will progress and which will improve. And we need more specific markers for identifying children with steatohepatitis who can progress to end stage liver disease.

Table comparing average± SD values of various parameters in the study group before and after study period

Value	Before	After
Age (years)	10.8±2.4	15.3±2.8*
BMI Kg/m2	31±4.4	34.6±7.8
ALT IU/Lit	139±95	56±33*
AST IU/Lit	72.8±4.9	31.5±9.4
TC (mg/dL)	165±37	151±22
TG (mg/dL)	149±87	106±77
LDL (mg/dL)	102±30	89±26
HDL (mg/dL)	35±8	39±11
HOMA	4.9±6.9	5.5±7.9
Quicki	0.3±0.1	0.3±0.08

* p value<0.05

PO1-263 Obesity, Fat I

Thyroid hormones and their relation with risk factors in obese and overweight children

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Objective: Although most of the thyroid hormone are within normal levels in obesity, they influence body weight, heart rate, serum lipids and metabolism of carbohydrate. The aim this study was determining if there is a relation between thyroid functions and cardio-metabolic risk factors for obesity.

Material-methods: In 250 overweight and obese children (128 girls,122 boys) the relationship between thyroid hormones (freeT3, freeT4, TSH) and risk factors; BMI, arterial blood pressure, waist circumference, fasting blood sugar, insulin levels, lipid profile. We compared these risk factors and thyroid hormones who have higher or lower than TSH 5mIU/ml.

Results: High TSH level were found 14% of overweight and obese children (TSH normal, n=215; TSH >5 mU/ml, n=35). A correlation analysis was made between thyroid function tests and other characteristics; body mass index, arterial blood pressure, waist circumference and lipid profile. Very weak positive correlations were found between TSH and total cholesterol (r:0.135, p:0.04). A weak but negative correlation between free T4 and BMI (r:-0.155, p:0.014), HDL (r:-2.17, p:0.001), LDL(r:-0.186, p:0.005) were found. There was a moderate correlation with waist circumference and freeT4 (r:-0.334, p<0.001). We could not find a significant difference between other parameters and thyroid hormones. When the groups according to TSH levels (low or high) were compared, LDL levels were found significant different (99,6±30.2 mg/dl vs 119.1±44.2 mg/dl, p:0.036)**.**

Conclusion: We conclude that high TSH level is associated with dyslipidemia and elevated TSH levels as cardiometabolic risk factor may be used to clinical practice in obese and overweight children.

PO1-264 Obesity, Fat I

Association of obesity and hyperandrogenemia in Korean children and adolescent obese girls

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Objectives : This study was to know relationship between obesity and hyperandrogenemia (HA) and assess the degree of HA in Korean children and adolescent obese girls.
Methods : Subjects were 69 normal weight (body mass index (BMI) for age<85 percentile) and 78 obese (BMI for age ≥ 95 percentile) peripubertal girls. They were divided into 3 groups (Tanner stage 1=prepuberty, Tanner 2, 3=early puberty, Tanner 4, 5=overt puberty) according to their Tanner stage. Blood samples were taken early in the morning after at least 8hr of fasting.
Results : Compared with normal weight girls, mean total testosterone(T), free T, DHEA-S, and insulin levels were significantly higher in obese groups($P<0.05$). SHBG level was significantly lower in obese groups($P<0.001$). Higher total T, free T, DHEA-S, insulin and lower SHBG levels were showed significant difference in overt puberty. BMI was significantly correlated with total T(r=0.47), SHBG(r=-0.36), free T(r=0.51) and insulin(r=0.49) ($P<0.01$). Fasting insulin and LH were significantly correlated with total T, SHBG and free T ($P<0.01$). In overt puberty, BMI was significantly correlated with total T (r=0.32, $P<0.05$), SHBG (r=-0.34, $P<0.05$) and free T (r=0.43, $P<0.01$) and insulin(r=0.46, $P<0.01$). In overt puberty, fasting insulin was significantly correlated with free T(r=0.47) and SHBG(r=-0.47).
Conclusion : HA is pronounced in obese girls during overt puberty. Identifying girls at risk for HA may be an effective means of preventing some of the longterm complication associated with PCOS.

PO1-265 Pancreas I

Changing the phenotype of homozygous 11p15-p14 deletion syndrome: congenital deafness with mild or severe hyperinsulinemic hypoglycemia and MODY

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Persistent Hyperinsulinemic Hypoglcemia of Infancy (PHHI) is caused by mutations in genes involved in regulation of insulin secretion, seven of which have been identified (*ABCC8, KCNJ11, GLUD1, CGK, HADH, SLC16A1* and *HNF4A*).
Usher syndrome is a condition characterized by deafness and progressive vision loss. Individuals with Usher syndrome type I are typically born completely deaf or lose most of their hearing within the first year of life.
A homozygous, contiguous gene deletion in 11p15-p14 including 22 exons of *ABCC8* and 26 exons of *USH1C* causing infantile hyperinsulism, enteropathy, deafness and renal tubular dysfunction has been reported in three cases (OMIM: Homozygous 11p15-p14 Deletion Syndrome).
We report of four new patients from two consanguineous families with the same homozygous deletion, but with clinical heterogeneity. All patients have PHHI and deafness, 3 of them had severe neonatal hypoglycemia demanding near total pancreatectmoy with need of continuous medical tretament after surgery for their hypoglycemia.
The fourth patient had congenital deafness, but mild, transient neonatal hypoglycaemia only. This child developed diabetes at 11 years of age. S-insulin level was low, 49 uU/ml (72-150), C peptide level 1.4 ng/ml (1.1-5.0 ng/ml) and he had an abnormal OGTT with 2hr BG of 16 mmol/l. BMI was normal. HBA1C level increased from 6.0% to 8.5% (4.4-6.4%) and after 3 months trial of life style changes he continued to have postprandial hyperglycemia and persistently high HBA1C, after which we started him on glucophage and his HBA1C dropped to 6.8% with improvement in glucose control.
All four patients are thriving well and have normal neurodevelopment and do not have gastrointestinal symptoms nor renal tubular defect.
Genetic testing by PCR of *ABCC8* shown that only exon 23 to 39 are present. In the same way only exon 1 and 2 of the *USH1c* gene was shown to be present. By sequencing, a homozygous contiguous partial gene deletion was identified, starting in *USH1c* intron 2, c.90+592, and ending in *ABCC8 intron 21, USH1c*:c.(90+592)_*ABCC8*:c.(2694-528)del.
Conclusion: In homozygous 11p15-p14 Deletion Syndrome, enteropathy and renal tubular defects are not necessarily present suggesting that these features are not a result of the deletion. 11p15-p14 Deletion Syndrome should be suspected in all children with hyperinsulinemic hypoglycemia associated with deafness. Milder cases are at risk for development of diabetes (MODY).

PO1-266 Pancreas I

Network for pancreatic organ donors with diabetes (nPOD): donor demographics and early progress report

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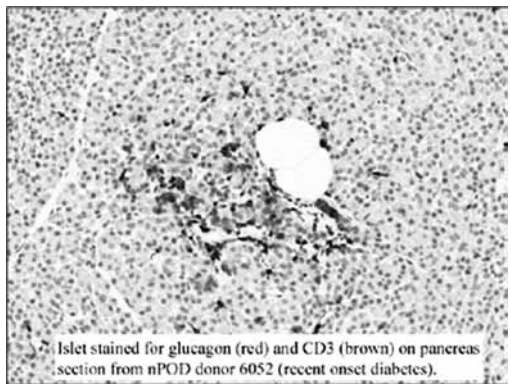
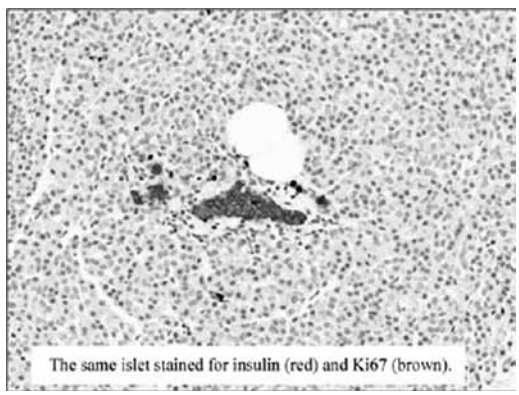
Incidence of type 1 diabetes (T1D) has been on the rise, worldwide, for many years. Despite therapeutic improvements, T1D patients suffer from a multitude of complications. In human T1D, little is known about mechanisms leading to autoimmunity and beta cell destruction. The study of pancreata from recently deceased T1D patients and at risk autoantibody positive subjects provides a unique opportunity to understand the pathology underlying this disorder. The Juvenile Diabetes Research Foundation established the Network for Pancreatic Organ Donors with Diabetes (nPOD) in 2007 to supply investigators with tissues (pancreas, spleen, lymph nodes). To date, pancreata have been obtained from 21 T1D (4-93 years old) and 31 nondiabetic donors (2-75 years old). Diabetes duration ranges from 1-76 years and age-at-onset 1-30 years. A comprehensive database was created consisting of patient information (age, gender, ethnicity, BMI, diabetes duration, onset age), immunology (HLA, autoantibody status), hospitalization (ICU time, ventilator time, meds) and organ collection factors (death-cross-clamp time, transit time) that may impact histopathology. Initial histologic analysis suggests that insulitis occurs during a narrow window of time surrounding onset of T1D. Figures 1 and 2 show adjacent sections of an islet from a T1D donor who died 1 year after onset.



Islet stained for glucagon (red) and CD3 (brown) on pancreas section from nPOD donor 6052 (recent onset diabetes).



The same islet stained for insulin (red) and Ki67 (brown).

The glucagon and insulin staining pattern indicates disruption of normal islet architecture resulting from T-cell (CD3) infiltration, not seen in longer duration cases (>1 year diabetes). Assignment of diabetes status (T1D vs Type 2 diabetes) prior to death didn't always correspond with postmortem data. nPOD is an extremely valuable resource for defining the histology of the pancreas in patients with diabetes and aiding in the classification and understanding the mechanisms leading to T1D.

PO1-267 Pancreas I

Maternally inherited ABCC8 mutation and a normal paternal ABCC8 gene in a case of severe neonatal hyperinsulinism

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Introduction: Congenital Hyperinsulinism (CHI), caused mostly by autosomal recessive mutations in ABCC8 gene, may appear as either focal or diffuse pancreatic lesions. Focal CHI is probably caused by a mutation in the paternal allele coinciding with the loss of the distal short arm of maternal chromosome 11, including the ABCC8 gene. We present an unusual case of a maternally inherited CHI, in an autosomal dominant negative fashion.

Clinical Data: A day old girl to non consanguineous parents developed severe hypoglycemia with hyperinsulinemia, responsive initially to continuous glucose infusion and then to G-tube feeding and somatostatin. There was no clinical response to diazoxide. A maternal aunt and cousin (confirmed to carry a heterozygous in-frame insertion ABCC8 gene mutation) had similar phenotypes as neonates.

Molecular Data: DNA was extracted from the patient, her parents, grandfather, another affected cousin and her mother. For the patient, all coding exons and exon/intron boundaries of *KCNJ11 and ABCC8*, coding for Kir6.2 and SUR1 components of K^+_{ATP} channel, were sequenced. A heterozygous in-frame insertion mutation in exon 37 of the ABCC8 gene was found in the patient and her unaffected mother, which was identical to the one present in their affected relative. No other maternal or paternal mutations were identified.

Co-transfection of Kir6.2 and the mutant SUR1 in COSm6 cells demonstrated expression on the cell surface. There was severe channel dysfunction and almost no current when exposed to MgADP (<10% of wild type); even in the heterozygous state. Only a very minimal response was achieved to diazoxide, indicating severe channel dysfunction, in both heterozygous and homozygous states.

Conclusions: This is the first case of maternally inherited ABCC8 dominant negative mutation, reported in familial CHI. Expression studies indicate that both homozygote and heterozygote mutant proteins are expressed on the cell membrane, but, they do not enable K^+ current through the membrane. Further epigenetic and molecular studies are in progress and may shed light on this unusual mode of inheritance; as some heterozygous individuals have no symptoms of hypoglycemia while others are severely ill. These findings exclude classical recessive and uniparental disomy (focal-HI) modes of inheritance and suggest the possibility of a novel mechanism for genetic CHI.

PO1-268 Pancreas I

Usefulness of 18-F-Dopa PET-CT in the diagnosis and treatment of hypoglycemia due to hyperinsulism

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Introduction: Congenital hyperinsulinism is the most frequent cause of refractary hypoglycemia with a high risk of neuroglucopenia and subsequent irreversible neurological damage. The differential diagnosis between difuse and focal hyperinsulinism is very important not only in the management but also in the prognosis of the patients. **Aim:** To evaluate the usefulness of 18-FluorDopa PET-CT in the investigation of hyperinsulinism and its impact on the patient outcome. **Material and Methods:** We present 11 patients diagnosed with hypoglycemia secondary to hyperinsulinism who underwent 18-FluorDopa PET-CT. In all of them the previously image studies (CT, MRI, Ultrasound) were negative. The study was performed without pharmacological therapy, in all, but 2 cases. **Results:**

Description of the results

Sex	Onset of symptoms	Treatment	Genetics	Age	PET result	Surgery	Outcome
M	13m	D	negative	14m	Difuse	No	Control
F	2 d	D, O	negative	6m	Difuse	No	With therapy
M	2d	D, N	ABCC8	16m	Difuse	No	With therapy
F	20h	H, O, D	pending	1m	Focal	Yes	No therapy
F	2d	O, D	pending	4m	Diffuse	No	With therapy
M	12h	H, O, D	ABCC8	14m	Focal	Yes	No therapy
M	24h	H,O, D, Hz	not done	9m	Diffuse	No	With therapy
F	12h	H, O, D, Hz	negative	2m	Diffuse	Yes	Dead (sepsis)
M	16m	D	pending	20m	Diffuse	No	With therapy
M	11y	D	pending	19y	Diffuse	No	With therapy
F	3m	D, Hz	pending	5m	Focal	No	With therapy

D: diazoxide, O: octreotide, N: nifedipine, H: hidrocortisone, Hz: hydroclortiazide, d: days, m: months, y: years.

Conclusions: It is essential to distinguish focal and difuse types of hyperinsulinisms in order to surgery approach. Some years ago invasive investigations were needed in order to know the place of the lesion. Genetic basis are not known in more than 50% of patients and there are very few focal cases that show a genetic alteration which modifies the therapeutical approach. 18-FluorDopa PET-CT is a very sensitive test to identify the focal form and it should be performed as soon as possible to improve the patient outcome.

PO1-269 Pancreas I

Beneficial effects of low glycemic index (GI) and glycemic load (GL) diets in glycemic control of diabetic type 1 children

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Introduction: Recent evidence suggest that the amount of carbohydrate ingested in a diabetic's diet, as well as its digestion and absorption rates, are related to postprandial glycemic response. However, findings are not always consistent, and consensus regarding the incorporation of the GI and GL concepts into dietary guidelines has not yet been reached. Objective: To evaluate whether GI and GL of the usual Brazilian diets influence the glycemic control of type 1 diabetic children. Methods: The diets of 139 children and adolescents (87 female) aged 12.9±3.6 years and having 6.9±3.6 years of DM1 (minimum of one year), followed-up at the Division of Pediatric Endocrinology of the Universitary Hospital were evaluated. We used a Quantitative Food Frequency Questionnaire, previously validated and tested in a pilot-project. The GI was estimated in accordance with the equation recommended by the FAO (1988). The GL was estimated by using the equation proposed by Foster-Powell and col (2002). Glycemic control was evaluated from the average of the two HbA1c

results in the six months prior to the diet evaluation. Results: Diets of individuals with good glycemic control had significantly lower (Tukey test, p=0.000) GI/GL (54.8±2.7/118.3±29.8) than those with regular (60.1±3.8/142.5±27.3) or poor glycemic control (60.3±4.1/153.7±40.7). Conclusion: This study showed beneficial effects of diets with low GI/GL in the glycemic control of children and adolescents with DM1.

PO1-270 Pancreas I

18F-DOPA PET/CT scan for pre-operative localization of focal lesions in 105 infants with hyperinsulinism

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Half of infants with medically-uncontrollable congenital hyperinsulinism have focal adenomatosis lesions in the pancreas due to isodisomy for a paternally-derived recessive mutation of the ATP-dependent potassium channel genes, ABCC8 or KCNJ11. Pre-operative diagnosis and localization is important to allow potential cure of the lesion by local resection. The purpose of this study was to prospectively determine the sensitivity and specificity of F-DOPA PET/CT in infants who required pancreatic surgery and had a histologic diagnosis of focal or diffuse hyperinsulinism.

Results were made available to the surgeon to use in searching for focal lesions after the first 5 cases. Patients with diffuse hyperinsulinism on intra-operative frozen section examination underwent near-total pancreatectomy. Patients with no evidence of diffuse hyperinsulinism underwent exploration and additional biopsies to detect and resect the focal lesion.

From 12/04 to 12/08, 105 infants with medically-uncontrollable congenital hyperinsulinism underwent pre-operative 18F-DOPA PET/CT imaging of the pancreas. Of these, 93 cases were evaluable for this study. 52 cases had focal adenomatosis. F-DOPA PET/CT identified a focal area of uptake in the pancreas in 46 of the focal cases. In 6 cases, focal lesions were not detected by F-DOPA PET/CT, but were found at surgery. Five of these were small lesions < 0.5 cm in diameter and all were identified and resected during surgery; one case had an extensive adenomatosis lesion occupying 80% of the pancreas. 14 of 52 focal cases required resection of the head of the pancreas and a Roux-en-Y anastomosis of the tail to the jejunum. In all of the diffuse cases, F-DOPA PET/CT scans were read as diffuse uptake.

In this series, F-DOPA PET/CT imaging had a high rate of success in detecting focal lesions and was 100% accurate in localization. These results indicate that F-DOPA PET/CT imaging should be considered in those infants with congenital hyperinsulinism who require surgery.

PO1-271 Pancreas I

Successful long-term treatment of congenital hyperinsulinism with lanreotide

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Congenital hyperinsulinism (CHI) is the most common course for persistent hypoglycaemia in infancy. Focal and diffuse CHI-forms are differentiated based on histological findings. While in focal forms curative surgery is the treatment of choice, it is discussed controversially in patients with diffuse disease. Medical treatment of diffuse CHI is difficult as the number of drugs is restricted and the response varies between the patients. Beside diazoxide and nifedipine, octreotide has been established as the most potent drug in the long-term treatment of CHI. However it has to be injected 3 to 4 times daily which is stressful for patients and parents and the blood glucose (BG) levels often remain unstable.

Lanreotide is a very-long-acting somatostatine analogon which is established in adults with neuroendocrine tumors, acromegaly or carcinoid syndrome since 2007.

We started for the first time individual treatment trials in two patients who had been on octreotide treatment before, to evaluate the use of lanreotide in patients with diffuse CHI.

Patient 1: Diffuse CHI from birth, compound-heterozygous mutation in ABCC8, diffuse uptake in DOPA-PET/CT, resistant to diazoxide (DZ), nifedipine (Ni) and partly responsive to octreotide (OCT) 4 times/day. Subtotal pancreatectomy at the age of 3 month with subsequent better response to octreotide. Treatment with lanreotide was started at the age of 6 month.

Patient 2: CHI from 1st day of life, resistant to DZ and Ni. Good response to high-dose OCT (>50 g/kg/d) 4 times/day. Compound heterozygous mutation in ABCC8, diffuse uptake in DOPA-PET/CT. Start of lanreotide treatment at the age of 3 month.

Results:

In the lanreotide-treated patients dose adaptation to 120 mg/month was necessary, glycaemia control has been improved compared to the octreotide treatment. Normal growth despite low IGF-1 levels (< 2,5 SDS) was seen in both patients, in patient 2 gall-bladder concrements have been reported which were treated with ursodeoxycholic acid (UDCA). No abdominal pain after administration of lanreotide was reported.

Conclusion:

1. Treatment with lanreotide reduces the load of therapy in the families (e.g.: reduces number of injections by 80-119 injections per month compared to 3 or 4 octreotide-injections per day).
2. No serious side effects have been observed during lanreotide treatment.
3. Acceptable glycaemic control can be reached by treatment with lanreotide (Somatuline Autogel) in patients with diffuse CHI.

PO1-272 Pancreas I

Localization of insulin producing tumors by 68Ga-DOTATOC PET/CT in patients with MEN1

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Insulinoma in the context of MEN-1 are rare causes of severe hyperinsulinemic hypoglycaemia in childhood and infancy. However localization of the insulinoma can be very challenging. Therefore the optimal diagnostic approach is important to detect the tumor and to perform successful surgical resection.

Methods: We report 3 patients with severe hyperinsulinemic hypoglycemia. The diagnostic work up for tumor localization include endosonography, 18F-DOPA-PET/CT, 68Ga-DOTATOC/PET and intra-arterial Ca-stimulation tests.

Results: None of the insulinomas in the 3 patients could be located by endosonography or 18F-DOPA-PET/CT. In two of them the tumor was localized after intra-arterial calcium stimulation test. However the tumor in these patients was also visible on the 68-Ga-DOTATOC PET/CT when we did a second look after the results of the Ca-stimulation test were known. Finally one tumor was localized by 68-Ga-DOTATOC. In all 3 patients the tumor was successfully resected and no further hypoglycaemic episodes were observed post-surgically. Molecular genetic analysis obtained heterozygous mutations in the MENIN gene in 2 patients, leading to the diagnosis of MEN1.

Conclusion: We report 3 patients with hyperinsulinemic hypoglycemia caused by insulinoma. No tumor was detected by 18-F-DOPA PET/CT. However all of them were visible in the 68-Ga-DOTATOC. Although 18F- DOPA PET/CT has been successfully established as localization tool in congenital hyperinsulinism, the sensitivity of this method in insulinoma later in life seems to be limited. In this small cohort 68-Ga-DOTATOC-PET-CT seems to be superior to the 18-F-DOPA-PET-CT.

PO1-273 Pancreas I

Diet soda increases early insulin and GLP-1 secretion in healthy volunteers

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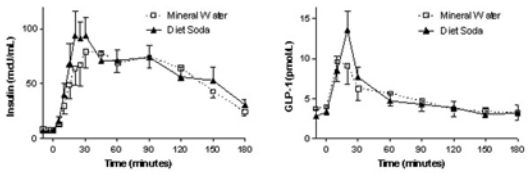
Background: Consumption of diet soda containing artificial sweeteners is common in children and adults. It is generally assumed that glucose metabolism is not altered, as these sodas contain minimal carbohydrate. However, recent data from animal studies indicate that sucralose (Splenda™) not only activates receptors in lingual taste buds but also in brush cells in the gut, which subsequently activate intestinal glucose transporters (GLUT2), resulting in more rapid glucose absorption. We thus hypothesized that 1) ingestion of diet soda before an oral glucose tolerance test (OGTT) would lead to faster glucose absorption in humans, and 2) in healthy volunteers, who can adjust insulin secretion to compensate for faster glucose absorption, diet soda ingestion should induce an earlier and higher insulin response.
Methods: In this pilot study, 14 healthy volunteers ages 13-23 years (mean 16.5 ± 3.5) underwent two 75g OGTTs. Ten minutes prior to the glucose load, subjects drank 8 oz of either sucralose-containing caffeine-free diet soda or mineral water, in randomized order. Each subject served as his/her own control. Glucose, insulin, and GLP-1 were measured for 180 minutes after the glucose load.
Results: There was no difference in serum glucose with diet soda vs. mineral water. The time course of insulin and GLP-1 secretion are shown below (mean ± SEM). Both insulin and GLP-1 were significantly higher at 20 minutes with diet soda compared to mineral water (insulin: p = 0.02; GLP-1: p = 0.02; Wilcoxon rank sum).



Conclusions: Ingestion of diet soda prior to an oral glucose load in healthy young volunteers appears to potentiate early secretion of the incretin hormone, GLP-1, and insulin. We postulate that this early rise in GLP-1 and insulin may be due to stimulation of gut taste receptors by artificial sweetener, resulting in more rapid glucose absorption. If these results can be confirmed, we further hypothesize that small elevations in insulin secretion due to artificial sweeteners may have public health implications, possibly contributing to hyperinsulinemia and weight gain.

PO1-274 Pancreas I

MODY2 natural evolution and treatment

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Introduction: Maturity-onset diabetes of the young type 2 (MODY2) is a monogenic diabetes due to heterozygous glucokinase mutations which result in mild hyperglycaemia from birth. MODY2 patients show little deterioration with age and nowadays the treatment option varies from diet to insulin.
Aim: To define the natural evolution of MODY2 and to assess the impact of molecular diagnosis on the treatment attitude, as well as to evaluate the short term effect of this treatment change on metabolic control.
Subjects and methods: We have analyzed clinical and biochemical data at clinical onset from a group of 136 MODY2 probands and 46 affected parents. To assess the natural evolution we re-analyzed 94 patients after 7.1 years follow up. To assess the treatment attitude we analyzed 85 MODY2 patients of whom we disposed of treatment option at clinical diagnosis and after molecular confirmation. To compare biochemical parameters between groups, Student and paired samples t-tests, Mann Whitney U-test and Wilkoxon signed rank test have been used.
Results: After 7.1 years probands present lower fasting glucose (117±11mg/dl vs 125±16mg/dl)(p:0.002) and higher HbA1c (6±0.5% vs 5.8±0.6%)(p:0.036). Affected parents (mean age: 31.3 years) present higher fasting glucose (134±17mg/dl vs 123±17mg/dl)(p:0.002) and higher HbA1c (6.1±0.7% vs 5.9±0.6%)(p:0.05) than probands at diagnosis (mean age: 9.5 years).
Before genetic diagnosis 9/85 patients received pharmacologic treatment [oral agents (OAA) n=2/insulin n=7]. 7 years after molecular diagnosis, only 3/85 received pharmacological treatment (OAA).
HbA1c levels were not significantly different between patients who were treated with pharmacological agents or diet at clinical diagnosis (6.14%±0.6 vs 5.84%±0.6) or after molecular confirmation (6.17±0.4 vs 5.99±0.5).
There were no differences between the HbA1c increment in the 6 patients who suspended insulin (0.18 % in 5.3 years) and patients who never received pharmacological treatment (0.19 % in 7.1 years).
Metabolic control of the 6 patients who suspended insulin did not worsen after 5.3 years (HbA1c 6.07%±0.7 *vs* 6.25%±0.4).
Conclusions: MODY2 patients suffer a slight HbA1c increment directly associated with age which is not clinically relevant in short term. Patients treated with diet did not present a worse metabolic control than those treated with pharmacological agents .

PO1-275 Pancreas I

First application in Italian children of 18F-DOPA-TC-PET to detect diffuse and focal forms of congenital hyperinsulinism of infancy

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Introduction: Congenital Hyperinsulinism of Infancy (CHI) is the most common cause of hypoglycaemia (1:50,000 births/year) in infants.
CHI may be associated histologically with two forms: diffuse insulin hypersecretion (Di-HI) or focal adenomatous hyperplasia (Fo-HI), with different molecular basis. The diffuse form is most frequently of autosomal recessive inheritance. The focal form is sporadic, due to a paternally inherited mutation in one of the genes encoding the subunits of the β-cell ATP-sensitive potassium channel (ABCC8 and KCNJ11) and somatic loss of maternal 11p15 alleles within a limited region of the pancreas.
The pre-operative detection of Fo-HI allows a limited pancreatic curative resection with no long-term side effects. Until recently, the localisation of insulin hypersecretion before surgery was only possible through pancreatic venous sampling (PVS), an invasive and technically challenging method. Positron Emission Tomography using fluorine-18-labeled L-dihydroxyphenylalanine ([18F] DOPA) has been reported to accurately distinguish between focal and diffuse forms of CHI, and can localise ectopic lesions.
Material and methods: This is the first study performed in Italy to discriminate focal and diffuse CHI with [18F] DOPA TC-PET. Eleven children (9 males and 2 females) of ages between 2 months and 11 years, were studied with [18F] DOPA TC-PET. Nine children were responders to pharmacological therapy (diazoxide), one patient was non responder, and another patient, previously submitted to surgery, still had persistent hypoglycaemia, even under therapy. For the first five patients diazoxide and octreotide were interrupted 48 and 24 hours respectively before the procedure. As suggested by recent literature results, the other patients continued medical therapy during the examination.
Results: [18F] DOPA TC-PET identified Fo-HI in 2 children, andDi-HI in the other patients. The histopathological results of two patients unresponsive to medical treatment who underwent surgery confirmed the TC-PET findings in both. Surprisingly, in one patient hypoglycaemia persisted despite near total pancreatectomy and the TC-PET scan showed an extensive area of captation, suggesting the growth of new pancreatic tissue.
Conclusion: These data confirm that 18F-DOPA TC-PET is an accurate and non-invasive technique that allows differential diagnosis between Fo-HI and Di-HI.

PO1-276 Pancreas I

Abnormal MRI findings in a patient with persistent hyperinsulinemic hypoglycemia of infancy (PHHI)

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Hyperinsulinism is one of the most common causes of hypoglycemia in early infants. Management of persistent hyperinsulinemic hypoglycemia of infancy (PHHI) which is characterized by inappropriate insulin secretion is obviously hard. Hypoglycemic events can cause severe brain damage in these infants. We present a patient with PHHI having remarkable magnetic resonance imaging (MRI) findings.

A seven-month-old boy was referred to our clinic for uncontrolled low blood glucose levels. His low blood glucose was noticed on the first day of life and he has been followed for 28 days in the neonatal intensive care unit of a university hospital before admission. Hypoglycemic convulsions were detected and anticonvulsive treatment was started in conjunction with diazoxide treatment. There was first degree consanguinity between parents. The history revealed that his five-year-old sister also has PHHI and still uses diazoxide, and his older brother has ectodermal dysplasia. His weight was 8.2 kg (25-50 percentiles), height was 69 cm (25-50 percentiles), and head circumference was 37.5 cm (<3 percentile). On physical examination; dysmorphic findings such as apathic face, pitosis, micrognathia, and sparse hair and eyebrows; a second degree systolic murmur on the mesocardiac area; and psychomotor retardation were noticed. Laboratory examination revealed a high insulin level of 39 mIU/ml at the time of hypoglycemia (30 mg/dl). There was no ketonemia. Complete blood count and routine biochemistry studies were unremarkable. Intravenous glucose infusion at a dose of 10 mg/kg/min was started immediately. Diazoxide therapy was continued at a dose of 20 mg/kg/day, and glucagon injection at a dose of 30 mcg/kg every six hour was also commenced. Diazoxide dose was reduced to 15 mg/kg/day because of the appearance of signs of fluid retention. Echocardiography showed a mild aortic and pulmonary stenosis, and left ventricular hypertrophy. MRI taken at the age of two months showed striking findings such as diffuse cortical atrophy especially in the frontooccipital lobes, diffuse cystic encephalomalasic lesions, and a thin corpus callosum. MRI findings four months later were not changed. Normogylcemia was maintained by diazoxide, nifedipine and glucagon.

Since hypoglycemia causes serious brain damage in the early periods of life, blood glucose should be monitored very closely and carefully in these infants. This case clearly demonstrates how hypoglycemia could affect the brain.

PO1-277 Pancreas I

Revision of type I diabetes in childhood during the last decade

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Type I diabetes in childhood is a chronic disease that has an important role in the daily consultation. There has been and increased in reported cases during the last year.

Methods. A retrospective study of diabetic debut from 1998-2008, assessing epidemiological, clinical, analytical treatment and subsequent developments. We analyzed the incidence, age, sex, month, year of debut, HbA1C at admission, 2, 4 and 6 years respectively, and type of insulin received. Statistical analysis was performed using the SPSS v11.

Results: 94 patients (≤ 14 years), 45 boys and 49 girls. The incidence has increased significantly in the last year to 2.49 casos/10000 children below 14 years old, the average age at onset was 8.82 ± 3.56 (range 0.9 and 13.9 years old) distributed in three groups: <5 years (14) (15%), between 5 and 10 years (39) (41.5%) and> 10 years of (41) (43.5%). There are two seasonal peaks, December, January and February, 33 cases (35.1%), and in August, September and October 31 cases (33%). There was 28 cases of Ketoacidosis (29.8%), mild (pH 7.25-7.3) 21.5% (6), moderate (pH 7-7.24) 75% (21) and severe (<7) 3.5% (1), The average HbA1C debut 11.44 ± 2.08. The average HbA1C at 2 years (73) was 7.26 ± 1.11, insulin dose 0.77 ± 0.31 U / kg / day; at 4 years (50) 7.82 ± 1.55, insulin 0.87 ± 0.29 U / kg / day<, at 6 years(40) 8.02 ± 1.54, insulin 0.87 ± 0.27 U / kg / day. About treatment; 61 patients (65%) receive insulin glargine and lispro at meals, 31 (33%) NPH insulin and lispro in three doses; two patients (2%) have insulin pump, with no significant differences in metabolic control. Conclusions: 1) The incidence of childhood diabetes has increased significantly in last years.

2) The existence of two clearly seasonal peaks.

4) There has been less cases of ketoacidosis, especially in severe forms.

5) The increase in HbA1C throughout the years.

6) The type of insulin has no effect on the diabetic control.

PO1-278 Pancreas I

A paternally inherited novel variant in the *ABCC8* gene associated with diffuse hyperinsulinemic hypoglycemia of infancy

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Background:

Hyperinsulinemic Hypoglycemia of Infancy (HHI) is the most common cause of persistent neonatal hypoglycemia. The etiology of HHI includes known loss of function mutations of the *KCNJ11* (Kir6.2) and *ABCC8* (SUR-1) genes in the beta cell ATP-sensitive K+ channels. These mutations may result in diffuse or focal disease.

Objective:

We report a case of a paternally inherited novel variant in the *ABCC8* gene causing diffuse HHI.

Case Report:

K.F. is a one year old boy who presented with a hypoglycemic seizure, BG 1.1mmol/L. PMHx revealed previous twitching episodes and tonic clonic seizures in the early morning hours which were misdiagnosed as febrile seizures. The patient had mild developmental delay. Family history was non-contributory. Physical exam was unremarkable apart from mild hypotonia. The patient required >10mg/kg/min of dextrose to maintain blood sugars >2.8mmol/L. Investigations showed non ketotic hypoglycemia, inappropriately normal insulin, low free fatty acids, normal cortisol, low GH; stimulated GH peak was 4µg/L. Abdominal U/S and MRI of the hypothalamus and pituitary gland were normal. F18- Dopa PET scan showed diffuse uptake in the pancreas. DNA molecular studies showed a heterozygous previously undescribed variant **c.2475+19G>A** in intron 20 of the *ABCC8* gene in both the patient and his father. Analysis of the *KCNJ11* gene was normal. Diazoxide, Nifedipine, and Octreotide therapies failed. Normal glucose levels were maintained with continuous NG tube feeds. The patient is scheduled to undergo subtotal pancreatectomy and the pancreatic tissue will be analyzed for loss of heterozygosity.

Conclusion:

This case is unique because heterozygous mutations of paternal origin in the *ABCC8* gene have previously been shown to cause focal disease; however our patient presented with a paternally inherited novel variant in the *ABCC8* gene associated with diffuse HHI.

PO1-279 Pancreas I

Once-daily insulin detemir in cystic fibrosis related diabetes (CFRD)

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Diabetes is common in cystic fibrosis and is associated with decline in weight and lung function. Affected children are often unwilling to have multiple daily insulin injections. We proposed that a single daily dose of insulin detemir would be anabolic, improve lung function, and be well accepted.

Eight patients (median age 13.5 yrs, range 10.7 to 17.3) newly diagnosed with CFRD were treated with pre-breakfast detemir (Levemir, Novo Nordisk) at median dose 0.1 units/kg/day (range 0.02 to 0.6) adjusted for blood glucose target range of 4-8 mmol/L (72-144 mg/dL). CFRD was diagnosed by oral glucose tolerance test in 6 asymptomatic patients (#1-6). Two (#7 and 8) had fasting hyperglycaemia, with poyluria and polydypsia. All had exocrine pancreatic

insufficiency.
After a median of 15 weeks treatment (range 5 to 47), mean weight standard deviation score (WtSDS) improved by +0.46 (95% CI, confidence interval, +0.19 to +0.74) compared with a decline -0.34 (95% CI -0.11 to -0.57) in the year prior to insulin treatment (P=0.003). Mean change in % predicted forced vital capacity (%FVC) was +7.8 (95% CI 1.0 to +15.6) compared with a decline -6.9 (95% CI -0.2 to -13.5) in the year prior (P = 0.002). Mean change in % predicted forced expiratory volume in 1 second (%FEV1) was +7.3 (95% CI 2.3 to +16.8) compared with a decline -6.9 (95% CI -0.2 to -13.5) in the year prior (P=0.005). There were no episodes of severe hypoglycaemia. Seven out of eight patients continue on once daily detemir. Patient #3 ceased insulin treatment following an improvement in his glycaemic control.

Pt	Sex	Wks on Insulin	U/kg/day	Δ Wt SDS	Δ %FVC	Δ %FEV1
				Yr Prior / Insulin Rx	Yr Prior / Insulin Rx	Yr Prior / Insulin Rx
1	M	6.3	0.13	-0.54 / +0.62	-17 / +19	-6 / +28
2	M	14.9	0.11	-0.18 / +0.07	-7 / +5	-10 / +7
3	M	15.5	0.02	-0.65 / +1.14	-21 / +9	-18 / -1
4	F	8.1	0.21	-0.06 / +0.40	-13 / -3	-5 / -8
5	M	19.6	0.07	-0.62 / +0.39	-14 / +22	+7 / +16
6	F	4.9	0.08	-0.51 / +0.17	-5 / -5	-12 / +1
7	M	47.1	0.62	-0.20 / +0.35	-10 / +7	-12 / +2
8	M	42.6	0.4	+0.07 / +0.57	-4 / +8	+1 / +13

Changes in WtSDS, %FVC & %FEV1 during insulin treatment are compared with the year prior to treatment

Conclusion: Once-daily detemir was well tolerated and resulted in significant weight gain and improved lung function.

PO1-280 Pancreas I

Mild glucose abnormalities in cystic fibrosis are preceded by poor weight gain

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Background:
Cystic Fibrosis Related Diabetes (CFRD), caused by progressive ß-cell loss, results in catabolism. It is usually diagnosed by WHO criteria, however, these were not based on CF-specific outcomes such as declining weight and lung function.
Aim:
To determine the relationship betweeen glycaemia and change in weight standard deviation score (SDS) and lung function over the preceding year.
Methods:
33 consecutive children (median age 13.1, range 10.2-18 yrs) underwent Oral Glucose Tolerance Test (OGTT) when clinically stable, as part of annual screening. 25 also agreed to Continuous Glucose Monitoring (CGM, Medtronic).
Results:
On OGTT, 18 patients (55%) had normal blood glucose (BG) by WHO criteria at 120 mins, 13 (39%) were impaired (7.8 – 11mmol/L), and 2 (6%) were diabetic (≥11.1mmol/L). None had fasting hyperglycaemia.
Peak BG occured at 30 mins in 6 patients, 60 minutes in 15, 90 minutes in 11, and 120 minutes in only one. Decline in weight SDS over the preceding yr (ΔwtSDS) was associated with higher peak BG (r=-0.41, p=0.02) and % time above 7.8mmol/l on CGM (r=-0.53, p=0.006), but not BG at 120 mins. Decline in %Predicted Forced Expiratory Volume in 1 second (Δ%FEV1) was associated with % time above 7.8mmol/l on CGM (r=-0.40, p=0.05). Decline in % Predicted Forced Vital Capacity (Δ%FVC) was associated with higher peak BG (r=-0.40, p=0.02) and % time above 7.8mmol/l on CGM (r=-0.45, p=0.02).
By Receiver Operating Characteristic (ROC) analysis, ≥4.5 % time above 7.8mmol/l on CGM detected negative ΔwtSDS with 89% sensitivity and 86% specificity (AUC 0.89, p=0.003). Peak BG ≥8.2 mmol/L gave 87% sensitivity and 70% specificity (AUC 0.76, p=0.02), while BG at 120 mins ≥11.1 mmol/L had only 10% sensitivity (AUC 0.59, p=0.41).
After excluding the 2 patients with CFRD by WHO criteria, we compared groups defined with cut-points from the ROC analysis. ΔwtSDS was significantly worse in patients with peak BG ≥8.2 mmol/L (-0.3+0.4 vs 0.0+0.4, p=0.04) and in those with ≥4.5% time above 7.8mmol/l on CGM (-0.3+0.4 vs 0.1+0.2, p=0.01). There was, however, no significant difference in ΔwtSDS comparing normoglycaemic and impaired groups defined by WHO criteria (-0.2+-0.4 vs -0.2+0.4, p=0.94).
Conclusions: Higher peak BG on OGTT and greater time on CGM above 7.8mmol/L (without CFRD) are associated with declining wtSDS and lung function, suggesting that early insulin treatment may be beneficial.

PO1-281 Systems Biology

Using a proteomic approach – protein expression patterns correlates with the growth response to growth hormone (GH) treatment in short prepubertal children

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Aim To use a pharmaco-proteomic approach to identify novel factors that correlate with the growth response to growth hormone (GH) treatment in short prepubertal children.
Background GH promotes many changes in protein expression in its target tissues. The broad range in GH response to GH treatment is due to individual variations in GH secretion and GH sensitivity. Models for predicting growth response to GH treatment have been constructed to optimize treatment.
Methods The study group consisted of 128 short GH-treated prepubertal children (39 GHD, 89 ISS) from the clinical trial (TRN 98-0198-003). Blood samples were collected at start and after 1 year of treatment. Serum protein expression profiles were analyzed by using SELDI-TOF on CM10, IMAC30 and H50 arrays. To ensure the significance of the regression analyses cross-validated regression analyses and random permutation tests were performed to find correlations between peak data and the 2 year growth response.
Results Best results were obtained when using peak data from only the CM10 array surface. At start of treatment significant correlations were found in the GHD group whereas after one year of treatment significant correlations were found in the total, the ISS and the GHD groups. Changes in peaks intensities during the first year of GH treatment correlated significantly with delta $Height_{SDS}$ 0-2 year in the total and GHD groups.

Predictive peaks against delta HeightSDS 0-2yr					
Timepoint	Group	No of peaks	R2	p	Peak (Da)
0 year	GHD	7	0.73	0.032	3160, 3318, 8767, 9135, 9642, 12872, 17390
1 year	Total	8	0.38	0.003	4470, 4628, 4793, 8817, 8885, 9019, 12872, 17146
1 year	ISS	8	0.47	0.015	3160, 4470, 6857, 8767, 8885, 9425, 12607, 12872
1 year	GHD	4	0.64	0.017	4408, 8696, 9019, 17146

Table continued on next page

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0-1 years	Total	8	0.35	0.003	4138, 8636, 8885, 9135, 9425, 14055, 28090, 29003
0-1 years	GHD	4	0.59	0.026	4138, 8817, 9019, 17262

Identification of proteins representing the specific peaks is currently ongoing using MALDI TOF.

Conclusion We show that analysis of serum protein expression patterns can be used to identify markers for growth response in short prepubertal children on GH treatment. In the future the knowledge obtained can be used for development of new clinical tools for diagnosis and GH treatment of the individual child.

PO1-282 Systems Biology

Hyponatremia in hospitalized children: review of 400 cases

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To determine the frequency, causes, and clinical significance of hyponatremia in children, we reviewed data of hyponatremic children over a 33-month period. Hyponatremia defined as serum sodium value of lower than 130 mEq/ L and it determined in 400 patients. An overall frequency of hyponatremia was 7.75 % for hospitalized children in our clinic. Two hundred and eighty children (70%) had hyponatremia on admission; other 120 children (30%) had hospital-acquired hyponatremia. While 234 patients (58.5%) were previously healthy children, 166 patients (40.1%) had chronic illnesses. Infectious diseases were the most common cause of hyponatremia on admission, and hospital-acquired hyponatremia. Eighty-three patients (20.7%) had a serum sodium concentration of lower than 120 mEq/ L 4 of them had lower than 100 mEq/ L. One hundred and five patients (25.2%) died during hospitalisation**.** Causes of hyponatremia were gastroenteritis and other acute infection diseases (48.25%) in both exitus and survivor groups. The prognosis was related to the underlying disorder and previously exiting chronic illness rather than hyponatremic state.

PO1-283 Systems Biology

Multiple endocrine neoplasia type 1 in childhood: how screening guidelines correlate with real life experience

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MEN1 is an autosomal-dominant disorder caused by mutations in the menin gene, a tumour suppressor gene. In contrast to MEN2, no correlation between MEN1 genotype and phenotype is found. MEN1 mutations appear to be non-penetrant < 8y. Guidelines suggest annual surveillance from 8-10y. Genetic testing identifies first-relatives at risk but no therapy is commenced until clinical/biochemical evidence of a disease. We describe 4 atypical cases of children with MEN1. Case 1, a 10y-old boy, had recurrent hypoglycaemia from 5y and was found to have an insulinoma diagnosed through prolonged fasting. His father was treated for hyperparathyroidism and prolactinoma. Father and son had a deletion on exon 2 of the menin gene (249-253 del GTCT). Case 2, a 10y-old girl, was referred for obesity (Ht 50th, Wt > 97th). Patient, mother (parathyroid and pancreatic adenomas) and uncle (pituitary adenoma) had a mutation in the intron 2 exon 3 splice site (IVS2-3 C>G). Initial investigation was normal. After 2y, Cushing disease was diagnosed (decreased growth velocity, weight gain, loss of diurnal cortisol variation, elevated ACTH and urinary free cortisol). Pituitary surgery was successfully performed. Case 3, a 11y-old boy, was investigated based on a family history of parathyroid adenomas and pancreatic tumours (mother, maternal grandmother), pituitary non-functioning adenoma (mother). Family screening confirmed a 2bp deletion in exon 2 (269-271 del AT). From 14y, he had intermittent asymptomatic hypercalcemia with high normal PTH. Case 4, a 12y-old girl (case's 3 sister; carrier of the same mutation)) was referred for short stature (prepubertal, Ht < 1st, Wt 5-10th). She had low IGF1 levels, normal GH hormone responses to stimulation tests, elevated FSH (144U/L) and LH (8.3U/L) and no other evidence of hormonal overproduction. Karyotype confirmed 46, X, del (X) (q22.3), a variant of Turner syndrome. In conclusion, in contrast to the care of children with MEN2 and young adults with MEN1, these 4 cases who underwent early genetic screening for menin mutation present to the paediatric endocrinologist with ethical, diagnostic and therapeutic dilemmas. The current guidelines for MEN1 need clarification regarding to screening, investigation and intervention of children 0-18y from families carrying MEN1 gene mutations.

PO1-284 Systems Biology

Proteomics in GH-treated short children – dissociation between proteins related to longitudinal growth, bone volume and bone mineralization

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Introduction Growth hormone (GH) promotes longitudinal growth and several metabolic functions including bone mineralization, anabolic and lipolytic effects. Children with GH deficiency (GHD) have lower bone mineral density (BMD). GH therapy increases BMD and bone formation markers.

We hypothesize that longitudinal growth, bone volume and bone mineralization are regulated partly by common and partly by different mechanisms, and that this could be reflected by blood protein markers.

Aim We used a proteomic approach for analysis of the protein expression pattern in serum from children at start of GH treatment to identify treatment response markers related to longitudinal growth (change in height$_{SDS}$), bone volume (change in bone area, height adjusted) and bone mineralization (change in bone mineralization content (BMC), height adjusted).

Patients and Methods The study group consisted of 128 short GH-treated prepubertal children; 39 GHD, 89 idiopathic short stature, included in the clinical trial TRN 98-0198-003.

Serum protein expression profiles were analyzed using SELDI-TOF on 3 different ProteinChip surfaces. Peak data were analyzed using cross-validated regression methods and random permutation tests to identify treatment response markers related to bone growth, both longitudinal and volume, and mineralization measured by dual energy x-ray absorptiometry (DEXA). BMC and bone area values were adjusted for height to eliminate height influence of the results.

Results At start of treatment, models including protein markers predicting the changes in BMC, bone area and height$_{SDS}$ were identified ($p<0.05$). Out of 42 identified markers, 6 markers were unique in models related to change in BMC, 19 unique in models related to increase in bone volume and 7 markers were only found in models related to increase in height, whereas 2 were found in models related to changes in BMC, bone volume and height$_{SDS}$, 5 were found in models related to change in BMC and increase in bone volume and 2 were found in models related to increase in bone volume and height.

Conclusion For the first time several unique protein markers were identified that distinguished between the longitudinal growth, the increase in bone volume and bone mineralization indicating different mechanisms involved in different biological responses to GH. These novel protein biomarkers might in the future be used for prediction of bone mineralization in response to GH treatment independent of the GH effect on height.

PO1-285 Systems Biology

A selective glucocorticoid receptor modulator improves muscle function in a mouse model for Duchenne muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) is the most common degenerative neuromuscular condition in childhood. Muscle function improves with glucocorticoid (GC) therapy but with significant side-effects. A proposed mechanism by which glucocorticoids confer their benefits is through its anti-inflammatory action. Compound A (CpdA), a Selective Glucocorticoid Receptor Modulator may improve muscle function through its anti-inflammatory properties without causing metabolic side effects because of its lack of transactivation activity. Mdx (M) and wild type C57 (C) mice were given daily intraperitoneal (ip) injections of vehicle (MV and CV), Prednisone (PNS) (MP and CP) (1.5mcg/g), and CpdA (MA and CA) (5mcg/g) between days 14 and 23 when muscle necrosis peaks. An ip injection of Evans Blue Dye, taken up by necrotic muscle cells, was given 24 hours prior to muscle strength (grip pull test - GPT) and muscle performance (rota-rod run- RRR) assessment. The mice were then anaesthetized, blood collected via cardiac puncture for fasting blood glucose measurement and cytokine profiling, and the heart, tibialis anterior, and gastrocnemii muscles collected for histology, immunohistochemistry (NF-kB p50/p65 antibodies), and protein and gene expression studies. The CV mice performed significantly better in the GPT when compared to MV, MP, and MA mice, ($p<0.0001$, $p=0.005$, $p=0.032$, respectively). CV mice also performed better than MV and MP mice on the RRR ($p<0.0001$ and $p=0.001$, respectively) but not the MA mice ($p=0.270$). Importantly, when compared to MV mice, MP and MA mice performed significantly better in both the GPT ($p=0.004$ and $p=0.035$, respectively) and the RRR ($p=0.049$ and $p=0.053$, respectively)(see table 1).

Table 1: Comparison of weight gain, grip pull test performance, and Rota-rod performance

	% Weight Gain Day 14 to Day 23		Grip Pull Strength (grams) Average of best three				Rota-rod Performance Score			
	Mean	SEM	Mean	SEM	p-value†	p-value*	Mean	SEM	p-value†	p-value*
C57 Vehicle (n=10)	44	3.4	65.1	4.8			9.4	0.76		
Mdx Vehicle (n=10)	41	2.8	34.5	3.1	<0.0001		2.0	0.87	<0.0001	
Mdx Prednisone (n=10)	42	1.8	47.9	2.5	0.005	0.004	4.6	0.87	0.001	0.049
Mdx CpdA (n=4)	42	4.0	49.1	4.5	0.032	0.035	7.0	1.73	0.270	0.053

†Compared to C57 mice injected with vehicle
*Compared to mdx mice injected with vehicle

Thus, CpdA, like PNS, improves muscle function in mdx mice. If shown to have reduced side-effects relative to PNS, CpdA may have significant therapeutic potential in DMD.

PO1-286 Systems Biology

Cross-talk between the NF-kB signaling pathway and insulin-like growth factor binding protein (IGFBP)-3 in gastric cancer cells

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Since elevated NF-κB activity has been implicated in cell proliferation, angiogenesis, invasion and metastasis, and the growth inhibitory IGFBP-3 axis is impaired in cancer, we hypothesize that IGFBP-3 axis inhibits NF-κB-induced angiogenesis, invasion, metastasis and chemoresistance in gastric cancer. The aim of this study was to characterize the molecular mechanisms and biological significance of IGFBP-3-induced NF-κB suppression in gastric cancer. Recombinant adenovirus containing human IGFBP-3 cDNA was constructed by using standard technique, and overexpression of IGFBP-3 in gastric cancer cell line was performed by adenovirus gene transfer technique. Adenoviral transduced IGFBP-3 overexpression resulted in significant suppression of IκBa and p65 NF-κB phosphorylation as well as total protein levels in a MOI(multiplicity of infection)- and infection time-dependent manner. In addition, overexpression of IGFBP-3 induced reduction of angiogenic factors, ICAM-1 and VCAM-1 expressions. Etoposide-induced growth inhibition in gastric cancer cell was intensified by IGFBP-3 overexpression, in agreement with significant suppression of IκBa and p65 NF-κB phosphorylation, ICAM-1 and VCAM-1 expression. These findings suggest that IGFBP-3 inhibit NF-κB signaling cascade which regulates critical genes involved in anti-apoptosis, angiogenesis, metastasis and drug resistance in gastric cancer.

PO1-287 Systems Biology

Endocrine abnormalities in congenital disorders of glycosylation

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Introduction

Congenital disorders of glycosylation (CDG) are a family of genetic diseases caused by defects in the synthesis of the glycan moiety of glycoconjugates or in the attachment of glycans to macromolecules. Although endocrine abnormalities were originally described as one of the most striking features of the disorder, they have subsequently received little attention.

We present a girl with delayed pubertal development and review the endocrine findings of CDG patients in the literature.

Case report

We report on a 13;7 year old girl, height 144.7 cm (-2.3 SD, Prader), weight 33.1 kg (3 kg>P3) with CDG Type IIx and without pubertal development. In her medical history we found muscular hypotonia, a dilatative cardiomyopathy, and fasting hypoglycaemia. Chromosomal analysis was 46,XX, the uterus and ovaries were prepubertal on sonography, and the MRI of the brain was normal. We found the following endocrine abnormalities: prepubertal resp. hypogonadotrophic hypogonadism, low IGF1, low TBG, low transcortin, fasting hypoglycaemia and hyperinsulinism at normoglycaemia.

Review of the literature

Endocrine findings in CDG patients in the literature are: normal puberty or pubertas tarda by bioinaktive FSH or primary gonadal insufficiency; low TBG, already in a newborn, with normal TBG function and euthyreosis; low IGF1, IGFBP-3 and ALS with sporadically high STH; low transcortin with normal cortisol; sporadically high prolactin; hyperinsulinism and lowered insulin sensitivity.

Conclusion

Endocrine abnormalities as a feature of CDG appear to be often. They mostly pertain to the pubertal development and the cortisol- and thyroxin-binding proteins, with TBG deficiency as a potential early serum marker of CDG.

PO1-288 Systems Biology

Insulin sensitivity and metabolic alterations in IGFBP-3 deficient mice

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The insulin-like growth factor (IGF) axis is an important regulator of metabolism, and recent models have confirmed the role of circulating IGF-1. However, the contribution of its primary binding protein, IGFBP-3, to metabolism is largely unknown. We characterized the effects of genetic deletion of IGFBP-3 in response to diet-induced obesity on metabolic parameters across different ages in mice. At weaning KO mice are significantly heavier, but consume less food. However both of these differences are temporal and disappear with age. KO animals exhibit elevated fasting glucose and insulin, which were exacerbated on a high fat diet. Glucose intolerance was demonstrated by IPGTT. We also observed significantly higher plasma insulin levels in KO mice even in the absence of HF feeding in 70 wk old mice, providing further evidence that HF feeding was not the sole factor in causing fasting hyperinsulinemia. Euglycemic hyperinsunlinemic clamps revealed that Glucose infusion rate was slightly elevated in KO mice; Insulin-stimulated Glucose Delivery Rate tended to be higher in KO mice and Hepatic Glucose Production was elevated in KO mice. In summary, we have demonstrated a metabolic phenotype secondary to genetic deletion of IGFBP-3. Further study of this mouse may reveal new insights into the contribution of IGFBP-3 in glucose metabolism.

PO1-289 Systems Biology

Neuron-specific enolase evaluation in healthy children and in patients with neuroblastoma

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The glycolytic enzyme enolase occurs in a variety of dimeric isoforms comprising three immunologically different subunits termed α, β, and γ. The enolase isoforms αγ and γγ, which are referred to as neuron-specific enolase (NSE), are primarily detectable in high concentrations in neurons and neuroendocrine cells as well as in tumors originating from them, the most important tumor in pediatric patients is the neuroblastoma. Scant studies reporting NSE reference ranges measured by current methods are available for the pediatric population. Aims: to determine NSE values in a healthy children population and to establish their diagnostic usefulness in patients with neuroblastoma. Subjects and methods: Serum NSE was measured by ECLIA (Elecsys 2010; Roche) in 196 healthy children (112 F; 84 M) (chronological age range: 0.05-20 ys) and in19 children with different stages of neuroblastoma (chronological age range: 0.1- 8.3 ys). Statistical analysis: linear regression model including sex and age and analysis of variance. Results: NSE serum values were not significantly different in sex (p=0.77) but were significant in age (p=0.0001). Distribution of sample percentiles are shown in the table1. The median serum values were 19.97 ng/ml for the healthy children and 23.85, 70.8, and 546 ng/ml for stages I, III, and IV, respectively. Mean levels of NSE were statistically different among stages (p<0.001). Maximum simultaneous sensitivity and specificity was reached at a concentration of 30 ng/ml and was 85%. In patients with advanced stage disease NSE levels were associated with a worse prognosis. Conclusions: Serum NSE is a good marker for diagnosis in patients with neuroblastoma and could be useful in monitoring treatment and detecting relapse, in combination with clinical assessment, other biochemical markers, and images studies.

Table 1: Percentiles of NSE according to age group

Age (ys)	n	P(05)	P(25)	P(50)	P(75)	P(90)	P(95)
0.05-3.0	53	8.8	18.27	21.77	31.23	33.38	35.90
3.1 - 7.0	45	10.8	15.20	19.55	27.00	30.85	31.28
7.1 - 11.0	56	9.39	15.78	18.77	24.77	27.80	32.01
11.1 - 28	39	2.16	8.55	16.84	22.67	26.32	27.52

PO1-290 Thyroid I

A case of aspergillus thyroiditis in a child

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Background:
Aspergillus thyroiditis is rare and is even less common in children compared to adults. Immunocompromised patients are at increased risk of developing Aspergillus thyroiditis. Patients may be asymptomatic, hyperthyroid, hypothyroid, or have a thyroid mass.
Objective:
We report a case of a 12 year-old girl with a disseminated Aspergillus infection including asymptomatic thyroiditis.
Case Report:
This 12 year-old girl presented with fever, cough, headache and joint pain. She had a previous history of neuroblastoma diagnosed and treated four years earlier, and two subsequent cardiac transplants for initial Adriamycin induced cardiomyopathy and subsequent cardiac rejection. Investigations revealed thyroid nodules along with disseminated Aspergillosis. Serum biochemistry was consistent with hyperthyroidism with negative thyroid antibodies. A pertechnetate thyroid scan demonstrated low uptake. The patient denied any symptoms of hyperthyroidism and was clinically euthyroid. A biopsy of the thyroid nodules showed branching hyphae suggestive of Aspergillus.
Conclusion:
Hyperthyroidism secondary to Aspergillus thyroiditis is rare in children. As illustrated in our case, the clinical presentation may not be consistent with the serum thyroid hormone biochemistry. The close observation of thyroid related symptoms is important in cases of disseminated Aspergillosis. However, this case emphasizes the importance of following the thyroid biochemistry and imaging even in cases without the presence of thyroid related symptoms.

PO1-291 Thyroid I

Newborns from mothers with thyroid dysfunction: risk of false negatives at neonatal screening for congenital hypothyroidism

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Newborn screening for congenital hypothyroidism (CH) has been widely adopted as early detection and treatment of CH to prevent neurodevelopmental deficits. Thyroid dysgenesis is the most frequent cause of CH but maternal thyroid dysfunction in pregnancy is also correlated with CH and brain damage is found when thyroid hormone deficiency occurs during brain development and the severity is related to the degree and timing of maternal T4 deficiency.
The aim of the study was to evaluate the impact of maternal thyroid dysfunction on neonatal screening for CH and the risk of false negatives.
In the Emilia Romagna program we screened 118,517 neonates born during the period 2006-2008 by measuring blood spot TSH levels (cut off < 12 μU/ml until 1st October 2007 and then cut off < 10 μU/ml): there were 72 neonates with CH (1: 1646 newborns) and in 11 cases (15.27%) the mother presented thyroid dysfunction (5 mothers with hypothyroidism or hypothyroxinemia, 3 with autoimmune thyroiditis and 2 with Grave's disease). In the same period we examined 20 neonates with normal TSH level at the screening but born from mothers with thyroid dysfunction (9 mothers with hypothyroidism or hypothyroxinemia, 4 with autoimmune thyroiditis and 7 with Grave's disease); 6 neonates started substitutive therapy with L-thyroxine. This group was studied because the presence of maternal thyroid disfunction that often remain unknown during pregnancy.Conclusion: Also if neonatal screening programs for CH have changed the outcome we must consider the possibility of false negatives. In fact newborns from mothers with thyroid pathology should be carefully monitored in the first weeks of life also when TSH results normal at the screening test. Our study underlines the importance of thyroid function evaluation during pregnancy. Actually there is not a maternal thyroid screening in pregnancy in our country and the number of false negatives at neonatal screening for CH could be higher.

PO1-292 Thyroid I

Are current guidelines for monitoring infants with congenital hypothyroidism appropriate?

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Background: Euthyroidism in the first few years of life is integral to optimal neurodevelopmental outcome in congenital hypothyroidism. Current guidelines indicate that thyroid status should be monitored every 1-2 months between 0-6 months and every 2 months between 6-12 months. However, there is a paucity of data to support these recommendations.
Objectives: We examined whether current recommendations for thyroid status monitoring are adequate in the first year or whether monthly monitoring is necessary throughout the first year.
Subjects and Methods: We retrospectively reviewed charts of 70 children with congenital hypothyroidism, and collected data regarding initial TSH, frequency of follow-up, dose changes and thyroxine and TSH levels in the first year. The following criteria were used to indicate need for monthly monitoring: (i) dose change within a month of a previous visit; (ii) total/free T4 levels not in the upper half of the normal range within a month of a previous visit, (ii) any elevated TSH within a month of a previous visit associated with total/free T4 not in the upper half of the normal range, or (iii) TSH more than twice the upper limit of normal two months after a visit (if monitoring was every other month) associated with a total/free T4 not in the upper half of the normal range; and (iv) any TSH below 0.1 uU/ml.
Results: Monthly monitoring was justified in 75% of children in the first 6 months of life, and in 35% for the next 6 months. For the second 6 months, 76% of children who appeared to require monthly monitoring and 64% not requiring monthly monitoring had thyroid dysgenesis (p not significant). Children requiring monthly monitoring in the second 6 months had higher baseline TSH (326.2±59.1 versus 191.7±26.0 µU/ml, p=0.02) and lower baseline total T4 (5.6±0.6 versus 7.8±0.6 µg/dl, p=0.03) than those not requiring monthly monitoring. In contrast, starting dose of levothyroxine, gender and race did not predict requirement for monthly monitoring. Overall, 30% of patients in the first and second 6 months had a high TSH with a total or freeT4 not in the upper half of the normal range.
Conclusions: Many children with congenital hypothyroidism require monitoring monthly in the second half of the first year, rather than every other month, based on study criteria. Predictors of need for monthly monitoring include lower T4 and higher TSH at baseline. Current guidelines for thyroid status monitoring may need to be re-examined.

PO1-293 Thyroid I

Hyperthyroidism caused by a novel activating germline mutation of the thyrotropin receptor gene, case report and assessment of the literature

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Objective: The *de novo* occurrence of activating germline thyrotropin receptor (TSHR) gene mutations has been reported previously as the cause of sporadic non-autoimmune hyperthyroidism in 16 children. We report the case of a Hungarian boy carrying a novel constitutive activating germline mutation of the TSHR gene and we present the assessment of 42 cases from the literature with sporadic or familiar activating germline mutations of this gene.
Design: Case-report. The patient presented with thyreotoxicosis at the age of ten month. As his thyroid autoantibodies were negative our diagnosis was non-autoimmune hyperthyroidism. Until the age of 11 years he was treated with antithyroid drug, but then he underwent a thyroidectomy because of a large goiter compressing the trachea. After a 2-months hypothyroid period, hyperthyroidism appeared again. At the age of 15 years he was treated with 400MBq radioiodine therapy because the recurrent goiter caused trachea compression and consequently hampered respiration. Since then he is in euthyroid state and passed off normal puberty.
Main outcome: Molecular biological analysis of the *TSHR* gene was carried out. The direct genomic sequencing of the DNA obtained from peripheral blood leukocytes and thyroid tissue showed a heterozygous substitution of adenine to cytosine at position: 1888, causing a change of isoleucine to leucine at amino acid position 630.
Conclusions: Our patient is the first case harboring the I630L germline mutation of the *TSHR* gene associated with non-autoimmune hyperthyroidism. Earlier this mutation was characterized as a gain of function mutation and was identified as a somatic mutation in thyroid nodules.

PO1-294 Thyroid I

Phthalate exposure and adverse effects on growth factors and thyroid hormones

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Background:
Animal studies have shown effects of phthalates on growth and thyroid hormone levels. We aimed at assessing the influence of exposure to phthalates on growth parameters and plasma levels of thyroid hormones in children.
Materials and methods:
846 prepubertal children (342 girls), aged 4 – 9 years, in whom postnatal longitudinal growth data were examined, participated in the study. Determinations of plasma TSH, T4, free T4, T3, free T3, IGF-I and IGFBP-3 concentrations were performed, and metabolites of phthalates (MEP, MBP, MBzP, MCIOP (the major metabolite of DINP) and metabolites of DEHP (MEHP, MEHHP, MEOHP, MECPP)) were determined in urine samples by LC-MS/MS. Data were analysed by multiple regression analyses, including sex and age as covariates.
Results:
Concentrations of all phthalate metabolites were associated with impaired growth and negatively affected hormone levels in children. Height SDS (HSDS), difference between child and midparental height SDS ($\Delta HSDS_{corr}$), change in height SDS between 0 and 3 years ($\Delta HSDS_{0\text{-}3yrs}$) and between 1.5 years and current examination ($\Delta HSDS_{recent}$) as well as IGF-I and IGFBP-3 were negatively correlated with concentrations of all phthalate metabolites. In addition, all urinary phthalate metabolites were negatively associated with T3 and free T3 (but not TSH and T4).
We found highly gender-specific results. In boys, IGF-I and IGFBP-3 were negatively associated with DEHP-metabolites and with MCIOP. Accordingly, $\Delta HSDS_{recent}$ was significantly negatively associated with DEHP-metabolites. In girls, HSDS, $\Delta HSDS_{corr}$ and $\Delta HSDS_{0\text{-}3yrs}$ were highly significantly and negatively associated with all phthalate metabolites, as were T3 levels. Free T3 levels in girls were negatively associated with MEP only.
Conclusions:
Our data indicate a potential adverse effect of current phthalate exposure on growth, IGF-I and thyroid hormones in healthy children. The associations were markedly gender-specific as negative correlations between phthalate exposure and IGF-I as well as $\Delta HSDS_{recent}$ were seen only in boys, whereas negative associations between phthalate exposure and thyroid hormones were seen mainly in girls. The deleterious effects of phthalate exposure on current hormone levels may be acute. However, the effects on previous height changes suggest that measurement of current phthalate exposure to some degree reflects overall childhood exposure.

PO1-295 Thyroid I

Is over-treatment a greater threat for cognitive development in congenital hypothyroidism than under-treatment?

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Objectives: To evaluate the influence of initial and post-initial under-treatment and over-treatment on cognitive development in preadolescent children with congenital hypothyroidism (CH), in order to optimise therapy and eventual outcome.
Study design: In a longitudinal study 61 CH patients (27 severe, 34 mild CH) were psychologically tested at 1.8, 6 and 11 years. Initial (mild/severe CH, day start therapy, initial dosage of *levo*thyroxine) and post-initial treatment factors (under-/over-treatment, defined as ≥ 4 periods with fT_4 concentrations < –2 SD/> +2 SD the individual fT_4 set points), were related to the results of neuropsychological tests. All results were corrected for socio-educational level and ethnicity.
Results: Full scale standardized IQ of the CH children at 11 year was comparable to that of the controls (n=167) (97.1±13.5 *vs.*100±13.1). High initial dosages *levo*thyroxine (≥9.5 µg/kg) led to higher development scores at 1.8 years (107±16 *vs.* 101±12), but eventually at 11 years to lower IQ scores than lower initial dosages (<9.5 µg/kg) (94±14 *vs.* 100 ± 13) (F=4.4, p=.041). Fast normalization to the euthyroid state after birth yielded similar IQ scores at 11 years than slow normalization. Late institution of therapy (≥13 days) led throughout the study to lower development scores than early therapy (<13 days) (F=9.1, p=.004). Over-treatment during the first four years resulted in high IQ scores at six years (109±13 *vs.* 105±12 for the under-treated children and 100±14 for the adequately treated patients), but thereafter to a loss of about 16 IQ points at 11 years, significantly more (p=.007) than that found for the under-treated and adequately treated patients (5 and 3 IQ points respectively).
Conclusions: Our study suggests that initial high dose treatment must be considered as a form of over-treatment. Initial and post-initial over-treatment at first leads to a stimulation of brain development, but thereafter to a considerable loss of cognitive potential. The study suggests that in CH (mild) hypothyroidism during treatment is less damaging for the cerebral development than (mild) hyperthyroidism. Our finding that higher initial dosages of *levo*thyroxine lead eventually to lower IQ scores than lower starting regimens is supported by literature data on longitudinal studies from other centra (UK, New England, Toronto, Quebec). Therefore, we suggest to start substitution with a low dose of *levo*thyroxine, obviously as early as possible.

PO1-296 Thyroid I

Analysis of serum levels of ghrelin and obestatin in adolescents with Graves' disease and Hashimoto's thyroiditis

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Thyroid disease is leading to a change of weight – in hyperthyroidism body mass is reduced, but in hypothyroidism it is increased. Recently researches suggest that many new bioactive substances, like ghrelin and obestatin, play a role in regulation of body mass. These closely related hormones have different effects- ghrelin increases, but obestatin decreases the appetite.
The aim of the study was to evaluate ghrelin and obestatin levels in young patients with untreated Graves' disease, subclinical Hashimoto' thyroiditis and in children with nodular goiter in euthyroid clinical state.
The study group formed 78 patients of the 2nd Department of Children's Disease (Medical University in Bialystok) and Outpatient Endocrinology IHC in Warsaw suffering from Graves' disease (29 girls and 2 boys; aged from 6 to 21- mean 15,2 yrs) and Hashimoto's thyroiditis (29 girls and 3 boys; aged from 9 to 18- mean 14,5 yrs). The control group consisted of children with nodular goiter (euthyroid) – 13 girls and 2 boys; aged from 9 to 18 –mean 14,8 yrs. In all patients, ghrelin and obestatin levels were analyzed by–RIA' method (Phoenix Pharmaceuticals, USA).
In children and adolescents with hyperthyroidism in Graves' disease we found lower levels of ghrelin compared to group of children with nodular goiter and with subclinical hypothyroidism in Hashimoto's thyroiditis (123±23 vs 151±45; vs 140±36 pg/ml, p<0,02, NS). On the other hand obestatin levels was lower in children with untreated subclinical hypothyroidism in Hashimoto's thyroiditis compared to a group with nodular goiter or Hashimoto's thyroiditis in euthyroidism (203,28±59 vs 222,49±49; 267,24±70 pg/ml, p<0,03, p<0,02). In a group of untreated hyperthyroidism in Graves' disease we found relationship between ghrelin and fT3 (r= -0,36, p<0,4) and fT4 levels (r=- 0,45, p<0,01). In conclusion, we suggested that disturbances in thyroid hormones in thyroid diseases have an essential effect on changes of hormones controlled appetite: ghrelin (in hyperthyroidism) and obestatin (in hypothyroidism).

PO1-297 Thyroid I

Safety and efficacy of thyroidectomy in pediatric patients at a high-volume thyroid surgery center

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OBJECTIVE: Recent data suggest a 15% major complication rate following thyroid surgery in children, when surgery is not performed by high volume endocrine surgeons (defined as >30 thyroidectomies per year). We assessed surgical outcomes of patients undergoing thyroidectomy for management of Graves' disease at a high volume endocrine surgery center. METHODS: Between 2006 and 2009, about 1,100 thyroidectomies were performed at Yale University, of which 60 were performed for the treatment of Graves' disease in children and adults. We reviewed all pediatric cases undergoing thyroidectomy for Graves' disease over this period. RESULTS: Fifteen patients had total thyroidectomy for the treatment of Graves' disease. 64% of patients were female, and the median age was 5 years (range 3 to 16 years). Preoperatively, patients were treated with saturated potassium iodine solution for 10 to 20 days. Some patients were treated with calcitriol beginning three days before surgery. In patients not receiving preoperative calcitriol, the length of post-operative stay was 1.3 ± 0.4 days, and 60% of patients experienced hypocalcemia (<8.0 mg/dl), requiring intravenous calcium infusions. No patient receiving preoperative calcitriol required intravenous calcium infusions, and all patients were discharge one day after surgery. No patient developed thyroid storm, bleeding complications, recurrent laryngeal nerve neuropraxia, or permanent hypoparathyroidism. CONCLUSONS: We show that at a high volume endocrine surgery center, total thyroidectomy is an effective treatment of Graves' disease for young pediatric patients. Total thyroidectomy has a 60% incidence of transient hypoparathyroidism in young children, which can be prevented with preoperative calcitriol therapy.

PO1-298 Thyroid I

Abnormal thyroid function tests – could it be thyroid hormone resistance?

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INTRODUCTION
Thyroid Hormone Resistance (THR) is an autosomal dominant condition with an incidence of 1 in 40,000 people. This case series illustrates how variable the presentation may be and that affected individuals may be asymptomatic.
BACKGROUND

THR occurs because of reduced end organ response to circulating thyroid hormones. This condition is characterized by elevated concentrations of free thyroxine (FT_4) and free tri-iodothryonine (FT_3) *with inappropriately normal or slightly elevated Thyroid Stimulating Hormone (TSH) levels*. THR may be due to a Thyroid Receptor (TR) defect, usually the TRβ subtype.

CASE 1

A 6-year-old female presented with significant diarrhoea, failure to thrive and palpitations. Examination revealed a small goitre without eye signs. Thyroid Function Tests (TFT's) revealed an elevated FT_4 with a normal TSH. Parental TFT's were normal. Genetic testing confirmed THR. She is currently controlled with a regimen of maintenance 3,3,5 triiodo-thyroacetic acid (TRIAC) along with propylthiouracil and a β-blocker for exacerbations.

CASE 2

A 14-year-old girl had abnormal TFT's (elevated FT_4 with a normal TSH) detected when she was assessed by neurology services because of learning, co-ordination and socialisation problems. Examination revealed no goitre or eye signs. Genetic testing confirmed THR and her mother also carried the same genetic mutation. As she was asymptomatic from a thyroid viewpoint no treatment was commenced.

CASE 3

This 11-year-old boy is a younger sibling of Case 2 and was identified through screening once his sister and mother were confirmed to carry a TRβ gene mutation. TFT's were characteristic of TRH and genetic testing confirmed the diagnosis. No treatment was required as he was clinically asymptomatic.

CASE 4

A newborn was noted to have an elevated TSH on Newborn Screening. TFT's were consistent with THR (elevated FT_4 with a slightly elevated TSH). She was monitored for a number of days but remained well with no thyrotoxic symptoms. Her examination was normal. Genetic testing confirmed the diagnosis of THR. She currently requires no treatment.

CONCLUSION

Thyroid Hormone Resistance is a rare condition in the paediatric population and may present at any age. Patients may show clinical features of hyperthyroidism or may be asymptomatic. Treatment is dictated by symptoms. Genetic testing is now available for this condition. Once detected other family members should be screened with TFT's.

PO1-299 Thyroid I

TSHR gene analysis in paediatric subjects with NASH (non autoimmune subclinical hypothyroidism) not selected by neonatal screening

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Mutations in thyrotropin-stimulating hormone receptor (TSHR) are notoriously associated with congenital hypothyroidism, as well as with non autoimmune subclinical hypothyroidism (NASH), a mild form of THS resistance, that is not so well characterized by diagnostic procedures and clinical follow up. For this reason we performed the analysis of the TSHR gene in 38 children (0.5-18.0 years), normal at neonatal screening, affected by NASH (follow-up from 1 to 11.5 years) and enrolled in a hormonal and auxological follow-up in our Centre.

11 different mutations of the TSHR gene were identified in 11/38 patients, all resulted heterozygous. Two mutations resulted new: the nonsense mutation C31X, and the missense mutation P68S, that shows a decrease of TSH binding capacity but not in biological activity, this mutation is currently published with similar results. Eight mutations are known missense mutations and one is a seven nucleotides deletion in exon 5 causing the I152fsX157 frame-shift mutation. This mutation and D403N and M527T mutations were previously described only once in an Italian study. In all cases the carrier relatives were identified.

Currently, it is the highest prevalence (29%) of TSHR gene mutations in children and adolescent with NASH reported. Our data showed that patients carrying a TSHR mutation seems to have a more severe phenotype than patients without mutations although data are not significant (37.5% vs 29.6% in therapy: 3/3 vs 1/3 with hypoplastic gland); we confirmed the presence of phenotypic variability in patients with NASH due to TSHR gene mutations and the difficulty of clinical management.

Functional studies show that not all the mutations seem to cause an inactivation of the TSH receptor, this reveals a possible limit of the *in vitro* studies or of the knowledge of mechanisms involving TSHr, or that other candidate genes must be considered. Following these considerations a mutation classification is proposed.

PO1-300 Thyroid I

Reproductive outcome in girls with congenital and acquired autoimmune thyroid pathology

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Background. Abnormal menstrual patterns have been reported in adult women with thyroid pathology, however there are few data in literature on menstrual and gonadal function in adolescent and young adult patients followed in paediatric centers .**Objective**. To evaluate the reproductive outcome in 2 groups of patients diagnosed and followed in our center for congenital or autoimmune acquired thyroid pathology and in a normal control group. **Study design**. 25 girls with congenital hypothyroidism (CH) diagnosed by neonatal screening (CA 17.4 ± 3.3 yrs; Group 1), 44 girls referred for autoimmune thyroiditis (AT) (CA 16.5 ± 3.3 yrs; Group 2) and 42 age-matched normal controls (Group 3) underwent a physical examination, a semistructured interview to evaluate gynecologic history and pelvic ultrasonography (US). All girls were subdivided according their gynecological age (GA) ≤ or > 6 years. **Results.** The age at menarche was not significantly different in the groups examined and was comparable with maternal age at menarche. Table 1 reports the menstrual patterns in all subjects examined.

Menstrual function in subjects examined

	n°	Age (Years)	Years after menarche	Girls reporting regular menses	Girls reporting polimenorrhea
GA ≤ 6 yrs					
Group 1	15	15.4 ± 2.2	2.3 ± 1.6	12 (80%)	1 (7%)
Group 2	32	14.9 ± 1.9	2.8 ± 1.8	19 (59%)	4 (12%)
Group 3	23	14.3 ± 2.6	2.1 ± 1.8	13 (56.5%)	4 (17%)
GA > 6 yrs					
Group 1	10	20.7 ± 1.9	9.2 ± 2.0	7 (70%)	2 (20%)
Group 2	12	20.6 ± 2.6	8.8 ± 1.7	10 (83%)	1 (8%)
Group 3	19	20.2 ± 1.3	8.4 ± 1.3	14 (74%)	0

Among the girls with GA > 6 years the percentage of girls reporting polimenorrhea was greater in pathologic than in control subjects. In Group 2 menstrual patterns and US results did not differ significantly according to the age at diagnosis (prepubertal or pubertal) and the thyroid functional status (eu- or hypothyroidism). Uterine (53.1 ± 18.8 ml Group 1; 48.4 ± 13.6 ml Group 2; 49.6 ± 14.6 ml Group 3) and mean ovarian volume (6.7 ± 3.4 ml Group 1; 7.2 ± 4.4 ml Group 2; 6.8 ± 3.6 ml Group 3) were within normal ranges and comparable in all groups of girls with GA > 6 years.

Conclusions. Neither congenital nor acquired autoimmune thyroid pathology seems to significantly affect menstrual patterns and US results in adolescent and young adult patients.

PO1-301 Thyroid I

Phenotypic variablity of Pax8 mutations in congenital hypothyroidism: role of additional TSH receptor mutations?

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Molecular basis of congenital hypothyroidism (CH) due to thyroid dysgenesis (TD) involved up to now 5 genes Pax8, FOXE1/ TTF2, Nkx2.1/ TTF1, Nkx2.5 and the TSH Receptor (TSHR). Human mutations have been identified in each of them either in sporadic or familial cases. Several mutations of Pax8 and TSHR have been reported in isolated thyroid hypoplasia or dysfunction whereas mutations in FOXE1 and Nkx2.1 where reported in syndromic TD patients only. Therefore, we aimed to search for Pax8 mutations in a large cohort of isolated CH patients and to test the Pax8/ TSHR digenism hypothesis. Patients and methods: 118 CH patients were analysed by direct sequencing of the binding domain of Pax8 (exons 2 to 4). In the Pax8 mutated patients, we then sequenced the 10 coding exons of the TSHR. Results: A total of four Pax8 heterozygous mutations were identified in eight patients; The previously described R31H mutation was found in one parent affected by severe thyroid hypoplasia and one child with radiologically proven ectopy in 2 different families (n=4). Three novel missense mutations were identified in 4 sporadic CH cases with normal thyroid gland: the R31C de novo mutation (n=2), the P2T (n=1) and the I47T mutation (n=1), which was inherited from the mother with subclinical hypothyroidism. All these 3 mutations were located in the DNA binding domain and functional studies confirmed the alteration of the transativation's capacity of the R31C and I47T mutated proteins on the human thyroperoxydase enhancer/promoter. Among the eight patients with Pax8 mutation, extrathyroidal malformation was found in one patient who displayed unilateral kidney agenesis. TSHR analysis revealed 2 novel heterozygous mutations in 3 out of the 8 mutated patients, affected by either athyreosis, ectopy or normal sited gland with minor radiological distinctive features. Conclusion : Pax8 mutations are rare in CH patients but could appear with a range of phenotypical variation, including for the first time, radiologically proven ectopic thyroid gland. The combined Pax8/ TSHR heterozygous mutations found in 3 cases raise the question of the role of additionnal TSHR mutation and may suggest a digenism.

PO1-302 Thyroid I

Neonatal hypothyroidism secondary to congenital nephrotic syndrome of the Finnish type – case report

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Introduction: Congenital nephrotic syndrome (CNS) is a severe and rare nephropathy, being the Finnish type the more frequent presentation. It is a hereditary disease with autosomal recessive inheritance and it is characterized by intense proteinuria beginning in fetal period. Hypoalbuminemia, edema and hyperlipemia always appear from birth up to the third month of life. In this situation, hypothyroidism appears frequently early in life, due to urinary loss of thyroid hormones.

Case report: 3 months of age, female, consanguineous healthy parents, born at term, birth weight 2,445g, length 44cm, small for gestational age. She had a brother with CNS of the Finnish type, confirmed by renal biopsy, who died on his 26th day of life. On her 2nd day of life, she presented edema and laboratory examinations showed proteinuria (+++), hypoalbuminemia (1.8g/dL, reference 3.4-4.8) and hypercholesterolemia (8.6mmol/L, reference < 4.4). She developed intense proteinuria (433mg/kg/day) and at that time it was started enalapril (0.2mg/kg/day) and nutritional support measures were reinforced. Mean weight gain in the first 15 days was 42g/day (mainly due to edema). On day 15, there was a more severe hypoalbuminemia 1.3g/dl and thyroid evaluation showed T4: 21.9nmol/L (reference 64-154); Free T4: 7.7pmol/L (reference 9-26); TSH: 4.51 mU/L (reference 0.8-6.3); thyroxine-biding globulin -TBG: 5.4mg/L (reference 7.3-15); other evaluations: total cholesterol 7.7mmol/L; triglycerides 1.6mmol/L (reference < 1.24); BUN 24mg/dl; creatinine 0.02mg/dl. It was started levothyroxine in a dose of 10mcg/kg/day, which had to be subsequently increased up to 17mcg/kg/day due the urinary loss of thyroid hormones. The patient has presented appropriate neuropsicomotor development.

Comments: Definitive treatment for CNS of the Finnish type is renal transplantation, which is expected to be performed when the child reaches appropriate weight and length. Since development of hypothyroidism tends to occur early and fast, thyroid status evaluation must be started in the first week of life. This way, hypothyroidism diagnosis and treatment onset happen opportunely, so avoiding its neurological and other systems consequences as well as more comorbities for these infants.

PO1-303 Thyroid I

Accuracy of using early I^{123} uptake results for I^{131} ablation dose calculation in children with Graves' disease

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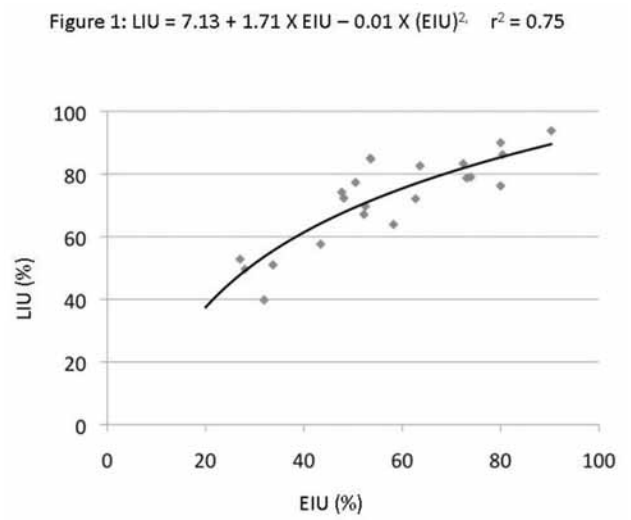
Background: In children with Graves' disease, I^{131} ablation has been increasingly used successfully as first line treatment in many centers. In adults, early radioiodine uptake with I^{123} at 4-6 hours (EIU) has replaced 24 hour uptake (LIU) for diagnosis and I^{131} ablation dose calculation.

Aim: To compare the accuracy of I^{123} EIU and LIU in calculating the I^{131} ablation dose in children with Graves' disease.

Methods: A total of 22 children with Graves' disease (age range: 10-17 years, mean: 13.9 $\pm$ 2.0 years) seen in the Helen DeVos Children's Hospital Pediatric Endocrinology Clinic between January 2005 and November 2008 were reviewed. The patients included in the study had a thyroid uptake following oral administration of 1 mCi of I^{123} at both 4-6 hours (EIU) and 24-26 hours (LIU) and all underwent thyroid ablation with a therapeutic dose of oral I^{131} following the uptake. The therapeutic doses were calculated based on LIU. The therapeutic dose which would have been calculated based on the EIU was compared to the actual dose.

Results: The mean I^{123} EIU was 57.1 $\pm$ 18.2 % and LIU was 72.1 $\pm$ 14.4 % ($p<0.05$). The curvilinear regression analyses of I^{123} EIU and LIU are shown in Figure I. The mean therapeutic dose of radioactive iodine given based on LIU was 9.3 $\pm$ 2.0 mCi and the mean calculated therapeutic radioactive iodine dose based on EIU would have been 12.4 $\pm$ 2.0 mCi ($p< 0.05$). All patients treated with I^{131} using LIU were treated successfully and became hypothyroid in 2-3 months.



Conclusions: In children with Graves' disease, I^{123} LIU is preferred over EIU for I^{131} ablative dose calculation, since the EIU can be lower and may be misleading. Using I^{123} EIU might lead to the use of higher calculated doses than needed in children.

PO1-304 Thyroid I

Serum thyroid hormones (TSH, T3, T4 and FreeT4) (TH): uncertainty in measurement (U), reference change value (RCV) and reference range in a pediatric population

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Primary care pediatricians often tend to screen for thyroid function abnormalities in children and adolescents with various non-specific complaints. U is a range of values containing the true value. RCV is useful for knowing when a result is different from a previous one. References ranges are necessary to understand laboratory results in individuals or in diagnosis-related groups(DRG). The Aims were to calculate U and RCV of TH, and to establish reference ranges as a function of age, in a pediatric population.

Laboratory data and clinical information of the data base of the Endocrinology Laboratory, between 2003 and 2007. Thyroid function was studied in 29901 children and adolescents. Patients with altered clinical and laboratory records were excluded. Data of 7581 children remained in the study, with the following age and n distribution: Gr1,0-3 months (m):241; Gr2,3-6 m:144; Gr3,6 m-1 year(y):274; Gr4,1-3 y:849; Gr5,3-6 y:977; Gr6,6-9 y:1266; Gr7,9-12 y:1493; Gr8,12-15 y:1256: Gr9,15-20 y:1081.

TSH, T3 and freeT4 were measured by MEIA, T4 by FPIA Axsym Abbott laboratories; ATPO and UATG by Chemiluminescence, IMMULITE; AFM and ATG by agglutination and TrAb by radioreceptor R&R. U was calculated by GUM-iso 1993 and RCV by Harris & Brown.

Results: Groups: Gr 50percentile(p)(1p-99p) reference ranges.

	TSH (µUI/ml)	T3 (ng/ml)	T4 (µg/dl)	Free T4(ng/dl)
Gr1	2.30(0.75-5.05)	1.57(0.89-2.40)	11.7(6.7-19.1)	1.34(1.01-2.08)
Gr2	2.05(0.65-4.94)	1.77(0.96-2.46)	11.3(6.7-18.8)	1.27(0.85-1.81)
Gr3	2.37(0.69-5.03)	1.71(0.96-2.38)	10.3(6.2-16.7)	1.26(0.81-1.90)
Gr4	2.46(0.70-5.70)	1.69(0.91-2.37)	10.3(6.5-16.1)	1.27(0.83-1.93)
Gr5	2.49(0.76-5.72)	1.62(0.99-2.27)	9.9(6.7-16.2)	1.29(0.91-1.94)
Gr6	2.36(0.75-5.30)	1.55(0.91-2.36)	9.7(6.5-13.5)	1.26(0.84-1.81)
Gr7	2.38(0.74-5.21)	1.50(0.77-2.12)	9.2(6.1-13.1)	1.24(0.81-1.96)
Gr8	2.28(0.63-5.00)	1.38(0.70-2.18)	8.3(5.3-12.6)	1.16(0.74-1.78)
Gr9	1.96(0.45-5.74)	1.20(0.69-1.88)	8.2(5.2-13.5)	1.15(0.71-1.94)
U (%)	20.09	19.52	18.83	20.67
RCV (%)	58.60	34.69	28.31	33.98

Conclusions: We are describing for the first time U and RCV apply in this methodology for TH. However futures studies will be required to establish the utility of these tools. We are presenting reference ranges and p by age for TH.

PO1-305 Thyroid I

Congenital hypothyroidism (CH) with goiter: findings in Argentinean patients

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CH with goiter may be caused by altered iodine orgnaification (IOD) due to defects in thyroid peroxidase (TPO) and less frequently in dual oxidase 2 (DUOX2) and mutations in the thyroglobulin gene (TG) originate defective Tg. We report the findings in 19 patients referred to our Endocrinology division with goiter and hypothyroidism (high TSH levels).All were grouped according to TG levels and Perchlorate discharge test(PDT) in G1: high TG levels and PDT >10% (n:9) that were assigned to the molecular study for IOD and G2 (n:10) low TG levels and PDT <10% who underwent molecular studies for TG defects.

All exons and intron exon boundaries of the TPO and DUOX 2 genes were sequenced in G1

IN G2 the promoter, complete coding and flanking regions of the TG gene were sequenced in 7/10 and in 3/10the exons with aberrant SSCP were sequenced

Results: G1: 6/9 patients harboured mutations in TPO gene : 2 compound heterozygous (pN129fsX208/pG387R; pQ72fsX86/pC808R) and 4 simple heterozygous (2 pN307T pP499L, pR396fsX472) 3 patients showed 4 different mutations in DUOX 2 gene as compound heterozygous (pQ36H/pS965fsX994, pG418fsX482/skipping of exon 20). G2: 3 patients were homozygous :1 for mutation R277X and 2 for pA2215D, 3 compound heterozygous (pR277X/R1511X in 2 and pR2223H/p R2317X) and 2 simple heterozygous pR277X.

Conclusion: Measurements TG and PDT were useful tools to characterize patients with CH and goiter optimizing the molecular approach Our findings an further investigation on genotype- phenotype relationships will probably help to explain differences in outcome and to ensure adequate care of these patients

PO1-306 Thyroid I

Thyroid disorders detected through primary congenital hypothyroidism (CH) neonatal screening

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Between 1985 and 2009 our neonatal CH screening program tested 1.155.496 newborn. Till 1997 an incidence of 1:3441 permanent CH and 1:15.069 transient were found. TC99 scan at neonatal confirmation or reevaluation at age 2 identified. 36% athyreotic, 46% ectopic and 18% with normally located thyroid (eutopic) CH patients.

Objective: To determine the proportion of thyroid disorders detected by neonatal screening since 1997 till 12/08.

Patients and methods : As screening was based on TSH determination on filter paper samples with IFMA method using 15 mU/l as cutoff level till 12/03 and 10mU/l thereafter, results were referred to 97-02 (P1) and 03-08(P2)

Results : In P1 from 310.755 newborn screened 13 transient CH (1:23.904) and 107 definitive CH (1:2.904) were detected. 28/107 remained unclassified and of the remaining 79: 24(30%) were athyreotic, 37(47%)ectopic and 18(23 %)eutopic

In P2 out of 342579 newborn studied 15 were transient (1:22.839) and 142 permanent CH (1:2.412). 31/142 remained unclassified and from the 111 studied 22(20%) were athyreotic, 47(42%) ectopic and 42(38%)eutopic. In P2, 14 of the 157 CH newborn had TSH between 10 and 15 mU/l (8/42 eutopic, 1/47 ectopic and 5/20 transient hypothyroids.

Conclusion Permanent CH is prevalent in our population with disgenesis as the mayor ethiologic feature. The lower incidence of athyreosis might be explained by more accurate techniques of thyroid imaging while the increasing

incidence of eutopic thyroid disorders is only partially explained by the lower TSH cutoff level used for detection. Other variables as the impact of genetic factors may be considered when analyzing this issue

PO1-307 Thyroid I

Paediatric population suffering from multiple endocrine neoplasia (MEN) type 2 in Spain: case review

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Mutations of the RET proto-oncogene (10q11.2) give rise to MEN 2. In 100% of cases, they present as Medullary Carcinoma of the Thyroid (MCT) in which parafollicular cells evolve from normal to neoplastic cells, with increased levels of calcitonin. The therapeutic recommendation is to perform a prophylactic thyroidectomy in asymptomatic relatives affected while they are still children
Subjects and Methods
Survey of members of the SEEP. Data: mutations in the RET proto-oncogene, clinical details, analytical results (calcitonin, PTH, catecholamines), age on diagnosis and prophylactic or therapeutic thyroidectomy, pathology and course
Statistics: non-parametric Kruskal-Wallis test
Results
After performing 70 determinations of the RET proto-oncogene. Four by compatible clinical signs: 2 type A (goitre 1, Hirschprung 1); 2 type B phenotype. By affected relatives 66: with 45 carriers of MEN 2A being detected (35 among children, 10 over 18 years of age) and 21 healthy individuals
MEN 2A GROUP: asymptomatic carriers 35. Prophylactic thyroidectomy in 33, details in attached Table (two patients under six months of age not subjected to surgery). The mean time from diagnosis to surgery: 1.4 ± 1.7 years (0-6.5). Age for prophylactic thyroidectomy: 6.2 ± 3.14 years (1.5-15.4).

Pathology (prophylactic thyroidectomy)	Age at diagnosis (years)	Diagnosis to surgery (years)	Age for thyroidectomy (years)	RET Proto-oncogene Mutation
Healthy tissue 7	3.5±1.9 (2-7.4)	1.2±1.2 (0-3.2)	4.6±2.1 (2-8.8)	634.72% 804.14% 618.14%
Hyperplasia 12	6±4.1 (0-14.8)	1.1±1.1 (0-3.7)	7.1±3.5 (2.1-15.4)	634.75% 609.8.3% 618.16.7%
Microcarcinoma13	4.3±1.9 (0.8-7)	1.9±2.2 (0-6.5)	6.1±3.1 (1.6-13.4)	634. 92.3%
Macrocarcinoma 1	8.5	0	8.5	634. 100%

p<0.05

Currently, 100% remain free of disease with normal values for calcitonin, PTH and catecholamines
MEN 2B GROUP: mutation 918 in both cases (100%). Therapeutic thyroidectomy (at ages 10 and 14 years), raised levels of calcitonin and metastasis persist
Conclusions. Genetic studies of relatives affected with MEN 2 makes it possible to carry out early prophylactic treatment of cases. Prophylactic thyroidectomy in MEN 2A is recommended prior to 5 years of age, depending on type of mutation, and wherever possible, even before two years of age; the recommended age in the MEN 2B group is before one year

PO1-308 Thyroid I

Attention abilities in preadolescents and adolescents with congenital hypothyroidism

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Despite a documented normal cognitive development, children with Congenital Hypothyroidism (CH), detected by neonatal screening and early treated, may show subtle mental deficits, particularly in visuospatial processing and attention abilities. A longitudinal study was performed to investigate attention performances in CH children at preadolescent and adolescent age; to analyze the influence of severity of CH at diagnosis and thyroid hormones levels at time of testing on attention skills. 21 CH patients (10 boys), positive at neonatal screening, early treated (<30 days with L-T4 dosage > 8 mcg/kg) and followed by the Pediatric Department were evaluated at the age of 8-10 and at 12-16. Age-sex matched controls were evaluated at the same ages. Neuropsychological assessment included WISC-R, MF test at preadolescent age, Continuous Performance Test (CPT) and Benton Line Orientation (BLO) at adolescent age. Scores were retrospectively correlated to the severity of CH at diagnosis (TSH, FT4, T4, time of starting therapy) and to TSH and FT4 levels at time of testing. CH patients showed normal IQ scores at 8-10 yrs (IQ 104±10.8) and at 12-16 yrs (IQ 107±15.8). Younger children's performances on visuospatial tests (MF test) were significantly poorer than controls, as described in our previous study (Blasi, 2009); on the contrary, CH adolescents showed higher visuospatial abilities (BLO mean score:23±4.8; WISC-R Performance scale IQ:106±15.7), without significant differences from controls. A higher IQ score mildly correlated with an earlier time of starting replacement therapy (p=0.02; r=0.47); higher WISC-R freedom from distractibility correlated with TSH levels at diagnosis (p=0.01; r=0.5) and at the time of performance (p=0.04; r=0.44). On the CPT, CH subjects made more commission errors than controls (p<0.05), in particular A-Y errors (p<0.05). Omission errors mildly correlated with TSH levels at diagnosis (p=0.05; r=0.58) and with the time of starting treatment (p=0.01; r=-0.5). This study confirms a normal IQ in CH patients. The suboptimal performances on visuospatial abilities at a preadolescent age showed an improvement and a normalization at an adolescent age. This is possibly related to the maturation of the alternative neuronal pathway used by CH subjects. Besides, our work highlights that CH patients made more commission errors on CPT showing impulsiveness. Further studies are needed to investigate the correlated neural mechanisms and prognostic factors.

PO1-309 Thyroid I

Recognition of congenital hypothyroidism severity and etiology based on neonatal screening data

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Congenital hypothyroidism is a avoidable cause of severe mental retardation when early diagnosed and treated.
Objective: To determine if it is possible to identify different etiologies of congenital hypothyroidism (CH) and treatment prognosis based on data from the initial TSH levels and treatment dosage.
Methods: Confirmatory TSH serum levels, etiology (based on radionuclide imaging studies), initial and current levothyroxine treatment dosage were studied in children diagnosed with CH from 1987 to 2006 at Pediatric Endocrinology Unit at Unicamp. Subjects were divided into 3 groups based on confirmatory TSH levels: 1) TSH ≤ 20 μUI/mL, 2) from 21 to 49 μUI/mL, and 3) ≥ 50 μUI/mL. This study was approved by the local ethics committe.
Results: From 134 studied children, 30 did not perform thyroid radionucleotide scanning and were excluded. From the remaining 104 subjects, the TSH was

≤ 20 in 40 (38.5%), from 21 to 49 in 17 (16.3%) and ≥ 50 in 47 (45.2%). According to the etiology, 10 (9.6%) were diagnosed as athyreosis, 20 (19.2%) as ectopic thyroid, 28 (26.9%) as dyshormonogenesis, 15 (14.5%) as hypoplastic thyroid and 31 (29.8%) had normal thyroid gland at scintigraphy. There was a significant association between confirmatory TSH levels and etiology of CH (p = 0.0001) and all patients with athyreosis had TSH values higher than 50. Confirmatory TSH values did not influence the initial thyroxine treatment dosage (p = 0.157), although the current one was significantly different according to the etiology (p = 0.005).
Conclusions: Patients with confirmatory TSH values higher than 50 µUI/mL had a more severe thyroid dysgenesis.

PO1-310 Thyroid I

Iodine deficiency prevalence in pregnant women from Palencia Area Health Service (Spain)

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Background: The relation between the state of the mother´s iodine deficiency during pregnancy and its effect in the later development of the child has been shown.
Objective: To know the iodine level in pregnant women in the province of Palencia and to value the need to make recommendations or to plan improvement actions in our health system.
Material and methods: An observational study made between October 2006 and October 2007 of a cohort of pregnant women during their first three months of gestation. A selection of 200 women from rural and urban areas was estimated. They were given a questionnaire including lifestyle and eating habits. A physical exploration was done and 24 hours urine was taken in order to measure the iodine urinary levels. To control possible confusing variants a bivariant analysis and a logistic regression were done in the statistical study.
Results: 164 pregnant women completed the study. The median of iodine urinary levels was 92 µg/L (Pc. 25-75 of 71-139 µg/L). Only 22% of the pregnant women presented iodine urinary levels higher than 150 µg/L. 8,4% of the women presented iodine urinary levels lower than 50 µg/L. 46% had taken iodized salt. The presence of goiter is observed in 37% of the women, without geographical differences (p=0,17), nor intake of iodized salt (p=0,48) or iodine urinary levels. With reference to the cut-off point of 150 µg/L in iodine urinary levels, an association was found with the variable of iodized salt, obtaining a risk estimate through Odds Ratio of 3,5 (CI 95% 1,3-9,6).
Conclusions: From our study we can conclude that in the Palencia Area Health Service 78% of the pregnant women present a iodine urinary level lower than 150 µg/L, indicating a iodine intake below recommended limits. That is why, the substitution of common salt by iodized salt in the population, specially pregnant women, should be encouraged, generally speaking and from all institutions with health responsibilities. From the first days of pregnancy a iodine supplement of at least 150 -200 µg per day should be added.

PO1-311 Thyroid I

Monoallelic inactivation of the dual oxidase maturation factor 2: a novel cause of transient neonatal hypothyroidism?

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Background. DUOXA2 (dual oxidase maturation factor 2) is required to express DUOX2 enzymatic activity, which is crucial for iodide organification. Recently, a bilallelic DUOXA2 mutation has been reported to cause congenital hypothyroidism. We report a monoallelic mutation in the DUOXA2 gene, identified in two brothers with a transient form of infantile hypothyroidism.
Case histories. The first born brother was born a term after an uneventful pregnancy. At day 5 neonatal screening showed a TSH level of 38 mU/L. At 4 weeks of age congenital hypothyroidism (TSH: > 200 mU/L; FT4: < 0.1 ng/dl) was confirmed in the presence of a highly Tc99m captating eutopic thyroid gland (thyroglobulin > 300ng/ml). No anti-thyroid auto-antibodies were present in the infant and his mother, who showed a normal thyroid function. In the second born brother, no goitre was noted at birth and neonatal screening at day 5 showed a TSH value < 15mU/L. As his brother, he was breastfed. At the age of 3 weeks thyroid function tests were done because of increasing sleepiness: primary hypothyroidism (TSH > 100 mU/L and FT4 = 0.1 ng/dl) and a high thyroidal uptake of Tc 99m (thyroglobulin> 300ng/ml) was documented. At the age of 5 years, thyroxine therapy was stopped for 6 weeks in the youngest brother, for a diagnostic perchlorate test, which turned out normal, while TSH was 11 mU/L and FT4 1.1 ng/dl at that moment. Normal FT4 and a slightly elevated TSH value (8 mU/L) were observed 6 weeks after stopping thyroxine in his older brother.
Mutational screening. No abnormalities in the IYD and DUOX2 gene were found, but a heterozygous c.535T>G, p.Tyr179Asp mutation was found in exon 4 of the DUOXA2 gene by direct sequencing. This mutation was also found in the mother, whose thyroid function remained normal at retesting. This mutation in the 4th transmembrane domain has not been described previously and needs further functional studies.
Conclusion. Monoallelic DUOXA2 mutations are to be included in the cause of transient neonatal or infantile hypothyroidism and of euthyroid hyperthyrotropinemia in childhood. In contrast to monoallelic DUOX2 mutations, they apparently do not always cause significant TSH elevations during the first week of life and might thus lead to a negative neonatal TSH screening test.

PO1-312 Thyroid I

Congenital hypothyroidism due to ectopic sublingual thyroid gland in Prader-Willi syndrome

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Several endocrine abnormalities have been described in Prader-Willi syndrome (PWS), including GH deficiency and hypogonadotrophic hypogonadism. Published data on disorders of thyroid function are very limited.
We describe a case of a girl admitted at the age of 9 months because of elevated TSH, low FT4 and normal FT3. A ^{99m}Tc-pertechnetate thyroid scintigraphy showed an ectopic sublingual thyroid gland. Thus thyroxine replacement therapy was started.
Since birth, she had a weak cry and muscular hypotonia. She had a psychomotor delay and a characteristic face. A brain CT scan revealed a ventricular enlargement and craniosynostosis.
Severe scoliosis was diagnosed at age 3. At age 5, a decrease growth velocity, an increase weight gain and a poor improvement of developmental delay were observed, despite the thyroid hormone replacement therapy.
At age 7.3, a growth hormone (GH) deficiency was diagnosed; therefore GH therapy was started (stopped at age 12.9). A brain MRI showed an empty sella with a small pituitary gland.
At age 9, PWS was suspected based on the phenotype. Genetic tests, only performed at age16 (mother refused previously), confirmed a maternal uniparental disomy of chromosome 15. She had no spontaneous menarche. At age 24.4, a bioenteric intragastric balloon (BIB) was inserted for treatment of morbid obesity losing 13 kg within 9 months.
At the last examination (25.1 years) she had: height 143.8 cm (-3.07 SDS); weight 84 kg, body mass index (BMI) 41.2 kg/m². She is on therapy with L-thyroxine, oestrogen and progesterone.
Most of the previous studies reported that thyroid function appears to be normal in most PWS patients and that there is a similar frequency of hypothyroidism in PWS subjects as in the general population. On the contrary, two other studies *(Tauber et al 2000 and Miller et al 2008)* reported a higher frequency in PWS population. However hypothyroidism in PWS seems to be mostly of

central origin.
This is the second reported case of hypothyroidism due to an ectopic sublingual thyroid gland *(Sher C, 2002)* in PWS. This case is also worth reporting to show that the diagnosis of PWS may have been delayed because mental retardation, obesity and short stature were attributed to hypothyroidism.
In conclusion PWS should be suspected in obese children with hypothyroidism who do not improve adequately on thyroid hormone treatment. Furthermore, PWS patients should have their thyroid function tested regularly.

PO1-313 Thyroid I

Thyroid and adrenocorticol status in extremely premature infants during the first five days of postnatal life

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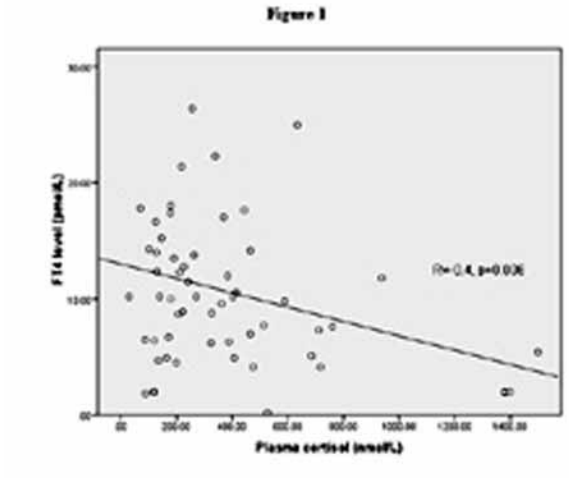
Background
Premature infants have low plasma free thyroxine (FT4) as well as transient adrenocortical insufficiency. Interactions between the thyroid and cortisol axes have been described in older children. Such an interaction could contribute to the poor neurological outcome of infants born extremely prematurely. The aim of this study is to examine the relationships between between the adrenocortical status and the thyroid status of extremely preterm infants in the first five days of postnatal life.
Method
We prospectively examined the relationships between early morning plasma cortisol, adrenocorticotrophic hormone (ACTH), TSH, FT4, gestation and clinical risk index for babies (CRIB) score within 5 days of birth in infants born below 28 weeks gestation. The relationship between plasma cortisol and FT4 and the other factors were examined. Multiple regression analysis was employed to identify the independent factors affecting plasma FT4 and early morning cortisol.
Results
There were 117 infants (69 males). Mean gestation was 25.4 weeks ± 1.3 (range 22^{+0} -27^{+6}). Mean birth weight was 812 ± 176 grams. Plasma cortisol correlated negatively with plasma FT4 (Figure 1), and gestation(r= -0.4, p=0.005).



There was no correlation between plasma cortisol and CRIB. FT4 correlated positively with ACTH (r=0.4, p=0.004), gestation (r=0.6, <0.0001) and correlated negatively with CRIB (r=-0.3, p=0.01). Multiple regression analysis examining the association with FT4 and cortisol (with CRIB,gestation, birthweight) demonstrated that gestation was the only independent factor affecting FT4 and cortisol concentrations.
Conclusion
Univariate analysis suggests that among infants born less than 28 weeks gestation there is an inverse relationship between the cortisol and thyroid axes in the first 5 days of postnatal life. However, gestation was the only independent variable associated with FT4 and cortisol concentrations. This suggests that immaturity is the dominant influence on endocrine status in these infants.

PO1-314 Thyroid I

Prevalence and treatment of childhood Graves' disease in the United States

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OBJECTIVE: To assess the prevalence and treatment practices of Graves' disease (GD) in the United States. METHOD: Data from claims data of Medicaid and commercially insured pediatric populations were used to estimate the prevalence of GD and treatment practices in children. Databases included 10.7 million children (ages 0–17 years) from 11 states with Medicaid coverage in 2003, and 4.3 million children from 50 states with commercial insurance during 2004–2005. RESULTS: GD (ICD-9-CM = 242.0) was found to be a relatively rare in children with a prevalence of approximately 1-2 cases per 10,000 children. Graves' disease accounts for about 16% of all goiter or thyrotoxicosis cases and about 5% of all disorders of the thyroid gland cases. There is a higher prevalence among females than among males (3:1). There is an increasing prevalence with age. 50% percent of children with GD were dispensed an antithyroid drug. Methimazole (MMI) was dispensed twice as often as propylthiouracil (PTU). When treatment practices over a one year period of a subset of commercially insured children (1.8 million) with a broader definition of GD (1,032 with ICD-9-CM 242.0 or 242.9) 38% received at least one treatment: 14% were found to be treated with MMI, 6% with PTU, 23% with levothyroxine, 3% with radioactive iodine, and 1% by surgery. CONCLUSIONS: These observations show that the incidence of GD (using the more specific disease definition) in the US is about 1-2 per 10,000 children. MMI is used twice as frequently as PTU. Among commercially insured youths, radioactive iodine is used three times as frequently as surgery.

PO1-315 Thyroid I

Extrathyroidal tissue mimicking a thyroid nodule

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Background: Thyroid nodules are rare in children, the incidence is estimated at 1 to 1.5% based on clinical examination, but the likelihood of malignancy is fourfold higher than in adults. The overall incidence of malignancy in thyroid nodules in children is 26.4%.
The thyroid arises from the fourth pharyngeal pouch, while the thymus develops from the third branchial pouch and migrates along the neck to reach its final mediastinal location. The thymus is composed of a variety of cells, the majority are epithelial cells and T lymphocytes. Ectopic localizations of the thymus along the thymopharyngeal pathway have been reported. Only a few intrathyroidal thymic nodules have been described in children, most were diagnosed after partial or complete thyroidectomy for suspicion of malignancy, only one case was diagnosed after fine-needle aspiration (FNA) biopsy.
Patient and Methods: We report here a case of a seven-year-old girl that presented with an asymptomatic solitary thyroid nodule. The ultrasound showed a nodule of 8 mm diameter in the left lobe and a mass of 34 mm in the left cervical region close to the thyroid. Her clinical and familial histories were unremarkable. Thyroid and parathyroid function tests were within the normal

range. The FNA biopsy of both masses showed lymphocytes without atypy and no abnormal epithelial cells. Flow cytometry indicated no polyclonal lymphocytic proliferation. A thymoma, a tumor of thymic epithelial origin, and a lymphoma were both excluded. The diagnosis of ectopic intrathyroidal thymus associated with a cervical remnant was made. The MRI of the neck and the chest confirmed the diagnosis and revealed an unexpected thyroglossal cyst. **Discussion and Conclusion**: Ectopic intrathyroidal thymus is a consequence of aberrant thymic migration during embryogenesis, as the thyroglossal cyst. Malignant tumors can arise from epithelial thymic cells presenting along the embryologic pathway. These tumors can occur in the thyroid and show several features of thymic differentiation, but they are less aggressive than thyroid carcinoma and a preoperative diagnosis can avoid an extensive thyroidectomy. In conclusion, ectopic thymic tissue should be considered in the differential diagnosis of a thyroid nodule. It is important to identify the thymic rests for what they are, but further research is necessary to understand the link between benign and malignant variants.

PO1-316 Thyroid I

Normalization of TSH serum levels in 468 neonates with congenital hypothyroidism

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Although the outcome in congenital hypothyroidism (CH) is substantially improved after introduction of newborn screening, affected children do still experience mild neuropsychologic deficits. It remains unclear whether editional genetic factors or suboptimal treatment regimen is responsible for this phenomenon. In a recent systematic literature search (Cochrane Database Syst. Rev. 2009) evidence that high dose is more benificial compared to a low dose initial thyroid hormone replacement was questioned. In this observational study treatment-related factors, e.g. age at onset of therapie, starting and subsequent dose levels and time to normalization of TSH has been evaluated. In 2000 the German working Committee of paeditric endocrinology (APE) has established a quality assurance program (AQUAPE). 468 patients with CH (303 girls, 165 boys) from 9 centres were longitudinal documented. The screening results of TSH are 37,5% < 100 mU/l, 30% 100 - 200 mU/l, 32,5% >200 mU/l. The initial dose of levothyroxine differed between between 10 - 16 µg/kg by 62% of newborn with CHT. The normalization of TSH levels occured in 51,1% within 5 - 12 days. Conclusion: (1) Initial high dose (> 10 µg thyroxin/ kg*day) normalized TSH before age 3 weeks. (2) Patients with CH should be documented in a quality assurance program (e.g. AQUAPE). (3) The longitudinal data could be used to compare the therapy of CH and the mental and psychomotor developmental outcomes.

PO1-317 Thyroid I

Congenital hypothyroidism due to thyroid dysgenesis in two infant boys with Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS, OMIM 194050) is an autosomal dominat disorder and it is characterized by a distinctive, "elfin" facial appearance, mental retardation, short stature, cardiovascular problems and hypercalcemia. Thyroid hypoplasia and subclinical hypothyroidism have been demonstrated in patients with WBS. Congenital hypothyroidism was, however, rarely reported in WBS. We present of two infant boys meeting WBS criteria associated with thyroid dysgenesis.

Patients and Methods: Case1: A 5month-old boy is presented atypical face. He has L-thyroxin treatment due to hypothyroidism since neonatal period. On physical examination typical "elfin" face and scrotal hyperpigmentation was found. Testis volumes were 1 ml. Biochemical analysis was in normal limits except hypercalcemia. TSH was 9.8uIU/mL, T4 was 7.74 ug/dL, fT4was 1.01 ug/dL. CAH was ruled out by ACTH stimulation test. Thyroid ultrasonography (USG) revealed hypoplastic gland for age (0.4 ml, normal: 1.76±0.27). Echocardiography showed severe aortic coarctation, bicuspid aortic valve, pulmonary stenosis and patent foramen ovale. The diagnosis of WBS was confirmed in infant by fluorescent in situ hybridization (FISH) analysis that showed the deletion of the 7q11.23 in one of the chromosome 7.

Case2: A 12 month-old boy presented with hypothyroidism. He was hospitalized due to jaundice and pale stool suspected cholestasis in neonatal period. Symptoms were relieved by phenobarbital treatment. At the same time hypothyroidism detected due to thyroid agenesis and L-thyroxin therapy was started. On admission, his physical examination was unremarkable except elfin face. Hormonal analysis revealed TSH 8.11uIU/mL, T4 7.22 ug/dL, fT4 1.0 ug/dL. Calcium was 11.1 mg/dL. Echocardiography revealed pulmonary stenosis. Denver developmental screening test revealed fine motor, broad motor and language milestone were retarded for his age. Karyotype analysis showed 46,XY. ish del (7)(q11.23q)(WBS-) and the diagnosis of WBS was confirmed.

Conclusion: The pathogenetic mechanism of the thyroid dysfunction was not understood completely in WBS. Mental retardation is commonly found in WBS. Untreated hypothyroidism also causes mental and motor retardation particularly in infancy period. Therefore physicians must be alert to meet infant with WBS and to investigate thyroid morphology and function.

PO1-318 Thyroid I

Fetal goitre and intrauterine treatment with thyroxine

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Introduction: Fetal goitre is a rare but potentially severe condition. It can cause polyhydramnios, tracheal compression with neonatal asphyxia after delivery and even death. Maternal thyroid disease, use of antithyroid medication or fetal dyshormonogenesis are the most common causes. It can be detected by ultrasonography, mainly in the second or third trimester, and confirmation of fetal hypothyroidism by determination of hormone levels is required.

Case Report: Healthy euthyroid 31-year-old primigravida, in whom fetal goiter was detected by ultrasonography at the 24th week (thyroid volume: 7.98 cc, normal value: 0.15 cc). Fetal hypothyroidism was confirmed by TSH determination (1.72 mIU/L, normal values: 0.05-0.99) and free-T_4 (0.16 ng/dL, normal values 0.1-0.77) in amniotic fluid. Thyroxine intrauterine treatment (8 doses ranging from 10 to 20 mcg/kg) was started, adjusting the dose by hormone levels in amniotic fluid. Progressive reduction in the goitre was observed by ultrasonography until reaching normal size. Caesarean section was performed at the 33rd week owing to suspected chorioamnionitis. Our patient was a newborn girl with normal physical examination, birth weight 2420 g, without palpable goitre. Six hours after delivery these thyroid hormone values were found in peripheral blood: T_4: 2.75 mcg/dL (mean ± SD: 14.55 ± 4.52) and TSH: 63.8 mIU/L (mean ± SD: 13.36 ± 6.49). Ultrasonography after birth showed increased thyroid size and scintigraphy a high thyroid uptake, confirming the suspected dyshormonogenesis. The genetic study remains unknown. Postnatal outcome with thyroxine treatment is completely normal.

Discussion: Some studies have demonstrated a good correlation between thyroid function and thyroid hormone levels in amniotic fluid. Measurement of these levels can be used for the diagnosis of fetal goitre and treatment monitoring. Given the low incidence of this disease, the doses and frequency of intra-amniotic levothyroxine treatment are not well established. This therapy can cause complications such as preterm delivery, chorioamnionitis or miscarriage.

Conclusions: Early detection and intra-amniotic treatment of fetal goitre with

hypothyroidism are indicated to avoid complications due to thyroid size and neurodevelopmental disorders.

PO1-319 Thyroid I

Persistent hyperthyrotropinemia in congenital hypothyroidism – successful combination treatment with levothyroxine and liothyronine

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Background: Recommended therapy for congenital hypothyroidism (CH) is based on levothyroxine, monitored at regular intervals by measuring serum TSH and Free T4. Some children develop persistent hyperthyrotropinemia despite high normal FT4 and good compliance. Since serum TSH levels reflect brain exposure to thyroid hormones it is considered important to normalize TSH. However, this might require increasing thyroxine to above the upper normal limit which may be harmful.
Aim: To evaluate the utility of adding liothyronine (= T3, cytomel ®) to levothyroxine in order to achieve normal TSH levels in children with CH.
Patients and methods: 8 female patients diagnosed by neonatal screening with CH had persistently high levels of TSH for 0.3-7.7 years. All 8 patients had persistent levels of FT4 in the upper range of normal and for two patients tested the FT3 was normal (see table). Average levothyroxine dose was 3.1±0.8 mcg/kg/day. Good compliance was assumed by the repeated presence of high normal serum FT4 levels and by parents' testimony. Patients were given either 6.25 or 12.5 mcg liothyronine and the thyroxine dose was reduced appropriately. Data from the patients' files were collected retrospectively.
Results: TSH values on the combined regimen decreased in all patients and normalized in 6 of 8 patients. Average TSH, FT4 and FT3 levels on levothyroxine and combination therapy appear in the table. Patients initially given cytomel less frequently than once daily did not reach normal TSH levels until they were switched to a daily regimen. Average final dose of levothyroxine was 2.4±0.6 mcg/kg/day and liothyronine 0.4±0.3 mcg/kg/day. The combined final dose was equivalent to 3.9±0.9 mcg/kg/day of levothyroxine.

Thyroid hormone values on the two regimens

	Levothyroxine only	Combination: Levothyroxine & Liothyronine	P-values
TSH (mIU/L)	18.6±8.0	4.7±4.25	p<0.001
FT4 (pmol/l)	18.3±2.3	13.8±1.5	p<0.001
FT3 (pmol/l)	5.5±1.1	6.5±0.7	Not applicable*

* for FT3 -on single drug treatment - n=2, on combination - n=8, all others - n=8

Conclusions: Liothyronine-thyroxine combination therapy can achieve normal TSH levels without abnormally high serum FT4 or FT3 in children with congenital hypothyroidism. This could theoretically further improve neurodevelopmental outcome.

PO1-320 Thyroid I

Evaluation of changes in thyroid hormone levels and TSH in critically ill full-term newborns and its clinical application

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Changes of thyroid hormones and TSH have been described in very low-weight preterm newborns. Nevertheless, few reports accounted on thyoid axis changes in critically ill full-term newborns. In this study we evaluated the thyroid hormone profile in full-term critically ill newborns and correlated changes found in the thyroid axis with the severity of the neonatal illness and outcome. Study design: A cross-sectional, observational, and prospective study was performed in 94 full-term infants admitted to the neonatal intensive care unit over a 10-month period (July 2007- April 2008). Serum T3, T4, and TSH levels were measured at admission. Disease severity was evaluated through SNAP, lactic acid, respiratory assistance and number of organs affected. Results: As a whole group, sick newborns showed significantly lower T3 and T4 levels compared to healthy infants T3= - 0.97g/dl (95%CI: -0.89 to -1.13) and T4= -4.37 g/dl (95%CI: -2.95 to -5.78)]. Only 29/ 94 (31%) infants showed a normal profile; the remainder of the patients had isolated low T3 levels [37/94 (39%)], low T3 and T4 levels [20/94 (21%)] and low TSH, T3, and T4 [8 (9%)]. Of this latter group, 5/8 (62%) died showing a significantly higher risk of death (RR 10.75 95% IC 3.93 - 29). No differences in the initial T4 and T3 serum levels were found in relation with the type of pathology on admission. Conclusions: Most of our critically ill newborns presented significally thyroid axis changes which could be related to the severity of the underlying disease. In addition, these changes might be useful as a prognostic marker of the outcome of the patient. However, more studies are neccessary to clarify if these changes imply a protective response during a severe disease or if they are an adaptive mechanism that should be treated with thyroid hormone supplementation.

PO1-321 Thyroid I

3,5-diiodothyropropionic acid (DITPA) in the treatment of patients with MCT8 deficiency: a preliminary study

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Defective monocarboxylate 8 transporter (MCT8) causes X-linked psychomotor retardation and failure to thrive. Patients have elevated T3, low T4, low rT3 and normal/high TSH. The neuropsychiatric abnormalities are presumably due to a relative TH deficiency in brain, and the failure to thrive to T3 excess in peripheral tissues. Two identical twins, hemizygous for a novel mutation (P321L), were given increasing doses of L-T4 (max 8ug/kg/d, aiming to increase entry of T4 into the brain by increasing serum T4), and propylthiouracil (PTU; max 32mg/kg/d, to limit peripheral tissue T3 by reducing conversion of T4 to T3 through deiodinase 1). PTU prevented further increase of T3 caused by the rising T4 levels and normalized the T3/T4 ratio but had no effect on the weight standard deviation score (SDS). PTU and L-T4 doses were reduced and DITPA (a thyroid hormone analogue, known to enter mouse tissues independently of MCT8) was given in increasing doses (up to 4mg/kg/d). Over 3 months, there was significant weight gain (1.4 kg; +1SDS). T4 and T3 and rT3 levels normalized for the first time while still receiving reducing doses of L-T4 and PTU. Table shows data for both twins. Abnormal values are in bold.

Age mo	L-T4 & PTU ug/d&mg/d	DITPA mg/d	fT4 pmol/L	fT3 pmol/L	fT3/fT4 ratio	TSH mU/L	Wt SDS
Normal Range			10.3-23.2	2.9-8.3	0.12-0.55	0.4-3.6	
19	0 & 0	0	**7.4, 8.8**	**10.9, 12.4**	**1.5, 1.4**	2.9, 3.5	-3.10, -3.12
19	50 & 75	0	**26, 24.2**	**15.4, 16.4**	**0.59, 0.68**	**0.03, 0.05**	-3.16, -2.97
20	50 & 150	0	**22.8, 21.9**	**12.3, 12.6**	0.54, **0.58**	**0.02, 0.02**	-3.13, -2.88
21	75 & 200	0	**59.3, 58.0**	**12.6, 10.5**	0.21, 0.18	**0.01, 0.01**	-3.32, -2.72
22	75 & 250	0	**49.3, 53.8**	**13.3, 12.6**	0.27, 0.23	**<0.01, <0.01**	-3.38, -3.15
23	75 & 300	0	**55.9, 42.9**	**11.3, 13.2**	0.20, 0.31	**<0.01, <0.01**	-3.02, -3.02
24.5	50 & 300	20	**59.2, 57.7**	7.7, **8.6**	0.13, 0.15	**<0.01, <0.01**	-2.66, -2.43
25.5	50 & 200	30	**53.5, 44.5**	6.4, 5.7	0.12, 0.13	**<0.01, <0.01**	-2.52, -2.31
26	25 & 200	40	21.1, 23.6	3.9, 5.3	0.18, 0.22	**0.01, 0.01**	-2.05, -2.02

PO1-322 Thyroid I

Urine and milk iodine concentration in healthy and congenitally hypothyroid neonates and their mothers

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Introduction:
Considering the high prevalence of CH in Iran, in this study we evaluated the role of ID or iodine excess in the etiology of CH.
Methods and Materials:
In a cross sectional study urinary iodine concentration (UIC) in newborns with CH as well as UIC and milk iodine concentration (MIC) of their mothers were measured and compared with a control group. Lower, mid and upper range of UIC for neonates and lactating mothers was considered to be <150µg/l, 150–230 µg/l and >230 µg/l and lower, mid and upper range of MIC was considered to be <150 µg/l, 150-180 µg/l and >180µg/l respectively.
Results:
The median of UIC in subjects with CH (n=68) and healthy subjects (n=179) was 30.50 and 29.50 µg/dl, respectively (P>0.05). The median of UIC in case and control group was 15 and 13 µg/dl, respectively (P>0.05). The median of MIC in case group was higher than control group (21µg/dl vs. 17 µg/dl, P<0.05).There was positive correlation between newborns UIC and MIC and there was not any correlation between newborns UIC and serum TSH, maternal UIC and maternal MIC, newborn UIC and serum TSH.
Discussion:
There is no inadequacy in iodine intake of the studied population. Iodine excess could be a possible risk factor for CH in our study, but there were findings such as lack of correlation between maternal MIC and UIC, the median of neonatal UIC which was similar in two groups, so, making conclusion in this field should be done with some caution and need further studies.

PO1-323 Thyroid I

Differential diagnosis of thyroid nodules: ectopic intrathyroidal thymic tissue in two children

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Background
Ectopic intrathyroidal thymus tissue that forms mass lesions as a consequence of aberrant thymic migration during embryogenesis is rarely reported in the literature.
Patients
We report the cases of two girls 3 and 11 years old, who were referred for sonographic examination of solitary thyroidal nodules. The younger girl has had sonographic investigations every 3 month for one year. In both children ultrasound investigation showed a thyroid gland of normal size. In both girls a small fusiform lesion, which was homogeneously hypoechogenic with diffuse bright internal echoes, was demonstrated in the right lower pole of the thyroid. A connection to the mediastinum or to the cervical component of a normal elongated thymus could be depicted.
Conclusion
Typical sonographic appearance of thymic tissue and a connection between a thyroid nodule and the thymus points to an accessory thymic lobe, embedded in the thyroid, mimicking a solitary nodule.

PO1-324 Thyroid I

An unusual presentation of multiple endocrine neoplasia (MEN)

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Introduction: We describe a case of multiple endocrine neoplasia(MEN) in a young girl, to emphasise the importance of fine needle aspiration in young children presenting with multi-nodular goitre, who are euthyroid and antibodies negative with no known family history of thyroid carcinoma.
Case Report: - A 14 year old girl presented to the paediatric endocrine department along with her stepmother, with a two month history of neck swelling and no associated symptoms. Blood tests showed normal thyroid function and antibody negative. There was no family history of thyroid problems or carcinoma. Ultrasound scan showed multiple nodules in both lobes of thyroid that contained areas of calcification and cystic areas, and was reported as consistent with multi-nodular goitre. She was seen in clinic a year later with an increase in size of her neck swelling with no associated symptoms. Repeat blood tests showed she was euthyroid and antibody negative. She wanted the swelling removed for cosmetic reasons and was referred to the surgeons who repeated her ultrasound scan. The scan showed areas of low attenuation and punctate calcification, reported again as consistent with multi-nodular goitre. Aspiration cytology done before surgery revealed medullary thyroid carcinoma, and she underwent complete thyroidectomy. On the day of her surgery her father was also noted to have a neck swelling, and it had been present for several years. He was also investigated and found to have medullary thyroid carcinoma and underwent thyroidectomy. Family history from him revealed that his father and paternal grandmother had died from neck cancer. Genetic testing showed that the index case was positive for the RET proto-oncogene consistent with diagnosis of MEN 2A. Her younger half sister was also positive to the RET proto-oncogene and underwent complete thyroidectomy.
Conclusion: All children who present with goitre, are euthyroid and antibody negative, should be investigated to rule out carcinoma. Ultrasound findings of micro-calcification and hypo-echoic areas are consistently associated with malignancy and should have fine needle aspiration done. Absence of family history does not rule out the possibility of medullary carcinoma or multiple endocrine neoplasia type 2.

PO1-325 Thyroid I

Positron emission tomography in the management of papillary thyroid carcinoma in children: is there a role?

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Background:Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) is being more frequently used in the evaluation of patients with papillary thyroid carcinoma (PTC) who have serologic evidence of disease and a negative

radioiodine (RAI) scan. Imaging with FDG-PET may localize disease, and there is accruing evidence that, in adults, increased FDG uptake by metastatic lesions is associated with a poorer prognosis. The utility of FDG-PET in children with PTC has not been studied.
Objective: To determine if FDG-PET is a useful imaging modality in children with PTC who have evidence of persistent disease.
Methods: We identified children with PTC who had undergone FDG-PET for the evaluation of persistent disease. Inclusion criteria for this study included: 1) diagnosis of PTC ≤ 18 yrs. of age treated with surgery and RAI; and 2) evidence of PTC, defined as a detectable thyroglobulin (TG) level, a rising anti-TG antibody, or disease identified on another imaging modality.
Results: We identified 13 patients (9F/4M) who met the study criteria. Mean age of diagnosis was 11.7 yrs (range 7-16); mean age at the time of FDG-PET was 14.4 yrs (range 8-20). 12/13 subjects had a negative RAI scan, and all patients had serological evidence of cancer. FDG-PET identified disease in 6/13 children (46%), with only 1/13 subjects (8%) having tumor not previously recognized. The computerized tomography (CT) scan simultaneously obtained during the FDG-PET identified subcentimeter lung metastases in several patients, but these nodules were generally below the limits of resolution for FDG-PET detection. Even in those ultimately proven to have PTC in the lesions with FDG uptake, FDG-PET did not necessarily identify all sites of disease. All subjects with positive FDG uptake ultimately received further therapies, whereas 4/7 patients with a negative FDG-PET continued on TSH suppression alone.
Conclusion: In this limited retrospective study, FDG-PET does not appear to be a highly sensitive test for localizing tumor in children with PTC and evidence of persistent disease. This may be explained by the frequently indolent nature of pediatric PTC or by the fact that metastatic lesions < 1cm in size, as is often the case, are below the limits of resolution for FDG uptake. Similar to adults, FDG-PET may play a role in determining prognosis or the degree of intervention necessary. Further studies are needed to define further the role of FDG-PET in the management of childhood PTC.

PO1-326 Thyroid I

Non-thyroidal tissue on ultrasound of the neck in infants with congenital hypothyroidism due to lingual ectopia: a diagnostic trap?

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Introduction
Thyroid imaging in neonates with TSH elevation is valuable in confirming the diagnosis of congenital hypothyroidism (CH) at an early age. While ultrasound scanning (USS) or radio-isotope scanning (RIS) may be used, we have previously recommended that USS and RIS be used together. However, infants with proven ectopia on RIS are frequently reported as having possible thyroid tissue in the neck on USS and we have re-investigated this phenomenon.
Patients and methods
We reviewed: (1) the images of all infants with proven ectopia on RIS; (2) USS of infants with no uptake on RIS and low/undetectable fT4, seen from 2004-8. Isotope scans were carried out using 99mTc Pertechnetate after intravenous application of 10–16 MBq of tracer. USS was performed with 7 - 15 MHz paediatric hockeystick transducer.
Results
Images of 18 infants (14F) with lingual or sub-hyoid (n=1) ectopia were reviewed. Mean (range) birth weight, gestation and age at scanning were 3.59 (2.2-4.9) kg, 40 (37-42) weeks and 19 (7-42) days respectively. Median (range) fT4 and thyroglobulin were 8.15 (3.0-24.0) pmol/l and 181.5 (2.0-612) ug/l, respectively.
RIS review confirmed ectopia in all infants. Putative thyroid tissue in a normal position had originally been reported on USS in 8/18 (44.4%). USS images were unsuitable for re-evaluation in 3. Review of the remaining 15 infants showed bilateral tissue in the thyroid fossa in all. This was judged non-thyroid in nature in view of the lack of uptake of tracer in the normal thyroid position together with the presence of at least one of the following features: heterogeneous cf homogeneous tissue (13); no well defined isthmus (13); no clear glandular capsule (14) and anechoic cysts present in either one or both "lobes" of tissue (12). Similar findings on USS were recorded in 7 infants (3F) with no tracer uptake on RIS.
Conclusion Care should be taken in interpreting USS images in neonates with suspected CH in the absence of RIS images since lingual ectopia is usually undetectable on USS, and non-thyroidal tissue in the neck can be mistaken for thyroid hypoplasia/dyplasia *in situ.* In the single case of sub-hyoid ectopia, non-thyroidal tissue was present in the contra-lateral position. Infants with no detectable uptake on RIS, i.e. apparent athyreosis, have similar USS neck findings suggesting the presence of very small amounts of lingual thyroid tissue which is not visible at current RIS resolutions.

PO1-327 Thyroid I

Five micrograms of levothyroxine suppresses the hypothalamic-pituitary-thyroid (HPT) axis in premature infants with transient hypothyroxinemia

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Background;
Euthyrotropic transient hypothyroxinemia is common in premature infants. Because the severity of hypothyroxinemia correlates with poor prognosis, many clinical trials have been conducted. However, no trials have proved an improvement of psychomotor development until now. In this study, we evaluated endocrinological effects of levothyroxine (LT4) administration for very low birth weight (VLBW) infants with euthyrotropinemic hypothyroxinemia.
Patients and methods;
Twenty-four VLBW infants were included in this study. Infants were divided into two groups; Group A consisted of 11 infants who showed hypothyroxinemia (FT4<1.0ng/dl), and group B of 11 infants who showed normo-thyroxinemia (FT4>1.0ng/dl). Infants of group A were treated with 5 micrograms of LT4 and those of group B were not. Endocrinological data of group A and B were compared.
Results;
Administration of 5 microgram of LT4 increased serum FT4 from 0.59 ± 0.25 to 1.17 ± 0.20 ng/dl, and decreased serum thyrotropin from 4.7 ± 1.9 mIU/L to 1.5 ± 1.5 mIU/L. Inhibition of thyrotropin secretion was observed in 10 of 11 infants. FT3/FT4 ratio was decreased form 3.77 ± 1.40 to 2.60 ± 0.64 by LT4 administration in these subjects.
Discussion;
Vanhole C (1997) and Wassenaer AG (1977) have already reported a suppression of thyrotropin by the administration of L-T4, but their dose of L-T4 was much higher or the initiation of the therapy was much earlier. Our study showed that 5 micrograms of L-T4 after two weeks of age suppressed secretion of thyrotropin in most cases. We think this finding suggest following two speculations. Firstly, the hypothalamic-pituitary-thyroid (HPT) axis is fully developed in most of these subjects. Secondly, administration of 5 micrograms of LT4 to these subjects was not necessary at least in endocrinological point of view. Based on these considerations, exogenous administration of LT4 for most of the premature infants with transient hypothyroxinemia might have a risk for disturbing the HPT axis in contrast to improving their neurodevelopmental outcomes.

PO1-328 Thyroid I

Alteration of thyroid stimulating hormone and thyroid hormones according to etiology in congenital hypothyroidism

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Object :: According to etiology in congenital hypothyroidism(CHT), serum levels of the thyroid stimulating hormone(TSH) and thyroid hormone such as triiodothyronine(T3), thyroxine(T4), and free thyroxine(FT4) following adequate doses of L-thyroxine administration were variable. The serum level of these hormones was monitored and delineate the relation to the etiology of CHT. Methods : CHT infants were selected retrospectively on the bases of the serum level of TSH(>20mIU/mL) in neonatal screening from Jan. 2000 to Dec 2006. Total 60 neonates were selected and 19 neonates were thyroid dysgenesis(6 agenesis, 3 dysgenesis, and 10 ectopic thyroid), and 41 neonates were dyshormonogenesis. Serum levels of T3, T4, FT4, and TSH were monitored by radioimmunoassay(RIA) on 1, 2, 4, 6, and 12 months of age. The hormone level of thyroid dysgenesis infants(group A) were compared with those of dyshormonogenesis infants(group B). All of the infants were administered adequate doses of L-thyroxines. Results : Serum levels of T3, T4, and FT4 were normalized less than one month of age in both groups of infants. In the group A, serum levels of TSH in neonatal screening was 119.0±125.43 mIU/mL, on 2 month of age 22.7±29.34, on 6 months of age 9.4±11.11, and on 12 months of age 11.1±9.48. In group B, serum level of TSH on neonatal screening was 51.4±36.81 mIU/mL, on 2 month of age 4.8±5.57, on 6months of age 4.5±5.40, and on 6 months of age 4.3±4.44. Theses TSH levels on screening, 2 months of age, and 12 months of age were significantly different from a group A to group B.Conclusions : Serum levels of T3, T4, and FT4 were normalized within a month of age after adequate doses of L-thyroxine administration in infants with congenital thyroid dysgenesis and dyshormonogenesis, but the serum level of TSH was sustained above normal values more than 6 months of age in infants with thyroid dysgenesis.

PO1-329 Thyroid I

Heterozygous mutation in TPO gene resulting fetal goitrous hypothyroidism

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Fetal goitre can arise secondary to transplacental passage of maternal thyroid-stimulating antibody that causes fetal hyperthyroidism. Contrary to this, fetal goitre due to maternal treatment of hyperthyroidism or dyshormonogenesis of the thyroid system or endemic iodine deficiency cause fetal hypothyroidism. So undestanding the reason of the fetal goitre is crucial for the correct treatment. In this report, we present a case of fetal goitre succesfully managed by in utero treatment with thyroxin by the wrong diagnostic way.
A term 2950 g infant delivered from 29-year-old primigravida mother admitted to our medical center for tachypnea in the first postnatal day. From medical history it was learned that; fetal goitre was detected on 19th week (19x11mm) and maternal thyroid function tests were normal but maternal TSH-receptor antibody: 25 U/l (0-10), maternal anti-TPO: 297 u/ml (0-60) and urine iodine: 10.5 µg/dl (>10). Fetal thyroid functions were not studied. Intraamniotic thyroxin were given with the dose of 400 µg at the 32nd gestational week. During follow-up, fetal goitre was decreased. Mother had been diagnosed hyperthyroidism 3 years ago and with treatment her thyroid functions were normalized and her antithyroid therapy was discontinued one year before the pregnancy. The infant's physical examination was normal and fetal goitre was absent. His tacipnea was normalized with nasal CPAP and he went well. FreeT4 : 0.5 ng/dl (0.7-1.85) TSH: 660 uIU/ml (0.4-8.6), TSH-receptor antibody: 15 U/l (0-10), thyroglobulin: 3120 ng/ml (1.6-59.9), urine iodine: 13 µg/dl (>10). Thyroxin treatment was started and his development was normal at the second year of life. A heterozygous mutation in exon 4 (nt265c>t) which results in a premature STOP codon at position 89 (R89X) was found in thyroid peroxidase gene of the patient. The same mutation was found in the father where as his thyroid function tests were normal. The mother was not carrier of the mutation.
Conclusion:
1. The clinical appearance may be different in the persons having the same mutation of the TPO gene.
2. Fetal goitre may cause hypo or hyperthyroidism in the fetus depending on the etiology. Before injecting thyroxine into the amniotic fluid, fetal hypothyroidism should be proven.

PO1-330 Thyroid I

A rare cause of short stature in an adolescent girl: the association of celiac disease and panhypopituitarism

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Celiac disease (CD) is characterized by hepatosplenomegaly, anemia, short stature and osteoporosis. Short children with CD have normal growth hormone (GH) response. GH deficiency was rarely reported in CD. Panhypopituitarism is also rarely found in atypical CD. We present an adolescent girl with panhypopituitarism associated with CD.
A 16 $^{3/12}$ year-old girl presented with anorexia and short stature. She suffered reduced height velocity (1cm/y). On physical examination, height was 132 cm (height SDS -4.5), weight was 29.7 kg (<3p), bone age was 8 $^{6/12}$. Skin was pale. I/VI systolic murmur was heard on all cardiac focus. Breast development and pubic hair was prepubertal. Biochemical analysis revealed iron deficiency and mild elevated transaminases. Thyroid hormones and IGFBP-3 was normal. IGF-1 was 74 ng/mL(261-752). Ultrasonographic examination showed granulated liver parenchyma, reduced ovarian size for her age (1.6 mL right, 1.7 mL left) and infantile uterus. Gonadotrophins and estradiol was normal. Peak GH response was compatible with GH deficiency in clonidine and insulin tolerance tests (ITT). Stimulated cortisol was 8.35 ug/dL in ITT. Hypophysis magnetic resonance imaging showed hypoplastic pituitary gland. Antigliadin antibody (AGA) IgA and IgG, and anti endomysial antibody (EMA) were positive. Small intestine biopsy was done by endoscopic examination. Pathologic examination of intestine showed hyperplasia of crypts, lymphoplasmocyts infiltration and villous atrophy and celiac disease was diagnosed. Gluten free diet was started. Bone mineral density Z-score was -2.8 at lumbar vertebra. Intravenous iron was given because of the fact that oral absorption was inadequate. Growth hormone, hydrocortisone, alendronate, vitamin D and calcium-lactate were initiated.
CD is commonly associated with other autoimmune disease. The cause of panhypopituitarism in our case may be autoimmune hypophysitis. All children with short children must be evaluated for CD and panhypopituitarism.

PO1-331 Type 1 Diabetes I

Atypical diabetes in a patient with myotonic dystrophy type 2

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Background
Myotonic Dystrophy, type and 1 and 2, is associated with a variety of systemic features. Diabetes is described in these patients secondary to insulin insensitivity. We report a girl with Myotonic Dystrophy type 2 and Diabetes with low levels of insulin and negative GAD 65 and IA-2 antibodies.
Clinical Case
A 10 year old female patient developed hyperglycemia following an appendectomy that was complicated by an abdominal abscess. She was initially treated with insulin Glargine at bedtime .Insulin therapy was eventually discontinued due to normalization of glucose values. On physical exam her BMI was 11.8 and her weight was 19.2 Kg(<3 ile).Her initial HbA1C was 6.9, while her Hb was 7.3g/dL, and GAD65 and IA-2 antibodies were negative. Family history was remarkable for Myotonic Dystrophy in her father and several members of her paternal side including a cousin also diagnosed with type 1 diabetes at age 2. Two weeks after her discharge she developed recurrent hyperglycemia. Her fasting glucose was 276mg/dl with an insulin level of 4.4 (1.4-14).She was started on multiple daily injections program using insulin Aspart and insulin Glargine. Two years following her initial diagnosis her insulin dose was 0.8 units/Kg/day with a blood glucose of 137 mg/dL and C-peptide in 0.6 ng / mL (0.9 – 4.3). Genetic testing showed a repeat expansion mutation in the full expansion range consistent with Myotonic Dystrophy type 2.
Conclusion
The Diabetes developed by this patient does not have typical features of neither

type 2 nor type 1 except for the fact that her insulin level was low when done simultaneously with a high blood glucose. Our patient is also unique because her diabetes was more severe and was diagnosed at younger age than typically seen in Myotonic Dystrophy type 2.However the presence of an affected family member should raise the possibility of true association between Myotonic Dystrophy type 2 and an atypical form of insulin deficiency diabetes.

PO1-332 Type 1 Diabetes I

Glycoalbumin as a useful marker for the assessment of glycemic fluctuation in T1DM

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Purposes: To clarify the differences between A1C and glycoalbumin (GA) in the evaluation of glycemic control, since recent reports regarding to GA suggested to reflect glycemic fluctuation in comparison with A1C, as well as the difference of past average glycemic levels, 2 to 4 weeks and 4 to 8 weeks in GA and A1C respectively.
Methods: In 786 T1DM patients enrolled as the 3rd cohort of JSGIT, 352 patients were examined GA, A1C, GA/A1C ratio and ferritin at the time of registry, in relation with data of A1C pre 1 month and post 1 month. These relevant variables were examined for (a) the difference from control subjects (Fathers=147, Mothers=169, non-diabetic siblings=98), (b) differences between prepubertal girls, pubertal girls with menarche and boys inT1DM.
Results: A1C levels in medium (quartile ranges) were 7.3 (6.7-8.1) and 4.8 (4.7-5.0)%. GA were 23.2 (20.6-26.6) and 13.4 (12.8-14.0)%, in T1DM patients and controls subjects all together respectively. GA/A1C ratios in T1DM were significatly higher than controls, 3.19 (2.97-3.40) and 2.79 (2.67-2.92), respectively. In T1DM GA and GA/A1C in pubertal girls with menarche were significantly higher than those in prepubertal girls and boys, while A1C in pubertal girls was not different from those in prepubertal girls and boys. Ferritin level in pubertal girls with menarche was significantly lower than those in boys, but not different from prepubertal girls. Whereas A1C shows a positive linear regression with GA, GA/A1C was also revealed to have a significant positive corelation with A1C. When differences of A1C between pre 1 month and at registry (pre Δ A1C) were >+0.5 %, GA/A1C was 3.29 (0.31) in mean (SD), while patients with decreased pre Δ A1C <-0.5 % showed a significantly lower GA/A1C being 3.07 (0.32), $p<0.0005$
Conclusions: We have demonstrated the usefullness of simultaneous measurements of A1C and GA. GA/A1C ratio in T1DM was revealed to be higher than those in non-diabetic controls and became higher along with increased A1C levels, indicating the wider glycemic excursion. Although ferritin levels in iron anemic late-pregnant women were reported as a negative relation with GA/A1C ratio, pubertal girls with T1DM showed higher those ratios even with lower ferritin than prepubertal girls and boys. This may reflect poor diabetic control with wider glycemic changes in pubertal girls. The GA/A1C ratio may more precisely assess a glycemic trend either worsening or improving.

PO1-333 Type 1 Diabetes I

Incidence and risk factors of diabetic nephropathy in children and adolescents as per Republic of Uzbekistan national register

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Aim: to study incidence and risk factors of diabetic nephropathy (DN) in children and adolescents as per the Republic of Uzbekistan National Register.
Materials and methods: an information list (card) and appropriate software were developed on the basis of the diabetic nephropathy register. The cards were collected in every region of Uzbekistan to be introduced into a computer database.
Results: DN screening performed in children by Alikhanova N.M. in 1998 showed that 4+5 degree DN incidence in children and adolescents was 20.4% and 51%, respectively. In 2007 on the basis of specially developed register card data on incidence of DN in children and adolescents with 1 type diabetes mellitus was assessed in the Republic of Uzbekistan. 4 and 5 degree DN incidence in children was found 17.0% and 0.6%, to be 31.7% in adolescents and lower than the one in 1998, but higher that the one in the 2006-year Register. It was 11.2% and 20.7%, respectively, to show that there is 1.5 – time difference between actual and registered DN incidence. Inheritability by diabetes mellitus and by arterial pressure, mean daily hyperglycemia, increase in HbA1c, body mass and cholesterol, reduction of blood fibrinolytic activity and both daily and nocturnal increase in arterial pressure as well as growth in manifestations of autonomic neuropathy are among the risk factors of onset and progression of DN in children and adolescents. Cow milk feeding, low weight at birth and disease duration differed insignificantly.
Conclusions. Rate of registration of late diabetic complications, DN and chronic renal insufficiency (CRI) in particular remains low, though in 50% and 100% children and adolescents, respectively, CRI is the main cause of death.

PO1-334 Type 1 Diabetes I

Evaluation of the effect of type 1 diabetes mellitus on the auxological data of children

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Objective: We aimed to evaluate the effect of type 1 diabetes on growth and other auxological data in children.
Subjects and methods: The inclusion criteria were: (i) A follow-up duration of at least 1 year, (ii) not having achieved near-final height at the presentation, and (iii) not having an associated disorder (e.g. celiac disease, pubertal delay) that could affect growth. The patients followed in our center since the diagnosis were included in Group A while the others in Group B. The mean weight, height, and BMI SDS values of the patients at presentation and each following year were calculated and compared. Then, for each disease year, the patients in Group A were divided into two subgroups as Group A1 (those who maintained or improved their height SDS) and Group A2 (height SDS of whom deteriorated). The two groups were compared regarding clinical and laboratory characteristics.
Results: A total of 248 patients with type 1 diabetes were followed since the institution of our department in 1990. The study consisted of 130 patients (M/F:70/60) after excluding 118 patients because of not meeting the inclusion criteria. The mean age at the diagnosis was 8.4 ± 3.6 years and mean duration of follow-up was 4 ± 2.7 years. Group B consisted of 29 cases (22%, M/F:15/14), who presented after a mean of 3.8 ± 2.6 years (range, 9 months – 11 years) following diagnosis. The mean values of auxological variables of the patients in Group A (n=101, M/F:55/45) at the diagnosis were as follows: Weight SDS: -0.3±1.1, height SDS 0.3±1.1, BMI SDS -0.6±1.4. Their mean target height SDS was -0.2±0.8. The mean height SDS of the patients in Group A did not change significantly during the follow-up. Additionally, weight and BMI SD scores were similar during the course of the disease, except a significant rise observed at the end of first year compared to those at the diagnosis. The comparisons between Groups A1 and A2 revealed that presenting with diabetic ketoacidosis at the onset was significantly associated with maintaining or gaining height SDS at the 4th year of diagnosis (p=0.03). On the other hand, mean HbA1c level was modestly negatively correlated with Δ height SDS (=observed height SDS – height SDS at presentation) at the 3rd year of diagnosis (r= -0.3, p=0.03).
Conclusion: No significant deteriorative effect of type 1 diabetes on auxological parameters was observed. Some clinical and laboratory variables related with metabolic control were found to correlate with growth.

PO1-335 Type 1 Diabetes I

Low dose (0.05 units/kg/hour) is comparable with standard dose (0.1 units/kg/hour) intravenous insulin infusion for the initial treatment of diabetic ketoacidosis in children with type 1 diabetes – an observational study

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Objective

To compare low dose (0.05 units/kg/hour) with standard dose (0.1 units/kg/hour) intravenous insulin infusion for the treatment of diabetic ketoacidosis (DKA) in children with type 1 diabetes.

Study design

Data from five paediatric centres were compared in children who received 0.05 (41 episodes) or 0.1 units/kg/hour (52 episodes).

Results

In the low versus standard dose group, at six hours following admission; the fall in blood glucose levels (11.3 [95% confidence interval 8.6 to 13.9] v 11.8 [8.4 to 15.2] mmol/L, P= 0.86) and rise in pH (0.13 [0.09 to 0.18] v 0.11 [0.07 to 0.15], P=0.78) were similar. These changes were comparable between doses in relation to; severity of initial acidosis, children newly diagnosed with diabetes or aged less than five years. After adjustment for other clinical and biochemical covariates, insulin dose was unrelated to the change in pH and blood glucose levels at six hours following admission. Comparisons of safety data, particularly in relation to abnormal Glasgow Coma Score, and data in relation to duration of acidosis and hyperglycaemia were inconclusive.

Conclusion

In this observational study, low dose was as effective as standard dose intravenous insulin infusion in the initial treatment (less than six hours) of DKA in children with type 1 diabetes. A randomised controlled trial is required to show true equivalence between doses and to evaluate potential safety benefits.

PO1-336 Type 1 Diabetes I

The diagnostic accuracy of pubertal assessment and gonadotrophin releasing hormone testing in assessing the likelihood of requiring treatment in females presenting with early puberty – an observational study

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Aims

To determine the accuracy of clinical, radiological and gonadotrophin axis measurements in assessing the likelihood of requiring Gonadotrophin Releasing Hormone (GnRH) analogue treatment in females presenting with early puberty.

Methods

Stage of puberty, stimulated gonadotrophins and basal estradiol levels and pelvic ultrasound scan measurements were assessed in 103 females presenting with early puberty between 2000 and 2007 in the Regional Endocrine Clinic at the Royal Manchester Children's Hospital, UK.

Results

Age at presentation was 5.8 [Range 0.5 – 9.9] years. 34 (33%) females subsequently required GnRH analogue treatment for central precocious puberty. In regression analyses, clinical factors associated with GnRH analogue treatment were Tanner breast stage≥3 (Odds ratio 4.04 [95% confidence interval 1.39 to 11.74]) and pubic hair stage≥3 (2.61 [1.01 to 6.82]). With stimulation testing, peak Luteinizing Hormone levels ≥5.0mU/L (5.75 [2.18 to 15.70]) and a Luteinizing Hormone:Follicular Stimulating Hormone ratio >0.3 (5.75 [2.11 to 15.70]) best predicted need for treatment, although accuracy was poor, as determined by receiver operating characteristic curves. However when used in conjunction with Tanner breast and pubic hair stage ≥3, these gonadotrophin levels correctly identified 88% of cases requiring treatment. Using these criteria; the likelihood of requiring treatment increased by a factor of 43.3 [6.30 to 295.82] and negative predictive value was 89%, however positive predictive value was only 52%. Pelvic ultrasound measurements and bone age advancement did not increase the likelihood of treatment.

Conclusion

In females presenting with early puberty, a composite assessment score incorporating pubertal staging and stimulated gonadotrophins improves the ability to correctly identify those requiring intervention.

PO1-337 Type 1 Diabetes I

Adiponectin level and its relationship to carotid intima media thickness in children with type 1 diabetes

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Background: Adiponectin is an anti-inflammatory and antiatherogenic hormone. It inhibits neointimal thickening and vascular smooth muscle cell proliferation. **Aim:** The aim of this study to evaluate adiponectin level and its relation to carotid intima media thickness (cIMT) in children with T1DM. **Subjects and methods:** Forty-six diabetic children mean age (13.59±3.64 years). The mean duration of diabetes (4.35±2.19 years), the mean BMI (20.56±3.54) the mean HbA1C (8.35 ±2.92).Thirty six healthy control subjects matched in age, sex and BMI took part in this cross-sectional study. All children had normal blood pressure for age and sex and no microalbuminuria. Adiponectin, albumin/ creatinine ratio in early morning urine sample, lipid profile, and HbA1c, were measured. **Results:** Adiponectin level was significantly lower in children with T1 DM than control (9.49±1.74) and (10.31±1.45) respectively (P 0.02). Children with T1DM had significantly higher cIMT than control (0.57±0.08) and (0.43±0.07) respectively (P 0.00). Adiponectin level correlated negatively with cIMT (P0.01). Diabetic children with good metabolic control (A1C <7) had no significant difference in adiponectin level (10.22±0.81) compared to control (10.31±1.45) (P0.82).Although children with T1DM and good metabolic control had higher cIMT (0.49±0.064) than healthy subject (0.43±0.049) (P0.00). Adiponectin was significantly higher (10.22±0.81) and cIMT was significantly lower (0.49±0.064) in children with good metabolic control than those with poor metabolic control (9.27±1.74) (0.62±0.081).Carotid intima media thickness correlated significantly with adiponectin level in children with T1DM.The most fitting factor that can predict cIMT was duration of diabetes, BMI and adiponectin.

Factors predicting cIMT in children with T1DM

Variableres	Beta	t	P
Age	0.49	1.85	0.07
age at onset of DM	0.21	1.4	0.17
duration of DM	0.63	3.3	0.00*
BMI	0.41	3.62	0.00*
Hb A1c	0.47	1.41	0.17
Adiponetin	-0.41	2.79	0.01*
Total cholesterol	0.12	2.7	0.06
Triglycerides	0.04	0.18	0.86

Conclusion: children with T1DM had lower level of adiponectin than control. Those with good metabolic control had no significant difference in adiponectin level than healthy children.Subclinical macrovascular disease begins early in children with T1DM.

PO1-338 Type 1 Diabetes I

The development of celiac disease (CD) in children with type 1 diabetes is increased in families with higher income, lower rates of ill health and late age of weaning; a case controlled study

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Background

We aimed to determine the socio-economic factors associated with the devel-

opment of CD in children with type 1 diabetes.

Methods

In a case-controlled design, demographic data and infant feeding patterns were collected on 54 children with type 1 diabetes who subsequently developed CD, matched for age, sex and duration of diabetes with controls without CD.

Results

In families of CD cases compared with controls, indices of socio-economic deprivation differed significantly; overcrowding was less (P=0.018 for tertiles of overcrowding), parental employment rates were higher (P=0.042) and proportion living on low income was lowest (P= 0.003). In addition, prevalence of CD was greatest in families with lower rates of ill-health (Log-Rank P= 0.014, after ten years of diabetes). Numbers weaned onto solids after age six months were greater in CD cases (n=9 (16.7%) v n=0, χ^2=6.61, P=0.01). In a Cox model, after adjusting for diabetes duration, the development of CD was associated with; families with higher income (Hazard Ratio 5.87 [95% confidence intervals 1.11 – 31.13] for income tertiles) and lower rates of ill health (3.54 [1.52 – 8.23] for health deprivation tertiles) and age of weaning onto solids after age six months (3.11 [1.13- 8.53]). Breast feeding was not associated with CD.

Conclusion

The development of CD in children with type 1 diabetes is associated with less deprived socio-economic indices. This is in contrast to reported findings for those developing CD in the general population, indicating that different environmental agents may be important.

PO1-339 Type 1 Diabetes I

Diabetes mellitus in children, aged less than 15 years, in the autonomous community of Castilla-La Mancha (Spain)

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OBJECTIVES:

To determine the prevalence and incidence of Diabetes Mellitus (DM) in children aged less than 15 years, in the autonomous community of Castilla-La Mancha (CLM).

RESEARCH DESIGN AND METHODS: We performed an epidemiological, prospective, observational study. The mark-release-recapture method was used to calculate the completeness of ascertainment. The information was obtained from hospital registries and primary care central computerized database records. To determine the prevalence of diabetes, all the children younger than 15 years old, diagnosed of diabetes at 31st of May of 2007 were included. To determine the incidence, children diagnosed between 1st of June of 2007 and 31st of May of 2008 were included.

RESULTS:

CLM is a region in the centre of Spain, which estimated total population at 1st of January of 2008 was 2,001,643 people, 14.8% of them were younger than 15 years old (297,160 children). Prevalence of diabetes in that population was 422 cases: 1.4 cases/1000 children (56% boys). Prevalence increases with the age, corresponding 59% of the patients between 10 and 15 years, 33% between 5 and 10 years and 8% between 0 and 5 years old. The prevalence is higher in three of the five provinces of CLM: Ciudad Real, Albacete and Toledo (1.63‰, 1.59‰ and 1.39‰ respectively), in comparison with Guadalajara or Cuenca (0.94‰, 0.80‰ respectively). 81 children, 43 boys (53%) and 38 girls (47%), were diagnosed in the period cited above, what supposed an incidence of 27.6/100,000 people/year. The estimated number of patients by Chapman-Seber method was 82. The exhaustivity of both sources (S1, S2):98 (S1: 96; S2: 68). The highest incidence corresponded to the group of age between 10 and 15 years old. There were no differences in sex except in children less than 5 years, where we found that 67% of the new cases were boys. We found again higher incidence in provinces cited above.

CONCLUSIONS:

1. Prevalence and incidence of diabetes in children aged less than 15 years, in CLM is one of the highest found in recent Spanish studies
2. An increase of incidence is found in lower ages (less than 5 years old). But, like previous studies, the age of maximum incidence still corresponds to the older groups.
3. We found higher incidence in boys than girls in group aged less than five years.
4. Prevalence and incidence showed wide variability among the different provinces of CLM

PO1-340 Type 1 Diabetes I

Correlating islet cell antibody titers to the age of onset of type 1 diabetes in an ethnically diverse, prodominantly Hispanic population of children in central California

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The age distribution of islet cell antibody positivity rate in children and adults in a predominantly Caucasian population with new onset Type 1 Diabetes is well described, with GAD being higher in adults and IAA higher in very young children. We reviewed the charts of all new onset diabetes patients who presented to Children's Hospital Central California (CHCC) in 2006-2007. CHCC is a children's hospital in the central valley of California which serves a large rural/urban community of mixed but predominantly Hispanic ethnicity. We identified 113 children below the age of 18 yrs, who presented with new onset diabetes mellitus and who had positive antibody titers that were done at the Barbara Davis Center in Denver. We compared the rate of positive antibody (biochemical antibodies ICA 512, IAA, and GAD) titers in 4 different age groups (<5, 5-9, 10-15, >15 years) based on their ethnicity. There were 39 Caucasians, 63 Hispanics, and 11 children of other ethnic origin (Asian/African American). The antibody titers in the different ethnicities for the various age groups was comparable except for a higher degree of GAD positivity in the children <5 yrs (8/8)of Hispanic vs Caucasian (3/10) origin ($p<0.05$). Not unexpectedly, all the children below the age of 5 years of both Caucasian (10/10) and Hispanic (8/8) origin tested positive for IAA.

PO1-341 Type 1 Diabetes I

A case report: rapid and extreme weight gain after initiating insulin therapy in a 15-year-old with new onset type 1 diabetes

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We report a case of rapid weight gain in a 15-year-old Hispanic male with newly diagnosed antibody positive Type 1 diabetes. He was treated for his initial presentation of diabetic ketoacidosis with intravenous insulin and fluids. The family was taught carbohydrate counting and insulin therapy and he was discharged home on basal/bolus insulin therapy at about 0.5 unit/ kg/ day. Within 7 days, he had gained 28.7kg (63 pounds). Clinically he was in no distress but had edema predominantly of his lower extremities and lower abdomen. Laboratory and imaging studies were non contributory. His insulin doses were reduced. A diet history revealed a very high sodium intake exceeding 10 gms/day (176mEq/day). A low-sodium diet was initiated. Diuresis followed soon after his sodium intake was lowered. He lost 9.6kg (21 pounds) within 3 days. He tolerated the rapid weight change well. We believe, in addition to the edematous properties of insulin therapy, the very high sodium intake played a role in fluid retention in this teenager with new onset Type 1 DM. This case suggests that dietary sodium intake education in children and adolescents should be considered in addition to carbohydrate counting for new onset diabetes mellitus. In adults, the American Heart Association (AHA) recommends a lower sodium intake (less than 2000mg, 34.5 mEq) for diabetic patients

compared to 40 mEq in non diabetic adults. In our center, we now recommend that daily sodium intake should be no higher than the recommended amount for diabetic adults.

PO1-342 Type 1 Diabetes I

Characterisation of patients with new onset diabetes and prospective follow-up from birth to diabetes onset

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Background and aims. Type 1 diabetes (T1D) is preceded by islet autoimmunity. Aim of the study is to characterize patients who have developed T1D at onset and compare the autoimmunity status at onset to the prediabetic period.
Materials and Methods. 55 children who developed T1D between age 1 and age 16 years were investigated. Unique in this cohort was that all children were prospectively followed from birth with repeated blood sample collection. Antibodies to insulin (IAA), GAD, and IA2, were measured in all available samples until diabetes onset.
Results. At diabetes onset, 56% of cases were positive for 3 islet autoantibodies (ab), 33% of cases were positive for 2 islet abs, 11% were positive for 1 islet ab, and no cases were autoantibody negative. At diabetes onset, IAA was present in 91% of the cases, GADA in 73%, and IA-2A in 71% of the cases. 9 of the 18 children with 2 antibodies at onset had all three antibodies during the prediabetic period. 1 of the 6 children with 1 antibody at onset had 2 antibodies and none had 3 antibodies in the prediabetic period. Two offspring lost IAA before onset, 5 offspring lost GADA, and 3 offspring lost IA2A before onset, resectively. The maximum titer of IAA was found 2.7 ± 3.2 yrs prior to T1D onset (mean peak titer 69 U, mean onset titer 55 U), the maximum titer of GADA 2.4 ± 2.3 yrs (mean peak titer 191 U, mean onset titer 137 U), and the maximum titer of IA2A 2.0 ± 2.7 yrs (mean peak titer 170 U, mean onset titer 82 U) prior to T1D onset, respectively. Of the 55 cases, 22 had developed rapidly progressive diabetes defined by progression from first autoantibody positivity to T1D within 2 yrs. In those cases T1D onset age ranged from 0.7 to 9.9 yrs, indicating that they were younger than slow progressors (mean onset age 3.1 ± 2.0 yrs vs. 8.9 ± 3.4 yrs, $p<0.001$).
Conclusions. Maximum expression of autoantibodies usually occurs prior to diabetes onset. A subgroup of rapid progressors differs from slow progressors by younger age at onset and higher IAA titers.

PO1-343 Type 1 Diabetes I

In vitro characteristics of immune response to GAD in combination with IL-1b in siblings with predisposing to type 1 diabetes

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IL-1β is one of the key cytokines involved in the first stages of β-cells destruction in Type 1 diabetes (T1D). Moreover, IL-1β assists in lymphocytes activation and proliferation during immune response. We studied the functional activity of peripheral blood mononuclear cells (PBMC) in children with T1D and their siblings in the presence of IL-1β due to reveal the new immunological criteria of possible risk of disease. Materials and Methods: PBMC of 32 non-diabetic siblings of 27 children with T1D was investigated. According to patients with T1D manifestation age (5-9 or 10-14 yo) siblings were divided in 2 groups: with 3,6% and 2,3% cumulative risk of diabetes by age 20 years, respectively. Control group included 26 healthy children of the same age. Cultural method with [H^3] thymidine incorporation to estimate PBMC proliferative response and ICA-synthesizing activity to nonspecific mitogens (PHA, PWM) and specific autoantigen GAD ("Sigma", G2251) in the presence of IL-1β (NIBSC 86/680, UK) was used. ICA in culture supernatants was defined by ELISA kit "Isletest-ICA" (BIOMERICA, USA). Results: In contrast to T1D children, alteration in PBMC proliferative response to autoantigen GAD was not characterized for both non-diabetes siblings and control group. The increase of lymphocyte proliferation in T1D patients in comparison with 3,6% risk of diabetes siblings was established (p=0,04). However, in the presence of IL-1β we observed the increase of GAD-specific lymphocyte proliferation (p=0,04) and ICA synthesis impairment (p=0,03) only in non-diabetic siblings with high risk (3,6%) of diabetes. Conclusion: estimation of immune response to specific autoantigens (GAD) in presence of IL-1β could be used as a predicting criterion of diabetes high risk among the siblings of T1D children.

PO1-344 Type 1 Diabetes I

Phenotypical heterogeneity in Wolfram syndrome. A diagnosis to be searched for in cases of diabetes mellitus with negative autoantibodies

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Wolfram syndrome (WS) is referred as DIDMOAD [diabetes insipidus (DI), diabetes mellitus (DM), optic atrophy and deafness]. Various neurological symptoms may be present: hearing loss, ataxia, peripheral neuropathy, dementia, epilepsy and psychiatric illnesses. Autosomal recessive mutations in the gene WFS1 (4p16.1), encoding wolframin, a protein of the endoplasmic reticulum, explain 90% of cases.
We report 5 patients from 3 families diagnosed with WS in our cohort of 200 diabetic children, of whom 20 were autoantibody negative.
Family 1: Ketoacidosis revealed DM at 8 yrs of age in patient AF. His brother MF was diagnosed with DM a few months later, at the age of 5 yrs. Autoantibodies were negative. Screening for diabetic retinopathy was performed once a year at the parents' request. Two years after DM diagnosis, optic atrophy was detected in patient AF, prompting for the search of WFS1 mutation. Both children were compound heterozygous for mutations in the WFS1 gene: c.467C>T (T156M) and c.1260_1261insC (I421fsX542). Two years later, AF had a gradual decrease in visual acuity, whereas his brother had only diabetes.
Family 2: Ketoacidosis revealed DM in patient M at 4 yrs of age. Autoantibodies were negative. Despite good metabolic control, initial polyuria and polydipsia persisted, gradually increasing, leading to the diagnosis of DI at 6 yrs of age. She was found to be compound heterozygous for two mutations in the WFS1 gene: 1243-1245del3nt (V415del) and C 1980G (Y660X). At the age of 10 yrs, she had mild hearing loss.
Family 3: The patient MM was diagnosed with mitochondrial disease at the age of 4 (epilepsia, cardiomyopathy and tubulopathy), with a global decrease in respiratory chain function but no mitochondrial DNA mutation. DM was diagnosed at 6 yrs, optic atrophy at 14 yrs and mild hearing loss at 18 yrs. His brother KM was diagnosed with DM at 5 yrs, optic atrophy at 6 yrs, epilepsia and mild hearing loss at 13 yrs. Autoantibodies were negative. Screening for WFS1 mutation was performed (association of DM and optic atrophy). Both adolescents were found to be compound heterozygous for mutations in the WFS1 gene: c.1525_1539del15 (V509_Y513del) and c.2643_2646delCTTT (F882fsX950).
Patients with WS showed phenotypic heterogeneity. The diagnosis should be suspected in all subjects with DM and no autoantibodies. The prevalence of WS in children with DM could be underestimated, as it represented 5 out of our 20 autoantibody negative cases of DM (20%).

PO1-345 Type 1 Diabetes I

Continuous subcutaneous insulin infusion in type 1 diabetic adolescents with poor glycemic control under multiple daily injections: 1-year evaluation of HbA1C and acceptability

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Introduction. Diabetes in adolescence is often associated with poor glycemic control. In France, continuous subcutaneous insulin infusion (CSII) is reimbursed by the Health Care system in diabetic subjects with poor glycemic control despite "optimized" multiple daily injections regimen (MDI). We assessed metabolic control (HbA1C and severe hypoglycaemia frequency) and quality of life (QOL) in 32 diabetic adolescents, one year after switching from MDI to CSII.

Subjects and methods. We studied 32 type 1 diabetic adolescents aged 10 to 18 years, initially treated with MDI in the Paediatric Endocrinology Department from Angers University hospital. They switched to CSII between 2000 and 2006 and used insulin pump for at least 1 year. Weight, height, BMI, insulin doses, severe hypoglycaemia frequency (coma, convulsions) and HbA1c were recorded every 3 months up to 1 year after the switch to CSII. The study period extended from 18 months before to 12 months after switching to CSII. QOL was assessed by ped SQL.

Results. Patients were 13.0 ± 2.5 years old (19 boys, 13 girls) and were diabetic for 5.1 ± 2.7 years at the onset of CSII. Body mass index significantly increased from 0.9 ± 1.2 SD score to 1.4 ± 1.3 SDS one year after switching to CSII ($p = 0.002$). CSII was associated with a significant decrease in insulin dose from 1.1 ± 0.4 to 0.9 ± 0.3 U/kg/d ($p < 0.05$), severe hypoglycaemia events from 1.2 ± 2.3 to 0.5 ± 0.8 per patient and per year ($p < 0.05$), and HbA1c from 8.7 ± 0.8 to 8.3 ± 0.7 % ($p = 0.007$). Patients appreciated the reduction in injection number and the flexibility of CSII for meals and sport practice. However, they complained of discomfort during sleep, and of cutaneous infection at the catheter site. QOL assessment mostly highlighted difficulties as regard to capillary blood glucose controls and diet, independently of the insulin regimen. Nevertheless, concerns about hypoglycaemias, treatment efficiency and diabetes evolution were scarce. After 1 year, 94% of patients wished to go on with CSII.

Conclusion. CSII improved metabolic control in diabetic adolescents with poor equilibrium under MDI, with a satisfying acceptability.

PO1-346 Type 1 Diabetes I

The effects of variable numbers of tandem repeats in insulin gene on the development of type 1 diabetes in Korean

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The variable number of tandem repeats in the insulin gene (*INS* VNTR), which is located on chromosome 11p15.5, is thought to be the susceptibility locus of type 1 diabetes. However, the *INS* VNTR genotypes show an ethnic variety and the association between the *INS* VNTR and type 1 diabetes has not yet been clarified in Koreans. This study was conducted to evaluate the effect of the *INS* VNTR on the development of type 1 diabetes in Koreans.

A total of 352 Korean subjects who had been diagnosed as type 1 diabetes under the age of 18 and 356 control subjects were included in this study. The insulin -23*Hph*I A/T single nucleotide polymorphism (SNP) was used as a marker of class I and III alleles and was typed using PCR-restriction fragment length polymorphism. To know the subdivisions of the *INS* VNTR, surrounding polymorphisms at +1127, +1140, +2331 and +2336 were determined using direct sequencing of PCR product.

The frequency of the class I/I genotype was 97.44% in patients and 94.10% in controls (*OR 2.389, P*=0.039), and that of the I/III genotype was 2.56% in patients and 5.90% in controls. The frequency of ID/ID genotype decreased significantly in patients (*OR 0.618, P*=0.017), and that of the IC/IC genotype in patients was higher than that of the ID/ID genotype in controls (*P*=0.039). The frequency of the I/I genotype was higher in patients who were diagnosed at the age of the <7 years (the younger age-onset group) than in controls (*OR 8.7, P*=0.011) but the difference was not significantly different in patients who were diagnosed at the age of ≥ 7 years (the older age-onset group). In the older age-onset group, the ID/ID genotype significantly decreased (*OR 0.558, P*=0.02) and the IC allele significantly increased as compared to controls (*OR 0.618, P*=0.042), whereas these genotypes were not significantly different between the younger age-onset and controls.

In conclusion, the *INS* VNTR was associated with susceptibility of type 1 diabetes in Koreans. The I/I genotype was generally susceptible to type 1 diabetes. However, the ID/ID genotype was protective against type 1 diabetes and the IC allele tended to be susceptible to type 1 diabetes. Additionally, *INS* VNTR may have distinctive susceptibility according to age at the onset of type 1 diabetes.

PO1-347 Type 1 Diabetes I

Is there any changes in the presentation of type 1 diabetes within years

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We aimed to identify whether there are any changes in presentation of childhood type 1 diabetes in recent years.

The clinical and laboratory data of a total of 95 children (54%, boys) with newly diagnosed type 1 diabetes in our pediatric endocrinology department from January 2005 to December 2008 were analyzed retrospectively. Diabetic ketoacidosis (DKA) was classified as mild, moderate and severe according to initial pH (< 7.3, 7.2 or 7.1, respectively).

The mean age at diagnosis was 8.9 ± 3.9 (0.7-17.3) years. Average age and the frequency in the younger ages showed no change in years. A family history of type 1 diabetes was 11% in first- and 7% in second-degree relatives. Thirty three (35%) of all patients were diagnosed in autumn. And seasonal rate of presentation was not different across the years. The most frequent symptoms were polydipsia (92%), polyuria (90%), weight loss (66%) and nocturia (60%). Thirty seven (39%) children presented with DKA. Severe DKA was observed in 12%, moderate DKA in 15% and mild in 12%. In 23%, there were different degrees of loss of consciousness (GCS 7 to 14). GCS decreased as pH declined ($r=0.49$, $p<0.001$). Kussmaul breathing was observed in 30% of patients with DKA. There was no increase in the number of patients but the ratio of patients who presented with DKA rose within years ($p<0.05$). The median duration of presenting symptoms before admission was 15 days (1-150). In 18 (18.9%) children, diagnosis was delayed >24 h (median 3, range 2-7 days).

The general epidemiological characteristics of patients at presentation did not vary while the rate of presentation with DKA increased within years.

In order to decrease the rate of DKA, diabetes education programs should be implemented to increase the consciousness of health professionals and population.

Table. Characteristics of type 1 diabetes at presentation across the years

	2005	2006	2007	2008	Total (n)	p
Patient (n)	23	29	25	18	95	
Age (mean ± SD)	8.4 ± 3.9	9.1 ± 3.7	9.3 ± 3.7	8.5 ± 4.6		〉0.05
Sex n (%)						〉0.05
Girls	14 (60%)	10 (35%)	11 (44%)	9 (50%)	44	
Boys	9 (40%)	19 (65%)	14 (56%)	9 (50%)	51	
Season n (%)						〉0.05
Winter	9 (39%)	6 (21%)	5 (20%)	10 (56%)	30	
Spring	5 (22%)	5 (17%)	5 (20%)	2 (11%)	17	
Summer	2 (9%)	8 (28%)	4 (16%)	1 (6%)	15	
Autumn	7 (31%)	10 (34%)	11 (44%)	5 (30%)	33	
DKA n (%)	5 (22%)	10 (35%)	11 (44%)	11 (61%)	37	〈0.05

PO1-348 Type 1 Diabetes I

Treating diabetic children and adolescents with insulin pump (CSII): impact of health-care team experience on patients' metabolic outcome

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Continuous subcutaneous insulin infusion (CSII) has been used for more than 3 decades. We hypothesized that internal, self-developed experience of health-care-teams on CSII use through the years could possibly impact the quality of metabolic control achieved by patients. In our institution, the use of CSII was introduced in 2001. For the past 8 years, 75 insulin pumps have been implemented. The purpose of this study was to evaluate the impact of the professional experience "gained" by our team on the metabolic control of diabetic patients who received CSII and to identify factors involved in the quality of metabolic control achieved through CSII

Patients and Methods: We retrospectively analyzed the charts of 63 patients. Two groups were made based on the chronological start of CSII: Group 1, with "earlier" start, and Group 2, which started later on CSII. CSII was always installed after intensive training and education of patients/parents on the principles of CSII. Outpatient follow-up in both groups was invariably the same. Age at CSII start, gender, pubertal stage, HbA1c, number of glycemic controls per day, number of boluses and basal rates per day were compared between the groups. HbA1c was measured by using HPLC (Menarini) (nv 5.1± 0.31%). Statistical analysis was performed by SPSS.

Results

	General Group	Group 1 (n=29)	Group 2 (n=34)	p (Group 1 vs 2)
Age at CSII start	12.7±4.4	14.9±3.7	10.8±4.2	0.000
Glycaemic controls/ d	7.2±1.7	6.5±1.9	7.8±1.5	0.001
Mean HbA1c (previous y)	7,3±1.1*	7.5±1.1	7.1±1.2*	0.046
HbA1c at 6 months	7.1±0.8	7.4±0.9	6.9±0.7*	0.008
HbA1c at 1 y	7.0±0.7	7.3±0.8(n=27)	6.6±0.4* (n=30)	0.000
HbA1c at 2 y	7.1±0.6	7.3±0.6(n=22)	6.8±0.4(n= 11)	0.043
Mean of last 3 HbA1c	7.0±0.8*	7.4±0.9	6.8±0.7*	0.001
Duration of CSII (y)	1.9±1.3	2.7±1.5	1.2±0.7	0.000
Stop CSII %	9.5	17.2	2.9	

*p<0.05

Conclusions: Patients who started on CSII after 4 years of experience of our team (Group 2) had significantly better metabolic control, and were more satisfied (low drop-out rate) than those who started on CSII during our initial years of experience. Positive predictive factors for good control were: younger age, better starting HbA1c levels and performing more glycemic controls. This improvement could reflect better selection of patients to receive CSII.

PO1-349 Type 1 Diabetes I

Lipid levels in children and adolescents with type 1 diabetes are frequently outside current recommendations

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Dyslipidaemia is a potentially modifiable cardiovascular risk factor in young people with type 1 diabetes (T1D). Guidelines are largely consensus-based or extrapolated from other populations. More aggressive management is being recommended.

Aims: Determine in children and adolescents with T1D:- (i) proportion meeting national lipid screening guidelines (within 12mths of diagnosis, within last 2yrs if pubertal, within last 5yrs if pre-pubertal) (ii) proportion with lipid values outside target levels (total-cholesterol≥4.4mmmol/L, LDL≥2.6mmol/L, HDL≤1.1mmol/L, triglycerides≥1.7mmol/L) (iii) association between dyslipidaemia and glycaemic control.

Methods: *Design:*-Retrospective descriptive study. *Setting:*-Tertiary paediatric hospital diabetes clinic. *Population:*-T1D, >12mths duration, age 2-19yrs. *Measures:*-Gender, age, HbA1c, BMI z-score, time since last lipid screen, most recent lipid values and fasting status.

Results: 146 patients were included (70F/76M; mean±SD age 13.1±3.6yrs, diabetes duration 5.6±3.4yrs, HbA1c (mean of 12mths) 9.0±1.5%, BMI z-score 0.56±0.87). 114 patients (78.1%) met lipid screening guidelines, with 24 patients never screened. Nine patients (6.2%) had fasting lipid levels. Fasting status was uncertain in 33 (22.6%). Levels were outside targets for total-cholesterol, LDL, HDL, and triglycerides in 71/128 (55.5%), 53/122 (43.4%), 32/123 (26.0%) and 53/128 (41.4%) of patients respectively. In patients ≥8yrs, LDL was >3.4mmol/L in 20/113 (17.7%) (pharmacotherapy recommended after failure of lifestyle intervention[1]) and LDL was >4.1mmol/L in 5/113 (4.4%) (pharmacotherapy recommended[1]). One patient was on statin therapy. Patients with HbA1c>10% (n=22) had significantly higher LDL levels than those with HbA1c≤10% (n=100) (3.23±0.89mmol/L versus 2.40±0.75mmol/L, $P<0.001$). HbA1c predicted LDL, independent of age, gender, diabetes duration and BMI z-score ($P<0.001$).

Conclusions: Almost half the children and adolescents had LDL values outside recommended ranges. A substantial proportion met the criteria for considering pharmacotherapy. Confirmatory fasting samples would be recommended. Use of non-HDL values in non-fasting samples is advocated but not yet included in guidelines. Lipid-lowering agents may be increasingly indicated while patients are still in paediatric clinics. However, safety, efficacy and cost-effectiveness data is needed.

[1]Daniels SR *et al. Pediatrics* 2008;122:198-208

PO1-350 Type 1 Diabetes I

Retrospective analysis of insulin pump therapy on long-term control in children and adolescents with type 1 diabetes – intensive education at pump start does not guarantee improved control

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Background: Continuous subcutaneous insulin infusion (CSII) has become more frequently used in the pediatric population. Several reports have documented that CSII is safe and achieves better or equivalent glycemic control to that obtained in the adolescent group in the DCCT. However few studies report results beyond 1 - 2 years of therapy.

Aim: To assess the long-term hemoglobin A1c (A1c) results in children and adolescents with Type 1 DM following intensive family education prior to CSII initiation and during regular follow-up.

Methods: Charts were reviewed on all patients who received their initial education on CSII therapy at the Stollery Children's Hospital Pediatric Diabetes Education Centre, and who were followed for at least 1 year subsequently. Criteria for pump initiation included A1c <10.0%, blood glucose monitoring ≥ 4 times/day, and evidence of the ability to adjust insulin and accurately carbohydrate count. Education was performed by Certified Diabetes Educators. All families received 4 half-day group education sessions at the start of CSII. Follow-up individual education occurred at clinic visits every 1-6 months until 17 years of age. A1c was measured by DCA 2000 at each visit. Paired Student T-tests were used to test for difference in mean A1c between baseline and follow-up time points.

Results: 121 charts were reviewed. 110 had a recorded A1c done at pump start (mean 8.11 % ± 0.75); 83 had been seen in follow-up for ≥12 months and were included for subsequent analysis. The mean age and duration of DM at the start of CSII in the 83 patients were 9.96 yr (±2.45) and 3.75 yr (± 2.84) respectively. The mean A1c at pump start was 8.00 % (±0.73). The change in A1c over time is provided in the table.

Months from CSII Start	No. of Patients	A1c % (±SD)	P (versus baseline A1c)
0	83	8.00 (±0.73)	
12 - 15	78	8.04 (±0.73)	0.83
24 - 27	62	8.42 (±1.04)	0.006
36 - 39	45	8.41 (±0.91)	<0.005
42 - 45	35	8.86 (±0.99)	<0.001
48 - 60	23	8.50 (±0.98)	0.033

Conclusion: Despite comprehensive education at the start of CSII therapy and at frequent follow-up visits, maintaining an A1c at recommended targets is difficult to achieve for many families. Similar factors that present challenges to achieving optimal control on injections are likely to be responsible.

PO1-351 Type 1 Diabetes I

Expression of interleukin-21 receptor, interleukin-17 receptor and other common gamma-chain dependent cytokine receptors on peripheral T lymphocytes in type 1A diabetes and the association with HLA alleles and autoantibodies

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Introduction: Type 1A diabetes (TID) is an autoimmune disease characterized by the pancreatic infiltration of T and B lymphocytes and production of cytokines. Interleukin-21 (IL-21), an inflammatory cytokine is a member of the common gamma-chain dependent cytokine family, which also includes IL-2, IL-4, IL-7, IL-9 and IL-15. An increased expression of IL-21R in T lymphocytes of NOD mouse (animal model of autoimmune diabetes) has been described. There was also an augmented expression of this cytokine receptor in several autoimmune diseases in humans, including systemic sclerosis and rheumatoid arthritis. However, it was also demonstrated a decreased expression of IL-21R in SLE patients. There are no studies of the expression of these gamma-chain receptors in T1D patients, especially of IL-21R. Interleukin -17 is also a highly inflammatory cytokine with biological effects at the interface between the adaptive and innate immune systems. Its production is partially dependent of IL-21 action. A role for IL-17 in autoimmune diabetes has been suggested in animal models but is yet to be proved in humans.
To evaluate the expression of IL-17R, IL-21R and other common gamma-chain dependent cytokine receptors of patients with type 1 diabetes.
Material and methods: We studied the expression of IL-21R, IL-2Ra (CD25), IL-2Rb (CD122), IL-4R (CD124) and IL-7R (CD 127) on peripheral T lymphocytes in 35 T1D patients (aged 1-16 yrs) with recent diagnosis and 25 healthy controls (aged 1- 16 yrs) using flow citometry. The expression of IL-17R was studied in 23 of the 35 T1D patients. The average duration of T1D was 2 months, and all patients were receiving insulin by the time of blood withdraw. Autoantibodies (anti-GAD and anti-IA2) were assessed by radioimmunoassay.
Results: All TID patients had at least 1 positive autoantibody (AB). None of the control subjects presented positive AB. We detected in TID patients a statistically significant decrease in the proportional expression of IL-17R (p=0,0032), IL-21R (p=0,0016), CD 25 (p=0,0015) and CD122 (p=0,004) in CD4+ T cells when compared to controls. There was no difference in the expression of CD124 and CD127 neither in CD3+ T, CD4+ T or CD8+ T cells.
Conclusion: The abnormalities of IL-17R, IL-21R, CD25 and CD122 signaling might be involved in the pathogenesis and/or progression of type 1A diabetes.

PO1-352 Type 1 Diabetes I

Novel KCNJ11 mutation resulting in transient neonatal diabetes

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Neonatal diabetes is a rare but potentially fatal disorder, with an estimated prevalence of 1 in 300,000 to 1 in 500,000. Approximately 57% of the diagnoses exhibit transient diabetes mellitus, and often relapse in later years. We report a case of familial neonatal diabetes in a newborn girl who presented with elevated blood glucoses of 200-300 mg/dl in the first week of life. This infant's blood glucose levels were regularly assessed in the postnatal period secondary to a maternal history of neonatal diabetes. As an infant, the patient's 23 y.o. mother had been found to have hyperglycemia requiring insulin treatment until her diabetes resolved at 4 months of age. The mother then experienced a relapse of her diabetes at 16 y.o., with subsequent insulin dependence since that time. Our patient was admitted to the University of Virginia Hospital on day of life 8 to treat persistent hyperglycemia. She ultimately achieved euglycemia on an Animas continuous insulin infusion pump and went into remission of her diabetes at 2 months of age. Multiple gene evaluation for possible causes of neonatal diabetes mellitus was performed by Athena laboratories revealing a novel KCNJ11 nucleotide change (535G>A) in both the mother and child. KCNJ11 codes for the Kir6.2 protein subunit of the ATP-sensitive potassium channel, regulating insulin release from pancreatic beta cells. Activating mutations in this subunit can cause reduced sensitivity to intracellular ATP, thus decreasing potassium channel closure and impairing beta cell membrane depolarization and subsequent insulin release. Previously-described KCNJ11 mutations have responded well to treatment with sulfonylureas. Diagnosis in our child resulted in a recommendation for the child's mother to begin treatment with a sulfonylurea. This case of familial transient neonatal diabetes represents the first reported substitution of lysine for glutamic acid at residue 179 in the Kir6.2 protein, illustrating important insights into the genotype-phenotype relationship of KCNJ11 mutations.

PO1-353 Type 1 Diabetes I

Cardiovascular risk in diabetic children: results of a longitudinal study

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Cardiovascular disease due to atherosclerosis is a major cause of morbidity and mortality in adult diabetic patients. In diabetic children, longitudinal assessment of subclinical atherosclerosis by measurement of carotid artery intima medial thickness (IMT) has not been performed up to date. This study reports for the first time results of a longitudinal observation in this patient group.
Patients and Methods: Of 37/150 diabetic children in whom an increased (IMT) had been found in a previous study, 27 (mean age 14.6 ± 2.6y) could be reevaluated two years after the initial assessment. 5/27 pts. were on medication with ACE-inhibitors, all patients underwent detailed counselling of their life style, sports activity and nutritional habits.
Results: Mean IMT increased significantly (0.49 ± 0.02 mm vs. 0.51 ± 0.026 mm, $p < 0.05$) However, there was no significant change compared to reference values (mean IMT z-score 2.4 ± 0.3 vs. 2.6 ± 0.5). 13/27 pts. (48%) showed a progression of the IMT whereas in 14/27 pts. the IMT values remained stable. In this subgroup, pts. with IMT-progression showed a higher HbA1c (7.5 ± 0.8 vs. 7.1 ± 0.7, $p < 0.05$) and a slightly higher systolic blood pressure (120 ± 14.4 vs. 113.9 ± 12.1, $p = 0.08$).

Table 1: Patients characteristics at baseline and after 2 years

	Baseline	Follow-up
BMI-SDS	0.2 ± 0.7	0.3 ± 0.9
sBP (mmHg)	110.5 ± 10.9	116.8 ± 13.3
LDL-Cholesterol (mg/dl)	88.1 ± 18.3	76.9 ± 21.6 *
HDL-Cholesterol (mg/dl)	56.1±12.8	65.2 ± 14.8 *
HbA1c (%)	7.1 ±0.7	7.5 ± 0.8
IMT (mm)	0.49 ± 0.020	0.51 ± 0.026 *
IMT-SDS	2.4 ± 0.3	2.6 ±0.5

BMI-SDS: standard deviation score of the Body-mass-index; sBP: systolic blood pressure; IMT: Intima medial thickness; IMT-SDS: standard deviation score of the IMT

Conclusions: In a well selected group of diabetic children, mean IMT progression during a two years period did not progress significantly. Children with a higher HbA1c and a higher systolic blood pressure showed a progression of the IMT. Good diabetes control children may help to avoid subclinical atherosclerosis progression.

PO1-354 Type 1 Diabetes I

Predictors of poor glycemic control in children newly diagnosed with type 1 diabetes mellitus

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Introduction: Diabetes is one of the most common chronic diseases in children, and the best recognized risk factor for acute and chronic diabetic complications is poor glycemic control. Little is known on the correlation between biochemical and anthropometric parameters at the onset of diabetes and future glycemic control. The objective of this study was to identify predictors of subsequent poor glycemic control in children newly diagnosed with type 1 diabetes mellitus (T1DM), in order to select patients at risk and develop a more intensive treatment strategy.
Methods: Medical records of all patients diagnosed with T1DM between 2002 and 2005 at St. Christopher's Hospital for Children were reviewed. Inclusion criteria were: 1-18 years of age; at least one positive autoantibody (ICA, IA-2 or anti GAD65); 3) ≥ 3 years of follow up, with at least 2 visits per year. Poor glycemic control was defined as a mean Hb1Ac ≥ 9% during the last year of follow up.
Results: 58 patients (55 % males) were included in the study. Their mean follow-up period was 4.3 yrs. At diagnosis, their mean age was 8.0 ± 4.2 years; 65 % were Caucasians, 20 % African-American, and 15 % Hispanic. When we divided the patient sample in two groups according to the last-year HbA1C (< or ≥ 9%), those with higher HbA1C exhibited a higher HbA1C at diagnosis (12.0 ± 1.4 vs. 10.3 ± 0.4 %), but this difference did not reach statistical significance. 40 % of children with HbA1C ≥ 9% at the end of the follow-up had DKA at diagnosis and 50 % of them had positive family history for T1DM, compared to 25 % and 28 % of children with a HbA1C of < 9 %, respectively. We found no significant correlation (by Pearson correlation) between HbA1C values at diagnosis and at the end of the follow-up period. Using univariate and multivariate logistic regression models, we also found no correlation between poor glycemic control (HbA1C in the last year of follow-up of ≥ 9%) and the following variables at diagnosis: age, sex, race, BMI SDS, Tanner stage, family history for type 1 or type 2 DM, DKA at diagnosis, and daily insulin dose.
Conclusions: Our findings appear not to support any correlation between a number of parameters at diagnosis and subsequent glycemic control. On the other hand, the trend toward a possible correlation between HbA1C at diagnosis, DKA at diagnosis, family history for T1DM, and future poor glycemic control warrant prospective studies with a large population sample.

PO1-355 Type 1 Diabetes I

Longitudinal assessment of diabetes management through the transition from high school to young adulthood: initial results at 6 months post high school

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It is well-documented that diabetes management (DM) becomes poorer during adolescence and the transition to adulthood may be a critical time for adolescents (adol) with type 1 diabetes (T1D). Yet, no longitudinal studies have examined DM for late adol, and not much is known about factors that predict outcomes during this transition.
We are currently engaged in a longitudinal study tracking adol with T1D from the last 6mo of high school (HS) through the 1st yr post HS; participants complete web-based surveys every 3mo for 18mo assessing behavioral and health-related domains. This analysis focuses on data from the 1st study wave, comparing outcomes obtained just prior to HS graduation with those obtained >6mo after. Data collected includes the diabetes self management profile (DSMP, Harris et al 2000) designed to assess management of exercise, hypoglycemia, diet, glucose testing, insulin administration, and dose adjustment over the prior 3 mo. Higher scores indicate more careful DM, with a max score of 90.
31 adol had complete survey data at both time points (39% male (M), 93% Caucasian). Baseline (BL) mean age was 18.2 yr, with mean T1D duration of 9.3 yr (range 2.6-17). 21 were using insulin pumps (mean pump time 4.7 yr, range 1.2-8.1). A1c was 9.0±2.1% (n=23). Mean DSMP score at BL was 51±11 (56% of max). Although A1c did not vary by gender, females (F) had better DSMP scores than M, with higher scores for hypoglycemia mgmt and glucose testing. DSMP score did not differ with pump use.
The second survey was reported ~8 mo (range 180-280 days) after graduation. 30 were in school; 51% had moved out of parents' homes. Neither A1c (mean change = -0.2±1.2, n=13) nor DSMP score (mean change =0.7±7.8) were significantly changed, nor had any DSMP subscale measures changed significantly. Again, F had better DSMP scores than M, with higher scores for hypoglycemia management and glucose testing, although now M had higher exercise scores.
We are continuing to follow these adol and recruiting 2 more cohorts over the next 2 yrs. Multi-level regression modeling will be used to examine changes over time in outcome, personal, and environmental factors as well as relationships among these factors in the context of situational transitions. Identifying these key times and influential factors will provide information critical to designing future effective interventions to improve glycemic control and quality of life for adol transitioning to adulthood. Funding: R01 NR009810-01A1

PO1-356 Type 1 Diabetes I

Effects of insulin dose changes of children attending a diabetes camp

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Summer camps are popular for children with type 1 diabetes (T1D). Since camp is associated with increased activity compared to at home, insulin and dietary regimens must be adjusted for hypoglycemia prevention. However, there are few guidelines for insulin adjustments at camp.
We retrospectively analyzed insulin doses in children with T1D attending a week long residential diabetes camp (June 2007 and 2008) prior to camp, after a fixed adjustment (~10% decrease in dose of long-acting insulin and basal rates) at the beginning of camp, and at the end of the week before children returned to pre-camp regimens.
Information was available on 256 campers (44% M, mean age 11.6±2.0yr (range 7.4-15.8)). Children had T1D for a mean of 7.0±3.2yr. 55% used insulin pumps. Of those on injections, 77% were on Lantus® or Levemir®. Children on pumps had lower A1cs than children on injections (8.0±1.2% vs 8.5±1.2%, p=0.005).
During their 1st day at camp, children using pumps received 11.1±6.3% (range +10% to -35%) less basal insulin than home doses, whereas children on injections decreased long-acting insulin by only 8.2±12.8% (range +100% to -50%) (p<0.02). 79% of campers had no change in rapid mealtime insulin dosing compared to home (7% had increases). 60% of campers had at least one blood sugar <70 mg/dL during the first 24h at camp. Despite larger decreases in basal insulin, children on pumps were more likely to have low blood sugars during their first day at camp than those on injections (average of 1.4±1.3 vs 0.8±1.0 lows/camper, p<0.001). The number of lows increased with increasing camper age, but was not correlated to A1c prior to camp.
Staff adjusted insulin doses twice daily throughout camp. Overall, children did not have significant reductions in their total daily insulin dose from the first to the last day of camp. Specifically, 45% had further insulin dose reductions, 4% did not change, and 51% had an increased daily dose. However, in the last 24 hours, children had fewer lows (1.1±1.2 vs 0.7±0.9, p<0.001) than on the first day, and 51% had no low blood sugars on the last day.
This is the first study to characterize effectiveness of a fixed dose reduction in long-acting/basal insulin. Starting with a ~10% reduction appears reasonable; however, this may not be enough. Additional prospective research can further characterize variables to facilitate tailoring adjustments to minimize blood sugar variability and improve overall control in the camp setting.

PO1-357 Type 1 Diabetes I

Factors contributing to terminal digital preference in 91398 patients with diabetes mellitus in Germany and Austria: possible impact on therapeutic decisions

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Aims Correct blood pressure measurements are decisive for appropriate diabetes management. The accuracy of blood pressure (BP) readings might be negatively influenced by systematic errors such as terminal digital preference (TDP). TDP describes an observer's tendency to record the measurements using certain digits. As TDP might interfere with diabetes treatment, we used the binational Diabetes databank DPV to study the rate of and factors contributing to TDP.
Methods A total of 580578 BP readings were documented in 286 participating centers, 494301 measurements from patients with type 1 and 86277 from patients with type 2.
Results Total TDP for the digits "0" and "5" was 55±50% and more frequent in smaller centers, rehabilitation centers, in non academic and in outpatient centers. A lower rate for TDP was found in Pediatric centers and centers treating patients with type 1 diabetes, in Austrian and West German centers compared to East German centers. Within the last 14 years, TDP increased with age and declined in type 1diabetes, but not in type 2 diabetes centers. Levels of systolic BP were significantly lower in centers positive for TDP.
Conclusions TDP is common among German diabetes centers. Profound differences between centers depending on the type of diabetes and the regional differences were found. Pediatric centers appear to be less susceptible for TDP. As TDP might be associated with insufficient BP management BP readings need to be improved by training academic and non-academic employees and by using automated BP devices.

PO1-358 Type 1 Diabetes I

Trends in incidence of type 1 diabetes in childhood – 20 years of diabetes incidence registry (DIARY) Baden-Wuerttemberg, Germany

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Aim: To predict the frequency of diabetes in childhood and adolescence in Germany for the next 20 years.
Methods: Data on diabetes onset has been collected continuously for more than 20 years by means of a registry in the German federal state of Baden-Wuerttemberg (BW). These data pertain to patients below 15 years of age (n=5,108; documentation period, 1987-2006; completeness of data 98.1%). Each of the 31 hospitals and one diabetes centre in the state participated in the survey.
Results: (1) The mean gender- and age-specific incidence rate for the total period of documentation (1987-2006) was 15.3/100,000/year (95 % CI 14.8-15.7). (2) The annual incidence rate can be expressed as a quadratic function of the calender year x (y = (3.05+ 0.0778*(x-1986))^2, r = 0.95). (3) The incidence rate was also increasing quadratically with calendar year for all ages. The highest increase was observed in the age groups comprising 2- and 3-year-olds (+12% and +13% per year, respectively for the square roots of the incidences), while the lowest increase occurred in the group comprising 11-year-olds (+3% per year). (4) The prediction model is as follows: b0 + b1 * age + b3 * (age - 8) ^ 4 + (m0 + m1 * age + m3 * (age - 8) ^ 3) * (x-1986); b0 = 2.11, b1 = 0.152, b3 = -0.00062, m0 = 0.132, m1 = -0.0069, m3 = 0.000124; age: 1 to 15, year: 1 to 20 (n = 300; variance of the observations =1.056; variance of the residuals = 0.3056; R2=0.71)
Conclusions: The incidence rate of type 1 diabetes is rising in children and adolescents in Germany. We observed a characteristic shift towards a younger age.

PO1-359 Type 1 Diabetes I

CSII vs MDI therapy in childhood and adolescent diabetes: the results of 3 years follow-up

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An adequate glycemic control remains a real problem for children and adolescents with Diabetes Mellitus type 1. The uses of new insulin analogs and intensive insulintherapy is not always available to reach the target levels of glycemia and glycogemoglobin (HbA1c). At the present time, insulin pump is more effective tool for patients which can help to improve glycemic control.
Objective: to evaluate the results of metabolic control in two group patients, receiving continuous subcutaneous insulin infusion (CSII) and multiply daily injection (MDI) respectively.
Materials and Methods: 61 patients (28 M/ 33 F) with diabetes type 1 aged 5-22 yrs (12.8 ±5.4 yrs) and duration of disease 3-14 yrs (6.2 ±2.9 yrs) took part in this study. The patients were divided in two groups –CSII (n=31) and MDI (n=30), having no difference in age, duration of diabetes and HbA1c levels. The insulin pumps "Minimed 508", "MiniMed 712", and short-acting insulin analog (Aspart) were used in CSII group. Insulin analogs Glargine in bedtime and Aspart before main meals were used in MDI group. Glucose self-control was performed 4-6 times in day. All patients stayed in hospital for 10-14 days before start of study and visited clinic at least 1 time in 6 month during 3 years for correction of insulin therapy. HbA_{1c} level before and in 12, 24, 36 month after the start of study, the frequency of severe hypoglycemia and diabetic ketoacidosis were analyzed.
Results: The results of HbA1c are present in table.

Characteristics of trearment groups

Therapy	Age, years	HbA1c,% before treatment	HbA1c, % in 1 years	HbA1c, % in 2 years	HbA1c, % in 3 years
CSII (n=31)	12.9±5.6	9.9 (±1.77)	8.9 (±1.29)	9.2 (±2.3)	8.9 (±1.94)
MDI (n=30)	12.7±5.3	9.8 (±2.0)	9.1 (±1.8)	8.9 (±2.6)	9.2 (±1.5)
					p>0.05

None of patients suffered from episodes of severe hypoglycemia during the evaluation period. Diabetic ketoacidosis occurred in 2 patients due catheter occlusions in CSII group and 2 patient in MDI group due concomitant viral infections. All patients in CSII group marked convenience of pump uses, absence of injections, a flexibility in day regime.
Conclusion: there was no statistical difference in Hba1c level between two groups. However, patients with CSII marked convenience of insulin pump.

PO1-360 Type 1 Diabetes I

Fear of hypoglycemia in diabetes and metabolic control

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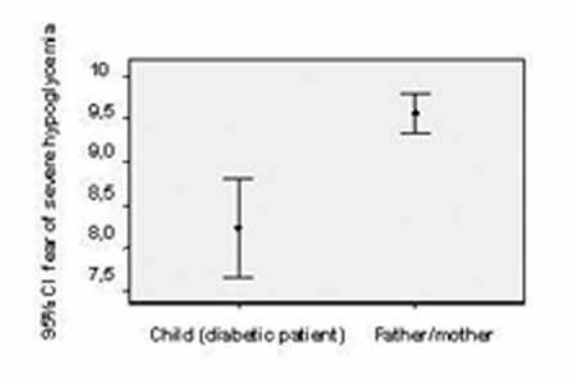
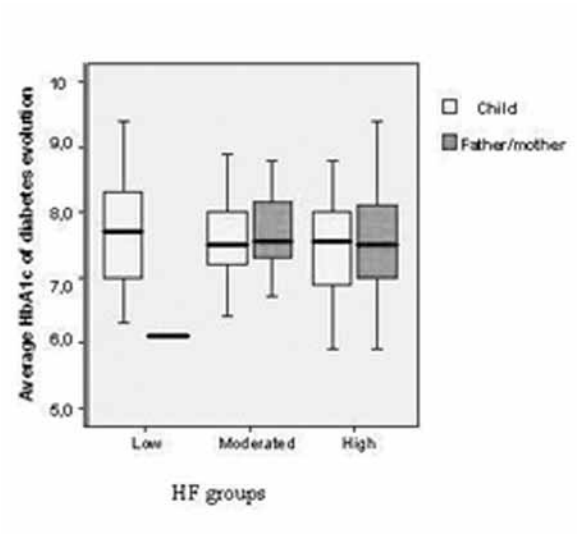
Adverse symptoms of hypoglycemia can cause "hypoglycaemia fear" (HF) in diabetic children as well as their families, and can produce an impact on diabetes metabolic control. Objectives: to value this hypothesis.
Methods.A cross-sectional survey was performed in type 1 diabetic children attended in our hospital (diabetes duration > 6 months and at least three capillary glycemia values/day). Affiliation data,average HbA1c of last year and HbA1c of the last visit were collected. Diabetic children (>7 years) and their parents were asked about HF according to analogical visual scale. For a better analysis, hypoglycemia was compared with other bothering aspects of diabetes: a game with fifteen picture cards was shown, and children had to order them from higher to lower concern.
Results. 65 parents and 68 diabetic children (35 boys, 33 girls) (age 11.7±2.3 years) accepted to participate. Average HbA_{1c} 7.6±1.36. Average punctuation

of HF was 8.24±3.27 for children and 9.57±0.95 for parents, which shows a statistically significant difference (p<0,001), (fig 1). By age groups (<7,8-13,14-18 years), fear to any type of hypoglycemia was statistically significant (p<0.05).





In comparison to other bothering aspects of diabetes, hypoglycemia did not take the preferential place. Most children located hypoglycemia in the second/third place.These results could explain that high HF does not correlate with a worst metabolic control as per parents and children is concerned (fig 2)
Conclusions. Hypoglycemia stills being an important reason to worry about, both for parents and children, being the parents the ones more concerned about it. However, HF does not suppose a worst metabolic control, maybe because currently there is a higher awareness related to chronic complications.

PO1-361 Type 1 Diabetes I

Evaluation of a local group structured educational programme for children with type 1 diabetes

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Introduction: In adults, there is evidence that the delivery of structured education for individuals with type 1 diabetes (T1D) facilitates self management to attain better glycaemic control. At Sandwell Hospital, we developed an interactive 3 hour structured group educational programme (SEP), delivered by a multi-disciplinary team (Paediatrician, a children's diabetic specialist nurse, a dietician and a child psychologist). The modules include: module for all those diagnosed in the last one year, basal bolus module, conventional insulin regime module, CSII module and Transition module. The modules promote self management skills including carbohydrate counting, illness management, adjustment of insulin doses to achieve target blood sugar, conflict resolution and improving self efficacy. All children and their parents attending the diabetic review clinic are invited to attend one appropriate module every year. In this retrospective study we have evaluated the impact of the SEP on diabetic control (HbA1c levels), rates of clinic non-attendance (DNA) and diabetic Ketoacidosis (DKA).
Method: We retrospectively retrieved relevant data from diabetic clinic electronic database to analyse the results. We evaluated the SEP run in 2007 (Jan 2007 to Jan 2008).
Results: A total of 101 children (ages 3-18 yrs) (mean age 13.6yrs) were invited to the SEP. 46% boys and 56% girls. Of those invited, 44/101 (43.5%) attended. There was no difference in the median age, ethnic distribution or sex distribution in those that attended (AT) compared to those that did not attend (NT). However, the AT group had significantly better diabetic control (median Hb1Ac 8.7%) prior to invitation than the NT group (median HbA1c 9.15%) (p=0.046). For the AT group, there was significant improvement in median Hb1Ac, three months afterwards (median HbA1c 7.8%) compared to that before the SEP (median HbA1c 8.7%). (p=0.0236). The improvement in median HbA1c continued at 6 (8.1%) and 9 months (8.5%) but this did not reach statistical significance. There was no significant difference in the rates of DNA between the two groups (p=0.522). Six children were admitted for DKA in 2008 (2 AT vs 4 NT)
Conclusion: Our data show that the SEP lead to short term significant improvement in glycaemic control. This highlights the need to regularly reinforce the principles of self management. Ways of encouraging the children with the poorer control to attend needs to be investigated.

PO1-362 Type 1 Diabetes I

Assessment of the different educational variables influencing the metabolic control in type I diabetic (T1D) young patients

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The **aim** of this study was to assess the different educational variables influencing the metabolic control in (T1D).
Material and methods: 84 T1D young patients aged between 10-19 years old attending to Endocrinology, Diabetes and Nutrition Service at "San Roque" Hospital in Paraná from 2006 to 2008 were evaluated by personal interviews and validated tests on their diabetes self care and family functioning style. The following variables, assumed as influencing the metabolic control were assessed:diabetological education, physical activity, nutrition, and family functioning style (Mc Master test). The metabolic control was estimated by the glycosylated haemoglobin A1C (HbA1C) levels. All the patients received insulin between 0.5-1.5 U/kg/d. To determine if there was any correlation between the independent variables and the metabolic control we used Spearman bivariate correlations.
Results:
Significant correlation between glicosilated haemoglobin values and nutrition (p=0,004), family functioning style (p=0,002), diabetological education (p=0,002) and physical activity (p=0,005) was observed. Risk factor for HbA1C levels higher than 8% were: inadequate nutrition (OR=3.5), inadequate family support (OR=4), poor diabetic education (OR=3.95) and insufficient or lack of physical activity (OR=3).
Conclusion:
The strongest risk factor for increased HbA1C levels (>8%) was the inadequate family support The present results might shed light on the rationale for the design of cost-effective educational programs.

PO1-363 Type 1 Diabetes I

Children and adolescents with type 1 diabetes mellitus have a higher frequency of parietal cell antibodies with signs of early atrophic gastritis compared to healthy controls

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Objective: Studies in adults have shown a higher prevalence of parietal cell antibodies (PCA; up to 20%) in patients with type 1 diabetes mellitus (T1DM) compared to healthy controls (1%). PCA are the principal markers of autoimmune gastritis (AG). AG itself is characterized by achlorhydria and hypergastrinemia leading to pernicious anaemia and iron deficiency. The aim of this study was to evaluate the frequency of PCA in children and adolescents with T1DM compared to healthy controls. Clinical and biochemical markers in PCA positive patients with T1DM were further investigated.

Research Design and Methods:

We studied 170 patients (87 males) with T1DM, mean age of 12.9 years (range, 2.6-19.5) with a mean duration of diabetes of 4.9 years (range, 1.0-16.3) and 101 healthy controls (49 males) mean age of 13.0 years (range, 3.4-18.6). PCA, free T4, free T3, TSH, TPOAb, and TgAb were measured in all patients. In addition, gastrin, pepsiongen I, iron, ferritin, vitamin B12 and folate were measured in T1DM patients only. Gastroscopies with multiple biopsies and *Helicobacter pylori* test were carried out in those T1DM patients with high (>100 U/ml) PCA levels.

Results: The frequency of PCA in patients with T1DM was 5.3% compared to 1.9% in healthy controls. We found a strong correlation between PCA and TPOAb as well as PCA and gastrin (p=0.001). Age, sex, diabetes duration, and age at diabetes onset were not identified as risk factors for developing PCA. Hypochromic microcytic anaemia was present in 4 out of 9 patients from the PCA positive group compared to 4 patients out of 160 from the PCA negative group (ns). Hypergastrinemia was found in 2 patients from the PCA positive group compared to none out of the PCA negative group. All patients had normal pepsinogen I levels. Histopathologically, one out of 4 patients, who underwent gastroscopy, showed beginning signs of AG.

Conclusion:

Compared to healthy controls, children and adolescents with T1DM are at increased risk for developing PCA, in particular if positive for TPOAb. Young T1DM patients with positive PCA have early signs of AG, as iron deficiency anaemia and hypergastrinemia. We therefore recommend screening for PCA at diabetes onset and later on in the adolescent years.

PO1-364 Type 1 Diabetes I

Potential celiac disease in tipe 1 diabetes: an Italian multicenter study

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Background: Celiac disease (CD) is frequently associated with Type 1 Diabetes Mellitus (T1DM) with a mean prevalence of 4,5%. Screening tests select subjects who need to confirm CD diagnosis by biopsy. Subjects with positive celiac-related antibodies and normal intestinal mucosa in the biopsy are defined as potential-CD (CD-pot).

Aim: to define the prevalence of CD-pot among a large T1DM population followed in paediatric centres and to characterize clinical features.

Methods: data are collected in 28 Italian Centres of Paediatric Diabetes. CD-pot patients have been selected by positivity of antiendomysium (EMA) and/or anti-tissue transglutaminase (TGase) antibodies and normal intestinal mucosa. Patients who did not perform biopsy are excluded by evaluation. Collected data are: age at present, at diagnosis of diabetes and at first positive CD-test, CD-related symptoms, presence of first-degree relatives with CD and co-morbidity with other autoimmune disorders.

Results: 8671 patients T1DM from 28 centres, 546 (6,3%) CD, 75 CD-pot (0,8%). Prevalence of CD-pot on all EMA/TGase positive patients was 8.3%. All 75 CD-pot patients (age 13,7±6,9 range: 3,0-27,4) presented EMA positivity, 70/75 Tgase positivity. In 64 patients (85%) CD-related symptoms were absent, in 11 (15%) were present abdominal pain (6), failure to thrive (3) and diarrhoea (2); 10 symptomatic patients observe a gluten-free diet. Age at diagnosis of T1DM was 5,92 ± 3,61 years (range: 1,-15,5) and at first serological positivity 8,67 ± 5,11 (range: 1,2-27,4): 46,6% were CD-tests positive within one year of diagnosis of diabetes, 36% in the successive 1-5 years and 17,3% >5 years (mean 2,82 years, range 0-24). Only 2/75 patients had a first degree relative with CD and 14/75 (18,6%) had another autoimmune disorder (12 patients thyroiditis, one multiple sclerosis and one thrombocytopenia).

Conclusions: In a large population of Italian T1DM patients followed by paediatric centres, prevalence of CD and CD-pot is 6,3% and 0,8% respectively. Prolonged studies of follow-up are necessary to define the evolution of the mucosa damage and the opportunity of the gluten-free diet.

PO1-365 Type 1 Diabetes I

Television viewing and computer use are associated with socioeconomic family status and glycaemic control in children, adolescents and young adults with type 1 diabetes

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Background: Aim of this study was to evaluate the relationship between television viewing, computer use, socioeconomic family status and glycaemic control in children, adolescents and young adults with type 1 diabetes.

Methods: The cross-sectional study included children, adolescents and young adults with type 1 diabetes up to the age of 21 years. Self-report questionnaires were used to determine the time spent watching television and using a computer and to assess the socioeconomic family status. Clinical data and HbA1c levels were determined during outpatient clinic visits.

Results: A total of 296 children, adolescents and young adults with type 1 diabetes participated in the study (median age 14.5 years, median diabetes duration 5.6 years, median HbA1c 8.3%). Time spent watching television and using computer was as follows: 27% watched television <1h daily, 57% watched television between 1 and 2 hours daily, 12% watched television between 3 and 4 hours daily and 1% watched television >4 hours daily. 48% spent <1h using the computer daily, 40% used the computer between 1 and 2 hours daily, 7% used the computer between 3 and 4 hours daily and 4% used the computer >4 hours daily. There was a significant increase in HbA1c levels with increasing television viewing (p=0.007) and computer use (p=0.006). Time spent watching television and using computer was not significantly associated with body mass index standard deviation score in children, adolescents and young adults with type 1 diabetes (p=0.09 and 0.08). Higher socioeconomic status (33% of families) was associated with better glycaemic control, whereas lower socioeconomic status (30% of families) was associated with poor control (p=0.004). Socioeconomic family status was associated inversely and significantly with time watching television and computer use (p=0.01).

Conclusions: Extensive television viewing and computer use is associated with poor glycaemic control in children and adolescents with type 1 diabetes.

PO1-366 Type 1 Diabetes I

Association of between the onset of type 1 diabetes and celiac disease

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Objective: The association of celiac disease(CD) and type1 diabetes mellitus(DM1) is well recognized. The purpose of this study was to determine the prevalence of CD in children with DM1 and indentify the association between the age at onset of diabetes and CD. **Methods:** A cross sectional study was carried-out on 135 children and adolescents aged 2-18 years with DM1 from 2006-2008 in children hospital, Tabriz-Iran. We measured IgG/IgA antigliadin(AGA), IgA antitissue transglutaminase(tTG) and IgA antiendomysial antibodies(EMA). Intestinal biopsies were performed in children who tested positive for 2 of the 3 classes of antibodies. Data were expressed as mean±SD & N(%).These were analyzed with the independent samples T-Test. A P.Value less than 0.05 was considered statistically significant. **Results:** Of the 135 children (meange 8.82±3.34), 28(20.7%) patient had positive testes for antibodies. Biopsy confirmed celiac disease was diagnosed in 9 (6.6%) Subjects.Mean age at onset of diabetes in biopsy confirmed celiac disease group was (7.31±2.32) and in non CD group was (7.83±4.40). There was no significant association between CD positive diabetic patients and non CD groups with regard to age at onset of diabetes(P=0.42)**Conclusions:** The prevalence of biopsy confirmed celiac disease in our study children with type1 diabetes is high, suggesting the need for regular screening assessment of diabetes children. Age at onset of DM1 was not associated with risk having celiac disease .

PO1-367 Type 2 Diabetes, Insulin Resistance I

Effect of ethnicity and puberty on insulin sensitivity as measured by HOMA-IR and QUICKI in obese children and adolescents with a normal OGTT

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Objectives: To compare the differences in the insulin sensitivity as a function of ethnicity and pubertal stage and to document reference values for homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) in obese children and adolescents with normal oral glucose tolerance test (OGTT).

Methods: Retrospective chart review from January 2007-December 2008. We calculated HOMA-IR and QUICKI using fasting insulin and glucose levels obtained through OGTT. There were 175 obese subjects (mean BMI z score: 2.37± 0.50), details are in table 1.

Results: 72% subjects were females, ethnic groups were 61.14% Caucasians, 25.71% African Americans (AA), 9.14 % Hispanic and 4.01% Asians. Acanthosis nigricans was present in 80.4 %, history of type- 2 diabetes in the first and the second degree relatives was 34.5 % and 79 % respectively. The values of HOMA-IR and QUICKI were compared for boys and girls according to Tanner stage, ethnicity and based on age groups < 7, 7-8.9, 9-10.9, 11-12.9, 13-14.9, 15-17.9 and >18 years.

Mean values for HOMA-IR and QUICKI for Tanner 1 were 2.87±1.8 and 0.14±0.008, for Tanner 2-3 were 4.79±2.85 and 0.13±0.04, and for Tanner 4-5 were 4.75±3.63 and 0.13±0.01 respectively.

In males there is a significant relationship ($p<0.001$) between age groups and the HOMA-IR and the QUICKI values with the highest insulin resistance between age groups 15-17.9 years. Males have higher HOMA-IR values and lower QUICKI values ($p<0.001$) as compared to females at age group 15-17.9 years. Tanner stage groups showed no significant relationship between ethnicity and HOMA-IR or QUICKI. Between age groups 13-14.9 years there was a significant relationship between ethnicity and HOMA-IR values ($p<0.01$), AA were more insulin resistant ($P<0.01$). There is a significant relationship ($p<0.05$) between Tanner stages and both QUICKI and HOMA, and within pubertal stages between Tanner stage 1 and 2-3 groups ($p<0.05$).

Conclusions: Ethnic sub-groups show similar values for surrogates of insulin sensitivity, with variable cut-off points in different age groups and pubertal stages. There is a significant increase in insulin resistance with the onset of puberty, males are more insulin resistant in late puberty.

Table 1	
Variable	Mean
Number	175
Age	12.13 ± 3.10
BMI Z score	2.37± 0.50
Tanner stage	3.15 ± 1.50
Fasting blood glucose	82.25±9.00
Fasting Insulin	20.73±15.54
HbA1c	5.51 ± 0.38

PO1-368 Type 2 Diabetes, Insulin Resistance I

Progressive accelerated deterioration of β-cell function in autoantibody positive obese youths with a clinical diagnosis of type 2 diabetes (CDx-T2DM)

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Background: We previously demonstrated that obese youth with a CDx-T2DM and positive islet-cell autoantibodies (Abs) have more severe impairment in insulin secretion but less insulin resistance compared with peers with negative Abs. In longitudinal studies of Latent Autoimmune Diabetes of Adults (LADA), the presence of Abs is a sensitive marker of future insulin dependence. We hypothesized that Ab^+ youths with a CDx-T2DM will demonstrate progressive deterioration in β-cell function.

Methods: We prospectively investigated 6 youths with Ab^+ CDx-T2DM, treated with Metformin and insulin, after a 13.3 ± 1.4 months of their initial evaluation which included body composition (DEXA), abdominal adiposity (CT-scan at L4-L5), in-vivo insulin sensitivity (3hr-hyperinsulinemic euglycemic clamp), and insulin secretion (2hr-hyperglycemic (~225mg/dl) clamp).

Results: (Mean ± SE).

	Baseline	Follow-up	Paired t-test P
Age (Years)	14.5 ± 0.9	15.6 ± 1.0	<0.001
Diabetes Duration (months)	13.3 ± 6.0	26.7 ± 6.0	<0.001
BMI (Kg/m²)	33.9 ± 3.7	33.8 ± 3.4	ns
% Body Fat	41.8 ± 2.6	43.5 ± 4.5	ns
Visceral abdominal fat (cm²)	103.9 ± 46.4	96.9 ± 44.5	0.04
Subcutaneous abdominal fat (cm²)	575.2 ± 85.0	571.2 ± 92.3	ns
HbA1c (%)	6.3 ± 0.5	6.8 ± 0.3	ns
Insulin Sensitivity (mg/kg/min per μu/ml)	2.6 ± 0.6	2.3 ± 0.4	ns
1st Phase Insulin (μu/ml)	48.0 ± 7.8	30.5 ± 6.6	0.021
2nd Phase Insulin (μu/ml)	76.1 ± 19.5	49.0 ± 16.22	0.002
Glucose Disposition Index (mg/kg/min)	114.9 ± 19.4	62.6 ± 12.7	0.045

There was ~35% decline in both 1st and 2nd phase insulin secretion over 12-15 months with 45% decline in β-cell function relative to insulin sensitivity (glucose disposition index).

Conclusion: Ab^+ CDx-T2DM youth show accelerated progressive decline of insulin secretion relative to insulin sensitivity without significant deterioration in the degree of insulin resistance. This observation is consistent with autoimmune β-cell destruction and reinforces the need to: 1) determine antibody status in obese youth clinically diagnosed with T2DM, and 2) institute insulin therapy early in the course of the disease.

PO1-369 Type 2 Diabetes, Insulin Resistance I

Prevalence of diabetes mellitus, impaired glucose regulation and associated risk factors in a population of the centre of Spain

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OBJETIVES:
1. To determine the prevalence of all kinds of diabetes mellitus (DM) in the autonomous community of Castilla-La Mancha (CLM) in population over 30 years old.
2. To estimate the prevalence of unknown DM, in the same population.
3. To estimate the impaired glucose regulation
4. To investigate the associated risk factors
RESEARCH DESIGN AND METHODS:
CLM is a region in the centre of Spain and has 1.894.667 people. A total of 3092 subjects over 30 years old were chosen in a random sampling of the population (stratified by age and sex), selecting 50 Zones of Health by a random method proportional to the size of the zone. An oral glucose tolerance test was performed (excluding known diabetic patients).Anthropometric and blood pressure measurements were performed, and history of smoking habits and medications was recorded. The study was performed between June, 2007 and June, 2008 by doctors and trained nurses teams
RESULTS: Final participation was 38% (1.181 subjects), and the final sample distribution by age and sex, was not significantly different to the population distribution. The overall prevalence of DM in people over 30 years old was 17.3 % (prevalencia of known diabetes 10 %; prevalencia of unknown diabetes 7.3 %). Prevalence of impaired glucose tolerance (IGT) was 5.3 %. Impaired fasting glucose (IFG) was 21.3 % under de ADA 2007 criteria, and 7% under the WHO 1999 criteria. The risk factors significantly associated with diabetes were age and obesity. Males has higher prevalence of DM (20.9 %, opposite to 14.5 % in women) and higher overall prevalence in IGT and IFG.
CONCLUSIONS:
1. We found a high prevalence of DM in CLM, higher than the most recent studies
2. The prevalence of IFG under the ADA 2007 criteria, is higher than under WHO 1999 criteria one
3. There is a high rate of unknown diabetes, that enhances the recommendation of strategies of precocious detection

PO1-370 Type 2 Diabetes, Insulin Resistance I

Alstrom syndrome causes a 'metabolic syndrome' phenotype in childhood with South Asians disproportionately affected

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Introduction
Alstrom syndrome is a recessively inherited genetic disorder (mutations in ALMS1) characterized by childhood obesity, congenital retinal dystrophy, and sensorineural deafness. Insulin resistant diabetes, hypertriglyceridemia and chronic renal failure are common complications in adults. We aimed to characterize the phenotype in a multi-ethnic cohort of UK children.
Methods
We systematically undertook clinical histories, physical examination and biochemical investigations on all 20 affected children (up to 18 completed years) attending the UK national Alstrom medical clinic from its inception in 2006 to 2009.
Results
The 20 children were from 15 families (median age 10.4yrs (range 2.2-15.9yrs); 13F; 9 South Asian). All had ALMS1 mutations; congenital retinal dystrophy; sensorineural deafness in the first decade; and all had BMI >98th centile for age and sex. 40% had dilated cardiomyopathy in early infancy. The obesity developed in infancy and was progressive. 80% had fasting hyperinsulinemia; 50% had insulin resistant diabetes (median age of onset 10.6yrs, range 18months-15yrs). 30% had hypertension (median age 10.6yrs); 70% had hypertriglyceridemia; 80% had non-alcoholic fatty liver disease; and 2 girls had polycystic ovarian syndrome. Metabolic complications were commoner in South Asian children compared to white children by a ratio of 2:1. Five children were treated with metformin, 3 with insulin and others with lifestyle and diet measures only.
Conclusions
Metabolic complications recognised in adults are already present in childhood, particularly from puberty. Current treatments appear ineffective in preventing progression to metabolic complications. South Asian children develop complications earlier and more severely than White UK children. The reasons may include known differences in insulin sensitivity.

PO1-371 Type 2 Diabetes, Insulin Resistance I

Prevalence of impaired glucose tolerance and diabetes type 2 in obese Italian children and adolescents

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The prevalence and magnitude of childhood obesity are increasing dramatically. Obesity can be associated with abnormalities of glucose metabolism. The aim of the study was to establish the prevalence of impaired glucose tolerance (IGT) and diabetes type 2 (DMT2) in a cohort of obese Italian children and adolescents.
A total of 350 obese children with BMI>97th percentile for age and sex (M/F 173/177, aged 10.96±3.3 yrs, pre-pubertal/pubertal 182/168) and 30 normal-weight subjects (M/F 12/18, aged 10.36±3.81 yrs, pre-pubertal/pubertal 16/14) underwent a standard OGTT and categorization of glucose tolerance status was made using the World Health Organization criteria. Moreover, impaired insulin sensitivity was defined as a HOMA-IR of 2.5 or higher for prepubertal patients and 4 for pubertal subjects.
Anthropometric and metabolic data of subjects are shown in table.

Anthropometric and metabolic of subjects			
Characteristics	Obese (n=350)	Normalweight (n=30)	p
BMI	29.41±5.47	18.43±2.93	<0.0001
Blood glucose fasting (mg/dl)	86.80±10.18	83.03±6.7	0.04
Blood glucose at 2 hrs (mg/dl)	112.16±24.84	103.4±16.11	0.05
Fasting insulin (μIU/ml)	17.48±22.51	5.33±3.24	0.01
HOMA-IR	3.93±6.04	1.1±0.67	0.003

Pathological HOMA-IR was present in 44% of obese patients and the prevalence of IGT or DMT 2 was 11.1% (39/350; M/F 16/23; prepubertal/pubertal 8/31) in particular IGF in10.0% and DMT2 in 1.1% of the pts. No normal-weight subjects met abnormalities of glucose metabolism.
All obese children with IGT or DMT2 presented impaired insulin sensitivity and compared with obese children with normal glucose tolerance they had significantly higher age (p=0.0007), BMI (p=0.01), HOMA-IR (p<0.0001), fasting glucose (p<0.0001).
Our data show that obese Italian children and adolescents have high prevalence of disturbance of glucose metabolism. The risk of the development of IGT and DMT2 is higher in pubertal subjects than in prepubertal, but the presence of impaired insulin sensitivity is already present in prepubertal age.

PO1-372 Type 2 Diabetes, Insulin Resistance I

Effect of long-term iron chelation therapy with deferasirax on glucose metabolism of young patients with β-thalassaemia major

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Disturbances of glucose metabolism are frequently reported even among young patients with β-thalassaemia major treated conventionally with frequent blood transfusions and iron chelation therapy. Deferasitrox (Exjade®) is a relatively new, once-daily, oral iron chelator that effectively controls iron-overload. The aim of this study was to evaluate glucose metabolism of young thalassaemic patients receiving long-term therapy with deferasirox.
Ten patients (4M and 6F) with β-thalassaemia major, with a mean age of 15.82 ± 2.76 years were enrolled in the study. All patients were treated with regular blood transfusions and were iron-chelated with deferasirox. The mean duration of exposure to deferasirox was 4.2 ± 1.03 years and the mean dose was 22.9 ± 4.7 mg/kg of body weight. In every patient, an oral glucose tolerance test (OGTT) was performed after a 12 h fasting period. Glucose was ingested at a dose of 1.75 g/kg body weight (max = 75g) and venous blood samples were collected at 0, 30, 60, 90 and 120 minutes for the measurement of plasma glucose and insulin. Diabetes Mellitus (DM) and Impaired Glucose Tolerance (IGT) were determined according to WHO's definition. Insulin resistance and β-cell function were derived from the HOmeostasis Model Assessment (HOMA), according to the following formulae:
Insulin resistance index (IR) = I_0 x (G_0 / 18) / 22.5
Beta Cell Function index (BC) = 20 x I_0 / [(G_0 / 18) – 3.5]
Serum ferritin concentrations were measured every trimester and mean ferritin values for each patient were calculated for one year prior to the study. In 8 patients an OGTT was performed at the time of the initiation of the deferasirox. According to the results of the OGTT, only one out of ten patients had IGT, whereas none was classified as having DM. Mean ferritin level was 792.20 ± 470.05 μg/L. No significant correlation was observed between HOMA indices and age or ferritin levels. As expected IR was strongly correlated with BC (r=0.842, p=0.02). When comparing the evolution of the HOMA indices, a statistically significant reduction of BC was observed (166.69 ± 195.38 vs 251.34 ± 94.46, p=0.05) followed by a concomitant reduction of insulin resistance (1.18 ± 0.58 vs 1.54 ± 0.42, p=0.11).
Our preliminary results show that iron chelation with deferasirox reduces hyperinsilunaemia and decreases insulin resistance and in this way helps the preservation of a normal glucose metabolism. However, more, longitudinal studies are needed for safer conclusions.

PO1-373 Type 2 Diabetes, Insulin Resistance I

RHIGF-1 and metformin therapy in a case of leprechaunism

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Introduction: Leprechaunism is a rare disease due to loss-of-function mutations in the insulin receptor gene (INSR). Response to metformin and rhIGF-1 in one case is presented.
Clinical observation: A 3-month-old girl with failure to thrive and dysmorphic features.
Personal history: Consanguineous parents. Born after 42 weeks´ gestation. Birth weight 1840 g(-3.5 SD). Hypoglycaemias and hyperglycaemias (max 190 mg/dL) were detected in the neonatal period.
Physical examination: Weight 2.9 Kg(-6.7 SD). Length 52 cm(–2.5 SD). Elfin-like face, hypertrichosis, acanthosis nigricans, sparse subcutaneous fat, tympanic abdominal distension and clitoridomegaly.
Complementary examinations:
·Glycaemia 278 mg/dL, HbA1C 6.2% (4.7-6.6). Insulin 1117 mIU/L.
·Capillary glycaemia profile: hypoglycaemias after 3-hour fast. Postprandial hyperglycaemias (max 280 mg/dL).
·INSR gene study: mutation IVS7+1G>A (intron 7) in homozygosis. Parents heterozygous.
Clinical course and treatment: postprandial hyperglycaemias increased progressively (300-400 mg/dL) without ketosis and with insulin levels up to 3000 mIU/L. At 5 months of life, metformin was started, producing a decrease in postprandial glycaemia (150-250 mg/dL). However, it was stopped at 8 months owing to severe gastrointestinal side effects. At 13 months of life, rhIGF-1 was started, hyperglycaemia improve but fasting tolerance decreased due to hypoglycaemias. rhIGF-1 had to be suspended 2 months later to avoid worsening of upper airway obstruction. Head tomography showed upper airway obstruction due to bone stenosis and hypertrophy of tooth buds and soft tissues.

Age (months)	Weight (Kg)	Glycaemia (mg/dL)	Insulin (mIU/L)	HbA1c (%)	Treatment
5	3.1	256	679	9.0	start of metformin
8	4.0	176	286	6.3	metformin
14	4.9	232	2678	7.0	start of rhIGF-1
16	5.3	144	725	7.4	rhIGF-1

The patient is currently 22 months old, has severe growth retardation (weight 5.8 Kg, -4.6 SD; length 67 cm, -5.6 SD), phenotypic anomalies have become progressively more evident, presents postprandial hyperglycaemias (max 350 mg/dL) and chronic respiratory failure.
Comments: Management of leprechaunism is difficult and unsatisfactory. Metformin and IGF-1 improve hyperglycaemias. However, metformin is poorly tolerated and rhIGF-1 worsens fasting hypoglycaemias and should be administered with caution owing to the risk of worsening respiratory airway obstruction associated with the leprechaunism phenotype.

PO1-374 Type 2 Diabetes, Insulin Resistance I

Population based incidence of type 1 vs type 2 diabetes in new South Wales, Australia from 2001 – 2007

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Background The incidence of type 2 diabetes (T2DM) in youth has increased worldwide in recent decades, in parallel with the rise in childhood obesity. T2DM represents more than 50% of incident cases of diabetes in some populations, however population-based prospective data are limited. The aims of this study were (i) to examine trends in the incidence of youth onset T2DM in New South Wales (NSW), where ~ 1/3 of Australia's population reside, and (ii) compare with rates of type 1 diabetes (T1DM).
Methods Cases of T2DM aged < 19 years were ascertained prospectively by the Australasian Paediatric Endocrine Group NSW Diabetes Register. The register was established in 1990 for T1DM and has ascertained T2DM since 2001. Independent secondary ascertainment was from the National Diabetes Register, based on registration for subsidised diabetes supplies. The diagnosis of T2DM was based on clinical features, high c-peptide and/or negative diabetes autoantibodies. Classification of ethnicity and denominator population data were obtained from the Australian Bureau of Statistics. Poisson regression analysis was used to model incidence by gender and over time.
Results There were 164 cases of T2DM (77 M, 47%) diagnosed between Jan 2001 – Dec 2007. Median age at onset was 14.5 years (interquartile range 12.8 – 16.3), median BMI 31.1kg/m^2 (26.1 – 36.5) and median BMI SDS 2.2 (1.7 – 2.6). Mean annual incidence in 10-18 year olds was 2.7 per 100 000 (95% CI 2.3 – 3.2), with no significant gender difference: males 2.5 vs females 3.0 per 100 000, rate ratio 1.3 (95% CI 0.9 to 1.7). Most were from minority ethnic groups: Asian 24%, Indigenous Australian 20%, North African/Middle Eastern 12% and Maori/Polynesian/Melanesian 10%, with white Australian 27%. Incidence was unchanged over the 7 year period. The incidence of T1DM over the same period was 20.9 per 100 000 (95% CI 19.8 – 22.2), increasing by ~3% per year (rate ratio 1.03, 1.0 – 1.06). T2DM represented 13% of incident cases in this age group.
Conclusions While T2DM represents > 10% of new onset diabetes in our population, is it less common than T1DM, and has not increased during this decade. Most cases are in minority ethnic groups. In contrast T1DM incidence

has continued to rise. We speculate that a plateau in obesity (1) may explain the static incidence of T2DM in our population.

1. Booth ML et al. Trends in the prevalence of overweight and obesity among young Australians, 1985, 1997, and 2004. *Obesity* 15:1089-95, 2007

PO1-375 Type 2 Diabetes, Insulin Resistance I

Survivors of bone marrow transplantation (BMT) in childhood with total body irradiation (TBI) have a high prevalence of glucose intolerance relative to their degree of adiposity

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Introduction

Survivors of BMT with TBI have increased risk of growth hormone deficiency(GHD), glucose intolerance(IGT), diabetes(DM), dyslipidaemia, and the metabolic syndrome. We aimed to examine glucose tolerance in this group and relate it to body composition and GH status.

Subjects 22 survivors (11 male) in late effects follow-up were identified. Mean (standard deviation) for age and time since BMT were 19.0(2.4) and 10.1(4.0) yrs respectively. All subjects were post pubertal, 13 had GHD, 10 were on GH treatment.

Methods All subjects had an OGTT. Fasting insulin(FI) and glucose(FG) were used to calculate HOMAR and HOMA%B. Subjects had body composition assessed by BMI, waist circumference, DEXA scan and bioimpedance. Fasting lipids and blood pressure were taken. 8 subjects also had VO2 peak tests as a measure of aerobic fitness.

Results

Results

	HOMAR	HOMA%B	BMISDS	WCSDS	body fat SDS bio-impedance	tri-glycerides (mm-ol/l)
mean(SD)	3.19(2.72)	210(132)	0.17(1.34)	1.3(1.1)	0.78(1.67)	2.12(1.23)

DM was found in 5(23%) and a further 8(36%) IGT on OGTT 120 minute glucose(G120) 7.8-11mmol/l. 2 subjects had DM on FG and 3 on G120 > 11.1mmol/l. None had impaired FG(5.6-7mmol/l). HOMAR is a measure of insulin resistance in the fasted state. HOMAR was < the normal value of 1 in only 3 subjects(15%). HOMA%B reflects beta cell activity and 16(80%) had values>100% suggesting increased beta cell activity as a compensatory mechanism in response to insulin resistance. HOMAR and FG correlated with time from BMT, $p<0.05$. HOMAR also correlated with triglycerides and negatively with fitness, $p<0.05$. HOMA%B correlated with WCSDS, $p<0.05$. HOMAR did not correlate with gender, GH status, IGF-1, body composition or WCSDS. The metabolic syndrome (as defined by body fat SDS>2 with 2 of the following: triglycerides>1.7mmol/l, HDL<1.03mmol/l, IGT, DM, systolic BP>130, diastolic BP>85)) was present in 3 subjects (14%)

Discussion In BMT survivors IGT occurred at a young age irrespective of body composition and GH status. This group appears different from published data on Leukaemia survivors who show increased HOMAR correlated to BMI. However, BMI is a poor indicator of adiposity after BMT. In addition, it may be that BMT leads to a variant of diabetes due to both insulin resistance and beta cell dysfunction, related to pancreatic damage from TBI. BMT survivors show an increased risk of the metabolic syndrome and early overt diabetes with long-term cardiovascular consequences.

PO1-376 Type 2 Diabetes, Insulin Resistance I

Glucose intolerance in children with Wilson disease

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AIMS: The liver has an important role in carbonhydrate metabolism. In the presence of hepatic disease, the metabolic homeostasis of glucose is impaired as a result of disorders such as insulin resistance, glucose intolerance and diabetes. Best of the our knowledge, this was the second study on carbonhydrate metabolism of children with Wilson disease have been published so far. In this study, we aimed to evaluate the effect of Wilson Disease on the glucose metabolism.

METHODS: Twenty-four children with newly diagnosed Wilson Disease were included. The diagnosis of Wilson Disease was made on the basis of clinical findings and laboratory abnormalities including serum ceruloplasmin, urinary copper excretion, copper content in liver biopsy. Of the patients, 13 (54.2 %) were male and 11 (45.8 %) were female. Mean age was 13.4+/-3.6 years (6.5 to 18 years). The severity of the disease was evaluated according to Child-Pugh, and Malatack's criteria. An oral glucose tolerance test (OGTT) was performed.

RESULTS: In patients with Wilson diasease, 21 (87.5 %) of the patients had normal, 3 (12.5%) had impaired glucose tolerance. Non of patients were diabetic Of the patients, 20 (83.3 %) were normal HOMA index and 4 (16.7 %) were impaired HOMA index. Using the plasma glucose concentration 120 minutes after glucose load as the dependent variable, is associated with the following variables: basal plasma glucose, age, and Child-Pugh, and Malatack's criteria. We could not find any correlation between the results of the OGTT, HOMA index and body mass index ($p>0.05$).

CONCLUSIONS: This is the second study investigating the carbonhydrate metabolism in children with Wilson disease. We found an extremely high prevalence impaired glucose tolerance. We emphasize the necessity of investigating the glucose intolerance children with Wilson disease.

PO1-377 Type 2 Diabetes, Insulin Resistance I

Decreased insulin sensitivity in young adults who underwent stem cell transplantation in childhood

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After stem cell transplantation (SCT) for leukemia during childhood, young adults may be at risk of developing the metabolic syndrome (MS), key components of which are decreased insulin sensitivity, impaired glucose tolerance (IGT), abdominal obesity, hypertension, and dyslipidemia. A putative mechanism may be growth hormone (GH) deficiency. We investigated 18 young adults (10 men, 8 women, median age 27.6 y) median 18.2 y after SCT. Insulin sensitivity was estimated by fasting insulin, the HOMA index, insulin during oral glucose tolerance test (OGTT), and the frequently sampled intravenous glucose tolerance test (calculated with MINMOD Millennium), which determined the sensitivity index SI and the acute insulin response AIR, and GT by fasting glucose and OGTT. GH was sampled every 30 minutes for 12 h during night. Body composition was measured with hip to waist ratio and whole-body dual-energy X-ray absorptiometry. We also analyzed blood pressure, triglycerides, and cholesterol. The results were compared with those of 14 healthy controls (7 men, 7 women, median age 27.4 y). All examinations showed a decreased insulin sensitivity in the SCT patients compared with controls, but there was no difference regarding GT. Patients had higher percentage total body fat and abdominal obesity. Three patients but no control had MS according to the International Diabetes Federation criteria. Peak GH was lower in patients, but did not correlate with insulin sensitivity (HOMA, SI).

We conclude that although only few patients had complete MS or IGT, most had decreased insulin sensitivity. This may herald future metabolic complications and warrants long-term follow-up. There was no relationship between GH secretion and insulin sensitivity.

Table 1			
	Patients (median/ ratio)	Controls (median/ ratio)	P
Fasting insulin (mU/L)	14.7	7.1	0.003
HOMA	4.3	1.3	0.004
HOMA > 2	13/18	1/14	<0.001
Insulin > 150 mU/L during OGTT	9/18	0/14	0.002
SI ([x10^{-4}/min]/ [μU/mL])	2.98	4.53	0.022
AIR (μU/[mL. min)]	717	364	<0.001
IGT	4/18	1/14	ns
Abdominal obesity	8/18	1/14	0.045
Body percent fat (percentile)	95	64.5	0.04
MS	3/18	0/14	ns
GH peak (mU/L)	8.4	19.3	0.001

HOMA=fasting insulin x blood glucose /22.5, IGT=fasting plasma glucose > 5.6 mmol/L or > 7.8 mmol/L at 2h during OGTT, abdominal obesity=waist to hip ratio >1.0 in males and >0.88 in females

PO1-378 Type 2 Diabetes, Insulin Resistance I

Protein carbonylation and GSTA4 expression in adipose tissue of obese and lean subjects

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Background: Obesity is associated with insulin resistance but the mechanisms controlling this relationship in humans are not well understood. Studies in model organisms suggest a linkage between reactive oxygen species (ROS) and insulin resistance. ROS oxidizes cellular lipids to produce reactive lipid aldehydes that in turn covalently modify cellular proteins in a process called carbonylation. Protein carbonylation is functionally implicated in the pathogenesis of other oxidative-stress-dependent diseases such as Parkinson's, Alzheimer's, and cancer. Mammalian cells defend against reactive lipid aldehydes by glutathionylation using glutathione-S-transferase A4 (GSTA4). Studies in obese insulin resistant mice demonstrate decreased GSTA4 expression is linked to increased level of protein carbonylation, mitochondrial dysfunction, ROS production, decreased insulin stimulated glucose transport and altered adipokine secretion. Thus, it is hypothesized that protein carbonylation may be operative in the insulin resistant phenotype in humans.
Methods: Samples of adipose tissue were obtained from grossly obese subjects undergoing bariatric surgery. RNA from bariatric samples and pooled RNA from lean, overweight and obese humans were used for real time PCR to measure GSTA4 expression. Soluble protein from adipose tissue from obese subjects was coupled with EZ-link biotin hydrazide to detect carbonylated proteins, separated by SDS-PAGE and immunoblotted with IR-labeled streptavidin.
Results: Expression of GSTA4 in overweight and obese subjects is 48% and 36%, respectively, of the level in lean subjects. GSTA4 expression in subcutaneous or visceral fat from bariatric surgery patients is 3% that of lean subjects. Multiple adipose proteins are shown to undergo carbonylation and the level of AFABP (a model target protein) carbonylation is comparable in subcutaneous and visceral adipose tissue. There is a negative correlation between the expression of GSTA4 in adipose tissue from bariatric surgery patients and carbonylation of AFABP.
Conclusions: GSTA4 expression is decreased in adipose tissue of obese individuals relative to lean controls and is correlated with increased carbonylation of AFABP. This suggests that a decreased capacity to counteract the effects of oxidative stress and the production of carbonylated proteins may be the factor leading to increased insulin resistance and type 2 diabetes in obese individuals.

PO1-379 Type 2 Diabetes, Insulin Resistance I

Simple estimates of β-cell secretion correlate with glycemic control at onset but not during therapy in pediatric type 2 diabetes mellitus

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Background: In the United States, Type 2 Diabetes Mellitus (T2DM) has been occurring with increasing frequency among obese children and adolescents, particularly among ethnic minorities such as African-Americans (AA). Pathogenesis of T2DM is believed to be due to impaired β-cell secretion in the face of obesity associated insulin resistance. Thus, we hypothesized that β-cell secretory ability would be predictive of glycemic control in children with T2DM.
Objective: To assess the relationship between glucose adjusted insulin and C-peptide levels with HbA1c levels at presentation and during therapy.
Methods: Clinical records of obese AA children with T2DM were retrospectively analyzed. Patients were included if they were obese (BMI>90%), had family history of T2DM, were diagnosed and followed up at Children' Hospital of New Orleans, and were under 19 years of age at diagnosis. Insulin and C-peptide levels drawn at the time of diagnosis and statistically adjusted for concurrent glucose (BG) were used to estimate β-cell secretion. Hemoglobin A1c (HbA1c) level was used as an indicator of glycemic control at diagnosis, 6, 12, 18, and 24 months post diagnosis. Anti-GAD65 antibody titers were measured in all subjects at diagnosis.
Results: Fifty-three patients met criteria for inclusion into the study. Eight were mildly anti-GAD65 positive ranging between 0.6-2.9 U/ml (<0.5 U/ml, normal). The female:male ratio was 3:2, average age= 13.1±2.5 years, BMI z score = 3.9±1.5. There was no difference in age, BMI, insulin, C-peptide, BG or HbA1c levels between GAD65 antibody positive or negative patients. HbA1c was correlated with insulin (r= -0.51, p<0.0001 n= 53) and C-peptide (r= -0.56, p<0.0001, n= 43) levels at presentation, but not at 6, 12, 18 and 24 months post diagnosis on therapy. Subsequent HbA1c levels on treatment were correlated with each other.
Conclusion:. 1) HbA1c at diagnosis of T2DM is highly correlated with random insulin and C-peptide levels adjusted for concurrent BG. Thus patients with better β-cell function have lower HbA1c at presentation 2) However, insulin and C-peptide levels at diagnosis are not predictive of glycemic control on therapy 3) GAD65 antibody status is not a determinant of initial insulin and C-peptide levels or of glycemic control. 4) On therapy, HbA1c levels are correlated with each other.

PO1-380 Type 2 Diabetes, Insulin Resistance I

Hypophyseal disorders in patients with genetically determined types of short stature in Uzbek population according to magnetic resonance tomography

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Stunting is a heterogeneous condition and studying of hypophyseal disorders in various genetically determined types of short stature with magnetic resonance tomography will help to reveal some aspects of pathogenesis of stunting.
Goal: to study variants of hypophyseal disorders in patients with genetically determined types of short stature in Uzbek population according to NMR data.
Materials and methods: We examined 121 patients with genetically determined types of short stature aged 7 to 16 years. 27 patients had isolated GH deficiency (GHD), 49 girls had Turner syndrome (TS), 11 boys had Noonan syndrome (NS), Russell-Silver syndrome was found in 13 patients, Sekkel syndrome in 12 patients and Lawrence-Moon-Barde-Bidle syndrome was detected in 9 patients. Family histories showed that parents of 47.1% of the patients examined were married to close relatives. All patients underwent the study of the pituitary body with NMR as well as clinical and hormonal examinations.
Results and discussion: NMR studies of the hypophysis revealed the "empty sella" syndrome (ESS) in 38.9% of the patients examined with genetically determined typed of short stature and ESS was detected more often in patients with GHD and TS. Hypophyseal abnormalities were found in 48.1 % of

patients with GHD, in 36.7% of girls with TS, in 36.3% of patients with NS, 33.3% of patients with Russell-Silver syndrome, 41.6% of patients had Sekkel syndrome and 44% of patients had Lawrence-Moon-Barde-Bidle syndrome. Patients with detected hypophyseal abnormalities were born during complicated labors such as breech or foot presentation or birth trauma. Hypophyseal microadenoma was seen in 4 TS girls and 2 boys with NS. Hypophyseal hypoplasia combined with ectopia of the neurohypophysis was diagnosed in 29.6% of patients with GHD. Hypophyseal hypoplasia alone was present in 55.5% of GHD patients, 38.7% of patients with Sekkel syndrome, 22.4% of TS girls, 45.4% of those with Noon syndrome and 30.7% of patients with Russell-Silver syndrome. **Conclusions:** The results of NMR suggest to a diagnostic value of NMR studies of the pituitary body for evaluation of the state of the hypothalamic-pituitary system and detection of pathogenetic hypophyseal disorders in patients with genetically determined types of short stature.

PO1-381 Type 2 Diabetes, Insulin Resistance I

Early insulin treatment of type 2 diabetes in adolescence: no sustained effect on HbA1c

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Starship Diabetes service cares for >95% of children with diabetes in Auckland, the largest multi-cultured city in NZ. Study period: 1 Jan 1995 to 31 Dec 2007. Patients were classified as having type 2 diabetes (T2D) based on a presentation and/or clinical course. Socio-economic status (SES) was classified using the NZ Index of Deprivation. All were negative for pre-type 1 diabetes Antibodies and/or had two or more risk factors for T2D.

Results

There was a marked increase in T2D: 0-3.5% (1995-8), to 14.9% (2003) and 10.3% (2007) of incident cases/year. **52** cases of T2D were studied, mean age at diagnosis 12.9 (1.8) years, female-to-male ratio 2.1. Mean BMI at presentation was 33.8 (6.6) kg/m^2, BMI Z score +2.30 (0.38). Acanthosis nigricans was noted in 92% of patients, HbA1c was 9.5 (2.5). The majority (57.7%) presented with classical diabetes symptoms, the remainder were detected incidentally. Both random blood glucose and HbA1c were higher in symptomatic patients (19.7 vs. 14.9mmol/L, $P = 0.05$ and 10.0% versus 8.6%, $P = 0.03$, respectively).

Maori and Pacific patients were overrepresented as were patients from more socio-economically deprived households, this higher level of deprivation amongst T2D patients was not accounted for by ethnicity. Thirty-four patients (68%) had at least one parent with diabetes (54% one parent, 14% both parents affected).

Treatment

Half of all patients were treated with insulin from diagnosis, with insulin more likely to be used in symptomatic patients ($P = 0.003$). A further 42% were treated with oral hypoglycaemic agents. Follow-up data was available for 48 of 52 patients, mean duration of follow-up was 2.4 (1.5) years.

During this time the mean BMI SDS did not differ or increase according to insulin or no insulin treatment.Although there appeared to be a difference in treatment outcomes at 12 months HbA1c 7.1 vs. 8.0 for insulin and no insulin at diagnosis; this effect disappeared over time and both groups had steadily increasing HbA1c with mean >9% in both groups. For those started on insulin, 30% were able to come of insulin or simply stopped taking it over 3-5 years, and those not on insulin at diagnosis 50% were transferred to insulin due to worsening metabolic control.

Conclusion

Despite early intensive insulin treatment there was no sustained benefit in terms of metabolic control in adolescents with T2D. Long term metabolic control remains poor with the current medical treatment options available to adolescents with T2DM.

PO1-382 Type 2 Diabetes, Insulin Resistance I

Medication induced diabetes during treatment of acute lymphoblastic leukemia: implications for future development of impaired glucose tolerance

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Medication induced diabetes (MID) is commonly seen during treatment of acute lymphoblastic leukemia (ALL). This transient form of secondary diabetes usually resolves after discontinuation of therapy. Survivors of ALL are at risk for increased prevalence of components of the metabolic syndrome (MS), including insulin resistance. However, the relationship between the development of MID during therapy and metabolic late effects in ALL survivors has not been well studied.

Aim of study: To investigate the connection between MID during ALL treatment and subsequent impaired glucose tolerance and the metabolic syndrome.

Study population: We studied survivors of B precursor or T cell ALL diagnosed in the years 1998-2004 and treated at our institution. A cohort of patients who developed MID (study group), and a randomly selected group of controls without MID were compared. Subjects were screened for components of the metabolic syndrome - waist circumference, blood pressure, lipid profile and an oral glucose tolerance test (OGTT).

Results: 45 patients were recruited - 14 in the study group and 31 in the control group. The groups had similar sex distribution (8/14 (57%) males in the study group vs. 19/31 (61%) in the control group, p>0.05), age at time of recruitment (15.6 ±3.9 years in the study group, vs. 14.7±3.9 years in the controls, p>0.05), BMI Z score (0.66 ±0.99 in the study group vs. 0.38±0.89 in the study group, p>0.05) and ethnic background. There was a tendency towards increased family history of type 2 diabetes in the study group (10/14 (71%) vs. 15/31 (48%), p=0.15). Subjects in the study group were diagnosed at an older age, (9.1 ± 3.3 vs. 6.3±3.8 years, p=0.04). Less time has elapsed between diagnosis and time of study in the study group (6.5±2.0 years in the study group vs. 8.3±1.5 years, p<0.01).Two subjects in the study group demonstrated impaired glucose tolerance (IGT) during the OGTT, vs. none in the control group (p=0.03). None of the subjects had impaired fasting glucose or T2DM. 12 (86%) study subjects had 1-2 components of the MS vs. 9 (41%) of the controls (p<0.001).

Conclusion: There was an increased prevalence of IGT in patients who developed MID during ALL therapy compared to those who did not develop it. This may suggest an increased risk for the development of IGT and the MS in survivors of ALL who demonstrate MID during treatment.

PO1-383 Type 2 Diabetes, Insulin Resistance I

Non-invasive skin intrinsic fluorescence can be a marker of type II diabetes in children

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BACKGROUND: Tissue accumulation of advanced glycation end-products (AGE) represents cumulative glycemic exposure and oxidative stress. In adults, measurement of AGE in skin has been shown to predict long-term glycemic control and risk of complications in patients with diabetes.

AIM: To identify if measurement of skin AGE via non-invasive skin intrinsic fluorescence (SIF) can be used to diagnose type 2 diabetes in children, and compare the same with known markers of diabetes, eg: HbA1C.

METHODS: SIF was measured in 3 groups: Type 2 diabetes patients (n=17, age 16.4 ±2.9), obese controls, with BMI>95% (n=54, age 13.3 ±1.75) and lean control group, BMI <85% (n=25, age 12.5±1.3). Other relevant clinical and biochemical parameters were also measured in these groups.

RESULTS: Three different indices: SIF Index I, II and III were reported. Indices II and III seemed better controlled for skin pigmentation and hence, ethnicity. All three were found to be significantly increased in Type 2 diabetes group. There were no significant differences in SIF when comparing lean and obese children. Strong positive correlation was found between HbA1C and SIF indices. (R2 58.8%, p=0.0005).
CONCLUSION: Measurement of noninvasive SIF can possibly be used in diagnosis and assessment of glycemic control of type 2 diabetes in children. Further studies are necessary in larger, multi-ethnic groups to validate this and also to compare incidence of complications with these indices.

SIF index in lean control, obese control and Type 2 Diabetes groups.

	Lean controls (N=25, F13,M12)	Obese Controls (N=54, F 25, M 29)	Type II Diabetes patients(N=17, F 8, M 9)
Age	12.5 (1.3)	13.3 (1.75)	16.4 (2.9)*#
SIF Index I	13 (2.05)	13.2 (2.6)	20 (8.7)*#
SIF Index II	0.0255 (0.003)	0.0263 (0.003)	0.034 (0.009)*#
SIF INdex III	0.035 (0.005)	0.035 (0.004)	0.045 (0.012)*#

* : p<0.05 when comparing lean controls and type 2 diabetes patients

PO1-384 Type 2 Diabetes, Insulin Resistance I

The utility of triglycerides/HDL as a marker of cardiovascular disease risk in pediatric obesity

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Background: Metabolic dyslipidemia (MetDys) (HDL and triglycerides (TG)), inflammation, and insulin resistance (IR) are associated with cardiovascular disease (CVD) risk. The ratio of TG to HDL is used in adults to predict CVD risk. Little has been published about its utility in pediatrics.
Objective: 1. To investigate the utility of TG/HDL as a marker of CVD risk in adolescents.
Methods: Cross-sectional study of pubertal, obese (BMI ≥95%ile) and lean adolescents (12-17 years). Categorical variables were compared between groups using Fisher's exact; Wilcoxon rank sum was used for continuous outcomes. Spearman correlation and linear regression were used for associations between continuous variables.
Results: Obese and lean groups were demographically similar.

Lipids and CVD Risk Factors in Pubertal Adolescents

	Obese (N=44)	Lean (N=34)	P-value
Age (yrs)	14.5 ± 1.4*	14.7 ± 1.3	0.5
Gender (Male)	36.4%	50.0%	0.26
Race (Afr Amer)	79.6%	76.5%	0.5
BMI (kg/m2)	33.2 ± 5.6	20.0 ± 1.9	<0.0001
HDL (mg/dl)	40.2 ± 8.4†	53.9 ± 11.2	0.0001
LDL (mg/dl)	107.2 ± 33.9	85.3 ± 17.1	0.04
TG (mg/dl)	82.9 ± 33.6	59.8 ± 21.4	0.009
Total cholesterol (mg/dl)	162.3 ± 34.7	150.9 ± 21.8	0.4
TG/HDL	2.20 ± 1.2	1.18 ± 0.6	0.0001
HOMA	4.12 ± 2.8¥	1.68 ± 0.9	<0.0001
QUICKI	0.133 ± 0.01	0.151 ± 0.01	<0.0001
hsCRP (ng/ml)	3.73 ± 3.5^	0.44 ± 0.29	0.0001
FFA (uM/L)	0.71 ± 0.23γ	0.52 ± 0.25	0.005

*Mean ± SD, †Lipids: Obese n=23, Lean n=24, ¥IR measures: O n=41, L n=29, ^hsCRP: O n=23, L n=11, γFFA: O n=23, L n=23

Obese adolescents had a more atherogenic lipid pattern including a higher TG/HDL, increased IR, and increased inflammation compared to controls. Moreover, HOMA and TG/HDL were significantly correlated (n=45, rho= 0.52, p=0.0003). Linear regression adjusting for gender, age, and race, showed a very significant relationship between between TG/HDL and HOMA (coeff 0.37, 95% CI: 0.23-0.50, p<0.0001). In addition, TG/HDL was significantly correlated with hs-CRP (n=34, rho= 0.50, p=0.003) and free fatty acids (n=46, rho= 0.31, p=0.038).
Conclusions: TG/HDL was strongly associated with IR and inflammatory factors, suggesting its utility in adolescents as a marker of CVD risk.

PO1-385 Type 2 Diabetes, Insulin Resistance I

Simultaneous presentation of new onset of diabetes mellitus and diabetes insipidus in an adolescent

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Hyperosmolar hyperglycemic nonketotic coma in pediatric patients is uncommon, but morbidity and mortality rates are high. We discuss a case of 16 year-old adolescent girl who presented with altered mental status, polyuria, severe dehydration and hyperglycemic hyperosmolar state with a simultaneous presentation of new onset type 2 diabetes mellitus (DM) and diabetes insipidus (DI).
At presentation patient's sodium was 163 mmol/L (corrected for hyperglycemia =183), glucose 1328 mg/dL, bicarbonate 17 mmol/L, BUN 54 mg/dL, creatinine 2.8 mg/dL, and urinary ketones were +1. Calculated serum osmolarity was 423 mOsm/L.
Treatment with IVF and insulin drip corrected hyperglycemia and dehydration, but polyuria and hypernatremia persisted which prompted evaluation for DI. The patient's urinary osmolarity remained low (155 mOsm/kg) and serum osmolarity was persistently high (307 mOsm/kg) with sodium levels in 150s mmol/L range. Antidiuretic hormone (vasopressin) levels were undetectable on two occasions. The patient responded to a trial dose of DDAVP with a decline in sodium level, serum osmolarity and urinary output and increase in urinary osmolarity. MRI of the brain showed discretely thickened pituitary infundibulum, measuring 4x4x8mm (reference range <2mm), and absence of the bright spot in the posterior pituitary. Subsequent studies revealed growth hormone and ACTH deficiencies. Biopsy of the pituitary stalk demonstrated lymphocytic hypophysitis.
This is a rare case of hyperosmolar hyperglycemic nonketotic state in an adolescent due to uncompensated water loss from polyuria related to both diabetes mellitus and diabetes insipidus.

PO1-386 Type 2 Diabetes, Insulin Resistance I

Sphingosine 1-phosphate activation of AKT kinase in adipocytes

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Background: Insulin signaling in adipocytes is partially mediated through the AKT kinase pathway. Insulin resistance at the level of the adipocyte leads to inability to stimulate glucose uptake and suppress lipolysis. Chronic inflammation links obesity and insulin resistance, with increased production of the cytokine tumor necrosis factor-α (TNF). The sphingolipid ceramide is also implicated in the development of insulin resistance as well as apoptosis. Sphingosine 1-phosphate (S1P), a downstream metabolite of ceramide, is a bioactive phospholipid that stimulates DNA synthesis, calcium mobilization, and mitogen-activated protein kinase activity in cells via S1P receptors (S1PRs). S1P can block apoptosis induced by both ceramide and TNF. S1P stimulates lipolysis in adipocytes and modulates insulin-stimulated leptin secretion. The ability of S1P to modulate the AKT kinase pathway in adipocytes in insulin-sensitive and insulin-resistant states has not been studied.
Objective: The objective of this study is to measure AKT activity and signaling in differentiated adipocytes treated with S1P.
Designs/Methods: 3T3-L1 cells were used as a model for adipocyte differentiation. 3T3-L1 cells were differentiated using standard cocktail of dexamethasone, insulin, and isobutylmethylxanthine. Adipocytes were treated with S1P (0.4 μM – 30 μM) and AKT kinase activity was measured. Western blot analysis was used to assess effects of S1P on phospho-AKT levels. S1PR levels were measured in preadipocytes and adipocytes by semi-quantitative PCR. Glycerol release assay was used to measure the ability of S1P to modulate TNF-induced lipolysis in adipocytes.

Results: S1PRs are expressed in 3T3-L1 adipocytes. AKT kinase activity in adipocytes was increased 2.8 fold in response to acute (10 min) stimulation with insulin (10 nM) and 2.5 and 2.9 fold in response to acute stimulation with 1 μM and 30 μM S1P, respectively. Activation of AKT was sustained for up to 30 min. The S1P analogue, dihydrosphingosine 1-phosphate, which acts exclusively through S1PRs, activated AKT in a manner similar to S1P, indicating that signaling is mediated through extracellular-ligand binding. Pretreatment with TNF interfered with AKT kinase activation by S1P.
Conclusions: S1P activates AKT kinase in adipocytes, and signaling occurs through S1PRs. These data indicate that S1P may modulate adipocyte function and the insulin resistant state by influencing the metabolic activity of the adipocytes.

LB-PO1-001 Late Breaking Submissions

Comprehensive genetic analysis of 183 unrelated patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Background: Genetic analysis is an important tool in the diagnosis and management of CAH. PCR based protocols identify the most common mutations and may not accurately identify genotype.
Study objective: To describe comprehensive CYP21A2 mutation analysis and genotype-phenotype and genotype-ethnicity correlation in a large cohort of CAH patients.
Methods: 183 unrelated families were studied (95 SW, 49 SV, 39 NC). Gene-specific PCR and multiplex mini-sequencing were performed. CYP21A2 was sequenced if only one mutation was detected. Southern blot was performed as needed. Genotype-phenotype and genotype-ethnicity correlation were analyzed.
Results: Targeted CYP21A2 analysis failed to identify mutations in 17 (9.3 %) patients and in 6 of 23 (26 %) patients reporting Hispanic ethnicity. Sequencing identified five novel mutations (IVs9-1G, IVS8+1G, Gln26insC, R426P, R408H) and other previously reported mutations. Five (3.0%) patients (1 SW, 4 SV) and four parents (1.7%) had 3 CYP21A2 alleles resulting in unusual haplotypes. *De novo* mutations (I172N, deletion) were identified in 2 (1.1%) patients. Maternal UPD occurred in 1 (0.6%) patient. Genotype accurately predicted phenotype in 92.4, 90.2 and 97.7 % of patients with SW, SV and NC mutations respectively. Gene deletions/ conversions were found in 30.9 % of alleles. Most common mutations according to ethnicity included: Western European: deletion (34.8%), In2G (24.9%) I172N (13.3%); Eastern European: deletion (21.7%), In2G (23.9%); Hispanic: deletion (31.1%), In2G (11.5%), V281L (13.1%); Ashkenazi Jewish: V281L (60.5%), deletion (17.6%), In2G (9.5%); Native American: In2G (30.3%), deletion (30.3%), I172N(13.6%); African American: In2G (30.8 %), Q318X (26.9%), I172N (11.5%), Asian: In2G (32.4%), R356W(29.4%),deletion (20.6%).
Conclusions: In our heterogeneous US cohort, deletions and large gene conversions were the most common mutations. Duplicated CYP21A2 haplotypes, *de novo* mutations and UPD were present in 3.0%, 1.1% and 0.6 % of patients respectively. Genotype-phenotype correlation was over 90% and was most accurate for the non-classic form. We also report five novel mutations. CYP21A2 analysis that targets most common mutations fails to identify mutations more often than expected, especially in patients of Hispanic ethnicity. Thus, CYP21A2 sequencing should be considered if CAH is highly suspected and targeted mutation analysis only identifies one mutated allele.

LB-PO1-002 Late Breaking Submissions

Assessment of adrenocorticotropin deficiency in children with Prader Willi syndrome using a low dose (1 mcg) synacthen test

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Many features of Prader Willi Syndrome (PWS) and functional imaging studies suggest an underlying defect in hypothalamic functions. It has been postulated that Adrenocorticotropin (ACTH) deficiency resulting from hypothalamic dysfunction may contribute to the increased unexplained death rate in PWS. A recent study using the metyrapone test reported a 60% prevalence of central hypoadrenalism in children with PWS. The aim of the present study was to examine the hypothalamic-pituitary-adrenal axis in children with PWS using a low dose Synacthen test (LDSST).
A cross-sectional study of 18 children (9 males, mean age 9.44 (±6.09) yr. with genetically confirmed PWS was undertaken. The mean BMI SD was 1.66 (± 1.06), 6 were in induced puberty, 8 were on Growth Hormone and 4 were on thyroxine. Baseline morning ACTH and cortisol were measured, following which, 1 mcg of Synacthen was administered intravenously. Post Synacthen cortisol levels were measured at 10, 20, 30 and 40 minutes. A 30 minute plasma cortisol level above 520 nmol/L is considered normal.
Mean baseline plasma ACTH and cortisol were 16.7 ng/L (± 12.7) (NR 9-50) and 215.4 nmol/L (±78.92) (NR 150-700) respectively. Peak plasma cortisol levels occurred at 30 minutes. The mean 30 minute cortisol level was 671.8 nmol/L (±100.8), and the average increase from baseline was 211%. None of the patients had stimulated cortisol level less than 520 nmol/L.
The cortisol response after a low dose Synacthen test in this cohort of PWS subjects suggests that ACTH deficiency is uncommon in this condition. Further studies are needed to clarify the discrepant results obtained using the low dose Synacthen and the metyrapone test.

LB-PO1-003 Late Breaking Submissions

Neonatal screening for congenital adrenal hyperplasia avoided adrenal crisis in a girl with HSD3B2 deficiency

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One of the objectives of newborn screening for Congenital Adrenal Hyperplasia (CAH) attributable to 21-OHase deficiency -the most common form of the disease- is to prevent mortality in boys who are being missing by normal clinical evaluation genitalia. In contrast, in a rare form of CAH the HSD3B2 deficiency, that impairs steroideogenesis in both the adrenal and gonads cause ambiguous genitalia in males and in general normal genitalia in girls. Therefore, there are very few cases of salt wasting HSD3B2 deficiency that have been diagnosed in females. We report a girl with HSD3B2 deficiency, who was detected by neonatal screening for CAH. A 14-day old girl, the first child of unrelated parents, delivered at term was refereed due to elevated screening blood spot 17-OHP level of :105 nmol/l performed at 72hs and 〉300nmol/l at 12 days (cut-off level:40 nmol/l) On physical examination she looked well with normal female genitalia. However, she had hyponatremia (Na: 125 mEq/l) and hyperkalemia (K: 6 mEq/l) compatible with mineralocorticoid deficiency confirmed with elevated PRA :130 ng ANG/ml/h (NV up to 3.3) and inadequately normal Aldosterone : 670 pg/ml. Serum 17-OHP after ether extraction was 60ng/ml.(NV less than 4 ng/ml).Treatment with gluco and mineralocorticoids was started. Despite the high levels of all the Δ4 steroids analyzed:17-OHP, Δ4A:

23 ng/ml and T: 8 ng/ml, the diagnosis of 21-OHase deficiency was unlikely to us due to normal genitalia in a 46 XX girl. This was confirmed by normal CYP21 gene. HSD3B2 deficiency was suspected and strongly suggested by elevated DHEAS levels: 24360 ng/ml. At 2y an ACTH test performed after 12 hs without treatment, revealed low levels of basal and stimulated Δ5-17P. It is possible that the short interval without treatment explained this finding. The definitive diagnosis was obtained by molecular probes.The DNA sequence of the DHS3B2 gene revealed a novel homozygous missense mutation in position 1237 of exon 4. This caused a change in AA 356 (V®D). We conclude: 1) HSD3B2 deficiency can be detected by 17-OHP at screening, thereby preventing adrenal crisis 2) The elevated levels of Δ4 steroids due to normal peripheral HSD3B1 activity may lead to misdiagnosis as CYP21deficiency. 3)The need of genetic molecular analysis in all cases of CAH, not only to establish a correct etiological diagnosis, especially when there is discrepancy between clinical findings and hormonal data, but also to a adequate genetic advice.

LB-PO1-004 Late Breaking Submissions

Discordance for diabetes mellitus in monozygotic twin boys with IPEX syndrome

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IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is a rare disorder caused by mutations in the FOXP3 gene that result in defective development of CD4+CD25+ regulatory T cells. Patients may manifest several autoimmune disorders including type 1 diabetes, entheropathy, dermatitis, thyroiditis, hemolytic anemia, membranous nephropathy and recurrent infections. Objective: To demonstrate a new mutation in FOXP3 gene in identical Brazilian twin brothers with IPEX syndrome and discordant phenotypes. Subjects and Methods: The boys were born from unrelated healthy parents after an uneventful pregnancy. **Twin 1** presented bronchiolitis at 2 months, recurrent respiratory infections, type 1 DM with ketoacidosis at 6 months, membranous glomerulonephritis diagnosed at 9 months of age. Failure to thrive and periods of watery diarrhea were maintained despite dietary changes. Laboratory work-up showed altered liver enzymes, positive anti-liver microsomal antibodies, positive anti-TPO, anti-TG and anti-insulin antibodies; upper endoscopy revealed eosinophilic esophagitis. **Twin 2** presented 3 pneumonia episodes needing hospitalization, the first one with 15 days of life, chronic diarrhea with failure to thrive and membranous glomerulonephritis. Auto-antibodies (RF, anti-TPO, anti-TG, anti-insulin) were present but diabetes was not manifested until he was 15 months old and died after another severe respiratory infection. High serum levels of total IgE and specific IgE for cow' milk protein were detected in both patients. Results: Flow cytometry analysis showed low expression of FoxP3 and molecular research showed a change in splicing site located immediately after the exon 1 (IVS1 210+1G>A), a novel mutation. Conclusion: IPEX syndrome is a cause of monogenic diabetes and perhaps exposure to different environmental factors in addition to genetic causes could explain the discordant phenotype for diabetes in these patients.

LB-PO1-005 Late Breaking Submissions

A novel mutation (E767Q) in the second extracellular loop of the calcium sensing receptor (CASR) gene in a Palestinian family with autosomal dominant hypoparathyroidism

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Background:
Autosomal Dominant Hypoparathyroidism (ADH) is a rare familial disorder, caused by activating mutation of the calcium sensing receptor (CASR), or mutations in the PTH gene that impair intracellular processing of the nascent protein. Patients with this inherited form of hypoparathyroidism are commonly asymptomatic and present at any age. Patients generally exhibit mild to moderate hypocalcemia, with serum PTH levels that are inappropriately low given the hypocalcemia.
We describe a novel mutation in the CASR gene in a Palestinian family with ADH.
Clinical Data:
A Palestinian infant, born to a non consanguineous parents, presented at 1 week of age with hypocalcemic seizures, hypercalciuria, hyperphosphatemia and inappropriately low PTH response. Screening of the parents, who are asymptomatic, revealed hypocalcemia in the father with inappropriately low PTH.
Molecular Data:
DNA sequencing of the CASR gene for the patient revealed a novel missense mutation in the second extracellular loop with replacement of G by C in codon 767 of exon 6 (GAG à CAG), predicting Glutamic acid to Glutamine substitution (E767Q) in the protein. The mutation was present in one allele and co-segregated with hypocalcemia. The father had the same mutation on one allele while the mother was negative.
Conclusion:
To our knowledge, this is the first description of this disease in a Palestinian family with molecular confirmation, allowing accurate genetic counseling, early diagnosis of affected kindreds, early therapeutic interventions and avoiding complications such as nephrocalcinosis.

LB-PO1-006 Late Breaking Submissions

Autosomal recessive transmission of familial hypocalciuric hypercalcemia (FHH) due to a unique homozygous loss-of-function mutation in the gene encoding the calcium sensing receptor (*CASR* – Gly768Val)

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FHH is usually transmitted as an autosomal dominant trait. Heterozygotes have asymptomatic hypercalcemia while newborns who are homozygous for inactivating mutations of *CASR* manifest severe symptomatic hypercalcemia with secondary hyperparathyroidism. Patient 1 is the firstborn male child of first cousins of Pakistani origin who was noted to be hypercalcemic (Ca 12.8 mg/dL) and hypophosphatemic (Phos 2.5 mg/dL) on day of life (DOL) #2; serum concentrations of parathyroid hormone (PTH 1076 pg/mL) were markedly elevated. On DOL #110, patient 1 under subtotal parathyroidectomy (3.9 glands). After transient hypocalcemia, the hypercalcemic state recurred. Patient 2 is the sister of Patient 1. She was found to be hypercalcemic (Ca 12.8 mg/dL) and hypophosphatemic (Phos 3.0 mg/dL) on DOL #1; serum PTH values were also greatly elevated (1834 pg/mL). Patient 2 underwent subtotal parathyroidectomy (3.5 glands) on DOL #173 with return to hypercalcemia by DOL #175. In both patients, the parathyroid glands were hyperplastic. Patient 2 also had osteopenia. The mother and father of these siblings were entirely asymptomatic, eucalcemic, and euphosphatemic with normal levels of PTH.
In patient 1, there was a homozygous G>T point mutation in *CASR* at nucleotide c.2303 resulting in replacement of a highly conserved glycine residue at codon 768 by valine (Gly768Val), a site immediately before the fifth transmembrane domain of the CaSR (C. Fratter, Oxford Radcliffe Hospitals, Genetics Laboratories, UK). Patient 2 was also homozygous for this mutation, while each parent was heterozygous for the Gly768Val mutation (Molecular Genetics Laboratory, All Children=s Hospital). Inasmuch as patients 1 and 2 had hypercalcemia and persistent secondary hyperparathyroidism, while their parents were completely normal both clinically and biochemically, we conclude that in this family FHH was transmitted as an autosomal recessive rather than autosomal dominant trait. In the heterozygous state, Gly768Val might be considered a benign polymorphism; however, when present in the homozygous state, the impairment in calcium sensing function of the mutated CaSR is clearly evident.

LB-PO1-007 Late Breaking Submissions

Vitamin D supplementation in the breastfed infants: preliminary results of a prospective trial

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Exclusive breast feeding of newborns has become popular because of the known benefits to mother and baby; however, there are concerns if breast milk has sufficient vitamin D to prevent rickets. The American Academy of Pediatrics (AAP) has recommended universal supplementation of breastfed infants with vitamin D (200 IU/day) from 2 months of age; this recommendation was recently increased to 400 IU/day from birth. However, there are few studies of vitamin D supplementation. We are therefore conducting a clinical trial to compare vitamin D supplementation with placebo control in breastfed children. After obtaining approval from the Ochsner Institution Review Board, normal newborns (breast milk intake >50 percent of total for >3 months) were randomized into three groups: no supplementation, 200 IU per day from 2 months, and 200 IU per day from birth. Blood samples and questioners were collected at birth, two, four, and six months of age. While this study is ongoing, we have already recruited 71 subjects, and 18 subjects have completed the study. Analysis of the preliminary data indicates little benefit to supplementation - no patients in the study have developed rickets, and we saw only mild, transient increases in alkaline phosphatase in the placebo vs. control group. The only statistically significant change in alkaline phosphatase levels occurred at 2 months (Vit.D 93+/-12, placebo 125+/-10 IU/l, $p<0.05$), even though there was no statistically significant difference in 25-vitamin D levels at that time point (Vit.D 97+/-18, placebo 62+/-8 nmol/l, NS). Statistically significant differences in 25-vitamin D levels were observed at the 4 month time point (Vit.D 137+/-37, placebo 63+/-9 nmol/l, $p<0.05$), but by 6 months the 25-vitamin D levels in the placebo group had risen to those of the treatment groups (Vit.D 132+/-29, placebo 117+/-28 nmol/l, NS). While serum calcium did not differ between groups, phosphate rose and PTH fell in the treatment groups (vs. baseline and vs. placebo), consistent with known effects of vitamin D. Given our previous report of vitamin D supplementation increasing the risk of urinary tract infections by 76% (Katikaneni R, et al., Clinical Pediatrics March 4, 2009), we have concerns about the current AAP recommendations for universal supplementation with vitamin D in breastfed infants.

LB-PO1-008 Late Breaking Submissions

Children with Prader-Willi syndrome exhibit more evident meal-induced responses in plasma ghrelin and PYY levels than obese and lean children

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Background: Ghrelin is an orexigenic 28-amino acid peptide produced by the stomach. Circulating ghrelin levels rise shortly before and fall shortly after every meal. Peptide YY (PYY), an anorexigenic 36-amino-acid peptide, is secreted primarily from the intestinal mucosa of the ileum and large intestine. Plasma PYY levels begin to rise within 15 min after starting to eat and plateau within approximately 90 min, remaining elevated for up to 6 h. Recently, some studies have tried to evaluate the potential role of ghrelin and PYY in the hyperphagia of patients with Prader-Willi syndrome (PWS). Aim of the study: to investigate ghrelin and PYY responses to a standard liquid high-fat meal in children with PWS.
Patients and Methods: circulating levels of total ghrelin (pg/ml)) and PYY levels (pg/ml) were assayed by RIA at morning fast and 45, 60, 90, 180 min after a standard meal (Ensure™ 6 ml/kg). We analyzed 16 PWS patients (11 boys, aged 6-14 yrs, including 10 receiving 0.02 mg/kg/die rhGH for 2-18 months), 10 morbid obese subjects (8 boys, aged 9-16 yrs; BMI: 32.06±1.63), and 16 normal-weight controls (5 boys; aged 7-14 yrs). Results: PWS children showed higher fasting levels of ghrelin than obese and lean controls (PWS: 898.0±238.1, GH treated PWS: 1093.8±120.4, Obese: 414.7±16.5, Controls: 474.4±53.9 - p: < 0.05). No significant difference of fasting PYY levels was found among groups (PWS: 91.2±16.2, GH treated PWS: 149.6±22.1, Obese: 123.3±8.3, Controls: 121.7±8.9). PWS children showed a more pronounced postprandial ghrelin drop and a higher postprandial PYY rise in comparison to obese and controls subjects. Fasting and postprandial levels of ghrelin and PYY were not different in PWS patients treated with GH, in comparison to not treated PWS. Fasting PYY levels correlated negatively with ghrelin levels only in PWS subjects ($p< 0.05$; $r = -0.68$).
Conclusion: we confirm fasting hyperghrelinemia in PWS subjects. An impaired postprandial suppression of plasma ghrelin was previously reported to be associated with a blunted postprandial PYY response in PWS adults. We found a meal-induced decrease in ghrelin, associated with an increase in PYY levels, in PWS children. These data would imply that the regulation of appetite/satiety is operative during childhood, but it probably progressively deteriorates and vanishes in adulthood, when hyperphagia and obesity progressively worsen.

LB-PO1-009 Late Breaking Submissions

A new luteinizing hormone receptor inactivating mutation with only a micropenis

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Background
Inactivating mutation in the luteinizing hormone receptor (LHR) is a rare autosomal recessive disorder causing mostly 46 XY Disorders of Sex Development (DSD) with female phenotype. Less severe LHR mutations caused milder phenotypes. Only 5 cases of micropenis have been described in the literature due to LHR mutations (1,2,3).
We describe a newborn with a micropenis whom resistance to continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone revealed the diagnosis of LHR mutation.
Clinical Case
The patient was a 3 months old boy, born to consanguineous parents. Penile length was 10 mm at birth with normal urethral opening and intra-scrotal testis. Basal testosterone was < 0.25 nmol/l before and after hCG stimulation. Basal LH and FSH were 2.6 UI/l and 5 UI/l. Serum anti-Müllerian hormone (AMH) was 1202 pmol/l and inhibin B 177 pg/ml. Because he was considered as congenital hypogonadotropic hypogonadism he received a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone (4). Mean testicular volume increased from 1 ml to 2 ml and penile length remained < 15 mm. Inhibin B value increased up to 1270 pg/ml, AMH to 1830 pmol/l and testosterone to 0.42 nmol/l. This minimal testosterone response and normal inhibin B/AMH suggested a resistance to the infused LH due to a LHR mutation.
Molecular study
Direct genomic sequencing of the complete LHR gene conding region revealed a new homozygote substitution in exon 11 yielding a Phe630Ser change in the intracellular domain of the receptor.
Conclusion
Inactivating mutation in the LHR can caused only a micropenis, and is probably underestimated.
Congenital hypogonadotropic hypogonadism diagnosis can be worn by mistake and the continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone can be a diagnostic test, to diagnose LHR mutations, probably underestimated.

LB-PO1-010 Late Breaking Submissions

Secular growth in Mexican children

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Precedents

Secular growth increment has been reported in many countries as results of better quality of health and life, but in Mexico there is not evidence of secular changes.

Objective

To verify if there is secular growth changes in Mexican children, comparing actual data with a growth study realized in 1950.

Material and Method

Prospective, descriptive, observational, comparative, and randomized study realized with healthy children attending public schools in Mexico City. Children were selected in a randomized method but they were included only if both parents are Mexicans, they have not chronic disease or pharmacological treatment in the past two weeks. Age; gender; height (Harpenden stadiometer); recumbent height; weight; arms span, inferior segment, hands, and feet length; cranial, thorax, waist, arm and leg circumferences; subcutaneous adipose fold thickness plicometry; genital volumes in males, and puberty development were consigned.

In this report we mention only data referring growth characteristics and the rest of the anthropometrical parameters will be discussed in other works.

Results

The males with height below mean (3rd to 50th percentiles) are 5 cm taller than those reported in 1950 ($p<0.050$), but there are not changes in those with height in percentile 90th. In women, those with height in percentile 10th are 3 cm taller, those in percentile 50th remains with the same height and those with height in percentile 90th are 2 cm shorter than those reported en 1950 ($p<0.05$)

Conclusions

In the last 50 years, secular growth changes are not similar for Mexican boys and girls. In both cases the lower height has increased 5 and 3 cm, respectively, the mean height are taller in boys, but remain similar in girls, and the higher heights remains similar in boys but has decreased in girls (2 cm) during this period of time.

LB-PO1-011 Late Breaking Submissions

Growth of Belgian and Norwegian children compared to the WHO growth standards: prevalence outside normal limits and the effect of breastfeeding

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Objective

New national growth references have recently been published in Belgium and Norway. The World Health Organization (WHO) recommends universal use of their growth standards from 2006, based on data from breastfed children. In the present study we have compared growth of Belgian and Norwegian children with the WHO growth standards.

Participants

Data from 6985 infants and young children 0 - 5 years of age from Belgium and Norway were analyzed. The proportion of children below -2 SD and above +2 SD of the WHO growth standards was calculated for length/height, weight, body mass index (BMI) and head circumference. In addition, the average SD-scores of exclusively breastfed children were compared with national reference data and with the WHO standards.

Results

Generally, the number of Belgian and Norwegian children below the -2 SD lines of the WHO growth standards for length/height, weight, BMI and head circumference was too low, and the number of children above +2 SD to high. The largest differences were observed for head circumference (0.97% of Belgian and 0.18% of Norwegian children below -2 SD, and 6.55% of Belgian and 6.40% of Norwegian children above +2 SD), and the smallest for length/height (1.25% of Belgian and 1.43% of Norwegian children below -2 SD, and 3.47% of Belgian and 2.81% of Norwegian children above +2 SD). The growth patterns of breastfed children of non-smoking mothers were in both countries more alike the local national growth references than alike the WHO growth standards.

Conclusions

In this study, we have demonstrated significant deviations in the proportion of children outside normal limits (± 2SD) of the WHO growth standards. Hence, adoption of the WHO growth charts may have important and unwanted clinical consequences. These findings advocate the use of national references in Belgium and Norway, also for breastfed children.

LB-PO1-012 Late Breaking Submissions

Laron syndrome due to a novel missense mutation p.W68L in the growth hormone receptor

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Laron syndrome is a rare autosomal recessive genetic dwarfism arising from growth hormone receptor (GHR) deficiency. More than 60 different mutations in the GHR have been reported for the disease worldwide. We report a patient of Indian ethnicity from a consanguineous marriage with a novel GHR mutation.

The patient was brought to the growth clinic at 13 months of age because of poor physical growth (weight 5.0 kg; length 58 cm). He had a round face with frontal bossing, mid facial hypopasia and blue sclera and small hands & feet. He had a high pitched voice. His base line growth hormone (GH) was 107 IU/ml (very high) and IGF-1 was <29 ng/ml (lower from detectable value, normal 1 year old: 50-357 ng/ml). There was fasting hypoglycaemia (BG – 2.4 mmol/L, normal – 3.0 mmol/L) and the GH-IGF-1 generation test failed to cause IGF-1 increase - IGF-1 was <29 ng/ml on the 4th day and 7th day following subcutaneous injection of GH.

GHR coding regions (exons 2-10) were PCR amplified and subjected to direct DNA sequencing. A homozygous novel missense mutation c.203G>T (p.W68L) was found in exon 4. The apparently normal parents are heterozygous for the same sequence change. The patient also had two sequence changes in exon 10, both of which are known polymorphisms. This patient represents the second case of Laron dwarfism from Malaysia.

LB-PO1-013 Late Breaking Submissions

Lipoatrophy in growth hormone deficient patients treated with a long-acting pegylated growth hormone

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Growth hormone (GH) secretion is important for growth, anabolism, lipid metabolism and maintenance of body composition. Adult growth hormone deficiency is deleterious in terms of altered body composition, increased cardiovascular risk and impaired quality of life; replacement of GH reverses many of these adverse changes. However, most recombinant human GH (rhGH) preparations must be given by daily subcutaneous injections. A long-acting GH molecule (PEG-GH) has been made by covalent binding of polyethylene glycol (PEG) to rhGH, enabling weekly injections. The objectives of this Pfizer-sponsored multicenter (34 centers), randomized,

double-blind placebo controlled, multiple-dose, parallel group study were to assess safety and efficacy after multiple weekly injections in adult GH deficient subjects. Subjects received six weekly injections of either PEG-GH or placebo. Subjects were randomized into 1 of 5 groups (Groups A to E). Groups A, B and C received 1, 3 and 4 mg PEG-GH, respectively, for the first three week period followed by 2, 6 and 8 mg PEG-GH, respectively, for the second three week period. Group D received 4 mg weekly for six weeks, and Group E received placebo for six weeks, respectively. One hundred and five subjects were assigned a treatment. Five cases of lipatrophy were reported after 3 to 6 injections in the same location -thigh- leading to a temporary suspension of the trial. The study restarted with an injection site rotation scheme, but additional cases of lipoatrophy were reported, leading to termination of the study. On the whole, lipoatrophy occurred in 10 of 53 females and in 3 of 52 males; this was independent of the number of injections, PEG-GH dose, IGF-I levels or BMI. All lipoatrophy lesions resolved with time, usually within 8-12 weeks. A single dose study was performed in children where one case of lipoatrophy was also seen. In conclusion, the unpredictable occurrence of lipoatrophy with weekly treatment may be a limiting factor for the development of long-acting GH molecules.

LB-PO1-014 Late Breaking Submissions

Effect of GH replacement therapy in boys with Dent's disease

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Dent's disease is X-linked recessive proximal tubulopathy, due to mutations in the CLCN5 gene. It is characterized by low molecular weight proteinuria, hypercalciuria nephrocalicinosis, phosphaturia and hypophosphatemia.
We rendered a case of a boy with proteinuria, hypercalciuria, phosphaturia, hypophosphatemia and small growth in comparison with the MPH, as well as with normal values of growth hormone. Molecular genetic analysis was performed and has showed S244L substitution on CNCL5. Because of the basic illness, the boy had been taking the conventional therapy for two years. During that time, the speed of growth decreased, the hypophosphatemia has aggravated, the value of IGF 1 fell and signs of hypophosphatemic rickets occurred. Phosphates and the growth hormone were included in the therapy. After two years of therapy, the median adult height was 1.1 SD higher than at the initiation of rhGH treatment; height velocity has four-fold increased. During the two-year rhGH treatment, the standardized sitting height has not increased. There were more than two-fold increases in IGF-1.
Global kidney functions, proteinuria and calciuria remained relatively stabile. IGF-1, serum phosphate and AP were within the normal range. TRP was lower. Bone age remained retarded.
Treating children who have Dent's disease and small growth with growth hormone can have positive effects on the final growth, but the utmost caution must be exercised because of the small number of patients and the short period of treatment.

LB-PO1-015 Late Breaking Submissions

Long-term efficacy of growth hormone therapy in prepubertal children with GH deficiency: results of a randomized dose response 4-year trial

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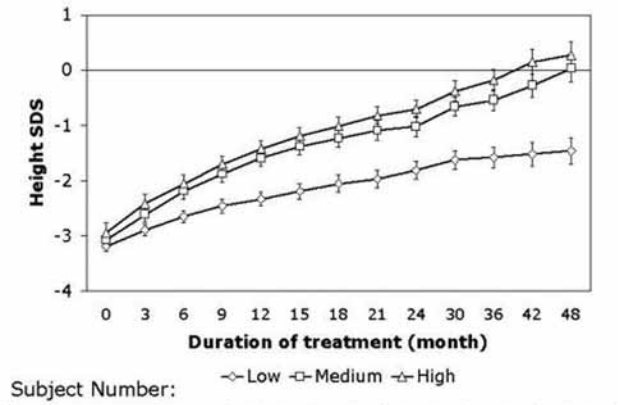
We previously published results of response to growth hormone therapy (GHT) using a wide range of recombinant human growth hormone (rhGH) doses (25-100 µg/kg/d) for up to 2 y in prepubertal children with growth hormone deficiency (GHD) (Cohen P. et al JCEM 2002; 87:90-98). Higher dose GHT (up to 100 µg/kg/d) is currently approved by the FDA for treatment of pubertal children with GHD. Longer-term clinical outcome data of higher dose GHT in prepubertal children are currently unavailable. We investigated the effects of a range of doses of rhGH in 111 prepubertal children with GHD (75M/36F, mean±SD age 7.75±2.83 y, height standard deviation score [HSDS] -3.0±0.9) who were randomized to receive low (L), medium (M) and high (H) dose (25, 50, and 100 µg/kg/d) rhGH for up to 5 years (median duration 42 mo). Fifty-two patients from the original study completed 48 mo of treatment. Cross-sectional HSDS data over 48 mo are presented in **Figure 1**. The M and H groups reached HSDS of +0.03 and +0.27 at 48 mo, compared to -1.46 SDS for the L group. The mean ΔHSDS at 48 mo for the L, M, and H groups were 1.91, 3.05, and 3.13. Corrected HSDS (CHSDS = HSDS - target height SDS) at 48 mo were -1.23, -0.18, and 0.86 for the L, M, and H groups. The M and H groups were significantly different from the L group ($p < 0.001$) for both HSDS and CHSDS, whereas no significant difference was found between the M and H dose groups. No significant difference in body mass index was observed among the 3 groups at 48 mo. In conclusion, prepubertal children with GHD who received a medium (50 µg/kg/d) or high (100 µg/kg/d) dose of rhGH achieved a HSDS equal to the normal population mean after 4 years of GHT, with both groups showing a greater HSDS compared to a lower dose (25 µg/kg/d) group. As there was no apparent difference in HSDS achieved between the medium and high dose groups, over long term-therapy, a steady weight-based rhGH dose of 100 µg/kg/d is unlikely required or appropriate for most prepubertal children. For children receiving GHT in the lower dose range, increasing the rhGH dose to mid-range may result in greater normalization of HSDS.



Subject Number:

Time	0	24 m	30 m	36 m	42 m	48 m
Low dose, n	37	34	30	27	21	17
Medium dose, n	38	37	34	31	27	21
High dose, n	36	33	30	29	22	14

Figure 1. Height SDS after 4 Years of GH Treatment

LB-PO1-016 Late Breaking Submissions

Continuous sc nightly infusion of IGF-1 stimulates growth in an acid labile subunit deficiency

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Deficiency of acid labile subunit decreases measurable IGF-1 and IGF BP3 levels. Short stature is associated with lack of this ternary complex. This patient was diagnosed with a novel homozygous 1308_1316dup9 mutation in the highly conserved leucine-rich repeat (LRR) 16 motif of exon 2 of IGFALS. (Fofanova-Gambetti et al. 2009. Horm Res 71:100). He was adopted from Guatemala and is of Maayan/Spanish descent.
Growth hormone stimulation tests were normal: Arginine GH peak 12.9 ug/l and Clonidine GH peak 25.5 ug/l. IGF1 was 49 ug/l (82-105) and IGF BP3 0.39 ug/ml (1.32-3.94). ALS was not measurable. He was treated with growth hormone prior to the diagnosis of ALS deficiency. Growth rate was not increased with growth hormone treatment up to 0.07 mg/kg/d or with IGF-1

injections divided BID or TID. Hypoglycemia occurred initially with increasing IGF-1 doses, but resolved quickly.
We hypothesized that continuous infusion of IGF-1 subcutaneously may improve growth velocity. A trial period of IGF-1 infusion delivered overnight by a continuous sc pump was initiated at a dose of 240 ug/kg over 8 hours. He required 7.5 mg Depo-Lupron monthly to delay epiphyseal fusion from age 10 years 4 months through the present age of 13 years 5 months.

Clinical Course					
Medication	Dose	Duration treatment (mo)	Annualized growth rate (cm)	Height (cm)	Bone age (yrs)
Growth hormone	0.03 mg/kg/d	3	1.3	121.5	10
Lupron				122.5	
Growth hormone	0.05 mg/kg/d	5	1.3	125.1	
	0.07 mg/kg/d	10	0.4	129.0	11.5-12
Off growth hormone	-----	3	1.0		
IGF-1	120 ug/kg BID	5	0.9	130.5	
	240 ug/kg BID	6	0.6	132	13
	240 ug/kg overnight	4	5.7	135.6 to 139.2	

Continuous overnight IGF-1 infusion appears to accelerate growth velocity over the short term. A pharmacokinetic/pharmacdynamic study is planned to evaluate IGF-1 serum levels during sc infusion as compared to sc injections in this patient.

LB-PO1-017 Late Breaking Submissions

Spontaneous accelerated growth in a 3 year old male with growth hormone deficiency

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2.7 yr male with growth failure for the past year but no growth records prior to immigration from Turkey. BW 3.9 kg and length 52 cm. Physical exam revealed mild obesity, frontal bossing, low nasal bridge, absence of 2 yr molars, nonpalpable testes, and phallic length 4 cm.
IGF-I undetectable, IGF BP3 0.9 mg/L (0.8-3.9). Cortisol 17.2 mcg/dL (3-25), free T4 .9 ng/dL (0.8-2), TSH 2.76 mIU/L (0.5-4.3), karyotype 46XY, prolactin 25 ng/mL (Tanner 1, ≤ 10). CBC, renal functions, sed rate, total protein and albumin normal. US: inguinal testes. Bone age: 1yr, 6m. Brain MRI: small pituitary.
Growth hormone (GH) therapy was refused. At 2.9 yr, repeat IGF-I undetectable, IGF-II 320 ng/mL (<3 yrs: 480 ± 85), random GH 0.4 ng/mL, prolactin 9 ng/mL (≤ 10).
At 3 yr peak GH to arginine & clonidine stimulation was 0.6 ng/mL, FBS 69 mg/dl, insulin level <1 uU/ml. His annualized growth velocity was 13.2 cm/yr (>>97%). At most recent follow-up at 3.4 yr, growth velocity was 7.4 cm/yr.

Auxological Data				
Age (yr)	Ht. (cm) (SDS)	Wt. (kg)(SDS)	BMI (%) (SDS)	Ht. Vel. (%)
2.7	81 (-3.7)	12.2 (-1.2)	18.6 (95.5) (1.7)	
2.9	83.5 (-3.2)	13.2 (-0.6)	18.9 (97.6 (1.98)	
3.0	85.5 (-2.7)	15.1 (-0.4)	20.7 (99.8) (2.95)	13.2 (> 97)
3.4	88 (-2.6)	14.6 (-0.2)	18.9 (98.4) (2.14)	7.5 (50)

This GH deficient patient demonstrated transient GV > 97% for age. Spontaneous normal growth with GH deficiency (GHD) may occur after resection of craniopharyngiomas or hypothalamic tumors and has been described in septo-optic dysplasia (SOD). Growth in fetal life and obesity occurs without GH. GHD with **accelerated** GV has been reported rarely in panhypopituitarism as well as obesity and SOD. This patient had no CNS anatomical defect, tumor or history of surgery. He was overweight for height.
The mechanism of normal or accelerated growth in GHD is not clear. Hypotheses include hyperinsulinism, elevated prolactin or leptin levels, GH variants, IGF-1, IGF-II or unidentified growth factors. Our patient did not have hyperinsulinism, had normal prolactin, undetectable IGF-I, low IGF-II and subnormal GH on provocative testing. In spite of this, he demonstrated accelerated growth followed by continued spontaneous growth. Psycho-social deprivation may also cause reversible growth failure but there was no history of inadequate nutrition or emotional support and the patient had demonstrable GHD at the time of accelerated growth velocity.

LB-PO1-018 Late Breaking Submissions

Growth hormone (GH) secretion, IGF-1 and IGFBP-3 can predict response to GH therapy

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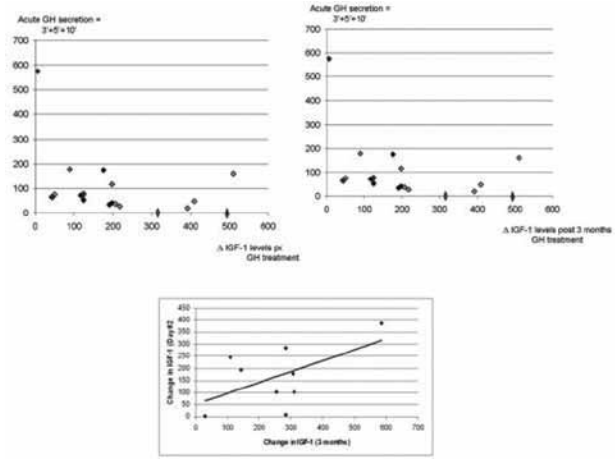
Hypothesis
We hypothesize that the combination of modified Growth Hormone (GH) stimulation & IGF-1 generation tests as a marker of GH secretion & resistance.
Methods
We studied 18 children (11 boys, 7 girls) with height (Ht) SDS <-2.0. We preformed a modified GHRH (GH releasing hormone) test, with samples of GH obtained at 3, 5, & 10 minutes in addition to 30, 60, 90 & 120 following GHRH (1 µg/kg IV). GH secretion was analyzed as sum of GH at 0.3.5.10 minutes, which represents an immediate release pool of GH. IGF-1 generation test was done with GH 0.05 mg/kg/dose (7 days) or treatment with GH 0.5 mg/kg/ week. IGF-1 levels were collected on day #2, # 8 & 3 months. The change in IGF-1 was determined as ΔIGF-1 as a marker of GH resistance. IGFBP-3 was collected in all children.
Results:
The mean Ht SDS at the start was - 2.91 ± 1.0 SDS, age 9.2 ±2.9 yrs, target Ht -1.0 ± 0.9, growth velocity 3.3 ± 1.4 cm/yr & IGF-1 < – 2.49 ± 1.2 SDS. 3 children had IGF-1 < - 2 SDS & had good response to GH therapy, IGF-1 – 1.7 SDS & IGF-1 response > 200 mg/dl (purple). 2 children had proven GH deficiency (green), 1 case of hypopituitarism, 1 case of GHRH receptor mutation. 3 had GH receptor mutation (red). 10 children with IGF-1 < – 2.5 SDS were divided to 2 subgroups according to their response to GH therapy: 5 responded poor to GH therapy with growth velocity on GH 0.5 mg/kg/week 5-7 cm/yr (blue) & 5 responded with growth rate of 10-15 cm/yr (dark blue). GH secretion and IGF-1 distributed as hyperbolic curve which physiologically represent that higher the resistance, higher secretion of pool of GH. Δ IGF-1 at day#2 correlated with Δ IGF-1 at day#8 and 3 months. ΔIGF-1 also correlated with growth velocity on 0.5 mg/kg/week for 6 months (r=0.69. p=0.001). IGFBP-3 was significantly lower in 5 children who didn't respond (blue) to GH therapy (2.1 ± 0.9) when compared with 5 who responded (3.4 ± 1.1) ($p < 0.05$) (dark blue).



Conclusion
ΔIGF- 1, GH secretion, IGFBP-3 can differentiate between children who will respond to GH therapy and, help to make diagnosis for primary IGF-1 deficiency, GH deficiency and neuro secretory dysfunction.

LB-PO1-019 Late Breaking Submissions

Seperation of auxological and metabolic effects of GH

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LB03002 (LB) is a novel once a week subcutaneous sustained release rhGH. 51 GH deficient children (7.3±2.1 years) with peak stimulated GH response ⟨7ng/ml in 2 provocative tests, HT SDS ≤-2.0; HV SDS ≤-1; IGF-1 SDS ≤-1) were randomly allocated to daily rhGH 0.03 mg/kg/day (n=12), or LB at 0.2 mg/kg/week (n=13), 0.5 mg/kg/week (n=13) and 0.7 mg/kg/week (n=13) respectively. All patients on LB arms were switched to LB 0.5 mg/kg/week in the second year. Daily rhGH was switched to LB 0.5 mg/kg/week at the end of 2 years.

Table 1: Selected growth and metabolic parameters (mean±SD)

	LB 0.2	LB 0.5	LB 0.7	Daily	p-value
Height velocity (cm/year)					
	9.67±1.51*	11.75±1.88	12.44±2.34	12.17±1.34	0.008
LDL (mmol/L)					
Baseline	3.65±1.13	3.04±0.95	3.46±1.11	2.79±0.63	0.124
Decrease first year**	0.98±0.75	0.51±0.79	0.74±1.16	0.40±0.72	0.361
Cholesterol (mmol/L)					
Baseline	5.54±1.39	4.79±1.00	5.45±1.55	4.56±0.68	
Decrease first year***	0.69 (0.25, 2.06)	0.31 (-0.28, 0.81)	0.51 (0.33, 0.91)	0.3 (-0.14, 0.82)	0.268

*Statistically significant difference**ANOVA (Mean±SD), *** ANOVA on ranks (median, 25%, 75%)

Despite a lower height velocity of the LB 0.2 group (p=0.008) versus daily rhGH, there were no relevant differences between treatment groups over the 3 year period in all assessed metabolic and lipid parameters (glucose, haemoglobin A1C, HOMA-IR, cholesterol, TG, LDL and HDL). None of the lipid parameters changed significantly over the treatment period except the LDL values which decreased significantly only during the first year. All LDL values were in the normal range (⟨4.2 mmol/l) at baseline, and after the first year decrease this was maintained, as the small fluctuations from there onwards were not significant. In summary the LDL values after 1 year of treatment were normalized regardless of the dose administered. IGF-1 increased significantly during the treatment years, but no difference between the treatment groups was demonstrated. No correlations were found between LDL decrease and HV SDS, IGF-1 increase, insulin or BMI SDS. It can be concluded that LB03002 has the same metabolic profile as seen with daily GH, and also that the metabolic and auxologic effect of GH is divergent with similar metabolic effect seen at a lower dose of GH.
'In cooperation with Biopartners' and LG Life Sciences' GH Study Group.

LB-PO1-020 Late Breaking Submissions

Association of the polymorphisms in the KiSS-1 and GPR54 gene with central precocious puberty in Korean girls

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Objective: Kisspeptins and GPR54 have recently emerged as indispensable factors for pubertal development. Upon the observations in primates, KiSS-1 and GPR54 mRNA expression increased at the hypothalamus during the transition from the juvenile to the mid-pubertal stage. We hypothesized polymorphisms located in the promoter region of the KiSS-1 and GPR54 genes are associated with the susceptibility of central precocious puberty (CPP).
Methods: One hundred fifty one Korean girls diagnosed with CPP and 172 normal Korean women as the control were recruited. To examine whether the single-nucleotide polymorphisms (SNPs) in the KiSS-1 and GPR54 gene on the 5' flanking region influences transcription factors, we compared transcription factor binding sites using the online program 'AliBaba 2.1' (http://www.gene regulation.com/pub/programs/alibaba2). Two SNPs (54656397 [C/T] and 54655861 [A/T]) in the KiSS-1 gene and one SNP (855765 [A/G]) in the GPR54 gene were predicted to bind transcription factors. The selected SNPs of KiSS-1 and GPR54 gene were genotyped using directly bidirectional sequencing.
Results: With regard to the SNP (54655861 [A/T]) in the KiSS-1 gene, CPP patient group had a higher frequency of A allele than control group (p=0.039). At this SNP site, a transcription factors (NF-1) can bind to the A-containing sequence, but any transcription factor can not bind to the T-containing sequence. The other two SNPs (54656397 [C/T]) in the KiSS-1 gene and 855765 [A/G] in the GPR54) showed no correlations.
Conclusions: The SNP (54655861 [A/T]) in the KiSS-1 may be associated with the susceptibility of CPP in Korean girl. Considering the limited sample size, further large-scale studies are required in other populations. NF-1 affects the transcription of hormone responsive genes, but the correlation and possible mechanism need further functional experiments.

LB-PO1-021 Late Breaking Submissions

Case report: prolactinomas secondary to amenorrhea in adolescence

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We report a female patient, aged 15 years and 10 months, born at term by cesarean section after an uneventful pregnancy, birth weight=2075g (-1.28 SDS) and lenght=47cm (-0.98 SDS). Mid parental height=165,5 cm (+0.33 SDS) and an unremarkable family history. She has gained excessive weight, although she received a nutritionally balanced diet. She has no past medical history. Neuropsychomotor development was adequate. Her menarche occurred at 12 years of age and she has been presenting regular menses for the last 3 years, but she has been in amenorrhea since the last six months. Physical examination revealed: chronological age=15y10m, weight=74.8kg (+1.51 SDS), height=160.7cm (-0.28 SDS), BMI=29.0 kg/sqm (+1.66 SDS), Tanner stage 5 (pubertal), and bilateral serous breast nipple discharge (galactorrhea). No phenotypic abnormalities were detected and thyroid examination was normal.
See table 1 for hormonal profile.

Hormonal profile		
	Case 1	Reference values
LH	1.41	0.7-17.3 mIU/mL
FSH	1.8	1.11-13.9mIU/mL
TSH	2.86	0.5-4.9 mIU/mL
FT4	1.19	0.83-1.44 ng/mL
Prolactin	165.73	2.8-29.2 ng/mL
ACTH	11.6	5-46 ng/mL
Cortisol	19.8	5-25 mcg/dL
Estradiol	10.28	30-200 pg/mL
IGF-1	324.0	226-903 ng/mL
Macroprolactin	Negative	

Table 1

Low-dose suppression test with dexamethasone resulted: ACTH= 3.2 pg/mL and cortisol=0.6 mcg/dL.
A magnetic resonance (MR) study of the hypothalamo-hypophyseal axis was performed: the sella appeared to be increased in size, as well as the adenohypophysis and an expansive lesion of about 1.0 cm was identified. The posterior neurohypophyseal hyperintense signal was found within the sella.
Diagnostic hypothesis: - Pituitary Macroadenoma (Prolactinoma)
Follow-up: The patient started carbegoline therapy at the age of 16y2m at a total dose of 1.0mg per week. She has currently been under therapy for 6 months, has already lost 10kg, and has been presenting regularity of menstrual cycles, without galactorrhea and with normal serum prolactin levels.

PO2-001 Adrenal II

A novel genetic diagnosis of glucocorticoid remediable aldosteronism

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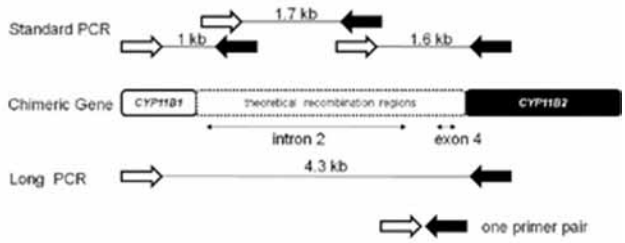
Background: Glucocorticoid remediable aldosteronism (GRA) is a rare form of inherited hypertension caused by the presence of a hybrid gene originating from a recombination between the 11β-hydroxylase (*CYP11B1*) and aldosterone synthase (*CYP11B2*) genes. The disorder is characterized by hyperaldosteronism and high levels of 18-hydroxycortisol (18-OHF) and 18-oxocortisol (18-oxoF), which are under ACTH control. Recently, identification of the *CYP11B1/CYP11B2* chimeric gene using a long PCR technique has reached definitive diagnosis of GRA.
Objective: To evaluate whether a standard PCR method for GRA allows definitive diagnosis.
Patients: The clinical and laboratory characteristics of affected children are shown on the Table1. Imaging studies showed no adrenal mass in both patients. Their clinical diagnosis was highly suspected of GRA.
Methods: We performed both long and standard PCR to amplify the chimeric gene and sequenced those PCR products. The recombination region between *CYP11B1 and CYP11B2* in GRA can be reported to be sitting anywhere from the start of intron 2 to the end of exon 4 of the chimeric gene. We set one primer pair for a long PCR as previously described and designed three primer pairs for a standard PCR to cover all theoretical recombination regions (Figure1).
Results: The long PCR yielded a 4.3 kilobase (kb) size of PCR product in each patient. The standard PCR did 1.6 and 1.7 kb size of products in patient 1 and patient 2, respectively. Sequencing those PCR products demonstrated that they possessed a chimeric gene with a recombination region in intron 2.
Conclusion: We established a standard PCR technique for diagnosis of GRA.

Table1

	Patient 1	Patient 2
Age(year) M/F	12/M	10/M
Blood Pressure (mmHg)	192/131	156/110
Renin	2.0 pg/ml *	<0.2 ng/ml/hr **
Serum Aldosterone (pg/ml)	538	406
Serum Potassium (mEq/l)	3.8	3.8
Urinary 18-OHF (mg/gCr)	0.38	1.72

*active renin concentration **plasma renin activity



PO2-002 Adrenal II

Is the inhibitory effect of metformin on androgen production mediated through AMP-activated protein kinase signaling?

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Metformin is widely used as insulin sensitizer in patients with type 2 diabetes and as modulator of the hormonal milieu in patients with the polycystic ovary syndrome (PCOS). Metformin is reported to suppress lipogenic enzymes in hepatocytes through activation of AMP-activated protein kinase (AMPK) signaling in hepatocytes. Furthermore, AMPK has been shown to regulate glucose uptake into muscles and hepatic glucose production.
By contrast, it remains unknown how metformin modulates androgen biosynthesis; keeping in mind that we only have little understanding of normal androgen biosynthesis overall. Additionally, whereas in patients therapeutic plasma concentrations of metformin are about 10 µM, corresponding effects of metformin are only observed at 10 mM in cell cultures. Recently, a role for organic cation transporters has been established in pharmacokinetic studies of metformin. Therefore the aims of our study were, first, to investigate whether metformin may modulate androgen production through the AMPK signaling pathway, and second, to study the role of human organic cation transporters (hOCTs) for metformin's action.
To study the effects of metformin on steroidogenesis, we used NCI-H295R cells as a model of the human adrenal cortex. Steroid production of the cells was assessed by substrate labeling and thin layer chromatography. RT-PCR experiments demonstrated that NCI cells and human adrenals express all three subunits of AMPK and hOCT1-3 and human plasma monoamine transporter (hPMAT) in variable amounts. High concentrations of metformin (10 mM) downregulated androgen production of NCI cells by inhibiting 3β-hydroxysteroid dehydrogenase type 2 (3βHSDII) and P450c17-17,20 lyase activities but not P450c17-OHase activity. Transfection of NCI cells with hOCT1 or hOCT2 before treatment with metformin prompted the same effects but at much lower concentrations suggesting better intracellular transport of metformin. Activation of the AMPK signaling in NCI cells by AICAR and inhibition by compound C revealed that AMPK modulates androgen production. However co-treatment with metformin did not change this profile markedly. In conclusion, metformin decreases P450c17-17,20 lyase and 3βHSDII activities in adrenal NCI cells. This effect may be modulated by drug availability depending on hOCT1/2 transporters. Androgen production of NCI cells seems to be regulated by AMPK but it may not be the direct target of metformin regulating androgen production.

PO2-003 Adrenal II

Activity of fetal adrenal in Japanese preterm infants using urine steroid profile by gas chromatography / mass spectrometry

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[Introduction] Previous studies showed that fetal adrenal in preterm infants secreted high level steroids continuously until term. The data on activity of fetal adrenal, however, have been limited in relation to gestational age at birth (GA) and corrected gestational age after birth (CGA).
[Objective] To investigate the dependency of timing and degree of maximum activity of fetal adrenal on GA or CGA.
[Subjects] We recruited 1605 newborn infants (male 829, female 776) with GA of 25-40w without neurological and endocrinological abnormalities. We excluded subjects treated with steroid, or born as small for gestational age. A total number of 2275 spot urine samples were collected for the study at the age of 3 - 139 days and kept at -20C°until assayed. All parents of the infants gave informed consent.
[Methods] As a marker of activity of fetal adrenal, urinary DHEA metabolites (D-Ms; DHEA, androstenediol, 16α-hydroxyDHEA, 16β-hydroxyDHEA, 16-oxoandrostenediol, and androstenetriol) were analyzed by gas chromatography / mass spectrometry as previously described. Median and range of the sum of D-Ms (mg/g creatinine) were calculated in combination of 4 groups of GA (25-28w, 29-32w, 33-36w, and 37-40w) and 5 groups of CGA (25-28w, 29-32w, 33-36w, 37-40w, and 41-44w).
[Results] Medians of the sum of D-Ms were shown in Table1. In all 4 groups of GA, the median of the sum of D-Ms at CGA of 37-40w (asterisked in Table 1) was highest. At CGA of 37-40 w, the shorter GA was, the higher the median of the sum of D-Ms was (25-28w > 29-32w > 33-36w > 37-40w, $p<0.05$).

Table1. Medians (sample numbers) of the sum of urinary DHEA metabolites (mg/g creatinine)

	CGA 25-28w	CGA 29-32w	CGA 33-36w	CGA 37-40w	CGA 41-44w
GA 25-28w	94.8 (46)	66.0 (55)	156.3 (50)	272.4 (18)*	96.2 (13)
GA 29-32w		101.5 (76)	105.3 (83)	150.8 (35)*	83.1 (17)
GA 33-36w			77.1 (256)	96.3 (64)*	91.5 (11)
GA 37-40w				37.8 (1255)*	26.6 (296)

GA:gestational age, CGA:corrected gestational age

[Discussion] Our results suggested that the timing of maximum activity of fetal adrenal depended on CGA, while degree of maximum activity of fetal adrenal depended on GA. To confirm these findings, we are currently performing longitudinal study.

PO2-004 Adrenal II

Concomitant P450 oxidoreductase (POR) and androgen receptor (AR) mutations result in neonatal 46,XY DSD and androgenization at adrenarche

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P450 oxidoreductase (POR) facilitates electron transfer to the steroidogenic enzymes CYP21A2, CYP19A1 and CYP17A1, a key regulator of androgen synthesis. POR deficiency is a unique form of congenital adrenal hyperplasia that can result in disordered sex development (DSD) in both 46,XX and 46,XY individuals. While impaired CYP17A1 activity due to mutant POR readily explains 46,XY DSD, androgenization of affected girls has been proposed to be explained by either impaired CYP19A1 activity or the presence of an alternative androgen pathway in early human life. Here we have investigated a patient with 46,XY DSD (micropenis resembling a clitoris, bifid scrotum with small, palpable testes). At age 2 weeks urinary androsterone excretion was undetectable. At 2 months serum androgens were low (DHEAS 0.23 µmol/L, n 0.3-0.9; androstenedione (A) 0.4 nmol/L, n 0.5-2.5; testosterone (T) <0.2 nmol/L (2.1 day 4 hCG), n 4-14). However, presentation with 46,XY DSD had already prompted *AR* sequencing, which revealed homozygosity for Q798E previously reported to affect AR function, but rectified by increased androgen exposure *in vitro*. The patient was raised as a girl and gonadectomized at 4 years. At 9 years the patient presented with rapid clitoral enlargement over the preceding 18 months. Serum DHEAS was raised (2.50 µmol/L, n 0.34-0.92), A was normal (0.73 nmol/l, n 0.3-1.7) and T was below the limit of detection. Urinary steroid profiling (GC/MS) showed normal androsterone excretion but otherwise a characteristic profile for POR deficiency. Baseline cortisol was normal (393 nmol/l) but only increased to 492 nmol/l 60 min after ACTH. *POR* sequencing revealed compound heterozygosity for missense mutation Y607C and frameshift mutation E601SfsX12. Yeast microsomal POR/CYP17A1 co-expression assays showed 71±2% residual 17-hydroxylase activity for Y607C, which had 52±3% residual classic pathway CYP17A1 17,20 lyase activity (17Preg>DHEA) while alternative pathway 17,20 lyase activity (5-pdiolone>androsterone) was largely preserved (73±2%). CYP19A1 activity was only mildly impaired (70±4%). In conclusion, mutant POR and AR cooperate to produce a 46,XY DSD phenotype. Androgenization at age 8 years in the gonadectomized patient suggests that the disruptive effect of mutant POR on adrenal androgen synthesis can be overcome at time of adrenarche and manifest with some androgen effect. Our case also highlights that patients with DSD require work-up of both gonadal and adrenal function.

PO2-005 Adrenal II

Dysregulation of glucocorticoid degradation pathways in PCOS

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Introduction: The Polycystic Ovary Syndrome (PCOS) involves a widespread dysregulation of glucocorticoid metabolism, including generalized hypersecretion of adrenocortical products.
Aim: We investigated the glucocorticoid degradation pathway: 5α and β reductase, 3αHSD, 20α and βHSD in young women with PCOS, using a fully-quantitative Gas Chromatography Mass Spectrometry (GCMS) method.
Subjects and Methods: The study group consisted of 12 young women, age 19.5 ± 3.1 years (mean ± SD), with BMI of 27.4 ± 6.4 kg/m^2, diagnosed with PCOS according to the Rotterdam criteria. The control group consisted of 14 healthy young women, age 28.6 ± 4.7 years, BMI 21.5 ± 3.3 kg/m^2. Urine samples were taken during the follicular phase of the menstrual cycle or on day 3-7 of the 7 day off-pill interval, in subjects receiving ORC. In women with PCOS who had no ovulations or menstruation, urine samples were taken on an arbitrary day. Samples were analyzed using (GC-MS).
Results - All glucocorticoid degradation metabolites were higher in the PCOS group versus controls. The activity of 11βHSD type 1, calculated by the ratio (THF+5αTHF)/THE, was reduced in PCOS women (0.61±0.32 vs. 0.91±0.32, p<0.046), and even more so when adding cortols (C) and cortolones (CL) to the equation (THF+5αTHF+aC+bC)/(THE+aCL+bCL) (0.43±0.24 vs. 0.74±0.23, p<0.0036). 5α-reductase activity was increased only when calculating the ratio of 11OH-An/11OH-Et (9.5±9.2 vs. 2.8±1.5, p<0.022), but not with the ratios (An/Et), (αTHB/THB), (αTHF/THF), and in no correlation with the BMI. The activity of 20αHSD (αC+ αCL)/(THF+5αTHF+THE) was markedly elevated in PCOS (0.45±0.20 vs. 0.16±0.06, p<4E-5), in negative correlation to BMI (p<0.05).
Conclusions: 1. PCOS is associated with dysregulation in glucocorticoid metabolism. 2. 5α-reductase activity is enhanced only through the 'backdoor' pathway in no correlation with the BMI. 3. We confirm previously reported decrease in 11βHSD1 activity. 4. Marked increase in 20α-HSD, hitherto recognized only as an important ovarian enzyme in progesterone metabolism, suggests an unknown derangement in PCOS.

PO2-006 Adrenal II

Neonatal screening for congenital adrenal hyperplasia: 2.5-yr experience in the two most populous regions of Russia

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Background: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is a life-threatening condition. To prevent its delayed diagnosis the neonatal screening was introduced in some countries. Since June 2006 CAH was included in the list of the disorders covered by the national screening program in Russia.
Objective: To analyse the incidence of 21-OHD and evaluate the effectiveness of neonatal screening for CAH in the two most populous regions of Russia, Moscow metropolitan area and Moscow oblast (total population ~18,000,000).
Methods: 17-Hydroxyprogesterone (17OHP) was measured by DELFIA assay in filter paper blood samples collected at day 4 in full-term and at day 7 in preterm infants. In the first 30 months a total of 471131 newborns were screened in these two regions. 4344 cases were recalled (0.92%). 63 were confirmed as having CAH (male:female ratio, 0.8; incidence, 1:7479). Only 37% of affected children (all of them females) would have been diagnosed by clinical signs only. 6 girls with Prader stage 4 virilisation were initially assigned as males. Mean age at diagnosis was 1 week for girls and 10-15 days for boys. Despite the screening hyperkaliemia or/and hyponatriemia was present in 25 patients

before initiation of treatment. 21-OHD was verified by molecular analysis in 55 patients. 49 cases were defined to have salt-losing form, 3 – simple virilising form, and 3 – non-classical form of CAH. In addition, 2 cases of CYP11B1 deficiency and 1 case of HSD3B2 deficiency were identified as result of the screening. None of them were suspected clinically.

Conclusions: Neonatal screening for CAH in the studied population proved to be effective. The results also indicate on a relatively high incidence of 21-OHD in Russia.

PO2-007 Adrenal II

The low-dose ACTH test does not identify mild insufficiency of the hypothalamic-pituitary-adrenal axis in children

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Undiagnosed insufficiency of the hypothalamic-pituitary-adrenal (HPA) axis can be life-threatening in patients stressed by illness or surgery. Adequate evaluation of the hypothalamic-pituitary-adrenal (HPA) axis requires provocative testing.

Objective: To investigate the sensitivity of published cortisol cut-off points of the low-dose adrenocorticotropin (ACTH) test (LDAT) in children with proven mild HPA axis insufficiency.

Patients and Methods: The HPA axis of 16 pediatric patients (age range: 5.5-15.7 yr) with established mild HPA axis insufficiency on the basis of a subnormally stimulated cortisol with the insulin tolerance test (ITT), normal morning basal cortisol concentrations, and lack of clinical signs of adrenal insufficiency was reinvestigated with the LDAT. No patient received glucocorticoid replacement therapy. The sensitivity of the LDAT was calculated on the basis of published stimulated cortisol cut-off points.

Results: The basal plasma cortisol concentrations did not differ significantly. The LDAT showed both a significantly higher cortisol peak and a greater cortisol rise compared with the ITT (both $P<0.001$). Assuming a 100% accuracy of the ITT, the LDAT yielded a low sensitivity of 6.3 - 37.5 % using published cortisol cut-off points as references.

Conclusion: Using published cortisol cut-off points, the LDAT showed a poor sensitivity to detect mild HPA axis insufficiency. We cannot recommend the use of the LDAT as a screening test of HPA axis impairment in such children. When deciding to perform the LDAT, a careful interpretation of the result is necessary and a high cut-off point should be used to avoid underdiagnosis.

PO2-008 Adrenal II

Adverse effect of phenytoin on glucocorticoid replacement in a child with adrenal insufficiency

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The typical treatment of primary adrenal insufficiency is the replacement of missing hormones by synthetic glucocorticoids and mineralocorticoids. The metabolism of these synthetic agents can be significantly accelerated through induction of microsomal liver enzymes by some drugs including phenytoin, barbiturates and rifampicin. We have recently observed an adverse interaction between phenytoin and hydrocortisone in a patient with adrenal insufficiency as glucocorticoids deficiency caused by increased metabolism of hydrocortisone by phenytoin.

A 14 year-old boy diagnosed 4 years ago with primary adrenal insufficiency presented with one-year history of intermittent fatigue and vomiting. Initial treatment included hydrocortisone (15 mg/m^2/day) and fludrocortisone (0.1 mg/day). His plasma ACTH levels had been within usual range for 2 years. Because of epileptic seizures, phenytoin was initiated 200 mg/daily on the second year of his treatment. After that, ACTH levels were markedly elevated while hydrocortisone was gradually increased to 30 mg/m^2/day. Fludrocortisone supplementation did not change significantly. The patient suffered from 3 episodes of vomiting, hypoglycemia and hyponatremia during 2-year follow-up. Serum potassium levels were normal or low during these episodes. At the last episode, serum Na was 126 mEq/L, potassium 3.26 mEq/L, glucose 73 mg/dl and ACTH >1250 pg/ml. Therapy with phenytoin was switched to levetirasetam, an anti-epileptic drug which was metabolized through kidneys, not liver. Fifteen days after stopping phenytoin, his serum ACTH concentration returned to normal range.

Administration of phenytoin to our patient with adrenal insufficiency led to adverse effects such as unsuppressible ACTH levels despite of high doses of hydrocortisone, and hypoglycemia and hyponatremia episodes without concurrent hyperkalemia, which imply isolated glucocorticoids insufficiency. The addition of phenytoin markedly increased hydrocortisone requirement and deteriorated our patient's metabolic control. Therefore, we suggest that drugs such as phenytoin affecting hepatic clearance of synthetic glucocorticoids and mineralocorticoids should not be preferred for therapy of patients with adrenal insufficiency. If their use is vital, patients should be closely monitored.

PO2-009 Adrenal II

Atypical Cushings presentation in a child with Carney's complex

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Background: Carney's complex (CNC) is an autosomal dominant disorder that includes a complex of myxomas, lentigines, endocrine overactivity, and a variety of other tumors (schwannomas, Sertoli cell, thyroid, and pituitary, and/or primary pigmented nodular adrenalcortical disease (PPNAD)).

Clinical presentation: We report a 4 yr old male, referred for evaluation of possible Cushings syndrome (CS) associated with CNC. CS in children typically presents with growth failure associated normal or increased weight gain. The child's medical status was further complicated by a history of: bile duct paucity, pancreatic insufficiency, hypercholesterolemia, fatty liver, failure to thrive, anemia, developmental delay, osteoporosis, & multiple fractures. Surgical history included several GI procedures.

Family history: The patient and his family carried a PRKAR1A mutation; PRKAR1A is the gene that is mutated in more than 70% of patient with Carney complex

Evaluation: See table for growth data. Physical exam was significant for: lentigines on face, groin, and forearm; & some typical Cushings features (moon facies, mild facial plethora, buffalo hump, supraclavicular fat). Biochemical testing showed abnormal diurnal cortisol: 12AM: 6.6; 8AM: 20.8 (mcg/dL), low ACTH (7.7pg/ml), and normal 24-hour urine free cortisol (UFC). The diagnosis of ACTH-independent CS was confirmed by lack of suppression to the Liddle's test, consistent with PPNAD (paradoxical increase of UFC from 12.5 to 42.5 ug/d24). He underwent bilateral adrenalectomy, without complication, and started on glucocorticoid and mineralocorticoid replacement. Ten months post-surgery, improvement was noted in all anthropometric measurements. Growth hormone stimulation testing performed at 8 mos post-op showed normal response.

Growth data			
	Ht SDU	Wt SDU	GV SDU
Initial eval	-4.25	-1.4	-5.6
10 mos post-op	-4.13	-1.1	-1.7

SDU= std dev units; GV= growth velocity

Conclusion: This case illustrates that CS due to PPNAD is a rare disease that can be difficult to diagnose, even in the context of a known PRKAR1A mutation. The pt's co-diagnosis of pancreatic and liver disease contributed to a cachectic state, which likely contributed to the poor weight gain, even in the presence of abnormal endogenous glucocorticoid secretion. Post-bilateral adrenalectomy and subsequent eucortisolemic levels, resulted in improvement in growth parameters, confirming the importance of abnormal endogenous cortisol production on growth.

PO2-010 Adrenal II

Effect of growth conditions on androgen synthesis in human adrenal H295R cells

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Adrenal androgens (mainly DHEA and DHEA-S) are produced in cells of fetal adrenal and zona reticularis (ZR) of adult human adrenal cortex. Its synthesis displays specific developmental pattern, declining after the birth and increasing again after 6-8 years of life in process called adrenarche. Studies showed that presence of 17α-hydroxylase/17,20-lyase (CYP17;p450c17), microsomal cytochrome b5 (CYB5) and steroid sulfotransferase (SULT2A1), as well as absence of 3β-hydroxysteroid dehydrogenase (3β-HSDII) in the cells of ZR are essential for androgen synthesis. However, in contrast mineralo- and glucocorticoids, regulation of adrenal androgen production remains poorly understood. As adrenarche occurs only in humans and higher primates, suitable animal models for its study are missing. Human adrenal H295R cells are broadly used for studying of steroid hormone synthesis. In presented work we describe specific growth conditions which enhance androgen and weaken glucocorticoid synthesis. Serum withdrawal for more than 24 hours shifts steroidogenesis strongly towards androgen production due to decreased activity and expression of 3β-HSDII and enhanced activities of P450c17, particularly 17,20-lyase activity. The expression of CYP17 as well as CYB5 or P450 oxidoreductase remained unchanged. However, the increase in lyase activity was accompanied by stronger phosphorylation at serine residues. Signaling pathways under different growth conditions were analysed as well as effect of 8Br-cAMP stimulation on the expression and activities of P450c17 and 3βHSDII. In contrast to published studies we did not observe direct effect of short time 8Br-cAMP stimulation on 17,20-lyase activity. However, threonine phosphorylation of P450c17 seemed to be increased. In starvation conditions, stimulation with 8Br-cAMP increases DHEA but not cortisol after very short time. Cortisol increase was observed after long term treatment only, when it correlated with the increase in expression of HSD3B2.

In brief, we describe that under specific growth conditions H295R cells shift their steroid production from glucocorticoids towards androgens and enhance expression of the genes predominantly present in ZR. Thus, further studies performed under starvation conditions might help to detect important factors regulating androgen production and cell specific expression of key steroidogenic enzymes.

PO2-011 Adrenal II

Regulation of HSD3B2 gene expression in NCI-H295R cells by the transcription factors GATA-4/6 and Nur77

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HSD3B1/2 genes encode 3β-hydroxysteroid dehydrogenase (3βHSD) enzymes which are essential for steroidogenesis. HSD3B2 is specifically expressed in the gonads and the adult adrenal cortex. It is tightly regulated by hormonal stimuli and growth factors in a tissue-specific and developmentally characteristic fashion. Although several transcription factors and different signals have been suggested to regulate the activity of the HSD3B2 promoter, the precise mechanism of transcriptional regulation remains unclear.

Here, we analyzed the transcriptional regulation of the HSD3B2 gene in human adrenal H295R cells in basal and cyclic AMP stimulated condition. Thereby we focused on the role of the transcription factors GATA and Nur77. Nur77 is an orphan nuclear receptor that was recently described to play a crucial role in the zone-specific expression of the HSD3B2 gene in the fetal as well as in the adult human adrenal cortex. GATA factors are known regulators of several steroidogenic genes.

The promoter of the HSD3B2 gene is activated by co-expression of GATA transcription factors or Nur77 alone. The stimulatory effect of GATA and Nur77 is additive suggesting cooperation between these factors. Mutations of specific binding sites (cis-elements -120 bp NBRE (Nur response element) and -196 GATA response element) disrupted activation by Nur77 and GATA respectively. NBRE seems to be crucial for basal as well as cAMP- stimulated promoter activity, but mutation of the GATA site influences neither basal promoter activity nor after stimulation with cAMP. In addition, mutation of NBRE abolished not only Nur77, but also GATA-mediated activation of the HSD3B2 promoter suggesting that binding of Nur77 is essential for the stimulatory effect of GATA-4/6. Physical interaction between GATA and Nur77 transcription factors were found by immunoprecipitation experiments. Thus, we conclude that Nur77 receptor seems crucial for basal as well as cAMP-stimulated expression of the HSD3B2 gene in human adrenal cells. GATA-4/6 factors are not essential for HSD3B2 expression, but seem to enhance HSD3B2 expression - at least in part - by interaction with Nur77.

PO2-012 Adrenal II

A female case of neonatal ACTH-independent Cushing's syndrome associated with masculinization symptoms

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Introduction McCune-Albright syndrome(MAS) is associated with Cushing's syndrome(CS). Moreover, patients who develop this combination of syndromes are known to exhibit symptoms of precocious puberty due to autonomous hyperfunctioning of the endocrine glands.

Case Report A 2-month-old female infant. The infant's weight and height were 1940 g and 41.5 cm, respectively(at a gestational age of 33 weeks). Poor weight gain, moon-shaped face, and clitoral hypertrophy progressively manifested after birth. Mass screening revealed an elevated serum 17α-hydroxyprogesterone (17OHP) level (200 ng/ml). We also observed high levels of serum cortisol level (148 μg/dl), plasma renin activity (300 ng/ml/h), dehydroepiandrosterone sulfate (DHEA-S) (2870 μg/dl), aldosterone (1040 pg/ml), estradiol (E2) (208 pg/ml), testosterone (T) (27.9 ng/ml),and low level of plasma adrenocorticotrophic hormone (ACTH) level (4.3 pg/ml). The abdominal ultrasound examination revealed adrenal parenchymal masses bilaterally. The results of the high-dose-dexamethasone-suppression test were abnormal. Therefore, we diagnosed the patient's condition as an ACTH-independent CS caused by an adrenal tumor. The patient had to undergo tumorectomy at 52 days of age, because the tumor showed a tendency to progressively increase in volume. The bilateral tumors on the adrenal glands had nodular surfaces. The tumor on the right adrenal gland weighed 28.7 g; its dimensions were 60 mm (height) × 54 mm (width) × 27 mm (anteroposterior diameter). The tumor on the left adrenal gland weighed 15.4 g; its dimensions were 48 mm (height) × 42 mm (width) × 24 mm (anteroposterior diameter). Histological examination revealed that the tumors were nodular and hyperplastic, and they mainly consisted of cortical cells with eosinophilic cytoplasm. We did not observe any necrosis or significant images of nuclear division; moreover, the boundaries of the tumors were surrounded by a membranous structure, and there was no extracapsular infiltration or vascular invasion. The pathological diagnosis was of adrenocortical hyperplasia.

Summary Adrenal tumors are rarely observed during childhood, and the symptoms of CS are observed in 20–40% of all cases of adrenocortical tumors. Furthermore, since CS and the symptoms of precocious puberty were observed in the child, we suspected that the patient was suffering from MAS and performed a genetic examination to evaluate this possibility.

PO2-013 Adrenal II

Investigation of premature adrenarche reveals a high incidence of congenital adrenal hyperplasia (CAH)

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Background: Premature pubic hair development with or without other manifestations of androgen production need to be differentially diagnosed from conditions including congenital adrenal hyperplasia, and may be associated with the early development of puberty.
Aim and Methods: We aimed to study the characteristics at presentation, endocrine profile, prognostic factors and final diagnosis of patients who presented with premature pubic hair development. We identified 41 patients [33 female (80.5%), 8 male (19.5%)] who have been referred to our unit for investigation over the last five years and case notes were studied retrospectively.
Results: Patients presented at a mean age of 6.9±2.2 years, with a mean height SDS of 1.6±1.5 (range -1.6 to 5.3) and a mean BMI SDS of 1.2 ±1.3 (range -1.06 to 4.06). Presenting height SDS was not significantly different from the target height SDS ($p \geq 0.05$). Development of pubic hair was the presenting complaint in the majority (83%) followed by body odour (24.4%), acneiform changes (17.1%), accelerated growth (14.6%) and mood changes (4.9%). In our cohort premature adrenarche was confirmed in 31 children (75.6%), of whom 83.9% were girls and 16.1% boys. Virilising CAH due to *CYP21* mutations was diagnosed in 14.6% (n=6), CAH due to *CYP11B1* mutations in 7.3% (n=3), whilst one female patient (2.4%) was diagnosed with gonadotropin-dependent precocious puberty. Adrenal androgens were elevated in this cohort (DHEAS: 3±2.2 umol/l; A4: 5±7 nmol/l); only 7.3% of patients had DHEAS within the normal range for prepubertal children. Increased concentrations of testosterone correlated with the diagnosis of CAH ($p<0.05$). Bone age was advanced by more than 2SDS in 50% of patients with premature adrenarche. Although 9% (n=3) of presenting female patients had evidence of PCO on pelvic US, there was no correlation with the advanced bone age, or increased BMI SDS in this relatively small cohort. There was no significant difference in the age at presentation, Ht SDS, Wt SDS, or concentration of adrenal androgens between boys and girls diagnosed with premature adrenarche.
Conclusion: The increased percentage of CAH diagnosed in this cohort may result from selection bias, as more severe cases tend to be referred to tertiary centers for investigation. Long term follow up data are needed to elucidate the natural history of premature adrenarche.

PO2-014 Adrenal II

Urinary free steroids in newborn infants with 21-hydroxylase deficiency using stable isotope dilution gas chromatography / mass spectrometry

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[Introduction]
The biochemical diagnosis of 21-hydroxylase deficiency (21OHD) on the newborn infant requires 1-2 days by serum 17-hydroxyprogesterone measurement, urinary steroid profile using gas chromatography / mass spectrometry (GC/MS), or genetic analysis.
[Objectives]
The objectives of this study were 1) to establish a method for simultaneous measuring urinary free pregnenolone (P5), progesterone (P4), 16-hydroxyprogesterone (16OHP), 17-hydroxyprogesterone (17OHP), 21-deoxycortisone (21DOE), 21-deoxycortisol (21DOF), dehydroepiandrosterone (DHEA), androstengione (AD4), 11-hydroxyandrostendione (11OHAD4), testosterone (T), and dihydrotestosterone (DHT) by stable isotope dilution-GC/MS and 2) to measure the above urinary free steroid concentrations in newborn infants with 21OHD.
[Subjects]
1) Pooled urine spiked with steroids was used for validation. 2) Three patients with 21OHD (3 females; age 2-4 days) and 22 Japanese control (10 males and 12 females; age 3-4 days) participated in this study.
[Methods]
Free steroids were extracted from 1 ml urine by ethyl acetate, followed by purification using C18 mini column. The obtained samples were delivered with heptafluorobutyric anhydride. Finally, those samples were analyzed by GC/MS.
[Results]
1) The interassay coefficient of variation in pooled urine were 7-18%. The recovery rates from urine were 85-106%. The quantification limits were 0.1-5 ng/tube. The turnaround time was within 5 hours. 2) In the 21OHD patients, 16OHP, 17OHP, 21DOE, 21DOF, 11OHAD4, T, and DHT were higher than the maximum of control (table).

Urinary free steroids in newborn infants with 21OHD

	21OHD patients	The maximum of control
P5	56.5~164.0	54.5
P4	n.d.~7.3	3.4
16OHP	38.4~224.0	13.5
17OHP	18.4~128.0	11.2
21DOE	183.0~699.0	37.8
21DOF	234.0~669.0	35.9
DHEA	9.2~154.0	48.6
AD4	2.9~31.2	2.3
11OHAD4	56.3~1170.0	23.7
T	3.9~8.4	3.6
DHT	3.7~12.5	1.6

(μg/g creatinine. n.d.: not detected)

[Discussion]
1) We validated a method of simultaneous measuring urinary free steroids, judging from good repeatability, recovery rate, and sensitivity. 2) We were able to determine the characteristic urinary free steroid increases in the 21OHD. This method may have an advantage for the rapid diagnosis of 21OHD. We will determine sensitivity and specificity for the diagnosis of 21OHD using urinary free steroid measurements.

PO2-015 Adrenal II

Molecular defects of the *CYP21* gene in Greek Cypriot patients with congenital adrenal hyperplasia

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Background: A limited number of *CYP21* mutations account for the majority of all mutated alleles in Congenital Adrenal Hyperplasia (CAH), but a number of rare mutations are often identified.
Aim: To determine the mutations in the *CYP21* gene in Greek Cypriots with CAH and attempt a genotype-phenotype correlation.
Subjects and Methods: 36 patients with CAH (8 -SW, 3 - SV and 25 - NC form) were studied. The categorization of patients was based on clinical and hormonal findings. Molecular analysis was performed by MLPA and direct sequencing of PCR products of the *CYP21* gene.
Results: The classical form of CAH is rare (1:25000) in our population, - total number of births (309,470) with 12 new cases diagnosed over 30 years (1978 – 2007). The most frequent genetic defect in the classic SW and SV forms was the IVS2-13A/C (47.9 %) mutation, followed by the Del 8bp E3 (17.4 %). In the NC form, the most frequent mutations were V281L (64.7 %) and Del 8bpd E3 (19.6 %).
Genotypes were categorized in 4 mutation groups (null, A, B and C) according to their predicted functional consequences. All 3 patients in the null group (Del 8bp E3, F306insT, and Q318X) manifested the SW form. In mutation group A (IVS2-13A/C splice site mutation in homozygous or heterozygous form

with mutations from null group), six out of seven patients presented with the classical form of CAH except of a girl who carried the genotype Del 8 bp E3 / IVS2-13A/C +V281L and presented with premature adrenarche with advanced bone age. There was only one patient in group B with the genotype I172N / I172N, who had the SV form as expected. Finally in group C (homozygous for V281L or compound heterozygotes with V281L and P30L, Del 8bp E3, Q318X, P482S, P453S and V304M) 24 out of 25 exhibited the NC form. The only patient in group C manifesting mild SV form had the P30L/X genetic defect and the known polymorphism N493S in homozygosity. The rare mutation V304M was identified in two females, who presented with a hyperandrogenemia in adolescence and had the NC form. The two patients had the Del 8 bp E3 / V304M and the Del 8 bp E3 + V304M / V281L genotypes.

Conclusion:

The mutations of the *CYP21* gene in our population is comparable to those reported in similar ethnic groups. There is a good genotype-phenotype correlation. In some cases the polymorphic variant N493S may be a plausible disease causing mutation. Knowing the ethnic specificity of the CYP21 mutations is a diagnostic tool for all forms of CAH.

PO2-016 Adrenal II

Severe form of systemic pseudohypoaldosteronism type 1 – a novel mutation in the alpha subunit of ENaC

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Pseudohypoaldosteronism type 1 (PHA1) is a rare disease which is characterised by hyponatremia, hyperkalemia, metabolic acidosis and elevated levels of aldosterone and renin. Aldosterone resistance is caused either by a mutation of the mineralocorticoid receptor gene or the epithelial sodium channel (ENaC). The first one causes autosomal dominant PHA1, also called renal form, the second one is responsible for the systemic form of PHA1 and is inherited in an autosomal recessive pattern.

We present a male arabic preterm baby, spontaneously delivered in 33+1 week of gestation (weight: 2280 g, length 49 cm). The parents were related. At day 9 the boy was presented with failure to thrive, vomiting and fever. Hyponatremia (126 mmol/l) and hyperkalemia (8,9 mmol/l) but no metabolic acidosis was documented. The diagnostic work-up showed elevated levels of aldosterone 3000 ng/l (normal: 70-830 ng/l) and renin 1000 ng/l (normal: 5.9-132 ng/l) while 17-Hydroxyprogesterone, ACTH and cortisol were normal.

With the tentative diagnosis of PHA1 we started a high dosed sodium supplementation via a nasogastric tube. In spite of this intervention we observed recurrent episodes of hyperkalemia so we had to increase the therapy by adding resonium and glucose insuline infusions.

Recurrent infections of the lower respiratory tract made the hypothesis systemic form of PHA1 very likely.

The molecular sequencing of the genes for the epithelial sodium channel (ENaC) revealed a new mutation in the gene for the alpha subunit of the ENaC (SCNN1A). This mutation leads to an exchange of only one amino acid (c.1678 G>A / p.Gly560Ser). In severe cases the typical findings are stop codons or frameshift mutations.

At the age of 8 months potassium remained in the upper normal range under high dose sodium supplementation (40 mmol/kgxd), resonium and sodium bicarbonate given by percutaneous gastrostomy. The boy develops well nevertheless we observed life-threatening events which resulted from trivial infections. In conclusions PHA 1 has to be included in the differential diagnoses of severe hyponatremia in newborns. Most important for the outcome of patients with systemic PHA 1 is a secure access way for the supplementation of high sodium dosages. Consequent immunisation should be performed to avoid trivial infections.

PO2-017 Adrenal II

Pro12Ala polymorphism of *PPARG* in prepubertal children with premature adrenarche and association of 12Ala variant with growth in healthy children

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PPARγ2 participates in the regulation of adipogenesis and insulin sensitivity, and has connections to growth hormone - IGF system and ACTH - adrenal androgen axis. We hypothesized that Pro12Ala polymorphism leading to decreased receptor activity could modulate the pathogenesis of premature adrenarche (PA) that results in early activation of adrenal androgen production, and associates with hyperinsulinemia, increased IGF-1 levels and tendency to overweight.

We performed a cross-sectional association study in 73 prepubertal children with PA and age- and sex-matched 97 healthy control children. We genotyped the Pro12Ala polymorphism of *PPARG* with TaqMan Allelic Discrimination Assay. Growth data, baseline hormone levels and the values of oral glucose tolerance test (OGTT) were associated with genotypes using Student's t-test and univariate linear model with age and sex as covariates.

We found no difference in the genotype distributions of *PPARG* between PA and control children (minor allele proportion; control *vs. PA; 0.12 vs*. 0.17; 95% CI for the difference; -0.04, 0.13, *P*=0.3). Birth measures and weight-for-height were equal between the genotype groups in both control and PA children. The minor Ala12 variant was associated with lower current height SDS in the healthy controls [C/C, n=76 *vs*. C/G&G/G, n=21; mean (95% CI); 0.4 (0.2, 0.6) *vs. -0.1 (-0.5, 0.3), P*=0.045], but no similar association was seen in PA children. When we analyzed growth parameters between birth and the age of 5 yrs, the control subjects with minor variant showed lower height SDS than those with major variants at the age of 3 and 4 yrs (*P*<0.05). The minor Ala12 variant was associated with trends for lower serum IGF-1 level [nmol/L; 21 (19, 22) *vs. 18 (15, 21), P*=0.06] and lower serum insulin concentration at 120 min time point of OGGT in control children [mU/L; 23 (21, 26) *vs. 18 (13, 26), P*=0.09], but the minor variant could not be associated with glucose metabolism in PA children. Lipid profile, adrenal steroid and SHBG levels were similar between the genotype groups in both control and PA children.

In conclusion, Pro12Ala genotype of *PPARG* was not associated with PA, but the limited power of the study does not prove the negative result. The minor 12Ala variant was associated with lower height SDS and trends for lower insulin and IGF-1 levels in healthy prepubertal children indicating its possible effects on growth.

PO2-018 Adrenal II

A novel mutation causing 3 β hydroxysteroid dehydrogenase deficiency

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Introduction: 3 β Hydroxysteroid Dehydrogenase deficiency is a rare cause of defective glucocorticoid and mineralocorticoid synthesis that may be fatal if not diagnosed in infancy. In its classic form, genetic females may have mild virilization and clitoromegaly due to DHEA overproduction and its conversion to testosterone via extra-adrenal 3 βHSD1. Here we describe a novel mutation in 3βHSD2 gene causing glucocorticoid and mineralocorticoid deficiency with no virilization.

Patients/Methods: A 9.3 years old girl, born to non relative Palestinian Christian parents, was diagnosed with severe salt-losing crisis shortly after birth, (Na=99 meq/l). No clitoromegaly or fusion of the labia was observed. ACTH test at 0 and 60 minutes showed: ACTH 18.6 pmol/l, cortisol <27.6 -> <27.6 nmol/l, 17-OH progesterone 0.1 -> 0.1 nmol/l, 17-OH pregnenolone <1 -> 13.9 nmol/l, DHEAS 6.49 ->6.13 micromol/l, Androstenedione < 0.35 -> <0.35 micromol/l, Testosterone < 0.3
-> 0.3 nmol/l. She has been successfully treated with hydrocortisone and Flurinef. Currently she is pre pubertal with height and weight in the 25th and 75th percentiles respectively. Her physical examination does not reveal acne or

hirsutism. Genetic studies for the family were done.
Results: Genetic analysis of DNA from the proband and her parents using micro satellite marker in the region of βHSD2 gene on chromosome 1, found our patient to be homozygous and the parents heterozygous in the gene locus. βHSD2 gene sequencing revealed the patient to carry a novel homozygous mutation in exon 3
(439), C->A corresponding to A231D amino acid substitution in the βHSD2 protein.
Conclusions: We present a unique case of 3βHSD2 deficiency with severe hyponatremia and adrenocortical insufficiency, and no signs of virilization, caused by a novel mutation in exon 3 in a location that was described in the past to be responsible for enzyme activity. Functional studies may explain the absence of virilization and the severe hyponatremia that are special in this case.

PO2-019 Adrenal II

Study of cabergoline in treatment of childhood Cushing's disease

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Background:
Effectiveness of dopamine agonist in Cushing's disease (CD) is controversial. Data on its response in childhood Cushing's disease is limited to few case reports.
Aim:
The aim of this study was to analyse the effectiveness of cabergoline in patients with uncured CD in age group below 20 years of age.
Patients and Methods :
Twenty patients (5 cases 20 yrs and 15 cases > 20 yrs) with persistent CD after pituitary surgery with or without additional radiotherapy entered the study prospectively. Cabergoline was started at 1mg/week and increased by 1mg/week every month until a maximum dose of 5mg/week was reached or therapeutic response was documented. Response was defined as serum midnight cortisol <5mcg/dl and/or standard 48 hours low dose dexamethasone supressed cortisol < 1.8 mcg/dl. In responders, without prior radiotherapy, drug was continued to see the long term response (12 months). In responders with prior radiotherapy, drug was withdrawn to ascertain the cause of response between radiotherapy and cabergoline**Resuts:**
5 / 20 patients (1 / 5 in ≤ 20 yrs and 4 / 15 in > 20 yrs) responded to the drug. Response was seen at an average dose of 3.6 mg/week. Drug was continued in one RT nave responder (16 yrs of age) at a dose of 2mg/week for 12 months with continued response documented. His antihypertensive drugs could be withdrawn completely and serum testosterone increased from 3.3 ng/ml to 7.6 ng/ml at end of 1 year.
Conclusion:
Cabergoline is effective in controlling hypercortisolism in one fifth (20%)of the patients in childhood Cushing's disease which is comparable to response in adult population. The response is persistent till 12 months. Cabergoline can be considered in uncured cushing's disease patients. Amongst responsive patients, the drug can be continued without affecting rest of the pituitary functions unlike radiotherapy.

PO2-020 Adrenal II

Time-dependent changes in the sex ratio of children with 21-hydroxylase deficiency in a patient registry: effects of the implementation of a neonatal screening program in Germany

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Introduction: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is clinically suspected in female newborns with genital ambiguity, in children with a salt-losing adrenal crisis and older patients with clinical features of hyperandrogenemia. 21-hydroxylase deficiency is transmitted as an autosomal recessive disorder. Based on the inheritance, an equal male to female ratio has to be postulated among affected individuals. In large historical cohorts, however, a female preponderance was noted. This is thought to be due to a verification bias in favour of females with neonatal virilization. The presence of a German patient registry prompted us to test this hypothesis by comparing the sex ratio in this registry before and after implementation of neonatal CAH screening. **Materials and Methods:** Since January 2000 the German Working Group for Pediatric Endocrinology has established a registry for children with 21-hydroxylase deficiency for the purpose of quality management. 1119 patients (487 male, 632 female) from 35 centres in Germany were documented until December 31, 2008. 57% of the patients had salt-wasting, 16% were simple virilizers, 8% had the late-onset form, 3% were heterozygotes, and 16% were not classified into these or other diagnostic groups. In Germany, newborn CAH screening was started as a pilot project in 1982, and increasingly implemented between 1999 and 2005. We analysed the registry data with regard to the male to female ratio and the patients' screening status. **Results:** 586 registry patients were diagnosed without screening. In 211 patients neonatal screening was performed. In 322 patients no information about the screening status was available. In the unscreened cohort (n = 586) 239 males and 347 females were identified (female/male ratio 1.45). Among the screened population (n = 211) were 130 males and 81 females. The female/male ratio was 0.62, with more male patients diagnosed in the salt-wasting group. After implementation of neonatal screening for 21-hydroxylase deficiency the sex ratio had changed, the difference being significant ($p < 0.0001$). **Conclusions:** Between 2000 and 2008, the CAH registry of the German Working Group for Pediatric Endocrinology has documented a large cohort of children with 21-hydroxylase deficiency. Based on these data, it was possible to show that the sex ratio of children with CAH had shifted towards the expected equal sex ratio with the implementation of a neonatal screening.

PO2-021 Adrenal II

Enhanced 17hydroxyprogesterone levels in children with multiple sclerosis receving glatiramer

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Multiple Sclerosis (MS) is autoimmune disease with progressive demyelinization of CNS with rare incidence in paediatric age.
Diagnosing MS in a child is challenging due to: limited diagnostic criteria; their overlap with acute disseminated encephalomyelitis, mostly experiencing a relapsing-remitting course. Initial brain MRI shows more frequent involvement of the posterior fossa, and more confluent lesions that decrease over time in patients with prepubertal onset. Although disability progression is slower than in adults, MS paediatric onset leads to significant disability at a younger age. Lack of paediatric-onset MS precluded enrolment in clinical trials. Thus, children receive off-label adult therapies without clear evidence of effectiveness and with limited knowledge of tolerability.
Glatiramer acetate (GA, Copaxone) is an immunomodulatory drug used to treat MS.

CNS involvement can induce hypothalamic-pituitary disarray with altered endocrine patterns. No data are published in MS paediatric patients treated with GA.
MS patients show hypothalamic-pituitary-adrenal axis hyperactivity and display significantly higher ACTH, cortisol and DHEAS plasma concentrations and urine cortisol values than controls.
We investigated the regulation of the hypothalamo-pituitary-adrenal (HPA), hypothalamo-pituitary-thyroid (HPT), hypothalamo-pituitary-gonadal (HPG) axes and prolactin secretion in 10 adolescents (age diagnosis <14 years) with clinically and radiologically definite MS treated with GA.
In our patients TSH, fT3, fT4, prolactin, FSH, LH were in the normal range for gender and age. No patients showed thyroiditis echographic signs and/or thyroid nodes, nor positive anti-thyroglobulin and anti-peroxidase antibodies. The HPA axis showed: ACTH 55.5±15.19 pg/ml; cortisol 27.5±10.48 ug/ml; DHEAS 213.3±142.4 ng/ml; 17hydroxyprogesterone 3.4±2.03 ng/ml, significantly increased respect the normal basal levels. The study of the 21hydroxylase sequence is still under development.
Our preliminary data reveal up-regulation of the HPA axis in response even to mild stressors, as recovery or blood-sample collecting in paediatric patients with MS. A possible common mediator in up- regulating the HPA axis is IL-6, which is elevated in MS.This is the first study reporting complete hormonal profile in paediatric GA-treated MS with the original relieve of significantly increased 17hydroxyprogesterone levels. Further studies are necessary to understand the underling mechanisms of this assess.

PO2-022 Adrenal II

Mutational spectrum of steroid 21-hydroxylase (21-OH) gene. Genotype-phenotype correlation in 360 Argentinian congenital adrenal hyperplasia (CAH) patients

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Deficiency of 21-OH is the most common form of CAH.We determined the frequency of 11 most common mutations described in the CYP21A2 gene by allele-specific PCR and southern blot analysis.In non-detected alleles coding and exon-intron boundaries sequence was carried out by automated sequencing.The correlation between genotype and phenotype in 360 patients (690 unrelated chromosomes)with the classical (CF,192)and non-classical (NCF,168) forms of CAH-21OH was also analyzed.In2 (36%) in salt wasting (SW), I172N (37.5%) in simple virilizing (SV) of the CF, and V281L (54.7%) in the NCF were the most prevalent mutations.The frequencies found were V281L 25.5%,In2 20.86%,I172N 8.98%,Q318X 7.24%,R356W 3.76%,ClEx6 2.31%,R483fs 1.44%,Ex3 1.01%,P453S 1.01%,P30L 0.57%.Large rearrangement (DEL/CONV)frequency was lower than in most populations but similar to brazilian and mexican populations (10.86%).The screening of 11 most common mutations allowed for the detection of 88.1% of affected alleles (80.94% for the NCF and 94.4% for the CF).The 295 patients with both detected alleles were classified into 5 different genotype groups according to the predicted severity of the mutations as previously described by Wedell et al. JCEM,1994(null:0%; A:In2; B:I172N; C:>20%; D:20%, chimeric gene promCYP21P+P30L; as the allele determining phenotype).In group null 100% of cases presented SW form, in group A 82.7% the SW form, in group B 83.3% the SV form and in group C 99.2% the NCF.In group D (n=4) one female patient presented an early manifestation of NCF while in the other 3 patients a CF(SV) was diagnosed. Sequence analysis revealed the presence of 8 rare mutations previously described and in 2 patients a novel nonsense mutation Y49X was found (In2/Q41X, Q318X/Q41X).The presence of a premature stop codon would predict a truncated protein without enzymatic activity that correlates with the SW phenotype found in both patients.The high correlation between genotype and phenotype found in CAH-21OH patients confirms the usefulness of molecular studies to predict the clinical outcome and its application in genetic counseling. The analysis of 11 most common mutations in our population allowed to detect a high percentage of CYP21A2 affected alleles.The different detection level of the affected alleles between CF and NCF would suggest that heterocygote carriers could have been included in the NC group of patients, and it confirms the overestimation of the NC form by hormonal laboratory studies.

PO2-023 Adrenal II

Evaluation of clinical and molecular studies aimed to optimize glucocorticoid treatment and bone homeostasis in patients with congenital adrenal hyperplasia

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Chronic glucocorticoid (GC) therapy can affect statural growth and induce disorders in bone metabolism. Patients with congenital adrenal hyperplasia (CAH) need a lifelong GC replacement therapy to reduce overproduction of adrenal androgens and prevent its adverse effects. Careful dose adjustment is necessary to obtain optimal bone mass and adult height according to their genetic potential. Polymorphisms of vitamin D receptor (VDR) and estrogen receptor (ER) genes have been associated with variations in bone homeostasis and in the therapeutic response in different diseases. **AIMS:** To determine clinical and biochemical indicators of the outcome of GC and mineralocorticoid (MC) therapy in patients with different clinical forms of CAH. To analyze the frequencies of gene polymorphisms of VDR and ER in CAH patients, and to determine possible associations with bone turnover markers and IGF systems. **SUBJECTS AND METHODS:** Data of 42 patients with CAH due to 21-OH deficiency (age range 1.8 - 26.8 years, 23 female,19 male), treated with GC and MC in cases with the salt-wasting form, were studied. They were analyzed in two groups according to the presence of an adequate clinical auxological and biochemical control based on levels of 17-OHP, delta-4 A (DPC), DHEA-S, testosterone (ECLIA). Osteocalcin (Oc) and B-crosslaps (BCL) (ECLIA), IGF1 and IGFBP3 (DSL) were determined. Two polymorphic sites of VDR gen (Bsm I and Fok I) and one of the ER gen (PVU II) were explored using PCR-RFLP method in CAH patients and in 27 normal controls of the same age and sex. **RESULTS:** The distribution of clinical forms was similar between groups. No significant differences in the relative frequencies of the analyzed polymorphisms between groups and the healthy controls were observed. CAH **Fok I**:FF 0.45, ff 0.15, Ff 0.40 **Bsm I**: BB 0.19, bb 0.26, Bb 0.55, **Pvu II:** PP 0.17, pp 0.4, Pp 0.43, versus Controls FF 0.30, ff 0.18, Ff 0.52; BB 0.16, bb 0.32, Bb 0.52; PP 0.27, pp 0.35, Pp 0.38. No significant associations were found between these genotypes and bone turnover markers, IGF1 or IGFBP3.**CONCLUSIONS:** Our data suggest that the genotypes analyzed, with a distribution comparable to the normal population, are not enough to explain the different results obtained with the treatment. The contribution of the other bone turnover markers and of other associated genes, should be evaluated.

PO2-024 Adrenal II

Cryptorchidism in adrenal hypoplasia congenita: two unrelated cases with novel DAX 1 mutations

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Introduction:
Mutations of DAX-1 gene (*NR0B1*) are a well-recognized cause of adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism. Affected boys usually present with primary adrenal insufficiency in early infancy and impaired sexual development, which typically manifests at the time of puberty. Cryptorchidism is not mentioned in literature as a frequent component of this

syndrome, whereas it is expected.
Here we report two unrelated cases of AHC due to novel DAX 1 mutations. Both patients presented with cryptorchidism and late onset adrenal failure.
Objective:
We aim to describe clinical and molecular characteristics of two patients with atypical AHC.
Case reports:
Patient #1 was referred to the endocrinology unit at the age of 11 years with clinical symptoms of adrenal failure. Diagnosis was confirmed and the glucocorticoid and mineralocorticoid treatment started. It is known that he was born as the second child in a nonconsagenious family, his parents and two his sisters (18 and 4 years old) were healthy. The boy presented with unilateral cryptorchidism (the inguinal testis), which have had not been treated and spontaneously descended at the age of 11. During follow up a normal peak levels of LH (12.1 U/l) in response to LH-RH test and testosterone (11.5 nmol/l) in response to hGH test, testicular enlargement (5 ml) were observed at 12. *NR0B1*-gene sequence revealed a hemizygous mutation X471K, which encodes the stop-condone replacement and, as we know, have not been described yet.
Patient #2 was born as the first child in nonconsagenious family. He presented with inguinal testis, failure to thrive from the first days of life and mild skin pigmentation. At the age of 4 years he had an episode of pernicious vomiting during the concurrent infection, which led to diagnosis of adrenal insufficiency. AHC was suspected and confirmed by demonstration of a novel hemizygous c.730delC_p.R244G_fs_297X mutation in *NR0B1* gene. His gonadal function remains to be assessed at pubertal age.
Conclusions:
We suggest that the combination of cryptorchidism and adrenal insufficiency, even with its mild presentations, should lay to DAX1 defects to be suspected. However cryptorchidism does not necessarily correlate to arrested puberty in adolescence. There is a considerable phenotypic variability associated with DAX1 mutations, reflecting the complexity of influences including genetic and epigenetic factors.

PO2-025 Adrenal II

Genotype:phenotype relationships in familial glucocorticoid deficiency types 1 and 2

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Background Familial Glucocorticoid Deficiency (FGD) is a rare autosomal recessive disorder caused by resistance to the action of ACTH. Based on the underlying causal mutation the disease is categorised as type1 (mutations in the ACTH receptor [melanocortin 2 receptor (*MC2R*)]), type 2 (mutations in its accessory protein [melanocortin 2 receptor accessory protein (*MRAP*)]) or type 3 (unknown aetiology). *In vitro* functional studies have shown that the majority of MC2R mutants have impaired trafficking from the endoplasmic reticulum (ER) to the cell surface resulting in reduced receptor expression and ACTH signalling. Consequently there is often some protein with residual function. In contrast, MRAP is required in the earliest stages of MC2R processing and in the absence of MRAP protein the receptor is retained within the ER. The nine reported MRAP mutations are all predicted to produce proteins lacking the transmembrane domain essential for MC2R interaction.
Methods Forty patients with missense *MC2R* mutations and twenty-five patients with *MRAP* mutations were characterized with specific reference to their age, height, weight and levels of ACTH and cortisol at presentation.
Results: FGD type 1 presents with a variable age of onset, mean (± SD) 3.11 ± 3.40 yrs (range 0-16yrs), whilst FGD type 2 presents earlier with mean onset at 0.31 ± 0.51 yrs (range 0-1.6yrs), p = 0.01. The most common MC2R mutation, S74I displays a wide spectrum in age of presentation, with the mean age of onset 3.82 ± 3.81yrs (range 0-16) similar to other patients with FGD type 1. The height of patients with FGD type 1 has previously been noted to be unusually tall. In FGD type 1 height SDS at presentation was + 1.76 ± 1.52 (mean ± SD) and in FGD type 2 height SDS at presentation was + 0.12 ± 1.35, p = 0.001. No differences in baseline cortisol, ACTH levels or weight at presentation were seen between FGD types 1&2.
Conclusion: When the clinical features of FGD types 1 and 2 were compared, striking distinctions were discovered in the height and age of presentation, but not in other aspects of endocrinology or body weight. FGD2 patients presented earlier and this may correlate with the fact that without MRAP, the MC2R will not traffic efficiently to the cell surface leading to complete failure of receptor function. FGD1 patients were tall, whether this increased linear growth is due to prolonged ACTH excess or glucocorticoid deficiency prior to treatment remains unclear.

PO2-026 Adrenal II

Growth-restricted preterm newborns are predisposed to functional adrenal hyperandrogenism in adult life

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Girls with catch-up growth after being born small-for-gestational-age are predisposed to early adrenarche and functional adrenal hyperandrogenism. For individuals born very preterm, it is unknown whether birth weight and early postnatal weight gain contribute to variations in adrenal gland markers in adult life, nor is it known whether these hormones are permanently influenced by prenatal and neonatal glucocorticoid therapy.
Our objective was to examine the effects of birth weight, early postnatal weight gain, and prenatal and neonatal glucocorticoid therapy on adrenal gland markers in 19-year-old individuals born before 32 weeks of gestation.
Subjects came from the prospective Project On Preterm and Small-for-gestational age infants (POPS). At 19 years of age, serum cortisol, DHEAS and androstenedione levels were measured in 393 participants.
In both men and women, birth weight SDS was negatively associated with serum DHEAS levels at 19 years of age: -0.758 (95% CI: -1.247 to -0.268) and -0.841 (-1.260 to -0.423) µmol/L for each 1 SD increase in birth weight in men and women, respectively. Birth weight SDS tended to associate negatively with serum androstenedione levels (-0.200 (-0.442 to 0.042) and -0.272 (-0.570 to 0.027) nmol/L per SD for men and women, respectively). These associations became statistically significant after adjustment for possible confounders. Early postnatal weight gain was not associated with any of the outcomes. In women but not in men, serum androstenedione levels were higher after prenatal glucocorticoid therapy (0.816 (0.022 to 1.609) nmol/L). Also for neonatal glucocorticoid therapy, a positive trend was observed with serum androstenedione levels in women only (0.907 (-0.085 to 1.898) nmol/L), which relation became statistically significant after adjustment for possible confounders.
We conclude that poor intrauterine growth predisposes to functional adrenal hyperandrogenism in individuals born before 32 weeks of gestation. Perinatal glucocorticoid therapy may influence adrenal gland markers in adult life in a sex-specific manner.

PO2-027 Adrenal II

Glucocorticoid deficiency in Smith-Lemli-Opitz (SLO) syndrome

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The final step in the cholesterol synthesis is regulated by the 7-dehydrocholesterol reductase (7DHCR). Mutations of 7DHCR gene cause cholesterol deficiency and up to 50fold increase of the main precursors 7- and 8-Dehydrocholesterol (7- and 8-DHC). The clinical picture is characterized by different degrees of mental retardation and a distinctive phenotype including sexual ambiguity - first described as Smith-Lemli-Opitz (SLO) syndrome (MIM 270 400). As part of a controlled clinical trial with the HMG-CoA inhibitor simvastatin (SV) we investigated 10 SLO children (age range: 3months to 18.5 y.) with a mild to moderate phenotype according to clinical score and ratio of (7+8DHC)/cholesterol (median 0.11 (range 0.03 to 1.2). In this sub study we

measured the steroid profile in 24-hour collected urine using gas chromatography-mass spectrometry (GC-MS), serum concentrations of ACTH and cortisol. **Results:** Sum of major urinary cortisol metabolites (a-THF, THF, THE, a-cortol, ß-cortol, a-cortolone, ß-cortolone) was < 5th centile in 13 of 21 samples. In contrast, normal serum cortisol (by immunoassay) but elevated ACTH was found in 5/10 children (>9 pg/ml). Substitution with hydrocortisone (HC) was started in one child with an ACTH peak of 98.6 pg ACTH /ml, in the remaining 4 / 10 we recommended HC in stress situations. In one adult we collected 9 samples over 3 years (age 18.5 to 22 y.) with a considerable intra-individual variation of cortisol metabolites but all below reference values. **Conclusions:** (1) A reduced excretion of adrenal glucocorticoid metabolites demonstrates a defect in steroid metabolism in 5/10 children with SLO. (2) In addition, elevated ACTH indicates cortisol deficiency in SLO. (3) In contrast, normal immunoassayable serum cortisol does not reflect glucocorticoid deficiency in SLO patients.

PO2-028 Adrenal II

The *CYP3A7*1C* variant is associated with lower cortisone acetate doses in the treatment of patients with 21-hydroxylase deficiency

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Glucocorticoid (GC) replacement leads to significant improvement in the prognosis of patients with classical form of 21-hydroxylase deficiency (CAH). Although, the replacement doses were well established, there is a great inter-individual variability and fine adjustment is necessary to avoid under or over-treatment. This variability in the drug metabolism could be genetically determined, involving the liver cytochrome P450s and/or their transcription factors. Objective: to evaluate if polymorphisms in the *CYP3A7 and PXR*, genes are responsible for the interindividual variations in the glucocorticoid doses necessary to control steroid levels in CAH patients. Patients: 107 CAH patients were selected to evaluate the polymorphism frequencies. The glucocorticoid (GC) doses were only evaluated in those (n=54) with adequate control, defined as normal androgen levels according to age in 4 measurements/year. Methods: all patients received cortisone acetate, and the salt wasters also received fludrocortisone. The mean GC doses (mg/m²/d) were evaluated retrospectively between 0-2 yrs, 4-6 yrs and 10-12 yrs. To identify the *CYP3A7*1C and PXR* variants, genomic DNA was submitted to direct sequencing. Statistical analysis: The mean GC doses in each age period were compared between patients with and without allelic variants. ANOVA test was used for statistical analysis. **Results**: The *CYP3A7*1C* variant was found in 2.7% of the 214 alleles, respectively. The P27S, G36R and A370T *PXR* variants were found in 2.3%, 1.4% and 0.5% of the alleles, respectively. The mean ± 1SD of GC doses according to age and the presence of polymorphisms are shown in the Table 1.

Table 1. Cortisone doses according to the presence of polymorphisms

Age (yrs)	CYP3A7*1C	Wild CYP3A7	PXR variants	Wild PXR
0-2	17 ± 3	19 ± 4	14 ± 2	18 ± 4
4-6	13 ± 3	20 ± 4	22 ± 1	19 ± 4
10-12	17 ± 2	21 ± 3	24 ± 6	20 ± 3

Conclusions: for the first time, we analyzed the effect of the genetic variability of drug metabolism in CAH treatment. We found a significantly lower mean GC doses in patients carrying the *CYP3A7*1C* variant (p = 0.001). The analysis of the genes involved in the GC metabolism could contribute to the CAH treatment optimization. However, we did not find any influence of *PXR* variants in the GC doses, but we cannot rule out that this result was due to the rare *PXR* variant frequencies in our population.

PO2-029 Adrenal II

Growth and skeletal maturation in 51 male patients with congenital adrenal hyperplasia: association to CYP21A2 genotype

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Recent metaanalyses have shown that final height is compromised in the majority of CAH patients. This may be due to oversubstitution with corticosteroids in early life or alternatively due to inappropriate advancement of bone age in patients with late onset CAH. The aim of this study was to evaluate growth and skeletal maturation in CAH patients depending on their specific CYP21A2 mutation.

Methods and materials

We evaluated longitudinal data on height, weight and bone age retrospectively in our cohort of 51 males with CAH who had been treated with hydrocortisone (HC) and mineralocorticoids if saltwasting. The patients were divided in groups according to their genotype resulting in 38 patients with severe mutations; group null (deletion, Q318X, clusterE6, R356W, A362V, n=14) and group A (I2 splice, n=24) and in 13 patients with milder mutations; group B (I172N, n=7) and group C (P30L, V281L, n=6). Age at diagnosis was 0.01 years (0 to 5.25) (median range) in groups null and A, and 4.67 years (0 to 12.5) in groups B and C. Bone age were determined annually in most of patients. Auxological data were compared with Danish reference values.

Results

Early growth deceleration was observed during first year of life (HSDS = -1.2 (-2.9 to +1.2) in patients with severe mutations (groups null and A) which normalised during childhood. Height SDS decreased markedly in pubertal years resulting in a decreased final height of these patients (groups null and A) (FHSDS = -1.4 (-4.6 to +0.9). Boneage advancement (BA-CA) was -0.28 years (-1.02 to +0.98) in children aged 0-5 years and +2.2 years (-2.6 to +6.1) in children aged 5-10 years in groups null and A.

In patients with milder mutations (groups B+C) height SDS were normal in the first 1-2 years of life, and increased significantly until diagnosis and initiation of HC therapy. BA-CA was markedly increased at diagnosis +3.3 years (0.23 to 7.2), but decreased during childhood and puberty. Height SDS decreased throughout childhood and puberty resulting in impaired final height (FHSDS = -0.4 (-2.5 to +0.9).

In conclusion, we found markedly different childhood growth patterns according to genotype; early as well as pubertal growth impairment in patients with severe mutations, whereas growth and bone age were markedly increased at diagnosis, but decreased hereafter in patients with milder mutations. Importantly, we found significantly reduced FH in the majority of CAH males regardless of genotype and age at diagnosis.

PO2-030 Adrenal II

Reproductive hormones and testicular adrenal rest tumors in boys, adolescents and adult men with congenital adrenal hyperplasia: association with the CYP21A2 mutation

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder with impaired function of the adrenal cortex caused by mutations in the CYP21A2 gene. Testicular adrenal rest tumors (TART) may be prevalent in up 95% of CAH adults and may already appear during childhood. Reproductive function may be impaired in adults with CAH. Whether genotype sub-types can account for development of TART and reproductive function has not previously been investigated. We therefore investigated this by coupling clinical information of CAH patients including FSH, LH, testosterone, and inhibin B levels with information of their genetic mutation. In 49 male patients (age 2.6 - 40.3 years) with 21-hydroxylase deficiency testicular ultrasound examinations were performed and CYP21A2 genotypes determined. These were grouped according to the residual 21-hydroxylase activity: Group Null (complete enzyme impairment), group A (almost complete enzyme impairment), group B (severe enzyme

impairment) and group C (partial impairment). TART were observed in 27 out of 49 patients (55%). For the 23 patients younger than 18 years TART were present in 11 (48%), the youngest patient being 7.5 years old. The presence of TART was dependent of the CYP21A2 genotype: 27 of 37 patients (73%) with the most severe mutations (group Null and A) had TART whereas none of 12 patients with the milder mutations (group B and C) had TART. Testosterone and inhibin B levels were in the lower part of the reference range. We conclude that TART were most frequently detected in patients with severe CYP21A2 mutations, and may in such patients occur already in early childhood.

PO2-031 Autoimmunity

Transient hyperthyroidism in a 6 years girl with EBV infection: a peculiar case

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We report a case of transient hyperthyroidism during Epstein-Barr Virus (EBV) infection in a 6-year-old girl. The diagnosis of EBV was suspected, in addition to the typical symptoms, by the positivity of anti-EBV antibodies IG M, previously negative. Hyperthyroidism was diagnosed based on : a)elevated thyroid hormone levels with thyrotropin (TSH) suppression, b) tachycardia as symptoms of thyrotoxicosis in absence of fever.

Introduction

Viral infections, and in particular HCV, parvovirus B19, EBV, are also frequently cited as a major environmental factor involved in subacute thyroiditis and autoimmune thyroid disease. Antibodies against EBV viral capsid antigen (Ig G-VCA) and antibodies against early antigen (IG G-EA-D/DR) have been more often found in thyroiditis than in controls.

Case report

A 6 year-old girl was admitted to our Department for a fever of unknown origin, resistant to antinflammatory drugs and antibiotics. He presented cervical lymphoadenopathy, throat inflammation, moderate elevation of white blood count (WBC) and markers of inflammation (ESR, CPR), spleen and liver enlargement confirmed by ultrasound and high titer of anti-EBV IG M, previously negative. The suspicion of EBV infection was confirmed by the clinical picture and by the titers of antibodies against the virus. For the persistence of tachycardia in absence of fever thyroid function was investigated with these results: TSH levels suppressed (0,1 uUI/ml range 0,2-4,2) with high levels of free triiodothyronine (FT3) and free thyroxine (FT4) (FT3 8,96 pg/ml range 2-4,40; FT4 28,15 range 12-22). Antiperoxidase and antithyroglobulin antibodies were positive, but anti-TSH receptor was negative. Ultrasound examination showed an increased vascular flow of the whole gland based on power Doppler analysis. The clinical symptoms were remitted in the next weeks after the start of methimazole therapy (7,5 mg/die). After a two months follow-up the treatment was progressively interrupted for the remission of clinical symptoms and normal levels of FT4 and TSH. The antibodies titer became normal after four months after the viral infectious.

Conclusions

Our case underline the strong evidence that EBV infections are involved in the pathogenesis of some cases of Hashimoto's thyroiditis, expecially in predisposing individuals.

Further studies are needed to clarify the relationship between viruses and thyroid disease, in order to develop new strategies for prevention and /or treatment.

PO2-032 Autoimmunity

In search of new type 1 diabetes autoantigens

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Type I diabetes (T1D) is characterized by the presence of circulating autoantibodies (ICA, GADA, IA-2A, IAA; T1D-Abs) directed against beta cells autoantigenes. Search for T1D-Abs in patients with recent diagnosis of diabetes is utilized for diagnostic purposes, and in first-degree relatives as predictive markers of disease. Recently, a new autoantigen (ZnT8) was identified that is found in 26% of patients with T1D previously classified as autoantibodies negative. Because up to 10% of children with the clinical feature of T1D test negative for autoantibodies, we set up a search for new autoantigens.

We studied the autoantibody repertoire of subjects with diabetes of early onset (1-10 years of age), divided in three different groups: 1) T1D positive only to IAA (4 sera); 2) T1D positive only to ZnT8A (4 sera) and 3) Subjects with diabetes negative to all autoantigens (ICA, GADA, IA-2A, IAA, ZnT8A) and to the search for mutations in neonatal diabetes genes INS and KCNJ11 (5 sera). Sera from patients with diabetes due to insulin mutations were used as "negative" controls (group 4). The reactivity of these sera was tested against cytoplasmic/membrane-enriched protein fraction from human pancreatic islets or from exocrine pancreas.

After bidimensional electrophoresis and classical Western blot, image were acquired and spot detection/matching performed by Progenesis software. Spots revealed by control sera (group 4) were subtracted ("noise"). Matching revealed that among protein spots detected in pancreatic islet cytoplasm, 37 were in common between ZnT8A+ sera and those negative to 5 autoAbs (group 3), 24 were in common between IAA positive sera and group 3, and 14 spots were common to all three groups. We then proceeded to identifying these protein spots by MALDI MS/MS. Initial analysis revealed Rab GDP dissociation inhibitor beta among those spots detected by IAA+ sera and group 3 and Cyclofillin A among proteins detected by ZnT8A+ sera and group 3. ZnT8A+ sera only identified Protein disulfide isomerase/PDIA3. Quite surprisingly, tubulin was identified by all three groups of sera. Further analisys is needed to ascertain protein identity of spots from exocrine pancreas revealed by sera in order to exclude any source of contamination of pancreatic islets from this tissue. In conclusion, these preliminary results seem to indicate that our "immunoproteomic" method is feasible for novel autoantigens identification.

PO2-033 Autoimmunity

The endocrinological dysfunction after allogeneic HSCT in children

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INTRODUCTION

Endocrinological dysfunction is a well known complication after hematopoietic stem cell transplantation (HSCT) in children. Immunological mechanisms, toxicity of the conditioning regimen, especially total body irradiation, is most commonly postulated cause of endocrine abnormalities.

AIM

We prospectively analyzed endocrine function in children who had undergone allogeneic HSCT and survived more than 1 year after the procedure. We aimed to estimate frequency of endocrinological dysfunction and whether there is influence of immune recovery on the abnormalities.

PATIENTS AND METHODS

We evaluated the function of thyroid, gonads, pituitary and adrenals in 51 children, aged 1-18 years, who survived more than one year after transplan-

tation. The reasons for bone marrow transplantation were: severe aplastic anemia (SAA), acute lymphoblastic anemia, inborn errors, acute myeloblastic leukemia and others.
In the Endocrinological Dept. the high velocity and sexual maturation of the children were observed. Every year we evaluated thyroid-stimulating hormone (TSH), free thyroxine (fT4), tri-iodo tyrosine (TT3), anti-tyreoglobuline antibodies (a-TG) and anti-peroxidase antibodies (a-TPO) in the serum in all our patients. In children with thyroid diseases the estimation was performed adequately to therapy. Similarly FSH, LH, prolactin, estradiol and testosterone were estimated. In children with short stature, the GH level was detected. Glycemia, lipidogram, insulin and glucose levels in OGTT and circadian rhythm of cortisole secretion were estimated in obese children.
RESULTS
In seven children, autoimmune hypothyroidism was diagnosed; the children needed l-thyroxin replacement therapy. In three patients this feature was transient. One patient was diagnosed as having Graves' disease. Transient delay of growth velocity was observed in all the children in the first year after HSCT, but only in 2 boys they were permanent; however the growth hormone secretion was normal. In 6 children hypogonadism was developed – in 4 hypergonadotropic hypogonadism and in 2 hypogonadotropic hypogonadism. 3 children were treated for obesity without adrenal and pancreatic disorders.
CONCLUSIONS
1. Abnormalities of autoimmunological thyroid function are a most frequent complication in children after HSCT.
2. The frequent complication is hyper- and hypogodotropic gonad disorders.
3. The children after HSCT need permanent observation in the Endocrinological Outpatient Clinic

PO2-034 Autoimmunity

Novel homozygous *AIRE* mutation: hypoparathyroidism, ovarian failure, dental enamel hypoplasia and asthma

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autosomal-recessive disease caused by mutations in the autoimmune regulator gene (*AIRE*). The most common disease components -chronic mucocutaneous candidiasis, hypoparathyroidism and adrenocortical insufficiency- frequently appear in childhood but none of them is constant. Many other disease components occur such as hypogonadism, chronic active hepatitis, pernicious anemia, vitiligo and ectodermal dysplasia. A 14-year-old girl, born form consanguineous Moroccan healthy parents, presented to our pediatric endocrinology clinic for absent pubertal development. She was born at 28 weeks of gestational age. She required mechanical ventilation for respiratory distress in the neonatal period. At 7 months of age, she started to have bronchial hyperreactivity evolving towards asthma with several pneumonia. At 3 years of age, she was diagnosed with hypoparathyroidism in the setting of generalized seizures. Her deciduous and permanent teeth were discolored with thin and rough enamel, and susceptibility to decay. No mucocutaneous candidiasis was ever noted. She was treated with inhaled steroids since 7 months of age, calcium supplements and calcitriol since 3 years of age. Her physical examination showed a height of 168.1 cm, weight 51.25 kg, BMI 15.7 kg/m^2, pubertal stage A3P3M1, blood pressure 109/71 mm Hg and abnormal enamel. Laboratory investigations are shown in (table 1).

Laboratory findings	
s-Ca2+ Total (RR 9.1-10.2 mg/dl)	8.1
s-PO42- (NR 2.4- 4.4 mg/dl)	6.6
S- PTH (NR 10-93 pg/ml)	4<
Urinary Ca/Cr	0.01
LH (NR 3-8 mU/ml)	26
FSH (NR 5-15 mU/ml)	60
Estradiol (NR 40-200 pg /ml)	12
ACTH fasting 8 am (NR 10-80 pg/ml)	33
Cortisol fasting 8 am (NR 7-28 ug/dl)	18.1
TSH (NR 0.3-4 uU/ml)	1.4
freeT4 (NR 0.8-2 ng/dl)	1.5

A homozygous frameshift mutation in exon 6 of the *AIRE* gene was detected : c.798del (p.Gly 267ValfsX111).
In conclusion, we report on an adolescent female Moroccan patient with hypoparathyroidism, asthma, enamel hypoplasia and ovarian failure. She harbors a novel homozygous frame shift p.Gly 267ValfsX111 mutation in exon 6 of the *AIRE* gene.
This case illustrates the clinical diversity of APECED. APECED diagnosis allows to tackle potential life-threatening disease components such as adrenal insufficiency and to provide adequate follow-up. Moreover, it allows a presymptomatic diagnostic test for the sibs of the affected patient.

PO2-035 Autoimmunity

Autoimmunity and pituitary disease in children and young adults affected with intracranial tumors

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The presence of antipituitary antibodies (APAs) and/or anti-hypothalamic antibodies (AHAs) has been until now mainly investigated in idiopathic and secondary pituitary deficiencies. The prevalence and the role of autoimmunity in pituitary involvement in children and adolescents with intracranial tumors has not been systematically investigated.
Objectives: The aim of this study was to evaluate the presence of APAs and AHAs in patients with intracranial tumors in order to verify whether an autoimmune process can contribute to pituitary dysfunction.
Subjects and Methods: Sixty patients (27 males and 33 females; age 14.4±6.4 years) were evaluated by indirect immunofluorence for APAs and AHAs (immunostaining vasopressin cells) 7.4±5.4 years after tumor diagnosis: 20 craniopharyngiomas, 19 gliomas, 15 germ cells tumors, 3 adenomas, 3 Langerhans cell histiocytosis. Treatment consisted in surgery and/or radiotherapy and/or chemotherapy in all but 6 subjects. Six patients presented isolated pituitary deficiency and 39 multiple pituitary hormone deficiencies (MPHD), 36 of whom had central diabetes insipidus. Thirty sex-/age-matched healthy children and 50 healthy adults were used as controls and were negative for both autoandibodies.
Results: Circulating APAs and/or AHAs were found in 26 patients (43.3%) affected with intracranial tumors. Five patients were APA positive, 11 AHA positive and 10 were positive for both autoantibodies. APA and/or AHA positivity was equally distributed in craniopharyngiomas, germ cells tumors and gliomas, while it was significantly higher in Langerhans cell histiocytosis and absent in adenomas. AHA titer significantly decreased with increasing time from the diagnosis of central diabetes insipidus (P=0.04); similarly, APA and/or AHA titer decreased with increasing time from intracranial tumor diagnosis. A trend of association was found between the presence of APAs and/or AHAs and number of pituitary hormones deficiencies (P=0.19). No relationship was found between the prevalence of autoantibodies and different treatment protocols.
Conclusions: A high frequency of APAs and/or AHAs in patients with intracranial tumors mainly affected with MPHD suggests a possible relationship between intracranial tumors, hormones deficiencies and hypothalamic-pituitary autoimmunity that need, however, further investigation.

PO2-036 Autoimmunity

Identification of disease suseptibility genes in Hashimoto's thyroiditis using the obese strain of chickens as a model

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Hashimoto´s thyroiditis is a common autoimmune disorder in which epidemiologic investigations suggest a strong polygenic influence on the susceptibility for the disease. A number of genes, each contributing with a modestly increased risk, have been identified eg. HLA-DR, TSHR, CD40, CTLA4 and PTPN22. However, the hitherto identified risk genes do not fully explain the epidemiologically observed genetic influence.

The Obese strain (OS) of chickens develops a spontaneous autoimmune thyroiditis that mimics human Hashimoto's thyroiditis with mononuclear cell infiltration of the thyroid gland, thyroid autoantibodies and similar clinical manifestations. We have generated an intercross between OS chickens and their wild ancestor, the red junglefowl (RJF) and expanded the F2 generation into a study population of approx. 1000 individuals. Approximately 25% of the F2 individuals show signs of hypothyroidism. A genome wide scan using the Illumina system and 384 single nucleotide polymorphisms (SNPs) have been performed on all individuals. All chickens are being carefully phenotyped for thyroid hormone levels, autoantibodies, thyroid cell infiltrates and body weight at five time points during life. Statistical analyses, taking epistatic interactions into account, will identify relevant Quantitative Trait Loci (QTLs) for disease phenotypes. The relatively high frequency of affected individuals in the F2 generation ought to make gene mapping possible. QTL analysis is ongoing, and so far we have identified two significant disease-derived QTLs on chr 2 and chr 16. Further analysis will identify disease-causing genes in the chicken model and enable the study of their human orthologues in patients with Hashimoto´s thyroiditis.

PO2-037 Autoimmunity

Monogenic diabetes in children initially diagnosed with type 1 diabetes: a role for autoantibody testing

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We used negative autoantibody (ab) status at diagnosis (GAD, IA2, IAA, or ICA) to screen for unrecognised monogenic diabetes in patients thought to have T1DM. Known monogenic diabetes (n=5) was excluded. Among 460 current T1DM patients, ab at diagnosis were positive in 330, unknown in 103, and negative in 36. The ab-negative group had repeat ab profile (GAD, IA2 & ZnT8, but not IAA because of insulin treatment), non-fasting C-peptide, and DNA sequenced for KCNJ11, INS & HNF1A mutations and HLA typing (DQA & DQB). 19/36 were Persistently Ab-Negative (PAN) and 17/36 were positive on repeat testing (converters). HNF1A changes were found in 2 PAN patients. One was heterozygous for a previously described P112L mutation, causing MODY3. Her diabetes control improved after switching from insulin to gliclazide. Her diabetic mother carries the same mutation. The second was heterozygous for a novel H469Y variant, also carried by the patient's non-diabetic grandmother, aged 76, and therefore of no clinical significance. No mutations were found in converters.

HNF1A	Age / pH at Diagnosis	C-peptide (nmol/L) / Diabetes Duration	Insulin U/ kg/d	BMI kg/m2	HLA: DQA1-DQB1
P112L	9.9yrs / 7.42	0.98 / 2.2yrs	0.52	21.5	0401-0402 (DR8), 0501-0201 (DR3)
H469Y	13.6yrs / 7.26	0.20 / 2.1yrs	0.77	23.4	0301-0302 (DR4), 0301-0302 (DR4)

Excluding the MODY3 patient, HLA genotype was diabetogenic for T1DM in 15/18 (83%) PAN and in all converters. Among the PAN group, C-peptide was detectable in 6/15 (40%) with diabetogenic HLA vs 2/3 (67%) without (p=0.56). PAN patients were not distinguishable from those ab-positive (at diagnosis or repeat) by age at diagnosis, duration of diabetes, insulin dose/kg/day, pH at diagnosis, HbA1c, body mass index (BMI), or family history.

Conclusions: Ab screening revealed one patient with previously unsuspected HNF1A mutation (MODY3), representing 5% (1/19) of persistently ab-negative patients (*vs* none among patients converting from ab negative to positive). No KCNJ11 or INS abnormalities were found. The PAN group could not be distinguished from ab-positive patients by other characteristics and 83% have HLA-types predisposing to T1DM. As monogenic diabetes can often be treated with sulphonylureas rather than insulin, persistently negative ab should prompt careful re-evaluation of the diagnosis of T1DM.

PO2-038 Autoimmunity

Screening frequency of celiac disease and autoimmune thyroiditis in children and adolescents with type 1 diabetes mellitus in Turkey and comparison with last surveys

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OBJECTIVE- Type 1 diabetes mellitus (T1DM) is associated with celiac disease (CD) and Hashimoto thyroiditis (HT) because of autoimmunity. The aim of this study was to determine the frequency of CD and thyroid antibodies in children with T1DM for Turkey and to compare with last surveys.

RESEARCH DESIGN AND METHODS- One hundred thirty-four children and adolescents with T1DM (74 boys and 60 girls, mean age 8.16 ± 4.0 years) participated in the study. Data from 134 patients were analysed from 2003 until 2008 in Pediatric Endocrinology and Metabolism Unit of Gaziantep University Faculty of Medicine. Sex, age, weight, height, body mass indexes, the clinical findings at the diagnosis of T1DM, and HbA1c, FT4, FT3, TSH, antithyroid peroxidase (anti-TPO), antithyroglobuline (anti-TG) and tissue transglutaminase (TGA) antibody levels were evaluated.

RESULTS- All of the patients were screened at the beginning and six months interval for screening of CD and HT. We determined that eleven percent of the patients had positive antibodies for CD and nine percent of the patients had positive thyroid antibodies. Eight patients for anti-TG and anti-TPO, 12 patients for anti-TPO, 15 patients for TGA and 2 patients for anti-TG, anti-TPO and TGA were serologically positive.

CONCLUSIONS- Screening anti-TPO, anti-TG and TGA antibodies in patients with T1DM are very important for early diagnosis of CD and HT. The frequency of CD in diabetic children in this study and last survey was similar but frequency of HT was different. This may be related with genetic and environmental factors and younger age of our patients.

PO2-039 Autoimmunity

The evolution of thyroid autoimmunity as reflected in the frequency of thyroid autoantibodies in a newborn screening programme

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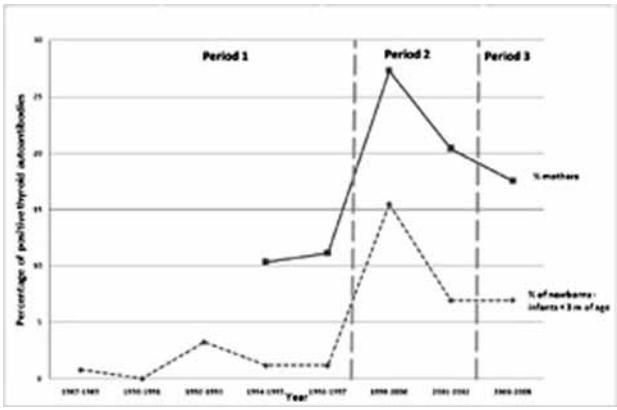
Recent data indicate a rise in thyroid autoimmunity especially in the pediatric population. The mechanisms involved are speculative. Since it is difficult to prospectively examine a certain target population for a long period of time for evidence based conclusions, we decided to retrospectively analyze data of thyroid autoantibodies (ThAbs) estimation collected within the protocol of a newborn screening programme during the past 22 years.
Patients. a) 1232 newborns and infants aged <3 months examined between 1987-2008 and b) 836 mothers examined from 1994-2008.
Methods. ThAbs (Ab-Tg and Ab-TPO) were determined in serum by a hemagglutination test between 1987 and 1997 (Period I), by IRMA between 1998 and 2002 (Period II) and by a chemiluminescence assay thereafter (Period III). Urinary iodine was also measured colorimetrically in a spot urine sample during the periods II and III.
Results.. The % of children with positive ThAbs (both or one) was 3.4% during Period I, reached a peak of 16% in the initial phase of Period II, declined to 7% at the 2nd part of this Period and remained at about this level in Period III. (Figure). A parallel change in ThAbs but of a higher magnitude was observed in the mothers studied. During Period I 10% of mothers were positive. This percentage reached a peak value of 28% in the first part of Period II, declined to 17.5% thereafter and remained around this value during Period III. Ab-TPO were the predominant autoimmune marker both in mothers and offsprings. The increase in Abs concentration may be partly attributed to improvements in specificity of the assays used, but most likely reflects a general increase in thyroid autoimmunity, since we observe differences in Period II under the same methodology. Median urinary iodine values per year during Periods II and III indicated iodine sufficiency (range 220-270 μg/L).



Conclusions. There are distinct alterations in thyroid autoimmunity over two decades. Although changes in iodine intake may be responsible for this evolution of autoimmunity, other, as yet unknown environmental factors, might have also contributed to this phenomenon.

PO2-040 Autoimmunity

Serum concentrations of proapoptotic cytokines TNF-related apoptosis-inducing ligand (TRAIL), osteoprotegerin (OPG) and receptor activator of nuclear factor kB ligand (RANKL) in children with autoimmune hypothyroidism and autoimmune hyperthyroidism

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Oryginality: OPG and RANKL have been implicated as potential links between T-cell activation, chronic inflammation and bone loss. TRAIL, the second ligand of OPG have been demonstrated in thyroid follicular cells and acts as an apoptosis inducing cytokine, leading to thyroid destruction and hypothyroidism (hypoT) in chronic autoimmune thyroiditis (AIT), whereas its role in Graves disease (GD), a disease leading primarily to hyperthyroidism (hyperT), due to TRAb activity, is not clearly established. Serum concentrations of OPG, RANKL and TRAIL in groups of these patients vs. control were evaluated at the onset of disease with ELISA tests.
Materials and Methods: We studied 26 children: 9 children with hypoT (7 girls and 2 boys), age 11.3+/-3.2 years; mean hormone values: TSH 44.9 uIU/mL, fT4 0.61 ng/dL, fT3 2.33 pg/mL; ATPO 2691.7 IU/mL; ATG 697,6 IU/ml, 12 children with hyperT (9 girls and 3 boys), age 12.7+/-5.1 years; mean hormone values: TSH 0.005 uIU/mL, fT4 4.16 ng/dL, fT3 18.95 pg/mL; TRAb 29.7 U/L; ATPO 2398 IU/ml; ATG 633.3 IU/ml. A group of 5 healthy subjects (2 girls and 3 boys, age 13.1+/-6.4 years) were studied as a control group.
Results: TRAIL levels were significantly higher in hyperT (98.04 pg/mL) compared to control (62.81pg/ml) (p=0.02). No significant difference was noticed in TRAIL serum concentrations in hypoT (89.4+/-30.9 pg/mL) vs. control and hyperT vs. hypoT. OPG level was significantly higher in hyperT (5,91 pmol/l) than in control (4,51 pmol/l)(p=0,019). In hypoT group (4,65 pmol/l) no significant difference vs. control was observed. There was significant difference between OPG in hypoT and hyperT (p=0,044). No significant difference was noticed in serum RANKL: hypoT (0,42 pmol/l) and hyperT (0,62 pmol/l) vs. control (0,36 pmol/l), and hyperT vs. hypoT. There was significant negative correlation with OPG level and age. OPG in hyperT decreased with age (p=0,012; r-Pearson=-0,7) and to a smaller extent in all groups: hyperT, hypoT, control (p=0,029; r-Pearson=-0,43).
Conclusions: These results confirm the relevance of TRAIL-mediated pathway in GD thus supporting that both, stimulation and apoptosis, are co-ocurring in GD of young patients with severe hyperthyroidism. Significantly increased OPG levels in hyperT group of GD may suggest the anti-osteoclastic activity compared to control and hypoT AIT group. However, the precise role of OPG/RANKL/TRAIL system in thyroid autoimmune diseases needs to be further evaluated.

PO2-041 Autoimmunity

Autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED) and response to immunosuppression

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Background
APECED occurs due to loss of function of the AIRE (autoimmune regulator) gene and results in loss of thymic tolerance to self antigens. Common features include candidiasis, hypoparathyroidism and hypoadrenalism. There is a paucity of literature on therapeutic options for severe manifestations of this condition. We present a profoundly affected young patient with a dramatic response

to immunosuppression with Mycophenolate mofetil (MMF).

Case

A 2.9 yo female child of non-consanguineous parents developed over 1 year period hypoparathyroidism (with hypocalcemia), keratoconjunctivitis, sialoadenitis, vitiligo and alopecia. She was found to have compound heterozygous 13 bp deletions on exon 8 of AIRE. Hypoparathyroidism was severe and managed initially with intravenous and subsequently oral calcium and calcitriol.

Her past medical history included several episodes of aspiration pneumonia, frequent diarrhea, intermittent urticarial eruptions (with lymphocytic vasculitis on biopsy) and frequent fevers of unknown origin. Between diarrheal illnesses, she had severe constipation, including small bowel obstruction at age 1.3 years. Evaluation revealed: normal TFTs; positive anti-thyroid peroxidase antibodies (abs); normal ACTH stimulation test; positive antiadrenal & anti-21-hydroxylase abs; hyperglycemia during illnesses with positive anti-GAD abs but negative ICA & anti-Insulin abs; Candida albicans and likely organic foodstuff on lung biopsy.

Her diarrhea deteriorated, and became associated with hypocalcemia that necessitated prolonged parenteral calcium therapy. Hypocalcemia deteriorated significantly after discontinuing parenteral calcium for even a few hours. She developed mineralocorticoid insufficiency, worsening Sicca symptoms, and recurrent life-threatening respiratory tract infections. After 7 months of hospital stay, immunosuppression was commenced initially with high dose oral Prednisone, then Azathioprine, and subsequently MMF monotherapy. IV calcium was discontinued after 1 week; diarrhea began to resolve; she was discharged within 1 month on oral calcium and calcitriol. Other improvements were noted in linear growth, salivation, lacrimation and repigmentation of her eyelashes. At 15 months of follow-up, her response to immunosuppression with MMF is excellent. However, she still has videofluoroscopic evidence of abnormal swallowing, cutaneous candidiasis and frequent pyrexial illnesses.

PO2-042 Autoimmunity

Malabsorption and severe diarrhea due to loss of gastrointestinal endocrine cells in patients with autoimmune polyendocrine syndromes

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Autoimmune polyendocrine disorders are characterized by both their genetic disorder and multiple endocrine failure, with other features such as gastrointestinal (GI) symptomes e.g. malabsorption, obstipation, watery diarrhea or steatorrhea. However, the pathogenesis of GI dysfunction in these patients is largely unkown. The importance of central and peripheral immunological tolerance in preventing autoimmunity is published for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyglandular syndrome 1, and immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). APECED is a rare autosomal recessive disorder typically presenting with chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenal failure variably accompanied by other symptoms and caused by a mutation in the autoimmune regulator gene (AIRE). IPEX is an extremely rare X-linked disorder with fulminant widespread autoimmunity, early onset type 1A diabetes and diarrhea caused by a mutation in the FOXP3 gene. Both monogenic diseases are associated with significant GI dysfunction which is difficult to control.

Here, we report two patients 7 and 11 years old with APECED and one 10 year old boy with IPEX-like syndrome with severe diarrhea, malabsorption or pernicious anemia. In contrast, to the only mild inflammation of the intestine in the APECED patients histology, they suffered from severe malabsorption and diarrhea. However, biopsies from the whole GI tract immunostained with chromogranin A displayed a widespread loss of gastrointestinal endocrine cells (GIECs) in all three patients with APECED or IPEX-like syndromes in all parts of the bowel. The secretory granules from GIECs contain peptides that act as systemic or paracrine mediators for the regulation of digestion and motility. Repeated biopsies in a two years follow-up confirmed the persistence of GIECs deletion, even under immunsuppressive therapy.

These observational data suggest that the loss of GIECs might be a common feature in autoimmune polyendocrine disorders with GI dysfunction probably due to an autoimmune attack against these cells. Unfortunately there seems to be no prevention of these process. However, realization of this pathology is helpful for the management of the GI-dysfunction in these patients.

PO2-043 Autoimmunity

Autoreactive T cells in patients with autoimmune adrenal diseases

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INTRODUCTION: The 21-hydroxylase protein is the major autoimmune (AI) target in adrenal deficiency as detected by autoantibodies. However, autoantibodies have no direct pathogenic role and there are several lines of evidence to indicate that T cells are directly involved in the adrenal gland destruction. One of these lines is the HLA-disease association with the A1-B8-DR3 haplotype, as well as DR3-DQ2 or DR4-DQ8 alleles conferring disease susceptibility. However, adrenal gland autoreactive T cells are not readily detected in patients and our goal was to detect circulating autoreactive T cells in patients with AI adrenal deficiency, and to identify their target antigen as well as the HLA molecules presenting these antigens.

METHODS: Using an ELISPOT assay, patients' or controls' T cells secreting IFNgamma in presence of antigens were visualized as spots. Forty-nine overlapping 20 aa-long peptides spanning the entire 21-hydroxylase protein were tested as antigens. Our cohort included patients with isolated Addison's disease (n=9) or associated with an autoimmune polyendocrine syndrome (APECED/APS1, n=3 and APS2, n=4).

RESULTS: **1-** The 49 peptides were tested in pools of 10 adjacent peptides. 11 out of 16 patients had autoreactive T cells against these pools compared to 4 out of 14 controls. The number of autoreactive T cells was 11 times higher in patients than in controls. Moreover patients' T cells responded to a mean of 2.7 pools compared to 1 pool in controls. **2-** Testing for individual peptides in responder patients (with isolated Addison's disease, APECED or APS2) revealed 3 major epitopes (peptide A, B and C). Reactivity to Peptide A was shared by 5 patients, to peptide B by 3 patients (including 2 brothers) and to peptide C by 2 patients. By adding a monoclonal anti-CD8 antibody, IFNgamma secretion induced by these 3 peptides was inhibited, suggesting that CD8 T cells were involved in their recognition. Four peptide A-responders had a common HLA class I molecule (presenting to CD8 T cells) which was HLA B8-1.

CONCLUSION: We conclude that autoreactive T cells reacting with 21-hydroxylase peptides are detectable in patients with AI adrenal diseases. This is the first identification of defined adrenal epitopes involved in AI adrenal disease. Discovery of HLA-B8 restricted epitopes offers new avenues for understanding disease pathogenesis and the course of destruction in patients at risk.

PO2-044 Autoimmunity

High intrafamiliar variability in APECED: study of the peripheral tolerance

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Background: Autoimmune Polyendocrinopathy Candidiasis Ectodermal Distrophy Syndrome (APECED) is an autosomal recessive disease, caused by mutations in the AutoImmune REgulator (AIRE) gene. Although APECED is a monogenic disease, clinical phenotype can reveal wide heterogeneity. This variability suggests that additional factors may influence the expression of the disease. Along with the central tolerance, primarily involved in pathogenesis

of APECED, several other peripheral mechanisms contribute to regulation of immune system. So far, only a few study have investigated peripheral tolerance in APECED patients.
Aim: To evaluate whether genetic, immunological and environmental factors may be involved in the modulation of the disease in two siblings with identical genotype and different phenotype.
Patients: Two siblings carrying the same complex homozygous mutation of AIRE (exon1: IVS1 + 1G>C; IVS1 + 5delG), showed a wide variability of phenotype: the older child (5 yrs) showed a severe phenotype complicated with uncommon manifestations, whereas the younger sister (4 yrs) had only a mild phenotype.
Methods: Perforin (PRF1) and HLA genes were amplified by PCR. APECED-related autoantibodies' were performed by indirect immunofluorescence or complement fixation or ELISA or RIA. The following infectious markers were evaluated: Herpes, EBV, HZV, Parvovirus B19. Peripheral tolerance mechanisms were evaluated by: resistance to FAS-induced apoptosis on PBMC activated with PHA, the number of $T^{CD4+CD25+}$ regulatory cells (Treg) through cytometer analysis and NK activity through the Wallac method.
Results: No alteration was found in PRF1. HLA aplotype and exposure to infectious triggers were not apparently associated to the severity of the disease. Autoantibodies' profile paralleled in both siblings to the clinical manifestations. The evaluation of Treg was comparable in both children (1.51 and 1.05%, respectively), but lower as compared to controls (4.33%). The NK activity was comparable between the 2 siblings and the controls. FAS-induced apoptosis was normal in both children (75 and 80 % respectively, n.v. <82%).
Conclusions: Peripheral tolerance mechanisms and infectious triggers evaluated in the current study, as well as HLA aplotype, do not seem to play a role in modulating the phenotype of the two siblings. Further studies are needed to identify additional factors, other than AIRE gene mutation, involved in the phenotypic variability of APECED.

PO2-045 Autoimmunity

High prevalence of exon 1 mutations in APECED patients from Campania, a region of Southern Italy

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Background: Autoimmune Polyendocrinopathy Candidiasis Ectodermal Distrophy Syndrome (APECED) is an autosomal recessive disease, caused by mutations in the AutoImmune REgulator (AIRE) gene. The prevalence of APECED worldwide is very low. Some different mutations have been identified to be peculiar to particular populations. In Italy the disease is also very rare but three hot spots areas have been identify. In Sardinia the typical AIRE mutation is characterized by a nonsense mutation on exon 3, defined as R139X. In Apulia a typical AIRE mutation is localized on exon 2 and is defined as W78R. In northern Italian populations R257X is very frequent and often associated with 1094-1106del113.
Aim: In this study we carried out mutation analysis of the AIRE gene in 6 patients affected with APECED originating from the region of Campania, an area of Southern Italy.
Patients and methods: Six children, originting from 5 different families of the region of Campania, were diagnosed as having APECED on the basis of the presence of at least two of the three major signs of the disease**.** Genomic DNA was estracted from peripheral leukocytes and the 14 exons of AIRE were amplified by PCR. A complete assessment of APECED-related autoantibodies' was performed by classical indirect immunofluorescence technique or complement fixation or ELISA or RIA, as appropriate.
Results: In all patients mutational analysis confirmed the diagnosis of APECED. All patients carried at least one mutation on exon 1:
- two siblings carried a complex homozygous mutation IVS1 + 1G>C; IVS1 + 5delG on exon 1;
- one patient was compound heterozygous for 47C>T + 232T>A (exons 1+8);
- one patient was compound heterozygous for 62C>T + 967-979 del (exons 1+8);
- one patient was compound heterozygous for 47C>T + 232T>C (exons 1+2);
- one patient was homozygous for T16M T16M on exon1.

The phenotypic expression of the disease showed wide variability, even between siblings with the same genotype. Circulating autoantibodies paralleled to the clinical symptoms in each patient.
Conclusion: Mutations on exon 1, in homozigosity or compound heterozygosisty, seem to be highly frequent in patients originating from Campania region. Although there is not a single typical mutation, the exon 1 could be suspected to represent a "hot spot" region for APECED patients originating from Campania. As already reported, genotype-phenotype analysis failed to reveal a clear genotype-phenotype correlation.

PO2-046 Autoimmunity

Autoantibodies against type 1 interferones and Th17-related cytokines in APS-1 patients

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Introduction
APS-1 (Autoimmune polyglandular syndrome type 1) is a rare autosomal recessive disorder associated with mutations in *AIRE* gene. It is characterised by at least two major clinical symptoms (hypoparathyroidism, Addison's disease and mucocutaneous candidiasis), and associated other endocrine or nonendocrine disorders. Usually, APS-1 begins in infancy with chronic mucocutaneous Candida infection. Auto-antibodies against type I interferons are reported to be highly specific for APS-1. Reduced production of Th17-associated cytokines has been described in heterogeneous group of chronic mucocutaneous candidiasis patients. But the pathogenic connection between the *AIRE* deficiency and susceptibility for Candida infections is not yet understood.
Objective
To review the diagnostic value of auto-antibodies against IFN-α2 in Slovenian APS-1 patients. To evaluate possible connection between the Th17-related cytokines (IL-22, IL-17F) and chronic candidiasis in APS-1 patients.
Patients and Methods
Six APS-1 patients were included into the study; one did not meet clinical APS-1 criteria. All patients had the history of the persistent Candida infections and had established pathological *AIRE* genotypes.
Binding and neutralizing anti-IFN-α2 autoantibodies were measured in plasma samples by ELISA and antiviral interferon neutralizing assay. Peripheral blood mononuclear cells were restimulated with Candida preparations and real-time RT-PCR was used to assess the expression of IL-22 and IL-17F.
Results
Anti-IFN-α2 auto-antibodies in high titres were detected in all patients, but not in age matching healthy control subjects. Expression of IL-22 and IL-17F in restimulated cells was significantly lower in APS-1 than in control group.
Conclusions
Autoantibodies against type 1 IFNs were detected in all patients, regardless their clinical presentation or *AIRE* genotype. Even in the 11 years old patient with mucocutaneous candidiasis as a single symptom and uncommon *AIRE* mutation (c.21-43dup23) these autoantibodies were strongly positive. This is confirming their diagnostic value in children with incomplete APS-1 clinical presentation.
Production of IL-22 and IL-17F in restimulated PBMC of APS-1 patients was significantly reduced. This possibly indicates the involvement of Th-17 like cells in pathogenesis of chronic candidiasis in APS-1 and implies the connection between *AIRE* deficiency and patients' disability to clear infection with Candida.

PO2-047 Autoimmunity

Recognition of HSP60 epitopes in a longitudinal cohort of patients with new onset type 1 diabetes mellitus; correlation with disease progression?

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Heat shock proteins (HSP) are under investigation for their immunomodulatory role in a range of autoimmune diseases, including type 1 diabetes mellitus (T1 DM). Our group previously identified a number of pan-DR binding HSP60 epitopes and recognition of these epitopes in juvenile idiopathic arthritis could be shown (Lancet 2005). In the present study we set out to establish whether these epitopes are recognised in patients with T1 DM, what type of inflammatory profile is associated with recognition and whether recognition is correlated with disease progression.

2 cohorts were used, the first consisted of 30 patients with longstanding DM, the second cohort consisted of 26 patients with new onset diabetes who were prospectively followed, blood samples were drawn at onset, 2-5 months and > 6 months after onset of DM. Depending on Hba1c and insulin need, all participants were classified as patients with or without a partial remission phase. Recognition of HSP 60 epitopes was measured in peripheral blood mononuclear cells by measuring proliferative responses as well as HSP60 peptide specific cytokine and chemokine production (measured by flow cytometry and Luminex).

Results: Peptide-induced cytokine and chemokine production (IL-1α, IL-1β, IL-6, IL-10, IL-17, IL-18, TNF-α, IFN-γ and MIP-1α, MDC, IL-8, IP10, OSM) could be established in patients with longstanding disease with a varying but predominant pro-inflammatory profile, irrespective of disease duration and age. However, no epitope-specific T cell proliferation could be detected in either cohort.

In the prospective cohort, patients without a partial remission phase showed increased IL10 and IFNy production during the course of the disease when analysed per patient. In a group comparison, IL10 production was higher 2-5 months after onset of the disease in both remitters and non-remitters compared to onset of T1 DM; IFNy was increased only in non-remitters. The number of FOXP3 positive cells was elevated after 2-5 months compared to onset in both the remission and non-remission group. Neither IL10, IFNy nor FOXP3 production at onset showed a significant correlation with subsequent progression (or occurence of partial remission) of the disease.

Conclusion: Epitope-specific cytokine production is induced by HSP60 epitopes, with an increase in cytokine production during the first months of the disease compared to onset of DM. The role of these HSP60 epitopes in the course of type 1 DM is yet to be determined.

PO2-048 Bone, Calcium II

Association between the T239M missense variation in the FGF23 gene and renal phosphate leak

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In the last years, new evidences changed the common concept of hypophosphatemic disorders from rare and specific conditions, like hypophosphatemic rickets, to a more common spectrum of diseases, for example, osteoporosis and nephrolitiasis.

Nephrolithiasis is a disorder affecting 10% of the western population. Approximately 20 % of patients with calcium nephrolithiasis and normal parathyroid function show a reduced rate of renal tubular phosphate reabsorption and reduced serum phosphate concentration.

Fibroblast growth factor 23 (FGF23), a hormone regulating phosphate homeostasis, plays a pivotal rule in this pathological pattern.

To determine whether genetic variation at the FGF23 locus is associated with renal phosphate handling abnormalities, we sequenced the entire regulative and coding regions of the FGF23 gene in 106 stone formers, 17 of which with renal phosphate leak, and 87 healthy controls.

We detected a sequence variation C716T in exon 3 in 7 out of 17 patients with renal phosphate leak. This variation changes the normal ACG triplet (encoding Threonine) at codon 239 to an ATG triplet (encoding Methionine) thus causing a missense variation that was designated T239M (rs7955866).

We found that prevalence of the T allele and of the CT genotype in stone formers with renal phosphate leak was significant higher compared to those observed in stone formers without renal phosphate leak [C vs T allele frequencies (p=0.024), genotype frequencies (p=0.002)], and in controls (p=0.03 and p=0.007, respectively). No significant differences were found for C716T allele and genotype frequencies between stone formers without renal phosphate leak and controls.

On the other hand, stone forming subjects and healthy controls with at least one C716T allelic variant showed reduced levels of serum phosphate (0.89 ± 0.09 *vs* 1.13 ± 0.18 mmol/l, p=0.03) and TmPi/GFR (0.76 ± 0.16 *vs* 1.49 ± 0.57 mmol/l, p<0.01) compared to subjects with wild-type FGF23 allele. No additional significant differences were detected.

In vitro functional studies are in progress, in order to firmly establish a causal relationship between the polymorphic variant described and the occurrence of the abnormalities in renal phosphate handling.

The identification of a genetic marker of calcium nephrolithiasis associated with hypophosphatemia could allow the elaboration of familial screening programs and pharmacological intervention (i.e. citrate administration) before clinical onset.

PO2-049 Bone, Calcium II

Pseudohypoparathyroidism type Ia and GNAS epigenetic defects: analysis of 40 patients with Albright's hereditary osteodystrophy and multihormone resistances

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The two main subtypes of pseudohypoparathyroidism (PHP), PHP-Ia and -Ib, are caused by mutations in GNAS exons 1-13 and methylation defects in the imprinted GNAS cluster, respectively. PHP-Ia patients show Albright hereditary osteodystrophy (AHO) and resistance toward PTH and additional hormones, while PHP-Ib patients do not have AHO and hormone resistance is limited to PTH and TSH. Recently, methylation defects have been detected in few patients with PHP-Ia and mild AHO, indicating a molecular overlap between the two forms.

Objectives: To screen patients with clinically diagnosed PHP-Ia for methylation defects and to investigate the presence of correlations between the molecular findings and AHO severity.

Patients and methods: We investigated differential methylation of GNAS regions and STX16 microdeletions in genomic DNA from 40 patients with clinical diagnosis of sporadic PHP-Ia, i.e. AHO with multi-hormone resistance, with no mutations in Gsa-coding *GNAS* exons.

Results: Molecular analysis showed GNAS cluster imprinting defects in 24 of the 40 PHP-Ia patients analyzed. No STX16 deletion was detected. Imprinting defects were not associated with the presence of severe or mild form of AHO.

Conclusions: We report the largest series of the literature of patients with PHP-Ia and no mutation in the Gsa gene. Our findings of frequent GNAS imprinting defects further confirm the existence of an overlap between molecular and clinical features of PHP-Ia and PHP-Ib, and highlight the necessity of a new clinical classification of the disease that takes into account the recent knowledge on the molecular basis underlying these defects

PO2-050 Bone, Calcium II

Inheritance and diagnosis of subcutaneous ossification within 2 families GNAS mutated: progressive osseous heteroplasia or pseudohypoparathyroidism type Ia?

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Subcutaneous ossifications occur in two rare GNAS gene-associated conditions, i.e. Progressive Osseous Heteroplasia (POH) and Pseudohypoparathyroidism Ia (PHP-Ia). The inheritance pattern of these diseases seems to be from paternal and maternal mutated allele, respectively. Clinically, in POH the subcutaneous ossifications are an isolated sign, whereas in PHP-Ia they are associated with the Albright Hereditary Osteodystrophy phenotype and multiple resistances to hormones that act via Gs-alpha protein pathway.

We described 2 GNAS gene-mutated families with peculiar inheritance pattern and clinical presentation overlapping the 2 conditions.

Family 1: M.E. at 3 months presented isolated subcutaneous ossifications spread at both legs and abdomen. She harboured a novel nonsense GNAS mutation (Q29X) in exon 1, inherited from the mother, which displayed an isolated brachydactiyly, and from a healthy grandmother. In a 8-year follow-up, TSH (at 11 months) and PTH (at 28 months) resistances and brachydactyly (at 5 yrs) appeared in the baby. The initial diagnosis of POH was thus converted in PHP-Ia; the inheritance pattern of the mutation in this family is indeed peculiar respect to the associated phenotypes.

Family 2: An.P. and Al.P. are two brothers in which subcutaneous ossifications spread at the lower limbs appeared in the first year of age; a 6 year follow-up has evidenced an increased PTH level and brachydactyly in An.P., brachydactyly in Al.P. Both probands harboured a nonsense GNAS mutation (Q35X) in exon 1, inherited from the healthy father.

In presence of subcutaneous ossifications, the familiar GNAS genetic analysis and the long term clinical follow-up can help in the accurate definition between the POH/PHP-Ia conditions

PO2-051 Bone, Calcium II

Reciprocal relations between adiponectin and bone mineral density in childhood-onset brain tumors

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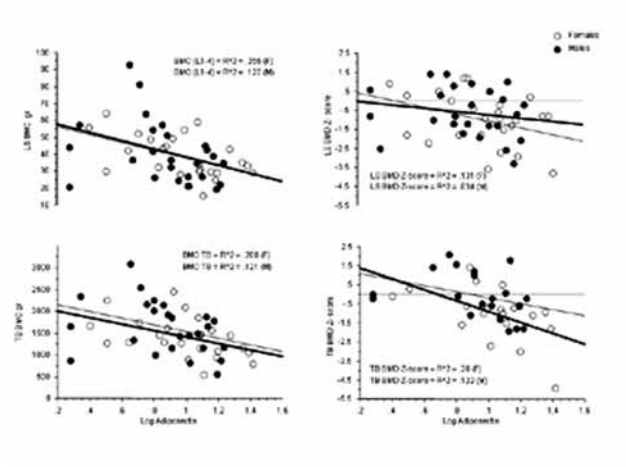
Background. Pediatric survivors of brain tumors are at risk of reduced bone mineral density (BMD). It has been shown that brain, bone and fat are intimately connected trough the sympathetic nerve system and adipokines. Indeed, adiponectin has been reported to be negatively associated to BMD in healthy and diabetic adults.

Objective. The aim of the study was to determine whether adiponectin is related to BMD in pediatric survivors of brain tumors; and whether such relationship is influenced by the severity of hypothalamic-pituitary dysfunction (HP) in patients with or without HP defects.

Methods. Serum concentrations of adiponectin, insulin and alkaline bone phosphatase (BALP) were measured in 50 subjects (25F, 25M; mean age 16.1±4.6yrs) treated for brain tumors (craniopharingioma n=7, embryonal tumor n=19,germinoma n=11,glioma n=9,other n=4) at the mean age of 7.7±3.8yrs. Thirteen subjects had a single HP defect and 29 had multiple HP defects. Measures for body composition (Fat Free Mass,FFM and Fat Mass,FM), bone mineral content (BMC) and BMD at the lumbar spine (LS) and Total Body (TB) were assessed by DXA.

Results. Females were significantly shorter (P=0.006) and had lower measures of FFM (P=0.002), BALP (P=0.004) and TBBMD Z-score (P=0.028) when compared with males. Adiponectin was significantly and inversely related to DXA bone measures regardless of gender.



On multiple regression models after controlling for age, height SDS, FFM, FM, insulin and PALP, adiponectin persisted significantly, independently and inversely associated to TBBMD Z-score in both females (R^2=0.143) and males (R^2=0.127) and to LSBMD Z-score in males (R^2=0.182). These relations were found regardless of the severity of HP involvement. Subjects without HP defects (n=8) had higher adiponectin levels and lower BMDZ-scores compared to subjects with HP defects.

Conclusions. Adiponectin is reciprocally related to DXA bone measures in pediatric survivors of brain tumors independently of adiposity and determinants of bone. The relation seems stronger in subjects with a preserved HP function supporting the hypothesis of a central role of adiponectin on bone homeostasis.

PO2-052 Bone, Calcium II

Adipokines may modify osteoblast Wnt signalling through Dickkopf-1, and promote osteoclastogenesis by altering RANK-ligand and osteoprotegerin signalling in obese children

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Introduction: We have recently presented data demonstrating that total body and regional bone mass relative to body size is reduced in obese children. Adipokines have been shown to play a role in bone metabolism. We hypothesised that

1) increased bone turnover would be associated with reduced bone mass

2) increased serum leptin would be associated with increased osteoclastogenic factors

3) reduced adiponectin would be associated with increased osteoclastogenic factors

Methods: We recruited 103 children following ethics approval. Bone turnover was assessed by serum P1NP (formation) and urinary CTX (resorption). Bone densitometry was used to measure total body and lumbar bone mineral content (BMC) and density (BMD), and total body fat mass (FM). Skeletal maturity was assessed using TW3 bone age.

Serum was analysed for the adipokines leptin, adiponectin; the osteoclastogenic factors TNF alpha and RANK-L; the decoy receptor for RANK-L, osteoprotegerin (OPG); and dickkopf-1 (DKK-1) an inhibitor of bone formation acting through down-regulation of wnt-signalling through LRP5 binding.

Results: After adjustment for height, weight and gender using multiple regression, FM was negatively related to total body BMC (p=0.05) and BMD (p=0.03) and lumbar BMC (p<0.0001) and BMD (p=0.04). P1NP (p=0.01) and CTX (p=0.0007) were significantly higher in obese children i.e. increased bone turnover.

Following correction for TW3 bone age and gender, leptin (p<0.0001) and adiponectin (p=0.006) were positively and negatively related to FM respectively. Leptin was inversely related to osteoprotegerin (p=0.01). Adiponectin was

inversely related to DKK-1 (p=0.04). TNF alpha was significantly associated with RANK-L (p=0.006) and DKK-1 (p=0.003).
Conclusions: We showed that in obese children increased bone turnover favouring bone resorption was associated with reduced total body and regional bone mass relative to body size. We also showed that in obese children there were alterations in osteokines favouring inhibition of osteoblastogenesis and supporting osteoclastogenesis. Obese children are at risk for significant skeletal ill health

PO2-053 Bone, Calcium II

A case with parathyroid adenoma presenting with mutiple brown tumors

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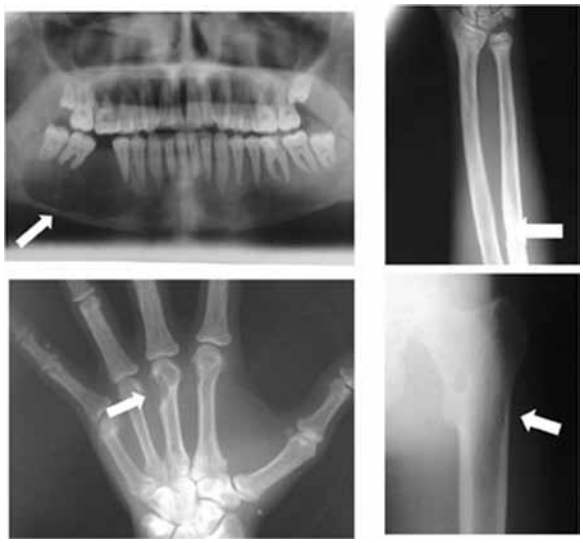
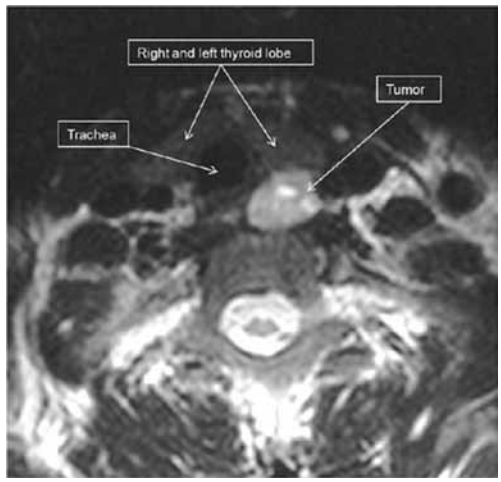
Introduction: Osteitis fibrosa cystica and brown tumors have become extremely rare clinical entities of primary hyperparathyroidism. We report an adolescent boy with multiple brown tumors as the manifestation of primary hyperparathyroidism.
Case history: A 16-year-old boy was referred because of swelling of the right mandible. He had a complaint of generalized weakness. Other physical findings were also unremarkable. In the laboratory: hemoglobin, white blood cell count, erythrocyte sedimentation rate, BUN, and Cr were in normal limits. Serum Ca, 14 mg/dL (8.6 to 10.0); P, 2.8 mg/dL (2.6 to 4.0); ALP, 616 IU/L (65 to 260); 25-OH vitamin D, 15.3 ng/mL (10 to 55); iPTH, 1160 pg/mL (10 to 65); and the 24-h urine calcium, 9.4 mg/kg/d (<4 mg/kg/d). Plain radiographs revealed multiple osteolytic lesions.



Ultrasonography (USG) of the neck and a parathyroid scintigraphy were normal. MRI revealed a tumor.



Radioisotopic bone scan and renal USG were normal. There was no family history of hyperparathyroidism. Serum prolactin, fasting insuline and C-peptide, and 24-h urine vanillylmandelic acid were within normal limits. Biopsy specimen taken from the mandible was consistent with brown tumor. The left inferior parathyroid gland was surgically removed. The lesion was histopathologically diagnosed as parathyroid adenoma. Postoperatively, the patient was managed with calcium and calcitriol. He remained recurrence-free at three months follow-up. At that time, the swelling of the right mandible tended to resolve spontaneously. Serum Ca, P, ALP, and iPTH were in normal limits.
Conclusion: Even though hyperparathyroidism with brown tumors in a single bone was uncommon in children, the most notable aspect of this case report was the presence of the multiple brown tumors, which is an extreme rare finding in parathyroid adenoma.

PO2-054 Bone, Calcium II

Identification of novel mutation in the calcium-sensing receptor (CaSR): keeping an eight year old girl away from the scapel

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Inactivating mutations of CaSR cause familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT). FHH is autosomal dominant, characterized by hypercalcemia, unsuppressed circulating parathyroid hormone (PTH) and inappropriately low urinary (U) calcium (Ca) secretion. Most FHH is asymptomatic and requires no surgical intervention. We identified a novel CaSR mutation in a patient referred to pediatric endocrinology prior to a planned parathyroidectomy. Careful family history and identification of a functionally significant CaSR mutation prevented unnecessary surgery. An 8-yo Caucasian female had intermittent knee pain for 6 mo. Total serum Ca was 9.9-11 mg/dL (normal 8-10.5); ionized Ca 1.4 nmol/L (1.11-1.30); phos 3.6 mg/dL (2.3-4.5); Mg 2.1 mg/dL (1.6-2.6); intact PTH 41.4 pg/ml (11-67); 25-OH vitamin D, 23 ng/ml (15-60). The fractional excretion of Ca was 0.0022 (24 hr U creat: 435 mg/day; UCa: 21 mg/day; Ca: 5.5 mg/dL; Ca/creat: 0.048 mg/mg). Her 51 yo father had serum total Ca: 11-12 and multiple intact PTHs in the 90's. He was diagnosed with primary hyperparathyroidism and subsequently underwent partial parathyroidectomy. His serum calcium remained elevated, and he was scheduled for total parathyroidectomy. Immunohistochemical analysis of his parathyroid glands showed a nodular pattern of CaSR protein on the cell surface of the chief cells compared with a smooth distribution in a normal control. DNA sequencing identified the in-frame heterozygous missense mutation c.1466 A>G changing Tyr489 to Cys in both father and daughter. This mutant was re-created by site-directed mutagenesis in a CaSR cDNA expression vector. When transiently expressed in HEK293 cells,

minimal amounts of mutant CaSR were targeted to the cell surface, as assayed by cell-surface biotinylation & immunoblotting and also by confocal fluorescent immunocytochemistry. Using single-cell microfluorimetry with Fura-2, 0.5-1.0 mM extracellular Ca induced robust intracellular calcium release with wild-type CaSR; but 5-6 mM extracellular Ca was needed to elicit responses in cells expressing the mutant, with amplitudes dampened by >80%. Tyr489 in CaSR is conserved in all species. The decreased cell surface expression of the mutant CaSR suggests that Tyr489 is important for protein conformation or protein targeting. The differential diagnosis of hypercalcemia can be difficult; careful clinical and laboratory studies can prevent needless parathyroidectomy.

PO2-055 Bone, Calcium II

Efficiency of alendronate treatment in children with secondary osteoporosis

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Background. Osteopenia/osteoporosis is being increasingly reported as a complication of many chronic diseases, even in children. Treatment and prevention of osteoporosis in pediatric patients is important in order to avoid bone fragility and to obtain an optimal peak bone mass. The use of biphosphonates in childhood has been limited. The purpose of this study was to investigate the efficacy of oral alendronate in children with secondary osteoporosis. Methods. A total of 46 children (28 males, 18 females) with secondary osteoporosis treated with oral alendronate between January 2003 and December 2008 in our institution were included. The oral dose was either 10 mg daily or 70 mg once weekly in patients with initial weights > 30 kg, and was 5 mg daily or 35 mg once weekly with weights ≤ 30 kg. The patients with juvenile osteoporosis and osteogenesis imperfecta were excluded. Bone mineral density (BMD) was measured with DEXA taken at 0, 6, 12 and 24th months of the treatment. The mean age of the patients was 11.01±4.11 (1.14 to 17.75) years. Of all children, 37% had neurologic diseases, 26% had connective tissue diseases with corticosteroid or methotrexate use, 24% had hematological diseases, 9% had gastrointestinal system diseases and 4% had immunologic diseases. Ten patients were using one or more antiepileptic drugs. None of them had fracture history and fractures during the treatment. Nine percent had backache, 9% had diffuse bone pain, 6% had bone pain on lower extremities and the remaining 76% had no pain. Mean height, weight and body mass index (BMI) SD scores were -2.43, -2.82 and -1.72, respectively. Serum Ca, P, ALP and PTH levels were all normal. Mean BMD-z score at baseline was calculated as -4.03±0.96 and it improved gradually at 6th, 12th and 24th months of the treatment with the corresponding BMD z-scores of -3.51±1.02, -2.82±1.09 and -2.43±0.93, respectively. The improvements were statistically significant. The drug was tolerated well and there was no withdrawal due to drug side effects. Conclusion. Our results confirm that oral alendronate is effective in improving BMD in children with chronic diseases having secondary osteoporosis and the drug is well tolerated in these groups of patients.

PO2-056 Bone, Calcium II

Bone involvement in patients with clusters of autoimmune diseases

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OBJECTIVE Complex autoimmune diseases are chronic conditions initiated by loss of immunological tolerance to self-antigens. Clustering of these diseases in the same individual is not unusual, suggesting that common immune mechanisms may facilitate immune mediated processes. Bone loss found in patients with either Type 1 diabetes (T1D), autoimmune thyroid disease (ATD) or celiac disease (CD) has been ascribed to metabolic or nutritional imbalance, but osteopenia could also be explained by a chronic inflammatory process leading to imbalanced osteoclast activity. We hypothesized that clustering of autoimmune diseases could increase the risk of bone impairment with respect to isolated autoimmune diseases.

RESEARCH DESIGN AND METHODS One-hundred-two patients (49 males) with T1D alone or associated with ATD and/or CD were studied; age 12.8±4.5 years; diabetes duration 5.9±3.9 years. ATD patients with subclinical or clinical hypothyroidism were on l-thyroxin treatment; CD patients were on gluten free diet (GFD) and were distinguished into categories of good or poor compliance to diet, according to EmA and/or anti-transglutaminase positivity. Each patient underwent anthropometric, biochemical, hormonal, gluco-metabolic (Hba1c) assessment. Phalangeal quantitative ultrasound (DBM Sonic, IGEA, Carpi, Modena, Italy) was performed. The average of amplitude-dependent speed of sound (AD-SoS, m/sec), measured in the last four fingers, was calculated and expressed as SDS. AD-SoS SDS ≤ -2 was considered as indicative of osteopenia.

RESULTS Osteopenia was equally distributed among children with T1D alone (8.1%), T1D associated with ATD (7.7%) or CD (4.3%), while it was found in 37.5% patients presenting with three autoimmune diseases. Poor compliance to GFD further increased osteopenia to 18.8% in patients with T1D and CD and 80% in patients with three autoimmune disorders. No difference among groups was found with regard to gender, age, time since T1D or CD diagnosis, height, BMI, serum total calcium, phosphorus, alkaline phosphatase and HbA1c. Thyroid function was normal.

CONCLUSIONS: Clustering of autoimmune diseases in the same individual is associated with high risk of osteopenia, independent on nutritional, metabolic or endocrine derangements. Poor compliance to GFD increases this risk in CD patients. More studies are needed to assess whether a chronic imbalance of inflammatory cytokines due to the sum of several autoimmune disorders might explain these finding.

PO2-057 Bone, Calcium II

Vitamin D toxicity in an era of vitamin D deficiency

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Over-the-counter vitamin supplements are frequently recommended for children, especially with the recent epidemic of vitamin D deficiency. However, since nonprescription supplements are not FDA-regulated, we often do not know if the contents stated on the labels of these vitamins are indicative of what are actually in them. We present a case of a 2 year old male, with no significant past medical history, who presented with decreased oral intake, 1.5 month history of intermittent vomiting, weight loss, recurrent abdominal pain, and irritability. Initial bloodwork confirmed on repeat evaluation revealed a calcium level of 20 mg/dL. The differential diagnosis of hypercalcemia includes: hyperparathyroidism, hypothyroidism, vitamin D toxicity, vitamin A toxicity, tuberculosis, sarcoidosis, adrenal insufficiency, malignancy, Williams Syndrome, and Jansen's Metaphyseal Dysplasia. An extensive work-up was negative for all of the above with the exception of vitamin D toxicity. Bloodwork revealed a normal 1,25-dihydroxyvitamin D level, but an extremely elevated 25-hydroxyvitamin D of 640 ng/mL, which is more than 6 times the upper limit of normal. His mother later revealed that he had been taking a vitamin D-containing supplement. Furthermore, we tested 25-hydroxyvitamin D and calcium levels in the patient's brother, who was also taking the same supplement. His brother had a high 25-hydroxyvitamin D level as well, but did not have hypercalcemia. We therefore concluded that the patient's hypercalcemia was due to vitamin D toxicity caused by the over-the-counter vitamin supplement that he was taking. His 25-hydroxyvitamin D and calcium levels subsequently decreased after stopping the supplement. Analysis of the contents of the vitamin supplement is pending.

PO2-058 Bone, Calcium II

Improved growth during the first year of monthly intravenous pamidronate therapy in prepubertal patients with osteogenesis imperfecta

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The aim was to study the effect of pamidronate (APD) therapy on linear growth in prepubertal patients with type I or type IV osteogenesis imperfecta (OI) during the first year of treatment (2 girls, age 2-9 yrs; 11 boys, age 2-11 yrs). APD treatment was given as a monthly infusion (10-30 mg/m^2 during the first six months and thereafter 30 mg/m^2). Height, sitting height and arm span were recorded before, at the beginning of and after 1 yr of APD therapy. Bone mineral density (BMD) was assessed by dual-energy X-ray absorptiometry (DXA). The height standard deviation score (HtSDS) was calculated based on the Karlberg 1976 height standards and by use of piecewise linear regression. To evaluate how growth was affected by APD, changes in HtSDS (ΔHtSDS), sitting height (ΔSHSDS) and leg length (ΔLLSDS) were calculated the year before (-1 to 0 yr) and the first year after (0 to +1 yr) the treatment was initiated. The mean age at treatment start was 7:9 (3:7-11:0) (yr:mth). The mean growth rates were 4.6±0.6 and 6.4±0.6 cm/year, over the last year before and the first year after the initiation of APD treatment. HtSDS was similar (-2.7±0.6 SDS) at -1 and 0 yr but improved to -2.3±0.5 SDS at 1 yr. ΔHtSDS was -0.02±0.06 the year before treatment start (-1 to 0 yr) and improved significantly over the 1st year (+0.37±0.11, p=0.006 vs. the year before APD). Similarly, a positive response in ΔSHSDS was observed during the 1st year of APD treatment (improved from -0.42±0.34 the year before to 0.50±0.18 during the first year of treatment, p=0.02). The effect on leg length followed a similar trend: ΔLLSDS improved slightly but not significantly during the first year of APD treatment (from -0.02±0.16 to 0.22±0.10). All patients had a positive response in BMD which improved from 0.316±0.025 to 0.456±0.020 g/cm^2 (p=0.0002) during the 1st year of treatment.

In conclusion, cyclic intravenous pamidronate therapy improved linear growth during the first year of treatment in a majority of prepubertal OI patients. The positive effect on growth was most pronounced in the upper body segment which may partially be due to the prevention of vertebral fractures. It appears that bisphosphonates do not have negative effects on linear bone growth but based on our current results no conclusions on the possible long-term effects can be drawn.

PO2-059 Bone, Calcium II

A novel mutation in the TBCE gene causing the hypoparathyroidism, retardation, and dysmorphism (HRD) syndrome demonstrates partial rescue of the TBCE protein

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The syndrome of hypoparathyroidism, retardation, and dysmorphism (HRD, MIM 241410) consists of permanent congenital hypoparathyroidism, severe prenatal and postnatal growth retardation, and profound developmental delay. The patients are susceptible to severe infections including life-threatening pneumococcal infections especially during infancy. HRD syndrome is the first reported disease caused by a defect in the tubulin folding and assembly pathway due to mutations of the tubulin cofactor E (TBCE) gene which encodes a chaperone required for α- and β tubulin dimerization and microtubules (MT) polymerization. MT metabolism performs one of the leading roles in regulating various features of the cytoskeleton, which plays a major role in many cell processes.

We have recently identified a novel homozygous mutation in the TBCE gene in a newborn patient with typical manifestations of HRD syndrome. The mutation: a deletion of 2 nucleotides (TA) in exon 4 causes splicing of exon 4 directly to exon 8, that keeps the reading frame of the protein. The TBCE protein is predicted to miss 102 amino acids in the leucine rich repeat domain. Primary fibroblasts of the patient revealed reduced density of MTs, intermediate and actin filaments as well as altered distribution of lysosomes and mitochondria.

This study demonstrates how an afflicted individual who would otherwise lack the capacity to make functional TBCE can survive, and point to a limiting capacity to fold tubulin heterodimers *de novo* as a contributing factor to disease pathogenesis.

PO2-060 Bone, Calcium II

Osteosclerotic variant of osteogenesis imperfecta with a mutation in the C-propeptide region of *COL1A1* gene

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Background: Osteogenesis imperfecta (OI) is a genetic connective tissue disorder, most cases of which caused by mutations in the genes encoding the proα1(I) and proα2(I) chains of type I collagen (*COL1A1 and COL1A2*). Pace et al. reported a patient of OI with dense bone in X-ray (defined as "osteosclerotic variant of OI") with the heterozygous mutation (c.4321G>T;p.Asp1441Tyr) in *COL1A1* gene. This mutation resides in the carboxyl-terminal propeptide (C-propeptide) region. This particular region consists of exons from 48 to 51, encodes non-triple helical, C-terminal region of the proα1(I), and has been thought to play a pivotal role in collagen assembly. We report here on three children of osteosclerotic variant of OI, two of whom had C-propeptide mutations of *COL1A1*.

Patients' report: The diagnosis of OI in three patients was convincing judging from clinical course which was summarized in the table. The discriminative radiological change was presence of dense bones. Dense bones were found in the vertebral bodies, metaphyses, and fracture sites of the long bones in variable severity and distribution. Despite severe multiple fractures of the limbs, no vertebral compression fractures were found in all patients.

clinical course

	Patient 1	Patient 2	Patient 3
Outcome	dead (day0)	dead (day0)	alive but severely handicapped (9-year-old)
Gender	F	F	F
Family history	-	-	+*
Consanguinity	-	-	-
Abnormal physical findings **	+	+	+
Multiple fractures at birth on X-ray	+	+	+

*The older brother of the proband was also diagnosed as OI and died during infancy. **Triangular face, hypoplastic thorax, and short and/or bent limbs

Molecular analysis of *COL1A1* gene: All the coding regions of *COL1A1* gene were amplified by PCR and directly sequenced. We detected c.4247delC;p.Thr1416fsX and c.4160C>T;p.Ala1387Val in the C-propeptide region in patient 1 and 3, respectively. We did not detect any mutations in the other coding regions of *COL1A1* gene in both patients. In patient 2, no mutation was found in the C-propeptide region of *COL1A1* gene.

Discussion: We propose a new clinical entity of OI with osteosclerotic variant, namely OI with dense bones having seemingly normal shaped vertebral bodies in X-ray. The characteristics of this entity are high lethality, dense bones in

variable severity and distribution, and genetic heterogeniety. In some cases, heterozygous mutation in the C-propeptide region of *COL1A1* gene is responsible, indicating an autosomal dominant inheritance.

PO2-061 Bone, Calcium II

Mutaions of the GNAS gene identified in two Japanese patients with pseudohypoparathyroidism 1a

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Pseudohypoparathyroidism is a genetic disorder characterized by clinical hypoparathyroidism caused by parathyroid hormone (PTH) resistance. Patients with pseudohypoparathyroidism 1a(PHP-1a) show resistance to other hormones such as thyroid hormone or gonadotropins as well as Albright's hereditary osteodystrophy (AHO), characterized by phenotypic signs of short stature, obesity, brachydactyly, ectopic ossifications and mental retardation. PHP-1a is caused by maternally inherited, heterozygous inactivating mutations in *GNAS* encoding Gsα.
Here, we report two mutations of the GNAS gene identified in two Japanese patients with sporadic PHP-1a.
The first patient was 3-yrs-old boy diagnosed as tuberous sclerosis from epileptic seizure, retinal hamartoma and cardiac rhabdomyoma at the age of 3 months. He was refered to our hospital because of hypocalcemia, hyperphosphatemia and elevated PTH level. He had a round face, brachydactyly and showed mental retardation. Ellsworth-Howard test did not show any responses of urinary cAMP and phosphate. A novel heterozygous IVS2+1G>A of the GNAS gene was identified in this patient.
The second patient was 7-yrs-old girl. She was refered to our hospital because of generalized seizure. She had a round face, short fourth fingers and showed mental retardation. Biochemical examination showed hypocalcemia, hyperphosphatemia and elevated PTH levels. There was no response of urinary cAMP and phosphate in Ellsworth-Howard test. A 264delG of the GNAS gene was identified in the second patient. This mutation produced a premature stop codon, resulting in the truncated protein.
In conclusion, we identified one novel and one previous reported mutation in two patients with PHP-1a.

PO2-062 Bone, Calcium II

Very high prevalence of vitamin D deficiency in infants in India

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Background: There is paucity of data regarding prevalence of vitamin D deficiency in healthy infants in India. We had earlier presented data showing high prevalence of hypovitaminosis D in mother-infant pairs during winter (E-PAS2008:633798.5) but the prevalence during summer had not been determined.
Objectives: To determine the prevalence of vitamin D deficiency, defined as serum 25-hydroxyvitaminD3 (25OHD3) <20 ng/ml, in healthy term breastfed infants at 3 months of age and their mothers, to assess for seasonal difference and to determine predictors of infants' vitamin D levels.
Methods: The study was conducted in New Delhi (28.4N, 77.1E) after taking ethical approval and informed consent. 98 infants aged 2.5 to 3.5 months, born at term with normal weight and their mothers were enrolled; 47 in winter (November- January) and 51 in summer (May-July). Details of infants' feeding, vitamin supplementation and sunlight exposure were taken and estimation of serum 25OHD3 and parathormone (PTH) was done. Seasonal difference in mean 25OHD3 levels was assessed by chi square test and predictors of infants' 25OHD3 by linear regression.
Results: 69 infants were exclusively breastfed and the others were also given animal milk 1-2 times/day. 34 infants were taking supplement containing 125 IU of vitamin D2/ day. Median and range of duration of sunlight exposure was 10 minutes/ day (0-120) and percentage body surface exposed was 40% (0-100). The prevalence (95% CI) of vitamin D deficiency was 86.5% (78.0, 92.6) in infants and 92.6% (85.4, 97.0) in mothers. Secondary hyperparathyroidism (PTH>46 pg/ml) was present in 48.5% infants and 53.7% mothers. Spearman's correlation coefficient between infants' 25OHD3 and PTH levels was 0.66 (p<0.001). The mean 25OHD3 levels in infants did not show significant seasonal difference (summer 11.5 ±7.3, winter 11.6 ±9.1, p=0.95) but that in mothers was significantly higher in summer (11.8 ±5.6) compared to winter (7.9 ±5.8, p <0.01). Multiple regression analysis revealed that intake of vitamin supplements, sunlight exposure and mother's 25OHD3 were independent predictors of infants' 25OHD3.
Conclusions: The prevalence of vitamin D deficiency was extremely high in healthy infants at age of 3 months and their mothers with secondary hyperparathyroidism in half of the study population. There was no seasonal difference in infants' 25OHD3 levels. Vitamin intake, sun exposure and maternal 25OHD3 were predictors of infants 25OHD3 levels.

PO2-063 Bone, Calcium II

High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes

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Normal 25-OH-vitamin-D (25-D) levels during childhood and adolescence are crucial to attain maximal peak bone mass. Since young adults with type 1 diabetes mostly have a lower peak bone mass than healthy individuals, optimal 25-D levels are of particular importance in children with type 1 diabetes. Here we report the levels of 25-D in children with type 1 diabetes seen at our outpatient clinic during one year. The data of 109 children (64 boys, 45 girls) aged 2.6-18.6 y with a duration of diabetes of 0.9-15.3 y were analysed. 62 children (57%) had 25-D deficiency, defined as 25-D levels below 50 nmol/l, and 31 children (28%) were 25-D insufficient, defined as 25-D levels of 52-74 nmol/l. There was a marked seasonality, the levels being below 50 nmol/l from november through april in most patients. The characteristics of the 25-D deficient children and the children with 25-D levels above 50 nmol/l were as follows:

	25-D > 50 nmol/l (n=47)	25-D≤50 nmol/l (n=62)
Age (y)	11.7+/-3.67	11.7+/-4.0
Diabetes duration (y)	4.8+/-3.22	5.1+/-3.77
HbA1c (4.1-5.7%)	8.0+/-0.89	8.1+/-1.46
Calcium (mmol)l	2.36+/-0.08	2.34+/-0.08
ionised Ca (mmol/l)	1.23+/-0.03	1.21+/-0.04*
Phosphate (mmol)l	1.39+/-0.17	1.42+/-0.21
ALP (U/l)	217+/-82	233+/-99
iPTH (10-73 pg/ml)	32.1+/-9.5	37.6+/14.3**
25-D (51-113 nmol/l)	72.4+/-19	28.0+/-13.0***

*p=.007,**p=0.04,***p<.0001 Mann-Whitney-U test. Data are given as mean +/- SD.

To summarise, we show that vitamin D deficiency is frequent in a cohort of type 1 diabetic children. Therefore, we think that vitamin D deficiency should be screened for and supplementation should be considered in children with low levels.

PO2-064 Bone, Calcium II

A case of transient peudohypoparathyroidism of the neonate

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Transient peudohypoparathyroidism (PHP) is one of rare causes of hypocalcemia during neonatal period and early infancy. Its biochemical characteristics include hypocalcemia, hyperphosphatemia, high tubular reabsorption of phosphorus (TRP) and elevated intact parathyroid hormone (iPTH) levels. A 30-day-old male infant presented with repeated episodes of generalized tonic seizure. Physical examination revealed no anomalous finding. Initial biochemical data showed calcium 7.35 mg/dL (inonized calcium 0.89 mmol/L), phosphorus 9.5 mg/dL, alkaline phosphatase 2,086 IU/L, iPTH 511.9 pg/mL

and TRP 97.2%. Serum magnesium and $1,25(OH)_2D_3$ were within normal range. He was treated with calcium supplementation and calcitriol for 3 months. All the biochemical data returned to normal limits during the treatment and remained within normal limits for 6 months after discontinuation of the treatment. We report a case of transient PHP of the neonate that presented with hypocalcemic seizures.

PO2-065 Bone, Calcium II

Phenotypical diversity in a family with 4 affected siblings of osteogenesis imperfecta type 1

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Backround: Osteogenesis imperfecta (OI) is a genetic disorder characterized by increased bone fragility and low bone mass. The majority of patients with the clinical diagnosis carry mutations in COL1A1 or COL1A2, the genes encoding for collagen type 1. Depending on the mutation, the phenotype can vary from mild to severe. Overall, genotype-phenotype correlations in OI are not fully understood and impose challenges in clinical decision making.
Methods: We report on a family with four affected members where the index patient with repeated fractures had to be treated with palmidronate while her triplet brother and sister were not.
Results: A 7 year old girl was presented to our department with repeated fractures of the tibia and evident blue sclera. Mutation analysis showed a heterocygous base exchange in Exon 12 on the COL1A2 gene. Because this girl is one of three tiplets genetic testing in the other two triplets was performed even though no clinical evidence of OI was present in the siblings. All three children carry the same mutation but differ dramatically in regards to phenotypical expression. Furthermore an 11 years old brother, who had two fractures on different long bones showed the same mutation. Hence, different clinical courses and different bone mass as shown in DEXA scans are present in the same family and require differential and selective clinical management.
Conclusion: Because of the different phenotypical consequences treatment strategies in families with osteogenesis imperfecta should be discussed carefully based upon genetic, clinical and phenytypic data.

PO2-066 Bone, Calcium II

Perinatal lethal hypophosphatasia with intrauterine bone deformity: a case report

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Hypophospatasia is a rare inherited disorder characterised by defective bone mineralisation and deficiency of tissue non specific alkalen phosphatese activity. Here, we report a 3 days old girl with perinatal lethal hypoposphatasia who presented with intrauterine bowling deformities of arms and legs.
Bone deformity was detected at intrauterine 5th month and she had delivered by cesarian sectio on the time. The family history was unremarkable. On physical examination; her weight was 2800 gr (<3. percentile), height 48 cm (<3. percentile), head circumference 32cm (3-10 percentile). She was hypoactive, hypotonic, widely open anterior fontanel, very small skull bones, blue sclerae, and bowling deformities in the extremities. Other physical findings were normal. Laboratory examination on admission showed that her serum calcium (Ca) level 8.6 mg/dl, ionized Ca 1,0 mmol/L, phosphorus 6.1 mg/dl, repeated alkalen phosphatese (ALP) levels 12 U/L and 8 U/L (N: 145-420), parathyroid hormone 67.8 pg/ml (N: 12-69), 25-hydroxy vitamin D 5µg/L (N: 10-50), urine Ca/creatinin ratio 0.02. Radiographic examination demonstrated very low dansity of skull and long bones such that most of skull bones were unvisible. Metaphysies of long bones had moth-eaten apperance and widen. Additonally, fractures and deformities were detected in extremities. Bone mineral density examination revealed tibial z-score as -5.3. The patient died of respiratory failure at 26 days old.
Perinatal hypophosphatasia is one of lethal skeletal displasias. It could be distinguished other severe forms of skeletal disorders with specific radiologic findings and low serum ALP levels. Currently, there is no known effective treatment for hypophosphatasia, however, various treatments have been attempted including zinc, magnesium and cortisone. Enzyme replacement therapy and bone marrow transplantation are acceptable promising therapeutic approaches.

PO2-067 Bone, Calcium II

Bone mineral density measured by quantitative ultrasound and bone turnover parameters in preterm infants

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Introduction: The aims of this study are to evaluate the role of quantitative ultrasound (QUS) in assessing the bone mineral density (BMD), and to analyze the relation between BMD and biochemical parameters in preterm infants.
Subjects and Methods: Serum levels of calcium, phosphorus, magnesium and alkaline phosphatase activity and plasma levels of 25-hydroxyvitamin D and urinary excretion of pyridinoline (Pyd) and deoxypyridinoline (D-Pyd) and speed of sound (SOS) of right tibia by QUS (Omnisense 7000P scanner) were measured at the first week and at term–corrected age in preterm 30 infants of gestational age below 34 weeks and within the first week in full-term healthy 25 infants.
Results: Although the mean SOS value of the term-corrected age infants was lower than term-matched healthy infants (2930±144.5 vs. 3017±107.8 m/s), the mean SOS values were not different between the term-corrected age and first postnatal week of the preterm infants (2994±137 vs 2930 ± 144.5 m /s; $p > 0.05$). The medians of serum Ca level and ALP activity were significantly higher at the term-corrected age than at the first week of delivery in preterm infants. Serum Mg [0.94 (0.7-1.1) vs. 0.84 (0.7-0.9) mmol/L, $p< 0.01$] and plasma 25OHD levels [23.7 (5.9-59.4) vs. 13.8 (6.2-57.2) mg/L, $p< 0.05$] and ALP activity [348 (146-1253) vs. 201 (87-340) U/L, respectively, $p< 0.01$], and urinary Pyd excretion (559±316 vs. 359±128, n=20, $p < 0.01$) were significantly higher, but SOS were lower at the term-corrected age in preterm infants than term healthy infants (2930±144.5 vs 3017 ±107.8 m /s; respectively, p< 0,05). Bone SOS was correlated inversely with ALP activity and Mg level and positively with gestational age (r: 0.31; $p <0.05$ and r: 0.48; $p < 0.01$).
Conclusion: Quantitative ultrasound is an easy to use and inexpensive tool for assessing BMD in preterm infants. BMD of preterm infants at term-corrected age was lower than term healthy infants. Bone SOS was inversely correlated to serum ALP activity and Mg levels.

PO2-068 Bone, Calcium II

Relationship between IGF/IGFBP system components and the macroscopic bone architecture in pediatric renal transplant recipients

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Background: The posttransplant bone disease in pediatric renal transplant recipients is characterized by an inadequately thin bone cortex in relation to muscular force (Rüth et al, Kidney Int 2004). A major hormonal modulator of periosteal growth is the IGF/IGFBP system. We therefore hypothesized that the reduced cortical thickness in these patients may be due to functional IGF deficiency.
Methods: 55 pediatric renal transplant recipients were investigated in a cross-sectional study. Bone mass, density, geometry, and strength of the radius as well as forearm muscle size and strength were analyzed by pQCT and by

hand-dynamometry. Serum levels of IGF system components were measured by specific radioimmunoassay; results were compared to 36 age- and gender-matched controls. **Results:** Standardized serum levels (SDS) of total IGF-I in patients (0.2±1.11 SDS) were comparable to controls (-0.26±0.93 SDS) as was IGF-II (patients, 1.16±0.78 SDS; controls, 1.33±0.87 SDS). Serum IGFBP-1 (33.7±29.7 *vs.* 27.7±17.4 ng/ml), IGFBP-4 (684±153 *vs.* 618±267 ng/ml) and IGFBP-5 (400±111 *vs.* 382±150 ng/ml) were not altered, while serum IGFBP-3 (4.60±1.1 *vs.* 3.66±0.82 mg/ml; $P<0.05$) and IGFBP-6 (34.3±24.5 *vs.* 12.5±4.8 ng/ml; $P<0.05$) were significantly increased. Age-adjusted cortical thickness was positively correlated with serum IGF-I ($r=0.407$, $P<0.05$).
Conclusions: The IGF/IGFBP system in pediatric renal transplant recipients is characterized by normal total IGF and increased IGFBP-3 and IGFBP-6 levels, resulting in a functional IGF deficiency. The positive correlation of IGF-I with cortical thickness underlines the importance of this hormonal system for the modeling of bone, particularly periosteal growth.

PO2-069 Bone, Calcium II

Diagnostic challenges in children with parathyroid adenomas: a case report

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A solitary parathyroid adenoma is rare in children and the diagnosis in pediatric patients can be difficult and often delayed. We report cases of 3 children ages 6 to 14 years with a solitary parathyroid adenoma who presented to our hospital with variable symptoms, laboratory studies and neck imaging results.
Case 1: A 14 year old female was admitted to the eating disorder unit for weight loss, bradycardia and hypotension associated with anorexia nervosa. Laboratory analysis revealed a calcium of 12.5 mg/dL, an ionized calcium of 1.63 mmol/L and a PTH of 159 pg/mL. She had a normal sestamibi scan, but a parathyroid ultrasound revealed a left parathyroid adenoma. She was then taken to the OR for removal of her adenoma. Post-op, she had transient hypocalcemia requiring IV calcium supplementation for 1 day.
Case 2: A 14 year old female presented to our office after hypercalcemia was discovered during work-up for a near-syncopal episode. Laboratory assessment revealed a calcium of 12.2 mg/dL, an ionized calcium of 1.53 mmol/L and PTH of 105 pg/mL. Both the parathyroid ultrasound and the sestamibi scan were normal. Due to persistently elevated calcium levels, she was taken to the OR and a left parathyroid adenoma was discovered and removed. She required calcium supplementation for only 1 day post-op, and her calcium levels have remained stable.
Case 3: A 6 year old male presented to the GI specialist with periumbilical abdominal pain and constipation for several months. Routine chemistry revealed a calcium of 12.9 mg/dL and an ionized calcium of 1.59 mmol/L. During hospital admission for work-up of his hypercalcemia, his calcium levels increased to 14.8 mg/dL. A parathyroid ultrasound revealed a parathyroid adenoma, though his sestamibi scan was normal. Due to persistently high calcium levels and increased symptomatology, he was taken to the OR and a solitary parathyroid adenoma was removed. Post-op he required supplemental calcium and vitamin D to maintain normal calcium levels. His pre-operative PTH level was elevated at 134 pg/mL.
There is a lack of studies in the non-invasive diagnosis of parathyroid adenomas in pediatrics. The sestamibi scan was normal in all three of the above cases and the parathyroid ultrasound was normal in one of the patients. These cases demonstrate the importance of maintaining a high degree of suspicion in diagnosing a parathyroid adenoma in a child.

PO2-070 Bone, Calcium II

Body composition and bone mineral density changes in adolescent girls with anorexia nervosa following weight and menstruation restitution

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Decreased bone mineral density (BMD) and changes in body composition, especially decreased fat mass, are important consequences of anorexia nervosa (AN). Weight and menstruation restitution are suspected to have a mayor role in bone health recovery in AN.
Aim of the study was to determine changes in body composition (fat and lean mass) and BMD (lumbar, hip, whole body) in adolescent girls with AN and secondary amenorrhea following weight and/or menstruation restitution.
Twenty adolescent girls (age 15.4 ± 0.3 years) with AN and secondary amenorrhea (SA) were studied at diagnosis and at the restitution of weight (10 % increase in BMI) and/or menstruation (> 3 menses in 6 months). Subjects were grouped into 3 groups (weight and menstruation restitution (W+M+), weight only restitution (W+M-) and no restitution (W-M-). Characteristics of girls at diagnosis didn't differ between groups. Body composition and BMD was determined by whole body DXA. Data are reported as means ± SE. Groups were compared using ANOVA.
At control groups didn't differ regarding duration of AN and time from the diagnosis. Groups W+M+ and W+M- compared to W-M- had significantly higher BMI (20.9 ± 1.2 and 19.1 ± 0.7 vs. 17.2 ± 0.7; p = 0.01), BMI SDS (0.0 ± 0.4 and -0.8 ± 0.3 vs. -1.3 ± 0.3; p = 0.04) and increase of BMI ratio (0.23 ± 0.01 and 0.22 ± 0.02 vs. 0.05 ± 0.02; p < 0.01). Increase in BMI was however not significantly different between groups W+M+ and W+M-. Restitution of weight (W+M+ and W+M-) was coupled with a significant increase in fat mass and fat mass ratio compared to W-M- (15.0 ± 1.3 kg (0.28 ± 0.01) and 11.4 ± 0.5 kg (0.22 ± 0.01) vs. 7.8 ± 0.6 kg (0.17 ± 0.01); p < 0.01 (p < 0.01)). Further, W+M+ had significantly higher increase in fat mass and fat mass ratio compared to W+M- (both p < 0.01). Lean mass remained non-significantly different between all groups. BMD and apparent BMD (aBMD) at control didn't differ significantly between groups at the lumbar spine, hip and whole body, neither was significant their change from the diagnosis of AN.
In conclusion, increase in fat mass in addition to increase in BMI correlates significantly with restitution of menstruation in AN adolescents with SA. However, despite weight and menstruation restitution no significant increases in BMD and aBMD were observed at the lumbar spine, hip or whole body.

PO2-071 Bone, Calcium II

Do bone mineral density, bone geometry and the functional muscle-bone unit explain bone fractures in healthy children? A study using peripheral quantitative computed tomography (pQCT)

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Fractures are very common in otherwise healthy children but fracture incidence is increasing. Previous studies used Dual-Energy X-ray Absorptiometry (DXA) to investigate the association between fracture risk and skeletal health in this age group showing an inverse correlation of areal bone mineral density (BMD) with fracture risk. In contrast to DXA, computed tomography measures true volumetric bone mineral density (vBMD) and reveals the three-dimensional bone geometry. In the present study pQCT was used to compare vBMD, bone geometry, and parameters reflecting the muscle-bone interaction to prove the hypothesis that alterations in bone mass and the impaired adaptation of bone strength to muscle forces explain the increasing fracture incidence.
The forearm of 220 healthy individuals (mean age 11.1 ± 3.2 yrs) was analyzed by pQCT (Stratec XCT 2000). Bone mineral content (BMC), vBMD, periosteal circumference (PC), cortical area (CA), strength strain index (SSI), muscle area

(MA), and grip force (GF, Jama dynamometer) were measured at the non-dominant forearm (pQCT, distal (4%) and proximal (65%) radius). Fracture history was assessed by questionnaire after a period of 5 ± 1.7 yr. Logistic multiple regression modeling was used to assess the influence of various independent variables on fracture risk.
During the observational period at least one fracture appeared in 78 children (35.5%). Individuals with and without fractures were not different in age, height, weight, and body mass index. BMD, BMC, and bone geometry as well as parameters describing muscle mass and muscle/bone interaction (Table) were not different between the groups.

	Male with fractures (n=44)	Male no fractures (n=64)	p value	Female with fractures (n=34)	Female no fractures (n=78)	p value
GF (N)	221±120	204±123	0.30	175±81	173±86	0.88
MA65% (mm^2)	2678±851	2497±889	0.15	2276±582	2182±576	0.47
BMC65% /MA65% (mg/mm^2)	2.5±0.3	2.5±0.33	0.61	2.7±0.4	2.7±0.3	0.77
SSI65%/ MA65% (mm)	7.8±1.5	7.6±1.3	0.60	7.7±1.7	7.6±1.6	0.79
PC65%/ MA65% (mm/mm^2)	1.4±0.3	1.5±0.4	0.20	1.6±0.4	1.6±0.4	0.59

Our results suggest that fracture risk in otherwise healthy children is not sufficiently explained by volumetric BMD, the skeletal phenotype and indices of the functional muscle-bone unit. These observations are in contrast to the results of other studies where DXA has been used to assess skeletal health.

PO2-072 Bone, Calcium II

Unveiling osteopenia in children with congenital adrenal hyperplasia (CAH): the use of bone age to re-index bone mineral density by DXA

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CAH (incidence worldwide 1/14,000) is the most common adrenal disorder in children and is due (>90%) to a mutation in the 21-hydroxylase gene(21OHD) that results in diminished/absent cortisol production and life-long glucocorticoid (GC) replacement at supraphysiologic levels.
Hypothesis: To determine if children with CAH are at risk for GC-Induced Osteoporosis (GIO).
Background: Studies of bone mineral density (BMD) in adults with CAH find increased risk of osteoporosis with age and GC dosing, but reports of BMD in growing children are less consistent. This is likely due to the often marked discrepancy between bone age (BA) and chronologic age (CA) in CAH. Although androgen excess in CAH may augment BMD, it also advances BA maturation relative to CA, thus yielding a theoretically "false normal" Z-$score_{CA}$ compared to a relatively "low" BMD Z-$score_{BA}$. The key component of this study is the re-indexing of Z-scores using BA (vs CA, HA (height age)).
Subjects: 13 Children as Research Subjects between 4-12 years of age and BA <14 years with the diagnosis of 21OHD CAH who had not completed linear growth and were ≤Tanner Stage II were enrolled after Assent/Consent was obtained in this IRB/CTRC-approved study conducted at UNC. There was no discrimination with regard to gender or ethnicity.
Methods: All children underwent a DXA scan (QDR, L1-L4, femur/hip; Hologic 12.3 software) for BMD and a BA radiograph (Greulich and Pyle). The critical analysis focused on BMD obtained by DXA scan for BMD Z-score indexed by CA, BA and HA.
Results: We found that the Z-score for the BMD re-indexed by BA is below the Z-score calculated using CA (ΔZ_{CA}-Z_{BA}). For example, the spine ΔZ_{CA}-Z_{BA} is -0.5 to -1.0 SD in 5/13 children; >-1.0 SD in 1/13; and ≥ -1.5 SD in 3/13. The absolute Z_{BA} was <-1.0 in 4/13 and ≤ -1.5 in 2/13 (osteopenia). (Note: A -1.0 delta Z-score correlates with ~1.5-2-fold increase in fracture rate).
Conclusion: The interpretation of BMD for a child with CAH depends on the "age" value used to determine the Z-score. Since the Z-score is a tool by which to assess potential risk for an individual relative to his/her peers, re-indexing may provide a valuable means to identify those children with CAH, and other conditions with advanced BA, for whom prophylaxis or treatment regimens for diminished BMD should be initiated.
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PO2-073 Bone, Calcium II

Normal peak bone mass in female rhythmic gymnasts during puberty: a role for leptin?

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Hypoleptinaemia has been reported in female peripubertal athletes. However, data is lacking on the variations in leptin and bone mass according to pubertal stage and the potential effect of leptin on bone mineralisation during this period. The aim of this study was to investigate the variations in leptin level and bone mineral density (BMD) in young elite female rhythmic gymnasts (FRG) according to the pubertal stage of development. In addition to leptin, IGF-1 and its binding protein (IGFBP-3), as well as sex hormones, were evaluated for their effects on bone mineral acquisition.
Plasma leptin levels were analysed in 43 FRG [mean age: 13.3±1.8 yr, range; 10.6-17.2; mean body mass index: 17.52±1.85 kg/m², range: 13.0-20.6; training volume range: 17.9-23.8 hours/week] according to pubertal stage (Tanner I: n=7; II: n=10; III: n=9; IV: n=8; V: n=9). IGF-1, sex hormones and bone biochemical markers (osteocalcin, bone alkaline phosphatase, type I-C telopeptide breakdown products) were also evaluated. BMD was measured by dual-energy X-ray absorptiometry at various bone sites.
Plasma leptin levels increased throughout pubertal growth and the values measured in Tanner stages IV and V (12.1±4.5 and 13.2±6.5 ng/ml) were significantly higher ($p<0.001$) compared with stages I and II (3.9±2.1 and 2.9±2.4 ng/ml). A gain in BMD was measured at all sites, particularly between stages II and IV. Although the prevalence of delayed menarche or menstrual disturbance was high in FRG, the T-scores for BMD appeared normal at Tanner stage V. Simple correlation analysis revealed that the BMD measured at all bone sites was significantly and positively correlated ($p<0.05$ to $p<0.001$) with plasma leptin levels, age, BMI, IGF-1, oestrogen and testosterone and negatively correlated with bone markers. However, multivariate analysis using a linear regression model by block (including age, anthropometric data and biological parameters) was performed to determine the factors independently associated with each BMD site, and only age, body fat mass and fat-free soft tissue remained independent predictors.
In FRG characterised by high training volume and low fat mass, plasma leptin levels increased throughout puberty, partially related to body composition changes. Despite the simultaneous increases in plasma leptin and BMD during pubertal growth, it was not possible to differentiate the leptin impact on bone independently from anthropometric parameters.

PO2-074 Bone, Calcium II

Hypercalcemia in infants with 1 alpha hydroxylase overactivity: case reports

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Hypercalcemia with elevated $1.25(OH)_2D$ plasma levels without any etiology such as skin aseptic necrobiosis or sarcoidosis, appears to be a specific entity. Eight cases were reported : 6 asymptomatic newborns and 2 symptomatic children with severe hypercalcemia. Familial occurrence was noted : 2 brothers; a son and his young mother.
Calcemia was elevated up to 4 mmol/l, plasma level of $1.25(OH)_2D$ was up to 500 pM/l (N 60-120), PTH was low, 25(OH)D in normal range, calciuria was elevated. Nephrocalcinosis was observed in the 2 symptomatic children.
Two infants were given Ketoconazole, Vitamin D supplementation was stopped and a low calcium diet was decided for the other cases.
Normally calcemia generally improves with age; an increase of both calcemia and calciuria was observed after summer time.
Theses cases suggest an 1 alpha-hydroxylase overactivity whose origin and genetics are currently under investigation.

PO2-075 Bone, Calcium II

Evaluation of bone mineral metabolism in patients with severe *osteogenesis imperfecta* treated with disodium pamidronate: the role of 25-OHVitamin D

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Introduction: The improvement of the bone quality of pediatric patients with severe osteogenesis imperfecta (OI) depends on the treatment with disodium pamidronate (DP) and of the suppression of plasma PTH to values lesser than 30pg/mL through the maintenance of plasma 25-OHVitamin D (calcidiol) values above 30ng/mL and the adequate calcium intake.
Aim: This study aimed to evaluate the bone mineral metabolism in patients with severe OI and the influence of the age on this metabolism.
Patients and methods: We enrolled 29 patients (16 girls) with severe OI. The laboratory investigation was performed 1 month after the cycle of DP and included the evaluation of plasmatic concentration of PTH, calcidiol, total calcium(TCa), ionized calcium(ICa), phosphorus(P), alkaline phosphatase(AP) and of calcium/creatinine ratio in urinary sample (UCa/Cr). The correlation between the variables and the comparison of the mean in patients younger and older than 3years-old were studied through Pearson's correlation coefficient and Student's t test, respectively. P-values <0.05 were significant.
Results: The age during the cycles varied between 0.16 and 16.32y. The mean number of cycles per patient was 5.9±3.2. There was a significant correlation between PTH-calcidiol(r=-0.27;p=0.009), PTH-TCa(r=-0.33;p=0.002), PTH-P(r=-0.25; p=0.021), PTH-UCa/Cr(r=-0.24;p=0.048) and calcidiol-P(r=0.28;p=0.009). The mean in patients younger and older than 3years-old were, respectively: PTH:21.74±27.83/28.50±18.23pg/mL; calcidiol:30.66±12.3/22.39±6.46ng/mL, TCa:10.01±0.45/9.57±0.48mg/dL, ICa: 1.33±0.07/1.30±0.05mmol/L, P:5.73±0.7/5.33±0.56mg/dL and UCa/Cr:0.28±0.22/0.19±0.20. There was a significant difference between the mean of calcidiol(p=0.0010), TCa(p=0.0001), ICa(p=0.0202) and P(p=0.0103), with higher values in patients younger than 3years-old. In the younger than 3years-old 43% and 84.3% of the patients had plasma values of calcidiol and PTH above 30ng/mL and lesser than 30pg/mL, respectively. In those older than 3years-old these values were 8.6% and 53%.
Conclusion: In our patients with severe OI the calcidiol contributed to the reduction of plasma PTH and to the increase in calcemia and phosphatemia, favouring the bone mineral metabolism. In these patients the monitoring of calcidiol allows the recognition of those that will benefit from the treatment with vitamin D. This care should be specially observed on those patients older than 3 years-old.

PO2-076 Bone, Calcium II

Decreased fracture rate and bone turnover after pamidronate infusion in children with cerebral palsy and severe osteoporosis

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Children with cerebral palsy often suffer from severe osteoporosis and fractures after low-density traumas. We evaluated pamidronate effects on fracture rate, bone mineral density and markers in 29 patients (15 M, 14 F) with a median age of 12,8 y (1,5-27) having cerebral palsy (congenital, n=22; acquired, n=7) referred to our department for osteoporosis with fracture(s) or pain. CT scan bone density showed a median Z score at -3, 25 (ranging -6 to 0,3 n=17). The femur was the main site of fracture (37/62).
Risks factors for osteoporosis were: absence of walking; low body mass index; sodium valproate therapy (n=10) or phenobarbital (n=1); delayed puberty or primary amenorrhea in 7/13; low calcium intake in 13/20. 16/29 received systematic vitamin D supplementation, thus had normal 25-OH-D and PTH levels. All children had increased urinary calcium or D pyridinoline excretion.
With an average pamidronate dose of 1, 4 mg/kg/3 months (range 1 to 8.8 mg/kg for 1 to 5 cure/year), the incidence of fracture decreased from 0.75 fractures. patient^{-1}.6 months^{-1} to 0.6 at M6 (n=21) ($p<0.005$) and to 0 between M6 and M12 (n=11) (p=0.02). Markers of bone resorption decreased (urinary calcium/creatinine: from 0.5±0.5 to 0.37±0.05; urinary D-pyridinoline/creatinine: from 151 to 117% of the normal age value). Side effects were fever at first infusion (5/29) and benign digestive symptoms (5/29).
Summary: Beside the decrease in bone turn-over markers, we observed a significant effect of low doses of pamidronate (~ 5.4 mg/kg/year) on fracture rate. Pamidronate appears as an effective and safe treatment in children with cerebral palsy, osteoporosis and fractures. The correction of associated risks factors is necessary.

PO2-077 Bone, Calcium II

Metacarpal thickness, width, length and medullary diameter in children: a sudden deceleration at age 7 to 11

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This paper presents and explores a reference database for metacarpal thickness (T), width (W), length (L) and medullary diameter (M) in children. 3121 left-hand X-rays (1661 from boys) from 231 healthy Caucasian subjects (119 boys) in the age range from 3 to 19 yrs were analysed by BoneXpert, a programme for fully automatic analysis of hand X-rays and assessment of bone age (BA, in yrs). In boys growth of T ($p<0.0001$, $p<0.0001$), W ($p<0.0001$, $p<0.0001$) and L ($p<0.0001$, $p<0.0001$) show a prepubertal decrease from BA 7 yrs until BA 13 yrs and then accelerates again. In girls the same is seen only for T starting at BA 8 yrs ($p<0.0001$, $p<0.0001$), whereas W and L show a progressive decline in growth rate ($p<0.0001$, $p<0.0001$). M shows steady growth until BA 10.5 and chronological age (CA, in yrs) 11 in girls and BA 13.5 (CA 13.5) in boys followed by a decrease in both. W is greater in boys than in girls from BA 6 onwards, while L is intermittently greater in girls from BA 9 to 13 ($p<0.0001$) and T likewise from BA 11 to 14 ($p<0.004$). BA is reflected best by L until puberty and by T and L during puberty. This reference data could be useful for further research into skeletal development and the management of hormone therapies in children. In conclusion, T, W, L and M show highly differentiated growth patterns, with sudden changes at around age 7 years and at puberty.

PO2-078 Disorders of Sexual Differentiation (DSD) I

Disorders of sex development: developing a multidisciplinary research infrastructure for the study and enhancement of health-related quality of life outcomes

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Disorders of sex development (DSD) are rare "congenital conditions in which chromosomal, gonadal, or anatomic sex development is atypical." ("Consensus Statement on Management of Intersex Disorders;" Pediatrics 2006;118; e488-e500. DOI: 10.1542/peds.2006-0738.) Previous workshops bringing together DSD researchers have identified gaps in clinical research agendas. Only a small cadre of outcomes researchers work in this area, and the little existing research on psychosocial and health-related quality of life has focused exclusively on diagnostic and phenotypic predictors of gender related outcomes, neglecting a broad range of outcomes and the potential impact of social environmental factors on these outcomes. In April 2009, we convened the *DSD Research and Quality Improvement Symposium.* Participants represented diverse stakeholders with varying experiences/perspectives, including, researchers, healthcare providers from interdisciplinary DSD clinical management teams, advocacy organizations, and patients and families affected by DSD. Our goal was to create an agenda for research and clinical care for persons affected by DSD to guide recommendations for future research and learning. Results to be shared include discussion of (1) Strategies to ensure representation of key stakeholder groups, including workshop format to ensure active engagement of all participants; (2) Strategies for developing and supporting a research consortium that will facilitate interdisciplinary collaboration; (3) Consensus on research priorities, including research necessary to enhance diagnosis, healthcare delivery, and outcomes of patients with DSD and their families; and (4) Current barriers/gaps in research and healthcare delivery (i.e., limited data, absence of consistently-applied standards in the diagnosis and treatment of DSD, gaps in research methodologies, issues of informed consent, etc.). The symposium helped inform the research process through facilitated interactions among researchers, healthcare professionals, and patient stakeholders. The integrated involvement of these constituencies will increase the likelihood that recommendations and initiatives flowing from the symposium will promote a balanced strategy. With participation of emerging multidisciplinary DSD teams, we created potential for a multi-site patient registry for DSD and opportunities to conduct prospective, longitudinal studies to examine medical and health-related quality of life outcomes.

PO2-079 Disorders of Sexual Differentiation (DSD) I

Recurrent HOXA5 promoter methylation in patients with androgen receptor gene mutations: first insights into the role of epigenomics in DSD

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Background
Male external genital differentiation is accompanied by implementation of a long term, male-specific gene expression pattern indicating androgen programming in cultured genital fibroblasts. We hypothesized the existence of an epigenetic background contributing to these observations.
Methods and Results
Screening of normal male scrotal - and labia majora fibroblast strains from 46,XY females with complete androgen insensitivity syndrome (AIS) due to androgen receptor (AR) mutations by Illumina Golden Gate ® methylation arrays exposed HOXA5 with significantly higher methylation in complete AIS. This was confirmed by pyrosequencing of 14 CpG positions within the HOXA5 promoter in these individuals. Moreover, analysis of 9 different normal male scrotum strains revealed a highly conserved HOXA5 methylation pattern with low HOXA5 methylation. In contrast, 14 different AIS individuals showed a highly variable methylation pattern with approximately half of the group showing significantly higher methylation than the control group. Individual HOXA5 methylation did not correlate with the degree of virilization. However, microarray gene expression analyses depicted significantly lower mRNA expression levels of 17 transcripts, namely PDLIM5 and KRT18 in only those AIS individuals with high HOXA5 methylation.
Conclusion:
The conservation of a low HOXA5 methylation pattern in normal males and its striking switch to extreme variability and high methylation in AIS suggest that low HOXA5 methylation may be androgen-mediated and part of a relevant embryonic sexual differentiation program. In the light of the variable genotype-phenotype relationship in AIS, the exclusive existence of high HOXA5 methylation in AIS but the absence of a clear-cut correlation of HOXA5 methylation with the AIS phenotype supports a polygenetic origin of phenotype modification in AIS.

PO2-080 Disorders of Sexual Differentiation (DSD) I

Absence of *NR5A1/SF1* mutations in 46,XX patients with familial primary ovarian failure

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Primary ovarian faillure (POF) is a condition characterized by a continuum impairment of ovarian function in women who are less than 40 years old. In the great majority of the cases the cause of POF remains unknown. A genetic basis of POF is supported by familial cases and for the description of inactivating mutations in several genes as *BMP15, FOXL2, POF1B, FSHR*. Recently, mutations in *NR5A1/SF1* have been identified in familial and sporadic cases of 46,XX POF (1). *NR5A1/SF1*, a nuclear receptor, is a key transcriptional regulator of genes involved in the hypothalamic-pituitary-steroidogenic axis and has an important role in gonad development. Mutations in *NR5A1* cause impairment of testis development accompanied or not by adrenal failure. The four families in which mutations in *NR5A1* were identified presented both 46,XY disorders of sex development and 46,XX POF, confirming the key role for this factor in testis and ovarian development and function. **Material and methods:** We studied 5 families (11 patients) that presented POF and one family with a 46,XX patient with POF and her sister with 46,XY complete gonadal dysgenesis. The entire coding region (exons 2-7) and splices site regions of *NR5A1* were PCR amplified and sequenced directly using a BigDie Terminator in ABI PRISM 3100 DNA sequencer. **Results:** The nonsynonymous variant p.Gly146Ala was identified in heterozygous state in two families. This already described polymorphism (c.437G®C) did not affect protein expression or stability although this variant had approximately 80% of the wild-type protein activity (2). None *NR5A1* mutations was identified in the remaining families including the family with the 46,XX and 46,XY patients.
Conclusion: *NR5A1* mutations are associated with a large spectrum of impaired ovarian, testes and adrenal development and function. Although the identification of *NR5A1* mutations represents an important step to elucidate the etiology of ovarian failure, other genetic cause of familial POF remains to be

identified.

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PO2-081 Disorders of Sexual Differentiation (DSD) I

MAMLD1 (mastermind-like domain containing 1) homozygous gain-of function missense mutation causing 46,XX disorder of sex development (DSD) in a virilized female

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Premature ovarian failure has been described in a heterozygous female for a microdeletion involving *MAMLD1*. We aimed to screen *MAMLD1* mutation in 46,XX DSD patients. Patients: 14 patients with 46,XX DSD due to gonadal dysgenesis were studied. Mutations in the coding region of *FOXL2, BMP15, STRA8, Nanos1, Nanos2* or *SF-1* were rule out. One of these patients presented an unexpected clitoral enlargement and had a normal *WnT4* coding region. She was 44 yr old, born from first cousin parents and had primary amenorrhea and failure of breast development. She had eunuchoid habitus and absence of hirsutism. Her height was 175 cm and Tanner IV pubic hair. External genitalia: clitoromegaly (4.2 cm), two perineal openings and unpalpable gonads. Basal hormonal levels: elevated FSH levels and normal LH, progesterone, 17OH-progesterone, androstenedione and testosterone levels (47 ng/dL) which did not increase after hCG stimulation (44 ng/dL). Laparoscopy: bilateral streak gonads, small uterus and bilateral fallopian tubes. Anatomopathologic findings: absence of left gonad, Fallopian tubes and a dysgenetic right gonad with hilars cells hyperplasia and persistence of wolffian rests. **Methods**: The entire coding region and the splicing sites flanking regions of *MAMLD1* were sequenced. Transactivation function of *MAMLD1* was analyzed by COS-1 transiently transfected with the reporter vectors p-Hes3-luc and p-Hes7-luc, the expression vectors WT and mutant *MAMLD1* and pRL-CMV vector as an internal control. Results: The mutation p.V432A, previously described in heterozygous state in a 46,XY DSD patient, was identified in homozygous state in the patient with clitoromegaly. This mutation localizes at exon 3 into the glutamine rich domain; it was absent in 190 normal alleles. Significantly higher transactivation activity than WT for p-Hes3 –luc (1.8-fold) and p-Hes7-luc (1.5-fold) was found **($p<0.05$). Discussion:** Molecular function of MAMLD1 remains unknown although it transactivates the promoter of a noncanonical Notch target gene Hes3 without demonstrative DNA-binding capacity. The virilization of this 46,XX DSD patient associated with the presence of hilar cells suggest an underexpression of *WnT4* or an overexpression of *DHH* but the relationship of these pathways with *MAMLD1* remains to be determined.**Conclusion**: We described the first homozygous gain-of-function mutation in MAMLD1 causing 46,XX DSD in a virilized female, indicating that this protein is involved in ovarian development.

PO2-082 Disorders of Sexual Differentiation (DSD) I

Gonadal pathology and tumor risk in relation to clinical characteristics in patients with 45,X/46,XY mosaicism

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Introduction: The decision to perform gonadectomy in patients with 45,X/46,XY mosaicism is often difficult: the increased gonadal tumor risk has to be balanced against the functional capacity of the gonad and the psychological burden of gonadectomy.

Methods: We combined detailed pathology investigations with clinical data of 27 45,X/46,XY individuals (44 gonadal specimen), divided into 3 groups according to the external masculinisation score (EMS). Differentiation of gonadal tissue was studied by morphology, and staining for AMH, FOXL2 and SOX9. Tumor risk was assessed based on gonadal differentiation, and the presence of malignant or premalignant (staining for OCT3/4 and/or stem cell factor) changes. In males with at least one gonad *in situ*, results of an HCG test or data on pubertal progression were recorded.

Results: Testis tubules are SOX9 positive and FOXL2 negative. In undifferentiated gonadal tissue (UGT), co-presence of SOX9 (Sertoli cell differentiation) or FOXL2 (granulosa cell differentiation) is common, with a predominance of FOXL2. No SOX9 or FOXL2 is found in streak gonads.

In individuals with EMS≥7, indices for (pre)malignancy were found in 1/13 gonads (7.6%), whereas in individuals with EMS<7, these indices were present in 8/14 gonads (57%). However, in girls with unambiguously female genitalia, indices for (pre)malignancy were not encountered.

Table 1

	#p	#g	Position	Differentiation	GC	(Pre)mal
			abd/ing/lsc/u	T/T+UGT/UGT/S/O/V		
EMS≥7	9	13	5/2/5/1	9/0/1/1/0/2	7	1
EMS<7	9	14	9/3/0/2	3/3/2/3/0/3	8	8
Female	9	17	17/0/0/0	0/0/0/13/0/4	0	0

EMS: external masculinisation score; #p: number of patients; #g: number of gonads; GC: number of tissue specimen in which germ cells are present; (pre)mal: number of tissue specimen in which (pre)malignant changes are present; abd: abdominal; ing: inguinal; lsc: labioscrotal; u: unknown; T: testis, UGT: undifferentiated gonadal tissue, S: streak; O: ovary; V: vanished

Outcome data in 8/13 males with at least one testis revealed moderate to good gonadal function.

Conclusions: The morphology of UGT is associated with the co-presence of SOX9 and FOXL2 positive cells, normally leading to testicular or ovarian differentiation respectively. In 45,X/46,XY persons with ambiguous genitalia, the EMS may be inversely correlated with tumor risk, in phenotypical females, this risk is very low. Testis function is at least partially preserved in 45,X/46,XY males.

PO2-083 Disorders of Sexual Differentiation (DSD) I

Advanced pubertal development in two adolescents with new SF-1 mutations

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Introduction: SF-1 is involved in gonadal and adrenal development, sex differentiation and steroidogenesis. 14 different SF-1 mutations have been described in 15 46,XY DSD patients. Often, a striking discrepancy between a severely undervirilised phenotype and intact testicular architecture and Wolffian duct development is noticed. Most patients have undergone early gonadectomy, so very little is known about testicular function during puberty.
Methods: We present detailed hormonal and pathological studies in two adolescents, with hitherto undescribed SF-1 mutations and advanced pubertal development.
Results: Case 1 is a phenotypical girl referred at the age of 13 because of severe virilisation in the past year. The karyotype was 46,XY, levels of FSH and LH were elevated and testosterone was in the normal male pubertal range. A heterozygous deletion of the first two exons of the SF-1 gene was identified. Gonadectomy specimen showed a left inguinal testis with Leydig cell hyperplasia, normal number of germ cells and incomplete spermatogenesis, and a right abdominal testis with scarce germ cells displaying premalignant changes. Case 2 is a 14 year old 46,XY boy, born with ambiguous genitalia. Initially, a female gender was assigned, but later, he was reassigned male after evaluation by a specialised pediatric urologist and psychologist. A heterozygous SF-1 mutation, introducing a stop codon in exon 1 was found. At the age of 13, bilateral testicular biopsies showed normal testes with normal number and maturation of germ cells. Spontaneous pubertal development started at 13.5 years, and clinical progression through puberty is hitherto normal. The pubertal rise in testosterone is accompanied by a modest increase in FSH. Penile length is increasing, gender identity is male. An attempt to preserve fertility by cryopreservation of ejaculated sperm or TESE will be performed in the near future.
Conclusions: SF-1 mutations present with wide phenotypic variability. However, a marked discrepancy between severe undervirilisation of external genitalia at birth, leading to female gender assignment in most cases, and the presence of intact testicular architecture and Wolffian structures is frequent. Despite failure of external virilisation during the fetal period, pubertal testis function and spermatogenesis seem to be partially conserved, at least in some patients. As in other forms of DSD, (pre)malignant changes may be present, especially in non-scrotal testes.

PO2-084 Disorders of Sexual Differentiation (DSD) I

Hormone profiles in adolescent and adult patients with complete androgen insensitivity syndrome (CAIS)

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Background: Although puberty related reference ranges for serum gonadotropins and sex steroids are available, there is uncertainty regarding reference ranges of hormone values in CAIS patients. Adolescent and adult patients with CAIS and gonads retained in situ show serum testosterone concentrations in the normal male reference range. Luteinising hormone (LH) is elevated above the normal range. Aromatization of androgens to estrogen results in breast development during puberty. Due to an increased risk of malignant degeneration gonadectomy with consecutive exogen estrogen administration has often been performed. Therefore data about normal values of gonadotrophins and sex steroids in postpubertal CAIS patients are rare.
Patients: Hormone profiles (testosterone, estradiol, sex-hormone-binding globulin SHBG, LH, follicle-stimulating hormone FSH) were studied in twelve patients with proven mutations in the androgen receptor (AR) gene. At that time patients were aged 15 to 47 years (median = 18) with a breast development corresponding Tanner stadium 5 with absent (PH1) or sparse (PH2) pubic hair. One patient of Italian origin showed Tanner stadium PH3. Gonadopexy had been performed in one patient, in the others the gonades had remained in situ.
Results: Basal testosterone values of 2.2 – 8.8 µg/l were within normal puberty related reference range for males (Tanner 5). Estradiol values (23.3 – 46.4 pg/ml) were measured within the normal male range for Tanner 5 or female range for Tanner 3. At the same time LH values (13.4 – 27 IU/l) were elevated above puberty related reference ranges with normal FSH values (2 – 11 IU/l) except for one 47-year-old patient who showed an increase in FSH to 25.6 IU/l with an LH of 27 IU/l. SHBG measurement (mean 54.4 nmol/l, range 27 – 96 nmol/l) was in the upper third of male or lower female reference range respectively.
Conclusion: The twelve patients show a specific hormone profile. Further studies of a higher number of patients might be able to reveal CAIS-specific reference ranges for gonadotropins and sex steroids.

PO2-085 Disorders of Sexual Differentiation (DSD) I

Male genital anomalies: epidemiology and influence of endocrine disruptors

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Background: With the increasing use of environmental chemicals that may be detrimental to normal male genital development in utero, an attempt was made to establish the prevalence of male genital anomalies in full term neonates and whether there is an influence of prenatal parental exposure to potential endocrine disrupting chemicals (EDCs) on these parameters.
Methods: A sample of 1000 male full term newborns was studied. A structured questionnaire gathered information on: age of parents, residence, occupation of the parents, contact with insecticides and pesticides and their type and frequency of contact, maternal exposure to cigarette smoke during pregnancy, maternal drug history during gestation, family history of hypospadias, cryptorchidism or other congenital anomalies, intake of foods containing phytoestrogens e.g. soy beans, olive oil, garlic, hummus, sesame seed and their frequency and history of IVF or infertility.
Results: The birth prevalence of genital anomalies was 1.7%; i.e. 17/1000 live birth. Hypospadias accounted for 88% of the cases. There was a significantly higher rate of anomalies among those who were exposed to endocrine disrupters when compared to non exposed newborns (7.4% versus 1.3%), $p<0.0001$, with an odds ratio of 6 (95% CI 2 - 16).
Conclusion: This study adds to the several epidemiologic studies that environmental factors are a possible cause for the observed increased incidence of abnormalities in male reproductive health

PO2-086 Disorders of Sexual Differentiation (DSD) I

Clinical course of micropenis associated with IUGR: a case report

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Disorders of male sexual development have an identifiable cause in approximately half of the cases leaving a large number of infants undiagnosed. Hypospadias has been associated with lower birth weights compared to controls. There is also increasing evidence of an association between infants with disorders of male sexual development and low birth weight. In a small

group of patients with unexplained micropenis one study showed testosterone administration resulted in definite penile growth (>40mm). We report an infant with severe intrauterine growth restriction (IUGR) and associated ambiguous genitalia with no other identified cause, who demonstrated significant spontaneous penile growth corresponding with general growth.
The infant was born at 30 weeks gestation. Amniocentesis demonstrated a 46XY fetus. Birth weight was 630 grams, in keeping with severe IUGR. Birth length was 30.7cm (<<3rd %). Physical examination revealed a wasted infant with severe hypospadias, and micropenis. The penis was 7mm, which was less than 2 standard deviations below the mean (-2 SD = 17mm). Testicles were palpable within the inguinal canals. Electrolytes were normal. Testosterone and DHT levels were normal suggesting normal steroidogenesis. At 2 months chronologic age LH and FSH were both normal at 4 U/L and 2 U/L respectively. MIF was normal making partial androgen insensitivity unlikely. An ultrasound showed no evidence of female reproductive organs. Repeat karyotype on lymphocytes was 46XY and a FISH probe against the SRY locus showed a normal, single signal. As he grew the penis grew to almost triple the initial length. At 6.5 months corrected age our patient's measurements were as follows: weight 6.17kg (<3rd %), length 63cm (3rd %), and penile length 2cm (<2.5 SD). It was decided to treat our patient with four monthly injections of Testosterone Enanthate 25mg both for treatment of micropenis and for easier surgical repair. Post therapy penile measurement (length 2.4cm) resulted in fair response.
This case demonstrates that significant spontaneous penile growth can be seen in infants with severe IUGR and micropenis. Testosterone treatment may still be desired to assist in easier surgical repair of associated hypospadias, however in our case the added benefit of this therapy was small.

PO2-087 Disorders of Sexual Differentiation (DSD) I

Promotor-specific dependency on the N/C-terminal interaction of the human androgen receptor – a reexamination

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Objective: The NH_2- and COOH-terminal (N/C) interaction of the human androgen receptor enhances transactivation in-vitro and stabilizes hormone binding. We analyzed whether this N/C-terminal interaction is crucial in the transactivation process of certain androgen-dependent promoters.
Methods: We selected two different mutations in the AR gene abolishing N/C-terminal interaction in two-hybrid assays, the naturally occurring mutation L712F and the artificial mutation ^{23}FQNAA^{27}, which derives from the ^{23}FQNLF^{27}-motif essential for N/C-interaction. These two mutations and their combination (^{23}FQNAA^{27} and L712F) were introduced into AR expression plasmids. Transfection into CHO/HeLa cells was performed using probasin-, $(ARE)_2$-TATA- and MMTV-promotors in front of luciferase reporter genes with increasing levels of DHT.
Results: The mutant L712F showed a constant transactivity of approximately 60% of wt transactivity at 10 nM DHT in all performed studies. This contrasts with the results obtained for the mutation ^{23}FQNAA^{27}. AR transactivation of the MMTV-promotor was similar to the mutant L712F but induction of both the probasin- and the $(ARE)_2$-TATA-promotor was significantly lower. The double mutation (^{23}FQNAA^{27} and L712F) lead to a stable reduction of transactivation of all used promotor constructs to 20-40% of wt transactivity.
Conclusion: The obtained data questions the classification of androgen-dependent promotor genes into N/C-dependent or non-N/C-dependent. The results for the two mutations differ in dependency of the promotor although both completely abolish N/C-terminal interaction. For the L712F mutant no difference in transactivation of an N/C-dependend (probasin) and non-N/C-dependent (MMTV) promotor could be detected. We assume that the differences in AR transactivation derive from a mutant-modified interaction with promotor-specific AR-coactivators.

PO2-088 Disorders of Sexual Differentiation (DSD) I

Ovotesticular disorder of sex development DSD: evaluation of 27 patients

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Background:
Ovotesticular disorders of sex development (OT-DSD) in humans are defined as the simultaneous presence of both testicular and ovarian tissue in a single individual.
Aims: to provide an extensive review of patients collected with OT-DSD showing clinical findings, cytogenetic studies and molecular analysis, diagnostic tools and treatment from 17 years experience at the Diabetes, Endocrine and Metabolism Pediatric Unit Cairo University.
Patients and methods:Full clinical examination, cytogenetic study, hormonal, laparoscopic, histological investigations, and surgical intervention of twenty-seven OT-DSD were performed wherever possible, as well as the decision about the sex of rearing.
Results:Median age of patients with OT-DSD at first presentation was 2 years. Meanwhile, genital ambiguity was the most frequent complaint (77.7%) followed by gynecomastia in 14.8% and inguinal mass in 7.4% -

Age group	No of cases (%)	Presentation	Sex of rearing n(%)Male sex	Sex of rearing n(%) Female sex	Re-assigned sex male	Re-assigned sex females
0-2 years	14 (51.85)	Ambiguous genitalia	10	4	1	1
2.1to12 years	9 (33.3)	• Ambiguous genitalia• Right inguinal mass•	7	2	1	0
Above 12years old	4 (18.8)	• Gynecomastia • Peviabdominal mass	4	0	0	0
Total	27 (100)		21(77.78	6(22.2)		

The most frequent karyotype was 46,XX in 77.68.% of patients, followed by 46 XO/XY, 46XY, 46XX/XY. SRY was negative in all 46,XX. The prevalent gonad was ovotestis (OT-37.%), followed by ovary (OV-31.48.%) and testis (TT-27.7% and the prevalent gonadal associations were OV+TT (40.74%), OT+OV (25.9%), OT+TT (18.5%) and OT+OT (14.8%). There was preference of male sex of rearing despite a severe degree of genital ambiguity. Two cases were reassigned as females at the age of 1.25 and 3 years, while one case reversed sex to male at the age of 2 years. Finally our cases assigned as 20 males and 7 females regardless of karyotyping. Moreover, two male patients were found to have dysgerminoma and gonadoblastoma at the age of 17 and 14 years old.
Conclusions: OT-DSD is a phenotypically and genetically a heterogeneous condition. Early diagnosis and sex assignment are essential to avoid psychological and social problems. For improving diagnostic standards and managements, satisfaction of sex of rearing the provision of centers of tertiary pediatric care is recommended.

PO2-089 Disorders of Sexual Differentiation (DSD) I

Late discovery of a disorder of sexual development in a child of African descent

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A 15 year-old African child was addressed to our consultation by a charity association to take care of a disorder of sexual development (DSD).
Ambiguous genitalia were noted at birth: penoscrotal hypospadias and no palpable gonads. The child was reared as a boy. He had no medical follow-up. Breast started to develop at 13 years of age.
Physical exam at the age of 15 y showed: weight on 60th and height on 50th percentiles; facial phenotype neither dysmorphic nor suggestive of a syndromic entity; Tanner stages B4 P4; phallic structure of 4 cm; penoscrotal meatus; completely fused labioscrotal folds, the right one being scrotum-like, the left one labia-like; a palpable gonad was found in the right fold only.
At uretroscopy, the meatus presented with a double orifice leading to a urinary bladder anteriorly and to a vagina posteriorly. At laparoscopy, a hemiuterus, a Fallopian tube and an intra-abdominal gonad, probably an ovotestis, were identified in the right pelvis. No gonad was found on the left side. An intra-abdominal scar suggested that the left gonad had been fixed in the right scrotum during a previous surgery.
Histological analysis of the right scrotal gonad showed seminiferous tubules with Sertoli and interstitial Leydig cells but no germinal cells. The intra-abdominal gonad was not biopsied.
Hormonal levels were as follows (N for males, Tanner 4): FSH 2.0 U/l (2-9.2), LH 2.2 U/l (0.4-7), total testosterone 1.6 mcg/l (2-6.2), estradiol (E2) 70 ng/l (10-36), 17-OHP 8.8 nmol/l (0.7-5.3), cortisol 334 nmol/l (>275), AMH 81.7 pmol/l (21-39), inhibin B 45 ng/l (55-309).
Blood karyotype was 46,XX. The same result was found in scrotal fibroblasts, gonadal tissue and buccal smear. No SRY was found in these cells using PCR and FISH. A chimerism was excluded. Multiplex ligation-dependent probe amplification analysis excluded deletion and duplication of WNT4, SOX9 and DAX1.
In conclusion, this patient seems to present a 46,XX SRY-negative ovotesticular DSD. The presence of testicular tissue is confirmed and the E2 level suggests that there is functional ovarian tissue. The presence of follicles should be confirmed by histology of the intra-abdominal gonad. R-spondin 1 mutations have been described in patients with SRY-negative complete female-to-male sex reversal. The mutation was associated with palmoplantar hyperkeratosis and predisposition to squamous cell carcinoma at advanced age. The R-spondin 1 gene remains a candidate gene to be sequenced in our patient.

PO2-090 Disorders of Sexual Differentiation (DSD) I

46 XY, deletion 9p. Association of gonadal dysgenesis, mental retardation and corpus callosum hypoplasia. ¿Contiguous genes?

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Studies uncovered a lot of genes involved in sex determination including the gen SRY, WT1, SF-1, DAX-1, SOX and others autosomal structural abnormalities including terminal deletions of 10q and a duplication of 1p.
We have a case of a boy who presents ambiguous genitalia. The external genitalia consisted of non-fused labioscrotal folds and perineal hypospadias. The testes were palpable in the inguinal canal. Serum testosterone was 1 ng/ml and rose to 2.317 ng/ml following stimulation by hCG. Serum 17-OH progesterone was 2.9 ng/m (NV: 0.6-3). Gynecological ecography did not show female internal structures. Karyotype result was 46XY. Early motor development was normal, but on reevaluation at 18 months of age developmental delay was apparent. Cerebral magnetic resonance revealed vermis and corpus callosum, and cortical atrophy. Surgery at 3 years revealed both dysgenetic gonads, without tumoral cells. A cystografy showed Müllerian duct derivatives. At 24 months of age, he showed delays in motor acquisitions and language development, and behavioral problems were noted. On physical examination at 3 1/2 years we noted bilateral 5th finger clinodactyly, upward slanting palpebral fissures together with mental retardation, midface hypoplasia, hypertelorism, epicanthus, flat nasal bridge, dysplastic ears and a long and fine upper lip. We investigate the karyotype and perform molecular analyses of distal 9p that revealed deletion 46,XY,del(9p)(p23-pter).ish tel(9p-), the region of DMRT1 and DMRT2 genes. The parental karyotypes were normal.
There are many genes involved in testicular differentiation. Deletions of theses genes can affect different tissues, involving hypogonadism, adrenal insufficiency, campomelic dysplasia, Wilms tumor and various more entities. It is very important to be aware accompanying signs or symptoms to make a correct diagnosis and management of each child. It is very interesting that in many cases of dystal 9p, deletions mental retardation, symptoms of autistic spectrum, behavioral and learning problems exists, some of them with cerebral cortical atrophy and corpus callosum hypoplasia. That suggest 9p is important only in testicular differentiation and that there are some genes responsible for the behavioral phenotype. It is also unclear if a mutation involving a single gene is responsible for 46,XY gonadal dysgenesis and autistic spectrum disorder or if they are a genetically distinct entity.

PO2-091 Disorders of Sexual Differentiation (DSD) I

Association of gonadal dysgenesis with or without Turner phenotype with the presence of Y chromosome and gonadal tumor

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Introduction: The frequency of gonadal tumors is higher in patients with dysgenic gonads and the presence of Y chromosome or Y-chromosome sequences as part of their genome. **Objective:** To verify the frequency of gonadal tumors in patients with gonadal dysgenesis with or without Turner Syndrome (TS) and the presence of Y chromosome. **Patients and Methods**: Among 260 TS cases diagnosed in our Laboratory, six were identified as having 45,X/46,XY mosaicism. In addition, 20 cases presented with a marker chromosome, 3 cases had a ring chromosome of unidentified origin and 73 had 45,X karyotype with negative X chromatin. Molecular investigations were carried out in those 96 cases searching for Y chromosome sequences such as *SRY, TSPY and DYZ3*. Nine cases of XY pure gonadal dysgenesis (XY PGD) had *SRY* gene sequenced. All TS cases with Y chromosome or Y-chromosome positive sequences and XY PGD cases were submitted to bilateral gonadectomy. The presence of gonadal neoplasia was investigated upon anatomopathologic studies. **Results:** Among the 96 TS cases that were evaluated for Y-chromosome sequences 10 were positive (5/20 with a marker chromosome; 2/3 with a ring chromosome; and 3/76 with 45,X karyotype). Those added to the 6 cases with 45,X/46,XY mosaicism totalized 16 cases in which detailed studies were performed. Gonadal neoplasia was not found in any of the 32 gonads analyzed. Seven out of the 9 XY PGD cases presented SRY gene mutations (deletion, delSp1A, E89K, G95R, N65H, W70X, P30I). Gonadal tumor was not found in 6 cases, whereas 1 case presented gonadoblastoma in the right, 1 had bilateral goandoblastoma and 1 was found to have bilateral dysgerminome. **Conclusion:** Gonadal tumors were observed only in cases of XY PGD (3/9); TS patients bearing the whole Y chromosome or Y-chromosome sequences did not present gonadal neoplasia.

PO2-092 Disorders of Sexual Differentiation (DSD) I

A new approach for rapid and reliable molecular diagnosis of patients with disorders of sex development

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Despite the discovery of the testis determining gene SRY and a subsequent suite of genes involved in sex determination, over 50% of children with 46, XY disorder of sex development (DSD) are still offered no accurate molecular diagnosis. Diagnosis is important prior to specific outcome studies, in addition to allowing more accurate quantification and research of future complications such as germ cell tumor risk and need for cancer surveillance.
There is currently no commercially available technique to screen for multiple causative genes at one time, and the difficulty of genetic screening contributes somewhat to the lack of accurate diagnoses in DSD.
We are implementing recently developed techniques in order to screen for pathogenic gene mutations in children with 46, XY DSD, via a high throughput approach. Multiplex ligation-dependent probe amplification (MLPA) is a rapid technique that allows high-resolution detection of gene copy number changes to exons of 100 bp or more in size. MLPA reliably allows several known causative gene mutations to be screened for simultaneously, thus reducing the time and costs involved. DNA melting curve analysis can be used to detect single base mutations in candidate genes if MLPA screening is negative for copy number changes. All positive findings are then confirmed with targeted DNA sequencing on independent DNA samples.
These techniques comprise a rapid, cost effective research and diagnostic system to screen for known pathogenic gene mutations in children with DSD. A current trial is successfully underway in a cohort of approximately 100 children with 46, XY DSD.

PO2-093 Disorders of Sexual Differentiation (DSD) I

Androgen receptor (AR) mutations are associated with variable phenotypes

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At least three factors influence the phenotype in patients with AR mutations: 1) mutant location 2) the substitution mutating individual residues 3) independent secondary factors, poorly defined. We present data for novel AR mutations and multiple substitutions at residues V581, D690 and A870. Complete data and phenotype were available for all substitutions at A870. A870E (CAIS) did not bind ligand, in agreement with the expected inactivating role of a mutation at this location. A870G (PAIS/MAIS) exhibited intermediate levels of ligand binding, promoter activation and significant loss of N/C interaction. Activity was consistent with ranges observed for other PAIS mutations. Ligand binding and transactivation for A870V (PAIS/MAIS/Gynacomastia only) were wild-type (WT), in contrast to a predicted loss of function. N/C interaction for A870V was elevated above WT levels; interpretation of this observation is unclear. The role of secondary factors on patient phenotype is highlighted by A870G and A870V. D690V (CAIS) and the highly conservative D690E (PAIS) had reduced transactivation, correlating to disease severity. Ligand binding affinity for D690V was elevated (1.6nM), insufficient to account for loss of transactivation or CAIS phenotype. D690E had normal binding affinity. Mutations in this AR loop can elevate ligand dissociation rate, measurement for D690 mutations is required. The alanine to glutamic acid substitution located close to the AF2 surface (A896E, PAIS) had little effect on ligand binding or transactivation, but N/C interaction was elevated. This may explain the mechanism of AR dysfunction for this mutation and indicate a pathogenic role in AIS. A novel helix 1 mutation E678G (PAIS) had an elevated Kd of 5.1nM, a useful tool to define the molecular mechanisms of function for this AR region.
Analysis was not restricted to mutations of the ligand binding domain. I603S (CAIS) and V581G (PAIS) in the DNA binding domain disrupted GRE promoter activation. Loss of function for I603S was severe and consistent with CAIS; dimerisation via the D-box is probably disrupted. V581G, identified in a PAIS patient raised male, had residual activity. Two other substitutions, described in patients raised female (V581L and V581F), were analysed. V581L was more active than V581G. The vast number of AR mutations associated with AIS is a rich source to understand disease mechanisms and AR function. However, there remains uncertainty sometimes in proving pathogenecity.

PO2-094 Disorders of Sexual Differentiation (DSD) I

Presentational and pubertal outcome characteristics in 2 disorders of androgen synthesis causing XY sex reversal

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Introduction: 17 beta-hydroxysteroid dehydrogenase type 3 (HSD17B3) and 5 alpha-reductase type 2 (SRD5A2) isoenzymes are essential to produce androgens for male development. Mutant enzymes cause variable XY sex reversal. Demographics, presentation, sex of rearing, androgen production and pubertal outcome are compared in these disorders.
Methods: Cases were identified from a large Disorders of Sex Development database comprising clinical details, biochemical results and mutation analyses.
Results: 21 subjects with HSD17B3 and 19 subjects with SRD5A2 mutations were identified.

Mutations		
	HSD17B3	SRD5A2
Homozygous	A56T,N130S,C248Y,E215D,M197K,V205E,S232L,IVS2+1 g>a,IVS8+9 t>a	A192T,G196S,E200K,A228T,R246Q,IVS1+1 t>a,726-1 g>a
Heterozygous	325+4 a>t and 525-17 g>a,325+4 a>g and V205E, S65W and 222delATC, 325+4 a>t and stop codon exon 4, C268Y het	Q126R and H231R, splice 755 and R246Q

Diagnosis was earlier in 5αRD (2.9 *vs*.5.3 yr) with more frequent ambiguous genitalia at birth (9/21 *vs*.3/19).The most common presentation of HSD17B3 was inguinal masses in early childhood (8/21 *vs*.5/19) followed by virilisation at puberty (5/21 *vs*.1/19).Sex of rearing in HSD17B3 was predominantly female (17 F, 2 M) one reassigned F to M after diagnosis at 5 weeks. Sex of rearing in 5αRD was 14 M,5 F, reassigned in 3 cases at diagnostic ages 10 months, 4 and 14 years.
After a short HCG test the median (range) testosterone: androstendione (T/A) ratio was 0.3(0.1-3.4) in HSD17B3 and 9.6(2.3-18.3) in 5αRD.T: DHT ratio was 4.8(0.9-18) in HSD17B3 and 21.4(10-36.3) in 5αRD.
We present puberty data on 9 females with HSD17B3 deficiency and 9 males with 5αRD deficiency (3 reassigned male). Three females were on oral estrogen replacement; Tanner stage was 2-3. Six males had surgery. Tanner stages were 2-5; testicular volume 4-20mls.One subject had gynaecomastia; 5 males were receiving androgens.
Conclusion: The phenotypic variability in both disorders often overlaps with the androgen insensitivity phenotype. Sex of rearing was usually male in 5αRD associated with a birth phenotype of an under virilised male. Median T:A and T:DHT ratios were raised compared to HSD17B3 but overlap was substantial. Puberty is neither concordant for the type of disorder or the cognate genotype thus making outcome unpredictable. This can only be addressed by more follow up data, including quality of life issues, to guide decisions on sex of rearing.

PO2-095 Disorders of Sexual Differentiation (DSD) I

Congenital adrenal hyperplasia genotyping strategy using MLPA and DNA sequencing: an Australian cohort

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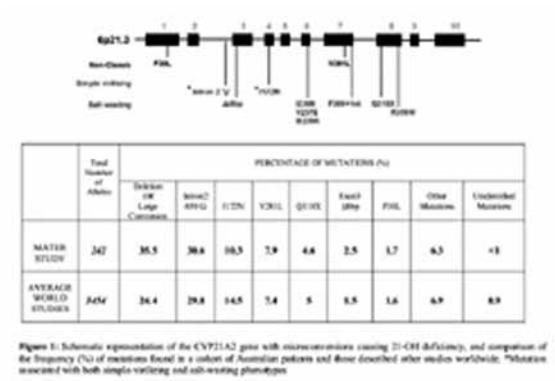
An estimated 90-95% of cases of Congenital Adrenal Hyperplasia (CAH), an autosomal recessive condition, result from reduced 21-hydroxylase (21-OH) activity. The 21-OH gene(*CYP21A2*) is located on chromosome 6p21.3 within the HLA histocompatibility complex adjacent to its highly homologous pseudogene (*CYP21A1P*). Recombination events between the two genes are responsible for up to 95% of mutations in the CYP21A2 gene. The resulting mutations include large deletions/conversions, (35%), microconversions (60%), and private non-pseudogene mutations (5%), and are associated with variable degrees of impairment in 21-OH activity. The least severe mutation determines the residual enzyme activity and phenotype: salt-wasting (SW), simple-virilizing (SV), or non-classical (NC) CAH. However, it seems that particular mutations can be associated with more than one phenotype giving rise to genotype-phenotype discordance. We describe our experience with a *CYP21A2* genotyping strategy using the combination of multiplex ligation-dependant probe amplification (MLPA) and DNA sequencing. MPLA is utilized for genomic quantification of *CYP21A2* exons and allows for the detection of large deletions and conversions. DNA sequencing is performed using two sets of primers - one binding to the promoter region and amplifying the complete gene and the other binding to the exon 6 cluster and amplifying the first six exons. This allows for the detection of both small- and large gene conversions. Genotyping of patients in an Australian cohort showed a greater frequency of deletions and large conversions compared to previously described studies (see figure 1).



In conclusion, combined MLPA and DNA sequencing is a useful Genotyping strategy for CAH and has allowed for the determination of differences in the frequency of mutation types in an Australian cohort relative to other populations.

PO2-096 Disorders of Sexual Differentiation (DSD) I

The effect of intramuscular testosterone enanthate treatment on stretched penile length in prepubertal boys with hypospadias

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Testosterone enanthate (TE) treatment is advocated for boys with micropenis, which was defined as significantly small penis without hypospadias, efficiently leading to the enlargement of penis. The TE is also administered to boys with hypospadias, ensuring the sufficient penile size to permit easier surgical repair of hypospadias. There have not been any studies comprehensively assessing the effect of TE treatment on penile size for hypospadias in prepubertal boys as compared with age-matched reference values.

The present study analyzed the responses of stretched penile length (SPL) to TE treatment in 17 Japanese boys with hypospadias at 0 to 5 years of age (median 1) who were seen at the outpatient clinic, Department of Pediatrics, Keio University Hospital, Tokyo, Japan from 1998 through 2008. Their SPLs ranged from 2.0 to 3.5 cm (2.8) and from -3.3 to 0.5 SD of the mean (-1.2) as compared with the age-matched Japanese standards. The severity of hypospadias varied from distal to perineal, based on the location of urethral meatus. The etiology of hypospadias among the 17 boys included sex chromosome DSD in five, *MAMLD1* deficiency in one, and unknown cause in others. PCR and direct sequence of genomic DNA did not identified any mutations in the *SRD5A2 and AR* genes. All boys received up to three intramuscular injections of TE 25 mg every four weeks (one injection in three boys, two in five, and three in nine). The TE treatment significantly increased SPL by 0.5 to 2.5 cm (0.9) and 0.9 to 5.1 SD (2.0) (cm, $P=0.0002$; SD, $P=0.0002$). There was no significant correlation between the effect of TE and either age, body surface area, BMI, or SPL before treatment. The effect of the first injection of TE in hypospadias [from 0.0 to 0.8 cm (0.3) and from 0.0 to 2.0 SD (0.775)] was significantly less than that in Japanese boys with micropenis at 0 to 5 years of age (3) [from 0.2 to 1.1 cm (0.6) and from 0.3 to 3.3 SD (1.2)] (cm, $P=0.0008$; SD, $P=0.02$). These data indicate that 1) the intramuscular injection of TE significantly increases SPL for hypospadias in prepubertal boys with no demonstrable *SRD5A2 or AR* gene mutation, 2) age, body surface area, BMI, and SPL before treatment are not significantly contributing factors to the effect of TE treatment, and 3) the effect of TE in hypospadias is significantly less than that in micropenis.

PO2-097 Disorders of Sexual Differentiation (DSD) I

Psychosexual development in children with disorders of sex development (DSD)

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Background: Psychosexual development is a complex process, influenced by biological and psychosocial factors. Children show a great variability in psychosexual development, both between and within gender-groups. Even so, there are relatively stable gender-related behaviors and self-perceptions, in which boys and girls differ distinctly. There is evidence that high concentrations of androgens lead to more male-typical behavior. Disorders of sex development (DSD) provide the opportunity to analyze the role of different factors on psychosexual development, because it comprise a clinically heterogeneous group of conditions with a variation in molecular-genetic etiology, levels of prenatal androgen effects, medical treatments, and phenotypes of genitalia. Methods: Within the clinical evaluation study of the German network DSD/Intersexuality we examined 166 children age 4 -12 with DSD. 111 children were reared as girls, 55 as boys. Gender identity was assessed with the Gender Identity Interview for Children, gender role behavior and cross-gender identification by the Child Behavior and Attitudes Questionnaire and the Gender Role Questionnaire. Toy preference was assessed with a toy to keep task. Results: Children without androgen effects demonstrated a tendency for female-typical behaviors, while children with androgen effects showed more male-typical behaviors. Boys with DSD did not differ from control boys. Girls with androgen effects showed higher scores for femininity and lower scores for masculinity than children with androgen effects reared as boys, but they differ from girls without androgen effects and from girls of a control group. Although girls with androgen effects showed more cross-gender behaviors than girls without androgen effects and boys with androgen effects, we found a high congruence between gender identity and gender of rearing. Discussion: The result that children with androgen effects tend towards more boy-typical behaviors - regardless of gender of rearing - gives evidence that prenatal androgen effects might have a stronger impact on psychosexual development than social aspects. Children with DSD did not show an increased risk for cross-gender behavior or gender identity confusion in general. However, girls with androgen effects showed higher rates of cross-gender behavior without an increased risk for gender identity disorder in these girls.

PO2-098 Disorders of Sexual Differentiation (DSD) I

Are polymorphisms of MAMLD1 a risk factor for hypospadias?

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Mastermind-like domain containing 1 (MAMLD1), also known as chromosome X open reading frame 6 (CXorf6), is a recently identified candidate gene for the development of male genitalia. Molecular studies showed that the mouse homolog is transiently expressed in fetal Sertoli and Leydig cells around the critical period for sex development and that knockdown results in significantly reduced testosterone production in this model. In human, we showed that almost 10% of patients with both severe and non-severe hypospadias exhibit mutations of MAMLD1. Subsequently, polymorphisms were strongly suspected to play a role in the development of hypospadias. The aim of this work was to determine whether polymorphisms of MAMLD1 are a genetic risk factor for hypospadias.

This study included 217 individuals: 87 patients presenting hypospadias with a range of severities and 130 normal controls. In the patients, abnormalities in androgen synthesis and action were ruled out by androgen receptor gene sequencing and/or hormonal measurement. Direct sequencing of the MAMLD 1 coding exons and their flanking splice sites was performed.

The polymorphism p.N589S was identified in 7/87 patients and in 12/130 controls (8% vs. 9.2%, p=ns). The polymorphism p.P286S was identified in 5/87 patients and in 12/130 controls (5.7% vs. 9.2%, p=ns). The S-S haplotype was present in 4/87 patients (1 glandular, 2 proximal, 1 penoscrotal and 1 perineal hypospadias) and in 7/130 controls (4.6% vs. 5.4%, p=ns).

Despite direct evidence of MAMLD1 implication in 46,XY disorders of sexual development or isolated hypospadias, the p.N589S and p.P286S polymorphisms are found in individuals both with and without hypospadias. Although previous studies reported the low incidence of these polymorphisms in the general population, we did not find that the combination of these alleles predisposes toward the development of hypospadias.

PO2-099 Disorders of Sexual Differentiation (DSD) I

Isolated micropenis with normal plasma testosterone can hide a molecular genetic defect of androgen pathway

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Micropenis is defined as a stretched penile length of less than 2.5 standard deviations for age. Etiologies of this condition include insufficient androgen production, partial androgen insensitivity syndrome and other rare causes such as GH deficiency. Often, the cause remains unknown. In cases of a deficit in androgen synthesis or action, micropenis is usually described along with other genital abnormalities including hypospadias and/or cryptorchidism, thus indicating a disorder of sex development. The aim of this study was to determine whether isolated micropenis with normal plasma testosterone could hide a molecular defect in the androgen pathway.

Eighteen boys (age 1 months - 14 years) with isolated micropenis were included in this study. The penile length ranged from 1 to 3.5 cm (from -2 to -4 SD for age). In these patients, normal LH and FSH values for age were found. All of them had a normal 46XY karyotype and normal plasma testosterone for age. SF1 (steroidogenic factor 1) and AR (androgen receptor) genes were sequenced. One hundred patients without any genital malformation were used as controls.

A mutation was identified in two patients. In one case, a p.K63Q mutation was present in the SF1 gene. Despite this genetic defect, a satisfactory clinical and biological response to the HCG test was observed (penis size from 2 to 4 cm, plasma testosterone from <0.2 to 6.17 ng/ml). This mutation was not found in controls and has not been previously described as a polymorphism. In another patient, a new mutation within exon 1 of the AR gene was present (p.P390S). The patient had a penile length of 2 cm at the age of 14 years (-4 SD) with otherwise normal genitalia and ongoing male puberty. Plasma testosterone response to the HCG test was 4 ng/ml, contrasting with only partial penile lengthening.

This is the first report of isolated micropenis as a revealing symptom of SF1 and AR mutations. Genetic defects in the androgen pathway can induce mild phenotypes, and any insufficient masculinisation in a boy, even without complete disorder of sexual development, justifies genetic exploration. Unidentified AR mutations in micropenis may explain the variable clinical response to androgen treatment.

PO2-100 Disorders of Sexual Differentiation (DSD) I

Psychological aspects and sexual behaviour of adolescents with disorders of sex development (DSD)

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For adolescents with DSD symptoms interfere in particular with adolescent developmental tasks. At the onset of puberty, the majority of these adolescents become aware of the developmental discrepancies in comparison with their peers. There is a lack of empirical data for adolescents despite specific demands and problems. We wanted to investigate the impact of the DSD in general and of specific interventions on the psychosocial well-being in adolescents. Methods: Within the clinical evaluation study of the German network DSD/Intersexuality we interviewed adolescents (13-16) with a specific battery of instruments between 2005 and 2007 in Germany, Austria and Switzerland. To measure health-related quality of life, mental health, coping and body image we used generic, standardised instruments (KINDLR, SDQ, CODI, BI-Scale). A new questionnaire contains specific questions concerning friends, sexuality, and the dealing with DSD. We collected medical data by the attending physician. Results: 60 adolescents with DSD participated. For statistical group comparisons we used four diagnostic subgroups: girls with 46,XX & overvirilization/CAH (N=29), girls with 46,XY & partial androgen effects (N= 18), girls with 46,XY without androgen effects (N= 7), boys with lack of virilization (N= 6). The general psychological well-being of adolescents with DSD on a group level is not impaired. However, sub-groups report impaired well-being in some aspects: Boys differ hardly from control boys in the different outcomes whereas girls with DSD differ from their female peers, especially in social relationships and sexual activities. Adolescents with DSD differ clearly in their coping strategies from adolescents with a chronic health condition. Adolescents who needed hormonal treatment to induce puberty report impaired well-being in nearly all outcomes than those who entered puberty spontaneously. Discussion: The majority of the adolescents cope adaptively with their condition and do not show severe psychological impairments. This suggests an age appropriate tendency to conform to peers. Being different is a source of fear and insecurity. In future interdisciplinary health care teams have to focus this pressure of conformance and to openly discuss it with the adolescent in context of treatment decisions.

PO2-101 Disorders of Sexual Differentiation (DSD) I

Investigation of the GBY locus in patients with Turner syndrome

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Genomic mapping studies in females with dysgenetic gonads and with a Y chromosome in their karyotype (DSD-Y group) have revealed that Y genes in proximal Yp and Yq are probably involved in the 30% risk for the development of gonadoblastomas (Gonadoblastoma Y locus; GBY). We carried out gene analyses for the presence of possible GBY candidate genes in patients with Turner syndrome with and without Y genes in their standard karyotype.

For this purpose we have established sensitive Y gene deletion PCR assays in the GBY region (TSPY) and the flanking azoospermia factor (AZF) regions, AZFa, AZFb, AZFc, known to be functionally important in the male germ line. The study was approved by the ethic committee of the University of Heidelberg and patients gave their informed consent to participate.

Our database currently includes 59 Turner syndrome patients. In only two of these patients we identified Y genes in their standard karyotype for chromosomal analysis. Karyotype analyses revealed 24 patients with 45,X0, 9 patients with 45,X0/46,XX, 6 patients with 45,X/46,Xi(X), 5 patients with 46,Xi(X), 4 patients with 45,X/46 XX marker, 1 patient with a partial deletion of one X chromosome, 2 patients with a 45,X/47,XXX karyotype and 8 patients with other mosaic constitutions.

We detected AZF genes only in the two patients in whom Y material was already obvious in their standard karyotype. Interestingly one of them had a negative result for the Y centromer and showed a deletion of AZFa, the other was positive for Y-centromer and had a deletion of AZF b and c. In 3 of the patients without Y-material in their standard chromosomal analyses Y-centromers were positive as well, but AZF genes were missing in all of them. All other patients were negative for the Y-centromer.

We conclude that in our patients group GBY-candidate-gene analysis did not add information concerning hidden Y-material indicating a potential risk for gonadoblastomas in Turner patients without obvious Y-material in their standard karyotype.

PO2-102 Disorders of Sexual Differentiation (DSD) I

A novel high throughput LC-MS/MS method for routine detection of androstendione, testosterone and dihydrotestosterone in pediatric plasma samples: age and sex specific reference data for children and its application in disorders of sex development

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Introduction

The measurement of gonadal hormones is of major importance for general paediatric endocrinology and especially for disorders of sex development (DSD). Various forms of DSD can be characterized on the hormonal level. We established a LC-MS/MS method for the simultaneous detection and quantification of androstenedione (A), testosterone (T) and dihydrotestosterone (DHT) as well as age and sex specific reference data for children.

Method

A plasma sample of 0.1 mL internal standard (17-hydroxy-progesterone-d_8 and cortisol-d_4) and 5 % phosphoric acid was extracted after centrifugation by solid phase extraction. Analysis was carried out with an UPLC-MS/MS system. For each hormone a specific MRM transition was determined. Left-over samples from routine paediatric blood tests were used for the reference data (138 males, 141 females) as a function of sex, age (0 to 18 years of age), pubertal stage, and testicular volume. Three DSD patients (17ßHSD3 deficiency, SF1 deficiency, PAIS) have been analyzed exemplarily.

Results

The assay is linear from 0.1 nmol/L to 200 nmol/L. The limit of detection was 0.1 nmol/L for each steroid. In males median A at birth was 0.6 nmol/L, median **T** was 0.7 nmol/L, median **DHT** was 0.1 nmol/L. A and T increased within the first two months (A 1.4 nmol/L, T 5.7 nmol/L). After that A, T, and DHT decreased and started to rise to 2 nmol/L A, 12.1 nmol/l T, and 1.35 nmol/L DHT at 18 years of age. In females median A at birth was 0.95 nmol/L, median **T** was 0.73 nmol/L, median **DHT** was 0.1 nmol/L. A, T, and DHT decreased and started to rise to 3,5 nmol/L A, 0.93 nmol/l T and 0.3 nmol/l DHT at 18 years of age. Levels for A, T, and DHT as function of pubertal stage and testicular volume are reported. A in 17ßHSD3 deficiency was highly elevated as was T in PAIS. Androgens were low with SF1 deficiency.

Conclusion

Our new LC-MS/MS method allows a rapid and precise analysis of A, T, and DHT in plasma samples. With our reference data for sex, age, pubertal stage and testicular volume we are able to diagnose different forms of DSD. The reference data allows analyzing plasma samples of children in daily routine as well as in research settings.

PO2-103 Disorders of Sexual Differentiation (DSD) I

Male gender assignment in a 46,XY infant with ambiguous genitalia, normal adrenal function and a novel heterozygous mutation in steroidogenic factor 1 (SF-1, NR5A1, Ad4BP) after 5 months of diagnostics and psychological counseling

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According to patients' inquiries mistakes in management of newborns with ambiguous genitalia may result in long lasting psychosocial disturbances and unfavorable medical outcome. Therefore, a multiprofessional team of a pediatric endocrinologist, surgeon and psychologist takes care of these patients and, as in the present complex sexual ambiguity, gender assignment can be delayed until a precise diagnosis and therapeutic concept have been set up with experts and parents.

Methods: Functional tests. Direct sequencing of exons 2 – 7 of NR5A1.

Results: A term newborn of non-consanguine Albanian parents presented with sexual ambiguity: small phallus 0.6 cm, ventral chordee, penoscrotal hypospadia, hypoplastic scrotum with midline fusion and small testes (left 0.5 ml descended/ right <0.5 ml high scrotal). Ultrasound revealed normally structured small testes and epididymis, but no uterus, and urethocystoscopy a 1.5 cm urogenital sinus and 2 cm vaginal remnant. Testosterone (T), inhibin B, LH and FSH were low at the 6th day of life (0.3 nmol/l, 61 pg/ml, <0.1 and 0.1 U/l, resp.), kariotype, DHEAS, Cortisol and ACTH being normal male (46 XY, 1.5 µmol/l, 51 nmol/l and 54 ng/l, resp.). T rose up to 2.2 nmol/l, but remained low after hCG stimulation (5000 mg/m^2 in 3 doses), with normal T/DHT ratio. Adrenal and gonadotropin stimulation tests were low-normal (peak cortisol 553 nmol/l after 125 µg ACTH, and LH 4.1, FSH 8 U/l after 25µg LHRH). Phallus size increased to 2.5 cm after 50 mg T enanthate monthly for 3 months, and to 2.9 cm after additional 75 mg. A novel heterozygous mutation was found in the SF-1 gene (exon 3, p.Cyst73Ser); functional characterization of this missense mutation is under investigation. After 5 months and extensive psychosocial counseling care team and parents uniformly agreed to assign male gender. Surgery resulted in a small, normally functioning penis with central meatus; orchidopexy of now inguinal testes is considered.

Conclusion: The severe undervirilization and Müllerian remnant due to gonadal dysgenesis in this 46,XY DSD patient reflect the dose-dependant loss of function of SF-1 in human gonadal development. So far patients with SF-1 mutations and similarly severe sexual ambiguousity have mostly been gonadectomized and raised as girls. Only long term follow-up in this boy will reveal a possible risk of gonadal tumors or adrenal insufficiency and whether puberty and fertility can be achieved. with adequate therapy.

PO2-104 Disorders of Sexual Differentiation (DSD) I

Partial androgen insensitivity in the neonatal period may hide 5α-reductase deficiency. Report of four new srd5a2 gene mutations

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The observation of ambiguous genitalia in the newborn signals a medical, surgical and psychological emergency. The most crucial decision will be the choice of sex assignment. Rapid and precise diagnosis is thus essential. In XY newborns with normal/high plasma testosterone (T), partial androgen insensitivity syndrome (PAIS) is usually the first diagnosis evoked and assumes an androgen receptor (AR) defect. The diagnosis of 5α-reductase deficiency is rarely considered. We report four new SRD5A2 gene mutations in newborns with ambiguous genitalia and normal plasma T values, diagnosed as PAIS. In four of the cases, normal sequences of the complete AR gene excluded this diagnosis and raised the hypothesis of 5α-reductase deficiency. Clinical, biological and molecular analyses are reported in the following table.

Patients	Age	Neonatal diagnosis	Phenotype	Basal testosterone/ post HCG (ng/ml)	Exon	SRD5A2 Mutation
1	6 days	PAIS	Female + clitoromegaly	3.2 / ND	1	c.122_123delAG
2	9 days	PAIS	Male + perineoscrotal hypospadias	2 / 7.2	4 / 5	p.A215V / p.X255Q
3	4 days	PAIS	Male + perineoscrotal hypospadias	1.3 / ND	1	p.S14R / p.V89L
4	1 month	PAIS	Female + clitoromegaly	0.5 / 3.8	2	p.G115N

The entire coding region (5 exons) of the SRD5A2 gene was assessed by PCR and direct sequencing analysis. For patient 1, we identified a homozygous 2bp deletion in exon 1 (c.122_123delAG). Patient 2 had new compound heterozygous mutations in exon 4 (p.A215V) and exon 5 (p.X255Q). Patients 3 and 4 presented two new substitutions in exons 1 and 2 (P3: [p.S14R]+[p.V89L], known as a polymorphism; P4: p.G115N, homozygous).

Our data confirm our previous experience and clearly demonstrate that a 5α-reductase defect should be considered in XY newborns with ambiguous genitalia and normal plasma T secretion. Early molecular diagnosis orients the crucial decision of the newborn's sex of rearing.

PO2-105 Genetics of Growth II

New SHOX mutations in patients with idiopathic short stature

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Mutations of SHOX (Short Stature Homeobox) gene are associated with the short stature in Turner syndrome, Leri-Weill dyschondrosteosis and in some patients (2 to 15%) with idiopathic short stature (ISS). Nevertheless, recently it has been characterized the SHOX gene enhancer, whose deletions has been shown to be responsible for the Leri-Weill syndrome.

We report the results of a study carried out on patients with ISS, investigated for the presence of SHOX gene and SHOX enhancer mutations.

Fifty-three patients of both sexes (2-18 years) entered this study. The inclusion criteria were: 1) height <3° centile; 2) normal stimulated GH values 3) absence of obvious skeletal anomalies 4) exclusion of chronic disorders causing short stature.

All 53 samples were examined for the presence of deletions or duplications within the SHOX gene and the PAR1 region by Multiplex Ligation-dependent Probe Amplification (MLPA), using the SHOX salsa P018B kit (MRC-Holland); they were also examined for the presence of point mutations and small deletions and insertions by direct sequencing of all the coding exons and the intron/exon boundaries of the gene isoform A (from exon 2 to exon 6a) and of its enhancer.

Sequencing analysis revealed no point mutations and small deletions or insertions that could account for the phenotype. On the contrary the molecular analysis of the entire PAR1 region by MLPA surprisingly revealed two large duplications. One patient presented a duplication of about 500Kb extending from exon 1 of the SHOX gene and encompassing its enhancer; another patient presented a smaller duplication involving only the enhancer. Both the duplications were further confirmed by using specific panels of microsatellites markers that resulted in triallelic patterns.

For all we know, these mutations had never been described before and they could have a role in the short stature of these patients. In fact, in both cases, the duplication of PAR1 could disrupt the normal cis/regulation of transcription caused by the closeness of the duplicated regions. Such proximity could impede the regular interaction of each enhancer to each appropriate promoter, resulting in the alteration, most likely a reduction, of the normal transcriptional activity. This could be the reason why the duplication involving also a regulative element such as the enhancer is associated to a short stature phenotype while an extra copy of only the SHOX gene seems to determine tall stature.

PO2-106 Genetics of Growth II

Prevalence of celiac disease in asymptomatic Indian children with idiopathic short stature

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Background: Celiac disease, an immune mediated enteropathy in genetically susceptible individuals, previously being thought to affect only Europeans is being increasingly recognized as a cause of chronic diarrhea in other parts of the world including India. However, unlike western countries, it is not commonly thought of and investigated as a cause of isolated short stature in India.

Objective: To determine prevalence of celiac disease among Indian children with idiopathic short stature.

Methods: All children who presented to the Pediatric Endocrine Clinic of our tertiary care referral institution during a one year period from June 2006-07 with short stature (height SDS <-2) and did not have any chronic systemic illness including diarrhea, abdominal pain or distension, anemia, familial, nutritional or endocrine cause of short stature were labeled as idiopathic short stature. They were investigated for celiac disease using antiendomysial (anti EMA) and anti tissue transglutaminase antibodies (anti-tTGA) and jejunal mucosal biopsy after taking informed consent from parents and assent from

the children. Ethical approval was obtained from the ethics committee of our Institute.

Results: 39 children (13 girls) with a mean age of 9.7 ± 3.8 years were diagnosed as idiopathic short stature during the study period. The mean hemoglobin and albumin of these children was 11.3 ± 1.7 mg/dl and 4.6 ± 0.7 g/dl and none had a family history of celiac disease. On investigation, three children (prevalence 7.7%) were found to have positive titers of anti EMA, high titers of anti-tTGA (419.7+118.5 IU/ml) and histological changes of celiac disease (Marsh class 2 in two and 1 in one). All three children were started on gluten free diet. After a mean follow-up of 1.5 years, their height SDS improved from -4.1, -4.2 and -2.8 to -3.2, -3.1 and -2.2 respectively.

Conclusion: Asymptomatic celiac disease was found to have a causal role in few cases of isolated short stature in Indian children. Institution of gluten free diet resulted in improvement of height SDS. Investigation for celiac disease should be a part of the routine work-up of short stature in asymptomatic Indian children.

PO2-107 Genetics of Growth II

Case report of an 11 year old male with gigantism and acromegaly without demonstrable growth hormone hypersecretion or increase in the insulin-like growth factor-1 axis

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Disorders of gigantism are exceedingly rare in pediatrics and commonly due to growth hormone (GH) excess of tumoral origin. We report a patient with striking features of gigantism and acromegaly, that appear not be due to discernible GH excess.

The subject was born at term weighing 4.1 kg (9lbs), and measuring 56 cm (22 in) in length. From infancy onward, height and weight gain have been excessive but height is beginning to level off at age 11 years. At present, he has exercise intolerance, daytime somnolence, and intermittent joint and bone pain. He also has copious night sweats, and has had an "apple cider" body odor from infancy. He has normal cognition for age. Physical exam includes hypertension (188/90 mm Hg), generalized obesity, tall stature, and acromegalic features: macrognathia, prognathia, widely spaced teeth, large tongue, frontal bossing, prominent brow, and ridges in the scalp, which are transverse in the back and longitudinal atop. He has acanthosis nigricans, striae and skin tags. He has large head, hands and feet, and some pitting ankle edema. He has a buried penis, 15 ml testes, pubic hair Tanner stage IV, moderate facial and axillary hair. Pertinent laboratory data appear below.

Age (years)	5.3	7.8	11.6
Height/weight/head size SDS	5.3/6.2	6.8/5.1	5.9/4.7/8.6
Bone age (years)	10.5	13.5	17
T4 (mcg/dl)	9.7 (5.5-12.8)	11.4 (4.5-10.9)	10.4 (7.6-13.7)
freeT4 (ng/dl)	1.3 (0.8-2.2)		1.4 (1-2.4)
T3 (ng/dl)			264 (60-181)
TSH (uIU/ml)	1.7 (0.3-5.5)		9.4 (0.28-4.3)
GH (ng/ml)			1.15 (0.08-4.7)
IGF-1 (ng/ml)	120 (52-297)	83 (64-345)	456 (139-395)
IGF-2 (ng/ml)		1098 (380-1140)	408 (245-737)
IGFBP-3 (mg/L)		3.9 (1.6-6.5)	4.2 (2-4.8)
ALS (mg/ml)			17 (5.6-16)
GHBP (pMol/L)			2607 (431-1892)
Testosterone total (ng/dl)	<30		75 (7-762)
Testosterone free (pg/ml)			19.2 (1-98)
Insulin (uU/ml)	44 (3-17)	76 (3-22)	

The radiologic work up has indicated normal CNS, hepatosplenomegaly and nephromegaly, and mild left ventricular hypertrophy of the heart. The presumptive diagnosis is that of acromegaloidism: this is defined by features of gigantism and acromegaly without excess GH, IGF-1, or IGFBP-3. He also has precocious pubertal development without evidence of excess early androgen production, and has evidence for the metabolic syndrome.

Auxologic and photographic data will be shown, and genomic and functional analysis of candidate genes will be presented.

PO2-108 Genetics of Growth II

Do children with Adams-Oliver syndrome require endocrinological follow-up?

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Introduction: Adams-Oliver syndrome (AOS) is a rare genetic condition characterized by aplasia cutis congenital and transverse limb defects. Cardiovascular malformations, as well as lesions in the central nervous system (CNS), with various impact on the psychomotor development have been described. Pathogenesis is not clear. Autosomal dominant inheritance with reduced penetrance is most often observed, however autosomal recessive inheritance has also been proposed. Due to the lack of data on hormonal disturbances in this syndrome, the aim of this report was to assess endocrine profile of 3 patients with AOS.

Patients and methods: At the first endocrinological evaluation, children were at the age of 2 years (patient 1, a boy), 3.5 yrs (patient 2, a girl) and 8.3 yrs (patient 3, a girl) and did not show pubertal signs. Mean follow-up period was 2.25 yrs. Concentrations of hormones (cortisol, IGF-1, LH, FSH, TSH, fT4, PRL - chemiluminescent immunomteric method), as well as parameters of carbohydrate metabolism were assayed in all cases. In one child growth hormone (GH) was assessed in the night profile and in two provocative tests. CNS imagining using computed tomography was performed in two patients and magnetic resonance (MR) in one subject.

Results: In the patient 1 manifesting delayed psychomotor development, Peters anomaly, epilepsis, deficits of the body mass and height and cryptorchidism - neuroimaging revealed hypoplasia of the midline structures, and low IGF-1 levels were found. Due to ongoing diagnostic procedures, the boy has not received hormonal treatment yet. In the patient 2 with normal development and normal body mass and height, with an incident of seizures which did not require pharmacotherapy - neuroimaging did not show any significant pathology and only subclinical hypothyroidism was found, followed by L-thyroxine administration. In the patient 3, with delayed psychomotor development and short stature - MR revealed the small pituitary and polimycrogyria; low IGF-1 levels and GH deficiency were found. The girl was qualified for the GH treatment, improving height velocity and gross coordination.

Conclusions: On the grounds of these observations, the extend of auxologic and hormonal deficits in children with AOS seems to be associated with CNS lesions. Hence, there are indications for performing neuroimaging in this group of patients. Their growth, puberty and psychomotor development should be monitored by an endocrinologist and a neurologist.

PO2-109 Genetics of Growth II

Clinical and biochemical characteristics of a male patient with defect in growth hormone signaling

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BACKGROUND: The syndrome of growth hormone insensitivity is characterized by phenotypic features consistent with the presence of a growth hormone deficiency, but with normal-to-elevated circulating concentrations of growth hormone and resistance to exogenous growth hormone therapy. Growth hormone insensitivity can be caused by defect in the growth hormone receptor (GHR) or in the post receptor signaling pathway. The GHR is a member of the cytokine receptor superfamily and has been shown to signal through Janus-family tyrosine kinase-signal transducer and activator of transcription (JAK-STAT) pathway. OBJECTIVE: To describe a male patient with severe short stature who had normal circulating growth hormone levels.

METHODS: We present the clinical, biochemical and magnetic resonance imaging (MRI) characteristics of a 16 year old male patient. RESULT: Physical examination revealed short stature with normal body proportions, an obviously younger appearance than that expected for his chronological age, normal IQ, no axillary or pubic hair and pre-pubertal genitalia. MRI showed empty sella with an ectopic neuropituitary gland located in proximal infundibular position. He had low levels of insulin like growth factor-1 (GIF-1), IGF binding-protein-3 (IGFBP-3) and acid labile subunit (ALS), associated with normal but not excessive growth hormone secretion. A trial of GH therapy was ineffective.

Biochemical features of the patient	
AGE	16
HEIGHT (cm)	126
WEIGHT (kg)	26
BMI	16.37
GLUCOSE (<110 mg/dl)	95
PROLACTIN (0-22 µg/L	82
THYROXINE (ng/dl)	4.4
TESTOSTERONE (ng/dl)	373
GH (mU/L)	0.34
GHBP (pmol/L)	1407
IGF-1 (ng/dl)	8
IGFBP-3 (ng/dl)	0.24
ALS (mg/L)	0.9

CONCLUSION: This case is most likely related to defect in post receptor signaling pathway i.e mutation in gene for STAT5b resulting in inactive truncated protein lacking most of the DNA binding-domain and SH2-domain or mutation in the growth hormone receptor gene that affects dimerization or the transmembrane and intracellular region

PO2-110 Genetics of Growth II

New IGF-I receptor (R) mutations in three patients with short stature: clinical and auxological characteristics

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The **aim** of this study presents the clinical and auxological characteristics of three patients with short stature and mutation in the IGF-I R gene. **Patients**. We have studied 65 short stature children without classical growth hormone deficiency and with familial antecedents of short stature. We have identified 3 patients with different mutations in the IGF-I R gene not described before. **Methods**. Direct sequencing of the IGF-I R gene, of the coding and splicing zones exons 2 to 21 by Abiprism 310 Applied Biosystem.

Clinical and auxological characteristics in the IGF-IR mutation patients			
	Case 1	Case 2	Case 3
Newborn length SDS	-4.91	-4.27	0.09
Newborn weight SDS	-3.46	-3.0	1.94
Newborn Head Circunference SDS	-5.7	-2.8	0.08
Father´s Height SDS	-0.83	-0.69	-1.54
Mother´s Height SDS	-1.6	-1.54	-4.02
Target Height SDS	-1.22	-1.11	-2.9
SGA in the mother	yes	yes	unknown
Head Circunference SDS (CA)	-4.1 (3.3)	-2.4 (12.1)	-0.7 (11.3)
IGF-I ng/mL (CA) (Tanner)	39 (3.3) (Tanner I)	348 (11.5) (Tanner II)	483 (9.3) (Tanner I)
Height SDS before rhGH (CA)	-3.19 (3.3)	-2.74	-1.72 (10.4)
Height SDS after rhGH (CA)	-2.89	--	-1.48
period of rhGH therapy (months)	20	--	7
rhGH dose (mg/kg/week)	0.21	--	0.20
Intelligence Quotient (CA)	111 (p50-75) (3)	95 (p10-25) (11)	normal (11)
mutation	Y487F	pL81F	IVS13(+30) delGT
location of the mutation	exon 7	exon 2	intron 13-14
mutation in the mother	yes	not studied yet	not studied yet

Case 2 did not receive rhGH treatment. Case 3 at 9.3 years of age his height SDS was -2.19 and his height SDS for bone age was less than -2 SDS with low adult height prognosis. CA: chronological age

Conclusions. Mutation in the IGF-I R gene are more frequent than previoulsy thought. These patients are characterized by strong antecedents of familial short stature, specially in the mother who also presented with SGA at birth, poor response to rhGH therapy and normal intelligence. It is frequent to observe, although it is not always present, the association with intrauterine growth retardation and microcephaly. The molecular functional study of these mutations will demonstrate whether they are pathogenic or not.

PO2-111 Genetics of Growth II

Post-receptor IGF-1 insensitivity restricted to the MAPK pathway in a Silver-Russell syndrome (SRS) patient with hypomethylation at the imprinting control region (ICR1) on chromosome 11p15

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Introduction: Hypomethylation of the paternal ICR1 is the most frequent molecular cause of SRS. Clinical evidence suggests that patients with this epimutation have mild IGF-1 insensitivity (1).
Objective: To assess *in vitro* IGF-1 action and signaling in fibroblasts from a SRS patient with IGF-1 insensitivity.
Subject: A 6.9 yr boy, born small for gestational age (birth weight and length SDS < -3.0) with typical SRS features, was evaluated by severe short stature. His height SDS was -5.4, laboratory evaluation disclosed elevated IGF-1 and IGFBP-3 levels (>2 SD) and exaggerated GH peak at stimulation test (32 mcg/ml). The patient was treated with rhGH (66 mcg/kg/d) with no clear improvement in growth rate, despite an additional increase in IGF-1 and IGFBP-3 levels, indicating IGF-1 insensitivity. Demethylation of ICR1 was shown by MLPA. The patient has a normal karyotype and normal *GHR, IGF1 and IGF1R* coding sequences.

Methods: Fibroblast cultures were established from skin biopsies taken from the forearm of the SRS patient. Cell lines from one nonsyndromic SGA child, one age-matched child and 2 adults were used as control. IGF-1 action was assessed by cell proliferation by colorimetric assay. IGF-1 signaling was assessed by AKT and MAPK phosphorylation after IGF-1 stimulation through SDS-PAGE of intracellular extract followed by immunoblotting with specific antibodies. The expression of *IGF1R and IGFBP3* gene was determined by Real-time quantitative PCR and the levels of the IGF-1R and IGBP-3 protein by direct immunoblotting.
Results: Fibroblast proliferation induced by IGF-1 was on average 50% lower in SRS cell lines in comparison with the controls ($p = 0.02$). The expression of IGF1R mRNA and the level of total amount of IGF-1R protein were similar in all cell lines, not explaining the IGF-1 insensitivity observed in the SRS cell line. Fibroblasts from the SRS patient presented a 14x increase in *IGFBP3* mRNA and 2x more IGFBP-3 secretion to culture serum medium. The SRS cell line presented normal AKT phospharilation, but a 65% lower p42/44-MAPK phosphorylation than other cell lines. Treatment with desIGF-1, which does not bind to IGFBPs, did not recovered proliferation response or MAPK phosphorylation.
Conclusions: IGF-1 insensitivity in our SRS patient with ICR1 hypomethylation is due to a post-receptor defect restricted to the MAPK pathway. In addition, increased IGFBP-3 does not seem to be directly mediating IGF-1 insensitivity in this patient.

PO2-112 Genetics of Growth II

A mutation in growth hormone receptor leading to partial growth hormone insensitivity syndrome

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Introduction: Growth Hormone Insensitivity Syndrome (GHIS) caused by mutations in the growth hormone receptor is a rare autosomal recessive disease leading to profound compromise of growth, variable dysmorphic features and hypoglycemia. GHIS is characterized by high serum growth hormone and low serum IGF-1 and IGFBP3 levels. Here we describe a unique case with growth hormone insensitivity still responding to growth hormone therapy.
Patients/ Methods: A 3 years old female, born to consanguineous Palestinian parents, birth weight 3040 grams and mild dysmorphic features, had increased frequency for food demand since age of four months. At 22, and 26 months of age she experienced two episodes of hypoglycemic seizure. Her physical examination revealed frontal bossing and flat nasal bridge. Her weight was 25%, length 3% (-2SDS), upper to lower segment ratio 1.7 and head circumference 95%.
Her laboratory tests during hypoglycemia revealed GH 947 pmol/l, cortisol 637 nmol/l, insulin 19 pmol/l, IGF-1 <3.25 nmol/l, IGFBP3 0.821 mg/l. Growth hormone stimulation by arginine showed a peak of 35 µg/l. IGF generation test led to increase in IGF-1 levels from 3.72 to 7.31 nmol/l. GH treatment at a dose of 0.03 mg/kg brought to cessation of the hypoglycemic events and improvement in the growth velocity from 2.7 cm/y to 6.7 cm/y.
Genetic analysis of DNA from the proband and her parents using micro satellite markers of growth hormone receptor gene locus on chromosome 5 found our patient to be homozygous and the parents heterozygous. No homozygosity was revealed at the GH gene locus. Sequencing of the Growth Hormone Receptor gene failed to amplify the 5' end of exon 10 of the GHR gene indicating a possible deletion in the intron exon boundary.
Conclusions: We present a unique case of growth hormone insensitivity, manifested by severe hypoglycemia, the height was not severely compromised, IGF-1 generation test was responsive to GH and a standard dose of growth hormone therapy was effective in increasing glucose levels and growth velocity. It suggests that mutations in the C-terminal of the GHR may specifically impair glucose metabolism and possibly enable partial response to GH rather than IGF-1 therapy.

PO2-113 Genetics of Growth II

Genotype-phenotype variations in a family with an unique X to 15 rearrangement

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Introduction: We present two sisters that, due to different segregation between chromosomes 15 and X, have unique phenotypes: a combined phenotype of Prader-Willi syndrome (PWS) and happloinsufficiency of the SHOX gene and isolated haploinsufficiency of the SHOX-gene.
Material: A healthy mother gave birth to two daughters born at term in 1997 and 2001, after normal pregnancies. Daughter 1 had normal birth weight, birth length and head circumference. At the age of 3, she is severely overweight (+ 4 SD) and her height is -1,5 SD with sitting height +3,5 SD and head +2 SD. Daughter 2 was severely hypotonic at birth, but had normal birth weight, length and head circumference. At the age of three months, she was diagnosed with PWS.
Methods: Karyotyping was performed by G-banding. The breakpoints of the rearrangement were cloned by FISH-mapping using BAC clones from the regions of interest. An MLPA methylation assay was used in order to determine the methylation status of the PWS-region.
Results: *Both sisters had an unbalanced translocation, and had lost the derivative chromosome 15. Karyotypes* were 45,X,der(X)t(X;15)(p22;q11),-15.
No known genes were located above the breakpoint on chromosome 15 and seven known genes including the *SHOX*-gene were located above the breakpoint on the X-chromosome. An abnormal methylation pattern of the PWS-region was present in daughter 2, indicating uniparental disomy (UPD) for chromosome 15, whereas the methylation pattern of the chromosome 15 in daughter 1 was normal. Chromosome analysis of the family members revealed a *de novo* balanced translocation between chromosome 15 and the X-chromosome in the mother, 46,XX,t(X;15)(p22;q11)
Discussion: Due to the unbalanced translocation, both the sisters have lost the terminal part of the short arm of chromosome X and thus have a haploinsufficiency for the *SHOX*-gene. Furthermore, daughter 2 shows maternal UPD for chromosome 15, consistent with the Prader-Willi syndrome. To our knowledge, this is the first report of a patient who in combination with PWS phenotype has also haploinsufficiency of the SHOX-gene.

PO2-114 Genetics of Growth II

Functional characterization of a heterozygous Leu1361Arg mutation of the insulin-like growth factor-I receptor in a patient with severe intrauterine growth retardation and absent postnatal catch up growth

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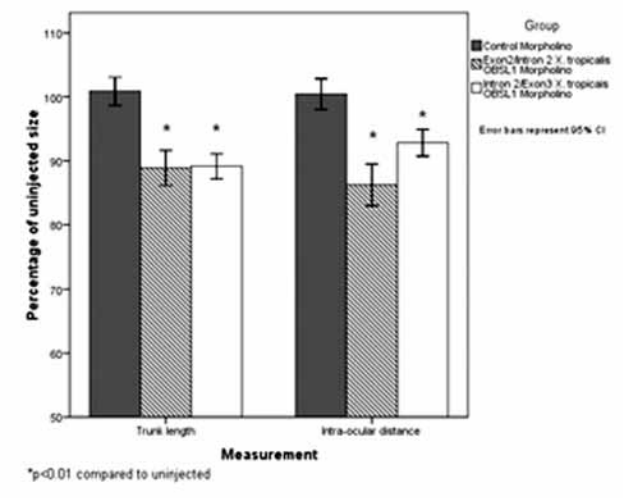
Mutations in the IGF1R gene can result in intrauterine growth retardation (IUGR) and failure to catch-up growth. Here we report on a novel IGF1R mutation in a female patient with IUGR and postnatal growth failure.
The aim of the study was to characterise the effect of the mutation using in vitro assays.
The girl was born after 30 weeks of pregnancy with a birth length of 31 cm (-8.9 SDS) and birth weight of 680 g (-4.0 BMI-SDS). Additional phenotypic characteristics are microcephaly, clinodactyly and retarded bone age. There was no catch up growth not even under GH treatment. At the age of 4.8 yr she had a height of 71.2 cm (-8.1 SDS) and a weight of 5600 g (-4.3 BMI-SDS). Basal GH and GH provocation tests were normal. In the course of GH therapy IGF1 serum levels rose steadily from less than -1.5 SDS at several occasions before treatment to 1.1 SDS. The lack of catch-up growth despite increasing IGF1 levels led us to assume IGF1 resistance.
Molecular genetic analysis of the IGF1R gene by dHPLC and DNA sequencing revealed a heterozygous nucleotide transversion resulting in an exchange of

leucine 1361 to arginine in a residue, which is highly conserved among IGF1R orthologs. The father as carrier of the mutant IGF1R allele was born with an inconspicuous length (50 cm) and weight (3100 g) and reached a final height of 179.8 cm.
In vitro assays with human fibroblasts from the patient were conducted to analyse the autophosphorylation of the mutant receptor after binding of IGF1. Interactions of IGF1R with downstream signalling proteins known to associate with the COOH-terminal tail of the receptor (GIPC, 14-3-3, PI3Kp85) were dissected with a yeast two-hybrid system. However, autophosphorylation of the mut IGF1R as well as protein interactions were not modified compared to wild type cells and receptor constructs. Also, molecular genetic analysis (sequencing and MLPA) of other genes of the somatotropic axis (GH, GHRH, GHRHR, GHR, STAT5B, IGF1) did not reveal any alteration at all.
So far, we have identified a non-conservative amino acid exchange at the COOH-terminal tail of the IGF1R in a severely growth retarded girl. The lack of co-segregation of the mutation with the phenotype as well as preliminary in vitro data suggest the existence of a second-site mutation, which alone or in an interplay with the IGF1R mutation causes this severe phenotype. Additional biochemical and genetic studies are on the way to identify this unknown factor.



PO2-115 Genetics of Growth II

Modelling the primordial growth disorder 3-M syndrome in xenopus tropicalis: early growth impairment is present in a non-placenting vertebrate

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Background: 3-M syndrome is an autosomal recessive disorder characterised by pre- and post-natal growth restriction, radiographic abnormalities (slender long bones and tall vertebrae) and a characteristic facial appearance (fleshy full lips, anteverted nares, triangular face, pointed chin). It is caused by nonsense mutations in the Cullin 7 and Obscurin like-1 (*OBSL1*) genes. Previous work has identified placental vascular abnormalities in the cullin 7 knockout mouse and in placental tissue derived from fetuses with 3-M syndrome. It has been suggested that the growth impairment seen in 3-M syndrome is due to poor placental function
Aim: The aim of this study was to model 3-M syndrome in a non-placenting vertebrate to assess whether the growth restriction is dependent on placental function.
Methods: Two morpholino oligonucleotides designed to alter splicing of X. tropicalis *Obsl1* pre-mRNA and a standard control morpholino were injected into fertilised X. tropicalis oocytes. The resultant embryos were allowed to grow until stage 50 (14 days of life) when the intra-ocular distance and trunk length were measured.
Results: The X. tropicalis ortholog of *OBSL1* was identified using tblastn and blastp searches on the Joint Genome Institute X. tropicalis website and consists of 19 exons encoding a transcript of 5079bp and a 1691 amino acid protein which is 36% identical and 50% similar to human OBSL1.
The control morpholino did not significantly alter size compared to uninjected embryos while morpholinos designed against the exon 2/intron2 and intron2/exon3 splice sites produced a significant reduction in trunk length and intra-ocular distance (see figure). RT-PCR of a 5' segment of X. tropicalis *Obsl1* cDNA demonstrated evidence of exon exclusion and intron retention for both active morpholinos. The morpholino injected tadpoles were phenotypically normal except for the growth restriction.
Conclusions: Early growth impairment in 3-M syndrome due to loss of OBSL1 is not solely dependent on placental function. Using non-placenting animals is a useful tool for evaluating the effects of placental function on genetic disorders with pre-natal growth impairment.

PO2-116 Genetics of Growth II

Clinical observations, molecular genetic analysis and treatment of sitosterolemia in infants and young children

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Background: Reports of infants and young children treated for sitosterolemia are rare. While ezetimibe is highly effective in adults presenting with sitosterolemia, its efficacy in young children has not been described.
Patients' profiles, methods and therapeutic outcomes We report clinical, biochemical and molecular genetic observations, and the treatment outcomes of 4 young Chinese children presenting with sitosterolemia, from 3 separate families, in whom we identified 1 new (Y329X) and 3 known (R446X, N437K, R389H) mutations. R389H mutation was found in 50% of the alleles we studied, a prevalence similar to that observed in Japanese patients suffering from sitosterolemia. In this study, we found a patient had no response to Ezetimibe at 3 months of age, but started to have a response at 2 years of age. The other patients also had various responses to treatment with ezetimibe.**Conclusions:** Children presenting with sitosterolemia who are <2 year of age might not respond well to treatment with ezetimibe, perhaps because of immaturity of the glucuronidation system.

Table Baseline lipid profiles, liver enzymes and blood cell counts of each study patient

		Patient no			
	Normal values	1	2	3	4
Age, y		8 years	18 months	3 months	23 months
Blood lipids					
Cholesterol, mg/dl					
Total	125-240	427	705	402	640
Low-density-lipoprotein	60-150	346	565	304	519
High-density-lipoprotein	35-84	59	64	42	64
Total triglycerides, mg/dl	20-200	111	149	395*	98
Liver enzymes					
Alanine transaminase, U/l	5-45	10	13	45	15
Aspartate aminotransferase, U/l	15-55	19	31	100	31
Blood count					
Erythrocytes, count/µl	3.7 x 10^9 -5.3 x 10^9	3.35 x10^9	4.25 x 10^9	3.98 x10^9	4.49 x 10^9
Hemoglobin, g/dl	11.5 -15.5	9.8	11.8	11	12.7
Mean corpuscular volume, fl	80-95	88.5	89.0	80.6	80.2
White blood cells, count/µl	4,500-17,500	7200	6,900	6700	11,200
Platelets, count/mm^3	150 x 10^6 - 350 x 10^6	211 x 10^6	289 x 10^6	506 x 10^6	566 x 10^6

*in the non-fasting state

PO2-117 Genetics of Growth II

Duplication of 17q21-25 is associated with growth hormone resistance

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Background: At present, only GH receptor and STAT5B defects are known causes of a distorted GH signal transduction pathway. A possible additional cause may be increased activity of one of the SOCS proteins, known inhibitors of the JAK-STAT signaling pathway.

Case: A 5.5 year old girl presented to our clinic with severe progressive short stature. She was born after an uncomplicated pregnancy of 42 weeks. Birth weight was 3433 gram (-0.8 SDS), head circumference was 37.0 cm.(1.2 SDS), the first measurement of length was at nearly 6 months of age: - 3.4 SDS.

At birth she was hypotonic, and had several dysmorphic features: flat nasal bridge, short neck, post-axial polydactyly at all extremities, club feet and short femur and humerus. The abdominal wall was flabby. The karyotype showed a de novo duplication of 17q21-25 in 26/30 metaphases, which was confirmed in bone and fibroblasts. In the first 5 years of life she underwent several orthopedic operations (for instability of the cervical vertebral column) and had frequent viral and bacterial infections. She initially showed some delay in motor development, but is currently performing normally at school.

At presentation, height was 93.2 cm (-4.7 SDS), BMI: 16.98 kg/m^2 (+ 0.9 SDS), head circumference: 48.3 cm (-1.5 SDS).

IGF-1 was 4.6 nmol/L (-3.1 SDS) and IGFBP-3 was 2.4 mg/L (-0.94 SDS). The GH peak after clonidine was 115 mU/L, with a baseline value of 30.5 mU/L and a minimum of 7.3 mU/L. The results of the IGF-1 generation test (0.7, 1.4, and 2.1 mg/m^2/day for 1 week) follow. IgA was decreased (0.2 g/L) and further immunological studies are in progress, as well as in vitro studies on JAK/STAT signaling after interferone and GH stimulation.

Discussion: We hypothesize that 17q21-25dup may result in overexpression of suppressor of cytokine signaling 3 (SOC3) (17q25.3) and STAT3 (17q21.2) and possibly other genes playing a role in the GH signaling pathway. SOCS is a known inhibitor of STAT5B. Overexpression of STAT3 could lead to heterodimerization of other STATs resulting in inhibition of the signaling pathway. Both abnormalities could lead to the clinical picture of GH resistance. The duplication of the GH1 gene (17q23.3) may contribute to the exaggerated GH secretion.

We propose that 17q21-25 duplication can be added to the list of possible causes of GH resistance.

PO2-118 Genetics of Growth II

A common polymorphism in the insulin-like growth factor 2 gene is associated with birth size and size at 3 years of age

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Introduction

Obesity and hypertension have been associated with birth size and early growth leading to the concept of the Early origins of Adult cardiovascular disease. Environmental influences have been proposed as the link but very few studies have considered underlying genetic explanations. We have previously demonstrated that a common polymorphism in the gene encoding Insulin like Growth Factor 2 (IGF2)(*Apa* I; alleles AA;AG;GG) is associated with measures of body composition in adults and now report findings in 449 infants followed longitudinally into childhood.

Methods

We genotyped 449 cord blood samples chosen at random from a cohort of 1650 singleton, white Caucasian pregnancies and related findings to size at birth (n=449), and growth at 3 years of age (n=216). One way analyses of variance (ANOVA), with the Tukey honest significant differences (HSD) post hoc test was used to determine differences between anthropometric measures and IGF2 genotype.

Results

The IGF2 AA genotype was significantly associated with lower birth weight and length and with lower weight and body mass index at 3 years of age.

Association of IGF2 (*Apa*1) gene polymorphism with intrauterine size; size at birth and 3 years of age

IGF2 *Apa*1 SNP	**GG**	**AG**	**AA**	***p***
Birth weight SDS	0.16(0.9) (n=236)	0.13(0.9) (n=180)	-0.22(0.78) (n=33)	0.025
Birth Length SDS	-0.10(1)	-0.13(1.2)	-0.52(0.97)	0.04
3 years Weight SDS	0.15(1.0) (n=118)	-0.03(0.9) (n=76)	-0.7(0.9) (n=22)	0.0005
3 year Height SDS	0.2(1.1)	0.2(0.9)	0.04(1.0)	0.44

Data shown as mean with standard deviation in parentheses

Conclusion

These data suggest that this IGF2 polymorphism in itself or as a marker of change in the locus associates with size at birth and 3 years of age. This polymorphism is associated with a 205g difference in birth weight (similar effect size to smoking in pregnancy) and a 1.7 kg difference in weight at 3 years of age suggesting an amplification of effect size with age.

PO2-119 Genetics of Growth II

How commonly are mutations in the sonic hedgehog signaling pathway found in individuals with septo-optic dysplasia and holoprosencephaly?

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Background: Septo-optic dysplasia (SOD) is diagnosed when ≥2 of; optic nerve hypoplasia, midline brain abnormalities and pituitary hormone insufficiencies are present. Holoprosencephaly (HPE) is characterized by incomplete separation of the cerebral hemispheres, and may reflect the more severe end of the SOD spectrum. A genetic diagnosis can be made in <1% of patients with SOD and 30% with HPE.

Sonic Hedgehog (Shh) plays a key role in the formation of the midline central nervous system (CNS) with haploinsufficiency of Shh disrupting midline CNS patterning and causing HPE. Ebstein et al identified an upstream regulator of forebrain Shh expression, Shh brain enhancer-2 (SBE2) and identified a pathogenic point mutation in one individual (474 HPE cases screened). We

hypothesized that mutations in SBE2 may also cause some cases of SOD, a condition also associated with abnormal forebrain patterning.
Growth-arrest specific 1 (Gas1) is a membrane bound glycoprotein that has an antagonistic effect on Shh signalling. Gas1(-/-) mice have microform HPE with multiple midline abnormalities. There are few data on the prevalence of mutations in Gas1 in patients with HPE.
Aim: To determine the frequency of pathogenic changes in Shh and Gas1 in individuals with HPE and SBE2 in individuals with SOD.
Methods: DNA was extracted from patients with HPE (n=44) and SOD (n=346). Primers were designed for amplification of the SHH, GAS1 and SBE2 exon sequences and standard PCR and sequencing protocols performed. Individuals with HPE (44) were screened for changes in SHH and Gas1 and patients with SOD (346) were screened for changes in SBE2.
Results: A novel coding sequence change in SHH was identified in two siblings with variants of HPE -c1295t>a pI431T. One presented with cleft lip and palate, corpus callosum hypoplasia and developmental delay; in the other the only abnormal clinical finding is a solitary median maxillary central incisor. Two single-nucleotide polymorphisms were identified in Gas1 c264c>t, p.A88A (n=4); c.291c>g, p.A97A (n=1). No mutations were identified in SBE2.
Conclusion: In this large SOD cohort no mutations in SBE-2 were identified. This upstream regulator of Shh expression is therefore unlikely to play a significant role in the pathogenesis of SOD. We identified a novel mutation in SHH in 2 siblings with HPE. No mutations in Gas1 were found in 44 individuals with HPE; changes in Gas1 are therefore not a common finding in individuals with HPE.

PO2-120 Genetics of Growth II

Exon 3 deletion polymorphism of the growth hormone receptor (GHR) and GH response: a meta-analysis

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Background: A polymorphism of the GHR gene involving a deletion of exon 3 (d3 GHR) has been implicated in determining an individual's growth response to GH. To test the relevance of this finding across a range of growth disorders treated in a number of countries, we have evaluated first year growth response to GH in published series genotyped for the d3 GHR polymorphism and from our own institution using meta-analytical approaches.
Methods: In our patients, GHR genotype was assessed by PCR and related to clinical parameters in a cohort of 97 children receiving GH therapy with a range of growth disorders (GHD, SGA/ISS and Turner syndrome [TS]). To identify published series, a systematic review using MEDLINE and EMBASE databases was supplemented by hand-searching conference proceedings. The primary endpoint of interest was change in height SDS over the first year (ΔHtSDS). When required, authors were contacted to obtain the relevant data. Results were combined using random-effects methods and expressed as standardized mean differences (SMD). Metaregression models were derived to test for study-level confounding factors.
Results: 24 datasets (1710 cases) from 14 studies were identified. The prevalence of the d3 GHR allele ranged from 32% to 82% (mean: 49%). All studies provided ΔHtSDS over the first year; the summary SMD for full length versus d3 GHR carriers was -0.09 (95% CIs: -0.3 to +0.11) [negative SMD indicates better response in D3 carriers]. A metaregression model for SMD revealed no significant variables, indicating that the overall contribution of genotype was weak. However there was significant clinical heterogeneity across studies and between diagnoses with the SMD in GHD being +0.1 (95% CIs: -0.22, +0.41), in SGA/ISS -0.22 (95% CIs: -0.59, +0.14) and in TS -0.24 (95% CIs: -0.56, +0.07). Adjustments for GH dose, mean age at start of therapy, prevalence of full length allele, and mean parental height SDS did not significantly influence the SMD metaregression model.
Conclusions: Across a range of studies with differing diagnoses, the evidence was weak that the GHR exon 3 genotype impacts upon ΔHtSDS as a primary index of response to GH therapy. However, the presence of heterogeneity across clinical settings suggests that the d3 GHR genotype may influence response in certain diagnoses such as SGA/ISS and TS.

PO2-121 Genetics of Growth II

Autosomal dominant GH-deficiency: GHRH stimulation increases in a cellular model the harmful dominant-negative GH isoform proportion while affecting *wt*-GH secretion

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An autosomal dominant form of isolated GHD (IGHD II) can result from heterozygous splice site mutations that weaken recognition of exon 3 leading to aberrant splicing of *GH-1* transcripts. *In vitro* and transgenic animal data suggest that the onset and severity of the IGHD II phenotype relates to the proportion of 17.5-kDa produced which depends on the position of splice site mutation.
To study more in detail the cellular and molecular mechanism of IGHD II, rat pituitary cell line stably expressing hGHRHR (GC-GHRHR) were singly transfected with either *wt*-GH or with different GH-splice site mutants (IVS+2, IVS+6 and ISE+28) known to cause IGHD II of various severity in patients. Upon GHRH stimulation, *wt*-GH produced almost exclusively 22-kDa GH isoform and undetectable amounts of 17.5-kDa while ISE+28 and especially IVS+2 and IVS+6 produced significantly increased amounts of 17.5-kDa isoform as assessed at mRNA- and protein level. This resulted also in a significant reduction of *wt*-GH secretion depending on the splice-site position involved. After inhibition of proteasome pathway responsible for the degradation of misfolded 17.5-kDa isoform, the difference in GH secretion was even more pronounced. Moreover, GC-GHRHR cells co-expressing *wt*-GH and each of the mutants (wt/IVS+2, wt/IVS+6, wt/ISE+28) displayed reduced GH secretion when compared to cells expressing only *wt*-GH (wt/wt) suggesting a dominant-negative effect of 17.5-kDa GH isoform on the secretion of 22-kDa GH. Furthermore, increased amounts of 17.5-kDa isoform produced after GHRH stimulation in cells singly transfected with GH-splice site mutants, affected endogenous production of rat GH, which was not observed when only *wt*-GH was expressed.
Taken together, the results we obtained in our cellular model of IGHD II support the hypothesis that after physiological GHRH stimulation the severity of IGHD II depends on the position of splice site mutation leading to the production of increased amounts of 17.5-kDa isoform, which block the secretion of *wt*-GH in the most severely affected cases. Due to the absence of negative feedback control in IGHD II, a chronic up-regulation of GHRH may lead to an increased stimulatory drive to somatotrophs to produce GH, from both the normal and mutant alleles resulting in an increased production of the 17.5-kDa GH isoform relative to the 22-kDa isoform accelerating auto-destruction of somatotrophs in a vicious cycle.

PO2-122 Genetics of Growth II

A case of DeGeorge syndrome (22q11.2) coupled with the 3q29 microduplication syndrome, neural tube defects (NTDs), seizure disorder and developmental delay

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Microdeletion and microduplication genetic syndromes (genomic disorders) are knownto be a significant cause of developmental delay and dysmorphology. The recent development of array Comparative Genomic Hybridization (aCGH) allowed the molecular and clinical characterization of several new genomic disorders. DiGeorge syndrome is a well recognized genomic disorder characterized by facial dysmorphism, growth and developmental delays, congenital heart disease, immune cytopenias and predisposition to psychosis. It is usually caused by a "3-5 Mb" de novo deletion of chromosome 22q11.2 which is usually confirmed by Fluorescent In Situ Hybridization analysis. Chromosome 3q29 duplication syndrome is a recently recognized genomic disorder associated with microcephaly, facial dysmorphism, obesity and developmental delay. It is caused by a (1.61-1.76 Mb) microduplication of chromosome 3q29 identifiable by aCGH. While spina bifida had been occasionally associated with DiGeorge syndrome, it is not recognized as a common feature of either syndrome.
Here, we report on a 3 9/12 years old girl who was born with myelomeningocele, hydrocephalus that required shunt placement and type II Arnold-Chiari malformation. Her postnatal course was complicated by severe developmental delay, hypotonia, excessive weight gain, lack of speech, neurogenic bladder, hydronephrosis, epilepsy. She had recurrent urinary and upper respiratory tract infections. She also suffered from two stroke like episodes associated with developmental regression. The cause for the developmental regression is unknown. Flow cytometry analysis showed mild B and T cell deficiency. Serum PTH and ionized calcium were normal. Oligonucleotide-aCGH analysis (Signature Genomics, LLC) of peripheral blood showed "2.47 Mb" deletion at chromosome 22q11.2 and "144.8 KB" microduplication at chromosome 3q29. Parental studies were not done. This unusual and complicated presentation including spina bifida is likely due to this child's complex genotype which was possible to examine in detail with aCGH. We therefore suggest that individuals with complex phenotypes including spina bifida, associated dysmorphism, and other anomalies should be evaluated for genomic abnormalities. Additional genetic testing such as chromosomal microarray analysis (aCGH) is warranted in such patients.

PO2-123 Genetics of Growth II

Three novel mutations in the growth hormone secretagogue receptor (*GHSR*) gene associated with constitutional delay in growth and puberty (CDGP) or isolated growth hormone deficiency (IGHD)

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Introduction: Ghrelin stimulates GH secretion by acting on GHSR. Recently, mutations in the *GHSR* gene were described in patients with IGHD and idiopathic short stature (ISS). **Objective:** Study the *GHSR* gene in children with growth impairment. **Methods:** The coding region of *GHSR* was directly sequenced in 71 patients with ISS and 17 with IGHD, with a eutopic posterior pituitary lobe and absence of mutations in the *GH1 and GHRHR*. **Results:** Three different heterozygous missense mutations within the *GHSR* exon 1 were identified in 2 patients with ISS/CDGP and in one with IGHD. These mutations are located in highly conserved regions and were not detected in 164 controls. The first mutation, a nucleotide substitution (c.505 G>A) resulting in an alanine to threonine substitution (p.A169T) in the 4th transmembrane-helix, was identified in a 17 yr-old male patient (bone age 14 y) starting puberty, with a height SDS of -2.8, normal weight (BMI SDS = -1.2) and IGF-1 SDS of -1.8. He achieved a final height SDS of -1.2, adequate for his target height. His father was unavailable for genetic studies and his mother and one brother have normal *GHSR*. The second mutation was found in a female patient who has a nucleotide substitution (c.545T>C) resulting in a valine to alanine substitution (p.V182A), in the 2nd extracellular loop. She started puberty at 13 y (bone age 11 y), with a height SDS of -2.5 and normal weight (BMI SDS = 1.0). She presented with a normal stimulated GH peak (7.9 µg/L) and IGF-1 SDS of -1.6. The same GHSR mutation was found in her father who was normal height. The third mutation, a nucleotide substitution (c.745G>T) resulting in a valine to leucine change (p.V249L) in the 3rd intracellular loop was found in a 2.8 yr-old girl with height SDS of -5.0 due to IGHD. She had low IGF-1 levels and the highest GH peak after stimulation tests was 1.0 µg/L. She was treated with hGH for 11 y, reaching a final height of 155 cm. Her father, who also presented with short stature (height SDS = -3.6), carries the same mutation, but her brother, who also has IGHD, carried normal alleles. **Conclusion:** We describe three novel *GHSR* mutations in patients with different severities of short stature and GH secretion status. The pharmacological characteristics of these variant GHSRs are currently being compared with those of the wild type receptor. This data will potentially reveal link between observed receptor properties and the range of observed phenotypes in affected individuals.

PO2-124 Genetics of Growth II

A study in 83 patients with pituitary stalk interruption syndrome: novel *HESX1* mutation and severe hormonal prognosis in malformative forms

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Pituitary stalk interruption syndrome (PSIS) represents a particular entity in the population of patients with GH or combined pituitary hormonal deficiencies (CPHD). Only rare cases have been assigned to a known genetic cause.
Objectives: To describe a cohort of PSIS patients, to compare subgroups with or without extra-pituitary malformations (EPM), to identify predictive factors of evolution and to screen pituitary transcription factor genes.
Methods: From the GENHYPOPIT database, we analyzed features of 83 patients from 80 pedigrees presenting PSIS and performed *HESX1 and LHX4* gene screening in 44 and 63 patients, respectively.
Results: In our population, mean age at diagnosis was 9.6 years with a 2.3/1 male predominance. PSIS was rarely familial (4.8%). PSIS patients with EPM (28.9%) presented significantly more anterior pituitary aplasia (8.7% vs. 0%) and GHD with at least two other hormonal abnormalities (87.5 vs. 69.5%) than patients without EPM. Posterior pituitary location along the stalk constituted a protective factor regarding severity of hormonal phenotype (p=0.02).An unreported *HESX1* causative mutation was found in a consanguineous family and 2 different *LHX4* mutations were identified in familial PSIS.
Conclusion: we characterized two types of PSIS patients: one presenting EPM suggesting an antenatal origin with severe hormonal and pituitary imaging status and a second with a milder phenotype. *HESX1 or LHX4* mutations accounted for less than 5% of cases in the overall population of PSIS and were identified in the context of consanguinity or in familial cases.

PO2-125 Genetics of Growth II

Heterozygous *IGFALS* gene mutations are present in both idiopathic short stature (ISS) and in normal children: impact on height and acid-labile subunit (ALS) levels

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Background: Human ALS deficiency, caused by inactivating mutations in the *IGFALS* gene, is characterized by moderate growth retardation and marked reduction of IGF-I and IGFBP-3 levels that remain low after GH treatment. Heterozygous (HZ) carriers for *IGFALS* gene mutations are frequently shorter than their wild type (WT) first degree relatives. Likely, milder cases of ALS deficiency having less detrimental gene mutations in one or both *IGFALS* alleles may be present in a subgroup of ISS children.
Objectives: The aim of this study was to search for *IGFALS* gene mutations in unselected ISS children and in control children selected by low normal levels of ALS, and to determine the impact of these mutations on height and ALS levels.
Subjects and Methods: We studied 46 normal children (ages 5.0-16.3 y) out of 190, presenting ALS levels <-0.5 SDS and 87 ISS children (ages 1.8-8.9 y). Serum levels of ALS were determined by RIA and expressed as SDS in relation to normal controls. The *IGFALS* gene was PCR amplified and entirely sequenced.
Results: Non-synonymous HZ *IGFALS* gene mutations were found in 6 ISS patients: E35GfsX16, G83S, R277H, A330D and A546V; as well as in 3 normal controls: V239M, N276S and R277H. Height SDS in HZ controls were: -0.35; 1.09; and -1.10, respectively. By extending this study to first degree relatives of ISS children, auxological, genetic and biochemical data were obtained from 11 siblings (1 HZ) and 8 parents (3 HZ). In HZ carriers (parents + children), height and ALS levels (mean SDS±SD) were lower than WT: -2.05±0.88 (n=10) vs. -0.08±0.83 (n=15), p<0.0001 for height; and -2.02±1.74 vs. –0.70±1.10, p=0.029 for ALS levels. When height deficit was evaluated within each family, the Δ height SDS in HZ (HZ HSDS - mean WT HSDS) was significantly lower than the hypothetical value 0 (median -1.71; range -0.61 to -2.32, p=0.002).
Conclusions: In families of ISS children, both height and ALS levels were lower in HZ carriers for *IGFALS* mutations compared to WT, suggesting that both gene alleles are required to express maximal ALS levels and to fulfill growth potential. Although the role of ALS on growth is not completely understood, HZ *IGFALS* gene mutations could be involved in the etiology of short stature in a subset of ISS children. The finding of *IGFALS* gene mutations in normal control children reveals that these mutations are also present in the normal population and could be the source of ALS deficient subjects in non-consanguineous families.

PO2-126 Genetics of Growth II

A patients with trichorinophalangeal syndrome type I (TRPS1) and a SHOX mutation

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TRPS1 syndrome is characterized by typical facial features (sparse, fine hair, bulbous nose, long philtrum), skeletal abnormalities and short stature.
Mutation of the SHOX gene causes short stature with highly variable clinical severity, ranging from isolated short stature to Leri-Weill dyschondrosteosis (LWD) and Langer syndrome.
We report a case of a pt with TRPS1 and LWD.
Case report: First born of non consanguineous parents at term. Birth-weight 2950g, length 46.5cm, OFC 34.6cm. She arrived to our attention at 6 yrs. At the physical examination: thin, sparse and slow-growing scalp hair, bulbous nose, high filtrum, Madelung deformity, short hands with brachyphalangy and tapered fingers, short stature with truncal prevalence. At the X-ray: cone-shaped epyphisis of proximal phalanges. At 7 yrs for pubertal signs she was treated with tryptorelin until 11.5 yrs. At 10 yrs height at the 3rd centile, TH at 50thcentile. Partial GH-deficit was diagnosed, GH therapy was started and continued until 12.5 yrs. FH 139.5cm. At 12 yrs vaginal septum (at echo and MRI) was diagnosed and surgically treated. Menarche at 13 yrs.
As the pt showed the typical signs of TRPS1 and disproportionate short stature with Madelung deformity, both TRPS1 and SHOX genes were studied.
Diagnostic tests: Genomic DNA analysis was performed by PCR and direct sequencing of all coding and splicing regions of both genes. 2 novel missense mutation were identified: the R908G in the exon 6 of the TRSP1 gene and the A234V in the exon 6a of the SHOX gene. The novel TRPS1 gene mutation maps in the conserved GATA type Zn-finger domain, one of the poly Zn-finger domains of TRPS1 that bind the DNA. The nature (positively charged Arg>neutral Gly) and the position of the R908G substitution probably cause a severe defect in the protein function. The novel SHOX gene mutation maps outside the conserved HMGbox domain, in the C-ter region that may be involved in the transcriptional regulation and the same substitution was reported affecting the previous residue, the A233, but a functional significance of these residues remains to be assessed.
Conclusion: This is the first report of a pt with TRPS1 and LWD, that presents the concomitance of a mutation in both TRPS1 and SHOX. The possible additive or sinergistic effect of the mutations must be studied, as these proteins are both expressed in chondrocytes and loss of these factors seems to accelerate plate fusion.

PO2-127 Genetics of Growth II

Relation of common IGFBP-1- and GHR- gene variants with glucose homeostasis in former extremely low birth weight preterm infants

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Background: Low birth weight predisposes to the development of insulin resistance and related disorders. In addition to auxological parameters such as rapid catch-up growth, low IGFBP-1 serum levels in childhood have been linked to an increased risk of insulin resistance later in life. Concerning postnatal growth, we previously reported the GH receptor (GHR) d3-variant to be associated with higher IGF-1 levels and catch-up growth in very low birth weight (< 1500 g) preterm infants. In children born small for gestational age, a common IGFBP-1 promoter polymorphism -575G/A has recently been linked to IGFBP-1 serum levels and has been suggested to be an additional player in the interplay between the IGF-IGFBP-axis and metabolism [Van der Kaay et al., JCEM 2009].
Design: We analyzed postnatal growth, metabolic parameters (fasting insulin, HOMA-R, HbA1c, and IGFBP-1), and genotypes for IGFBP-1 -575G/A (RFLP) and the GHR d3-variant (multiplex PCR) in 51 former extremely low birth weight preterm infants (ELBW; < 1000 g). Mean age on examination was 6.1 yrs (range 4.0-7.8 yrs).
Results: As expected, the GHRd3 carrier status was significantly associated with current length-SDS (fl/fl -1.2±1.0; [fl/d3+d3/d3] -0.5±1.0; corr. for target height, resp.; $p < 0.05$) and catch-up growth. IGFBP-1 -575G/A, on the other hand, did not influence any of the auxological parameters analyzed. None of the variables postnatal growth, GHRd3, and IGFBP-1 -575G/A significantly influenced fasting insulin or HOMA-R, although mean fasting insulin levels seemed to slightly increase with every GHRd3 allele inherited (fl/fl 2.9 U/l; fl/d3 3.5 U/l; d3/d3 4.0 U/l). However, we found significantly lower IGFBP-1 (fl/fl 53.9±35.4 µg/l; [fl/d3+d3/d3] 36.6±21.6 µg/l; $p < 0.05$) as well as higher HbA1c (fl/fl 5.0±0.4 µg/l; [fl/d3+d3/d3] 5.3±0.3 µg/l; $p < 0.05$) concentrations in GHRd3 carriers, a finding not seen in respect to IGFBP-1 -575G/A (IGFBP-1: GG 38.4±20.4 µg/l; [GA+AA] 45.2±31.7 µg/l; $p > 0.2$; HbA1c: GG 5.2±0.4; [GA+AA] 5.1±0.3 %; $p > 0.2$). Interestingly, IGFBP-1 and HbA1c levels also did not differ between children either with or without catch-up

growth (p > 0.2).
Conclusions: Our data link the GHRd3 variant to both catch-up growth and parameters of glucose homeostasis in ELBW infants. Whereas the impact of IGFBP-1 -575 G/A on glucose homeostasis seems to be only moderate in preterm infants, those carrying GHRd3 may bear an increased risk for the development of insulin resistance in adulthood.

PO2-128 Genetics of Growth II

Holoprosencephaly associated gene mutations as a possible cause of pituitary stalk interruption syndrome (PSIS)

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Background: Holoprosencephaly (HPE) is characterized by great phenotypic variability. The phenotypic spectrum ranges from minor defects like hypotelorism or single central incisor (SCI) to severe malformations. Potential pathogenetic mechanisms include genetic and environmental factors. Mutations in genes located in various chromosomal loci have been associated with HPE, Sonic Hedghog (SHH), TGIF, SIX3 and ZIC2 being the most extensively studied. PSIS syndrome constitutes a distinct midline defect of unknown pathogenetic mechanism. It seems however that genetic, as well as, environmental factors are implicated. Based on the observation that 3 of our patients with PSIS also had SCI, a characteristic found in certain HPE cases, we initiated a search for mutations in HPE associated genes.
Patients and methods: Thus far mutations in the TGIF and SHH genes have been looked for in 30 patients with MPHD associated with PSIS and ectopic neurohypophysis.
DNA was extracted from peripheral lymphocytes and the entire coding regions of TGIF gene (exons 2 to 4) and of the HSS gene (exons 1 and 2) were PCR amplified and sequenced. PCR amplification and sequencing of HSS exon 3 was unsuccessful due to its high GC content.
Results: One of our patients was found to carry a novel heterozygous C to T nucleotide transition at position 799 of the TGIF gene. This molecular defect results in a premature stop codon at Q267X in the repression domain 2b of exon 4 of the TGIF gene. This mutation results in a truncated protein (5 amino-acid shorter than the wild type peptide).
A second patient had an 18p deletion. The deleted part included the TGIF gene. Thus far no mutation has been detected in exons 1 and 2 of the SHH gene in our patients with PSIS.
Conclusions: Our data suggests that in certain cases of PSIS, mutations of the HPE genes may be implicated.

PO2-129 Genetics of Growth II

Presence of the exon 3 deleted isoform of GH receptor (GHRd3) does not influenced on clinical and laboratory characteristics, IGF-1 generation test and the response to rGH treatment in children with ISS

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Introduction. GHRd3 polymorphism has been reported to be associated with responsiveness to rGH therapy in children with variety range of growth disorders (GH deficiency, SGA, ISS and Turner syndrome) in some but not in all studies. Recently was reported that children with ISS carrying the *GHR* d3 allele present a higher GH sensitivity on IGF-1 generation than children homozygous for *GHR* fl allele. On other hand, this correlation did not observed in children with GH deficiency.
Aim. The aim of our study was to evaluate the influence of the GHRd3 polymorphism on clinical data and laboratory parameters (including IGF-1 generation test) and responsiveness to rGH treatment in children with ISS.
Materials and methods. The study included 39 prepubertal children with ISS (31 boys and 8 girls). Standard GH stimulation test (clonidin) and IGF-1 generation test (rGH 33 mkg/kg/day during 4 days) were performed in all children. The GHRd3 polymorphism was genotyped by PCR assay. Patients received rGH therapy at dose 40,8±10,3 mkg/kg/day with duration of treatment from 6 up to 12 months.
Results. The distribution of GHR genotype (56,4%- fl/fl, 41%- fl/d3 and 2,6%- d3/d3) was similar previously reported in 150 healthy (control) group in Russian population (Orlovski, 2004). Basal clinical data and laboratory findings in children with different genotypes were similar. Interestingly, that basal GH concentration was a statistically significant higher in children with *GHR* d3 allele than in children homozygous for *GHR* fl allele (1,18±1,20 for fl/fl *vs.* 0,74±1,03 for fl/d3 and d3/d3; *P*= 0,037). No statistically significant differences in changes of IGF-1 concentration were found in both groups neither at short-term generation test nor during 6-12 months of rGH treatment. Growth effect of treatment (height velocity, changes in height SDS) did not differ statistically among genotypes.
Conclusions. Analysis for GHRd3 polymorphism in children with ISS does not give sensitive predictions of phenotype and cannot predict growth response to rGH treatment.

PO2-130 Genetics of Growth II

Genotype-phenotype correlation in children with *SHOX* gene variants

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Background Haploinsufficiency of *Short stature Homeobox-containing gene* (*SHOX*) is known to cause short stature. Multiple mutations as well as allelic variants (Vs) have been described, but the clinical significance of Vs is not known.
Objectives To define the clinical and biochemical correlates of *SHOX* Vs in patients with short stature.
Methods We retrospectively reviewed records of patients (pts) referred for short stature over 6 years, with *SHOX* tested as part of clinical care at Esoterix Labs (CA). Pts with chromosomal or skeletal abnormalities (i.e. Turner Syndrome, Leri-Weill dyschondrosteosis) were excluded. *SHOX* Vs included partial gene deletions, mutations previously described as allelic variants, and previously undescribed mutations. We compared Height (Ht) SDS; IGF-1 SDS; and Bone Age/Chronologic Age ratio (BA/CA) between pts with *SHOX Vs and normal SHOX*. Growth Hormone (GH) stimulation tests were done using Arginine & L-Dopa, normal GH ≥10 ng/mL. Among GH treated pts, Ht SDS, IGF-1 SDS, Growth velocity (GV) and GH dose were compared at baseline, 6, 12 and 24 months. Hypothesis test of equal means and Fisher's exact test (SAS) were used for comparison.
Results Among 335 pts tested for *SHOX* mutation, 17 were excluded (12 chromosomal, 5 skeletal abnormalities, of whom 7 had whole *SHOX* gene deletions). Among 318 included pts, 83 had *SHOX* Vs (S+), with 19 different *SHOX* mutations. Nucleotide change c.277+17G>T was found in 43% of S+ group. There were no differences at baseline in age, Ht SDS, and BA/CA between S+ and pts with normal *SHOX* (S-).

Table 1. Baseline Comparison

Variable	S+ Mean (SD) n=83	S- Mean (SD) n=235	p
Age (yr)	9.78 (3.68)	10.61 (3.47)	0.06
Ht SDS	-1.96 (0.70)	-2.06 (0.75)	0.31
IGF-1 SDS	-1.46 (1.21)	-1.84 (1.36)	0.19
BA/CA	0.85 (0.11)	0.86 (0.11)	0.60

GH stimulation tests were performed in 57% of pts; there was no difference in % of GH deficient pts (S+ 42.6%, S- 39.8%, p=0.74). Among 38% of pts treated with GH, Ht SDS, IGF-1 SDS, GV and GH dose did not differ significantly at baseline, 6, 12 and 24 months (S+ n=31, 30, 24, 16; S- n= 91, 76, 66, 42, respectively). Subgroup analysis comparing c.277+17G>T and S- groups

among GH treated patients did not show statistical difference in the above parameters.
Conclusions *SHOX* Vs in children with short stature were common. There seem to be no auxologic or biochemical difference between pts with and without *SHOX* Vs.

PO2-131 Genetics of Growth II

Responsiveness to growth hormone therapy in SGA children in dependency of INS VNTR genotype

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Aims: Responsiveness to growth hormone therapy has measurable phenotypic variation resulting from both genetic and nongenetic effects. In the last years some genetic factors were detected to have effects on responsiveness to growth hormone therapy. The insulin-system is common as essential factor for prenatal and postnatal growth. Variations in the insulin-system are therefore of particular interest for responsiveness to growth hormone therapy in SGA patients. An allele length variation at the variable number tandem repeat (VNTR) locus, a 14- to 15-base pair (bp) oligonucleotide minisatellite in the promoter region of the insulin gene (INS), is known to have functional effects on INS transcription. INS VNTR allele lengths fall into 2 general classes: class I with an average of 570 bp and class III with an average of 2200 bp. The objective of our study is to investigate the association of INS VNTR genotype with responsiveness to growth hormone in the first 6 months of therapy.
Patients and methods: Auxiological and laboratory data from 44 patients with SGA (52.3 % female) at the begin of therapy and after 6 months were collected. We analysed the INS VNTR genotype. The INS VNTR distribution was 59.1 % for class I/I and I/III and 40.9 % for class III/III.
Results: [group 1 vs. group 2; mean ± SD; * = p<0,001; # = n.s.]
Baseline: N: 26 (14f/12m) vs. 18 (9f/9m) - age at onset therapy (yrs): 8.16 ± 3.12 vs. 7.68 ± 3.48 # - BW (g): 2021 ± 723 vs. 2075 ± 507 # - BL (cm): 42.9 ± 5.9 vs. 43.3 ± 4,3 # - GA (wks): 36.9 ± 3.8 vs. 37.6 ± 2.2 # - doses (µg/kg): 0.037 ± 0.01 vs. 0.042 ± 0.01 # - Ht (SDS): -3.38 ± 0.65 vs. -3.62 ± 0.84 # - Ht vel (SDS): -1.61 ± 1.77 vs. -1.43 ± 2.39 # - target ht (SDS): -0.94 ± 0.65 vs. -1,4 ± 0.78 # - IGF1 (SDS): -1.82 ± 1.42 vs. -1.06 ± 1.59 # - IGFBP3 (SDS): -0.71 ± 1.69 vs. -0.36 ± 2.25 #
After 6 months: Δ Ht (SDS): 1.95 ± 1.29 vs. 1.79 ± 2.40 # - Δ Ht vel (SDS): 0.27 ±Δ IGF1 (SDS): 2.52 ± 1.35 vs. 1.67 ± 1.09 # - Δ IGFBP3 (SDS): 1.31 ± 1.61 vs. 0.42 ± 2.93 #
Conclusion: In conclusion we see no correlation between INS VNTR genotype and responsiveness to growth hormone therapy.

PO2-132 Genetics of Growth II

Analysis of mineral status in children with Marfan syndrome

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Marfan syndrome(MS) is an inherited connective tissue disorder due to mutations in the fibrillin-1 gene. Fibrillin abnormality could influence mineralization. A decreased bone mineral density has been reported in adults with MS. Data on bone mineralization in children are still lacking.Aim of the study was to assess bone mineralization and bone metabolism in children with MS. BMC and BMD were evaluated in the left femoral neck and in the lumbar spine (L2-L4). Measurements were obtained with DXA (Hologic 1000). Bone metabolism was evaluated by means of bone turn-over hormones (PTH, osteocalcin, CTX, 25-OH-D3 vitamin and alkaline phosphatase).Twenty-five children with MS (age 8.9- SD 3.6, BMI 14.9- SD 2.3) and 12 healthy controls (age 10.9 SD 4.1. BMI 17.8 SD 2.18) were enrolled. A significant difference (P= 0.01) was shown in Z-score of lumbar spine (-0.73- SD 1 in MF children vs -0.33- SD 0.42 in controls) and a significant difference (P<0.05) in Z-score of femoral neck (-1.31-SD 1.02 in MF children vs -0.37-SD 1.23 in controls). The same significant difference(P<0.05) was in lumbar BMC determinations (20- SD 7.22 vs 31.36- SD 13.2), lumbar BMD (0.59- SD 0.10 vs 0.77- SD 0.18) and BMC of femoral neck (2.68-SD 0.71 vs vs 3.54- SD 1.08). No differences between the two groups were found in bone turn-over hormones. Compared to healthy children, patients with MS show a significant decrease in bone mineralization as demonstrated by the lower Z-scores of femoral neck and lumbar spine. The lower values of mineral measurements expressed as Z-score allow an appropriate estimation of mineral status in growing patients by using reference values for age. In the management of MF patients an assessment of mineral status by DXA should be taken into account.

PO2-133 Genetics of Growth II

Short stature caused by a novel heterozygous mutation in the *IGF-I* gene: clinical and biochemical findings and short-term effect of hGH treatment

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Patients with homozygous *IGF-I* deletions or mutations present with severe intrauterine growth retardation, extremely short stature, deafness, microcephaly and mental retardation, while heterozygous carriers only show a mild deficit of birth weight, height and head circumference (HC).
We present the clinical and biochemical characteristics (table) of 2 children (III-1 and III-2), their mother (II-2) and grandfather (I-1) with a heterozygous *IGF-I* mutation and the results of short-term treatment with hGH in these children.
Whereas both children presented with postnatal growth retardation, delayed bone age and low plasma IGF-I levels, only child III-1 was born small for gestational age (SGA). Case III-1 had feeding problems during early infancy, the GH peak after stimulation with clonidine was 32 mU/L and IGF-I in a generation test only increased on a high GH dose. The GH peak in case III-2 after arginine stimulation was 17 mU/L. Mother was born SGA, and had feeding problems in infancy. Grandfather was examined at the age of 65.2 yrs and was known with poor bone quality, overweight, early dementia and several other problems.
Sequencing of *IGF-I* revealed a heterozygous duplication of 4 nucleotides, resulting in a frame shift (c.243_246dup, p.Ser83GlnfsX13). Grandmother (I-2) and aunt (II-1) had a wild type (wt) *IGF-I* sequence. In vitro, synthetic mutant IGF-I did not bind to the IGF-I receptor or antagonize the growth-promoting effect of wt IGF-I. It did bind to serum IGFBPs, but without forming 150 kD complexes.
hGH treatment (1.4 mg/m^2/day) was started in cases III-1 and III-2 at 8 and 6 yrs, respectively. After 2 years height SDS increased from -3.9 to -2.8 (III-1), and from -4.5 to -3.0 (III-2). Plasma IGF-I SDS rose from -2.3 to 0.6 (III-1) and from -2.6 to 0.3 (III-2). IGFBP-3 did not change significantly.
In conclusion, *IGF-I* haploinsufficiency can be associated with low birth weight, short stature and low HC. Short children with a heterozygous *IGF-I* mutation can be successfully treated with GH.

Clinical and biochemical features						
	I-1	II-2	III-1	III-2	I-2	II-1
IGF-I gene	+/-	+/-	+/-	+/-	+/+	+/+
Gender	M	F	F	M	F	F
Age (yr)	65.2	35.5	8.2	6.2	64.5	37.2
Height (SDS)	-1.4	-3.5	-4.1	-4.6	-1.2	-2.0
HC (SDS)	-1.8*	-1.7*	-2.4*	-1.6*	0.1	0.4
BMI	28.6	31.3	14.1	13.5	27	40
Sitting height : Height (SDS)	1.7	2.2	1.6	2.5	0.7	1.7
IGF-I (SDS)	-2.0	-1.8	-2.3	-2.6	0.6	-0.5
IGFBP-3 (SDS)	0.1	-0.6	1.2	0.1	2.0	0.6

PO2-134 GH and IGF Use II

Hypotonia-cystinuria syndrome in an adult female

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Introduction. The hypotonia-Cystinuria is a recently described syndrome with the clinical picture of hypotonia, anorexia, failure to thrive, delayed motor development, craniofacial dysmorphy in infancy and by hyperphagia and obesity developing during childhood; all patients develop severe nephrolithiasis due to cystinuria. They all manifest absolute growth hormone deficiency or growth hormone dysfunction as has been described already in the Prader-Willi syndrome. A microdeletion of part of SLC3A1 (renal cystine transporter) and PREPL (propyl oligopeptidase-like) genes located at chom. 2p21 was found in all patients (18 of 13 families).

Casus. We describe an adult female suffering from the hypotonia-cystinuria syndrome who was diagnosed at the age of 22 years: height: 143.6 cm, facial dysmorphism, truncal obesity, severe nephrolithiasis due to cystinuria and ostoporosis. The bone age was 17Y6M and the pubertal development was normal with absence of hypogonadism. Serum IGF-1: 140 ng/mL (Ref. value: 223-471).

Trial with growth hormone therapy. Growth hormone was given in a dose of 0.6 mg/day SC. In 5 months she growed 2 cm and the osteoporosis improved as has been showed by Bone Mass Density measurements (DEXA).

BMD Lumbar Spine (DEXA)		
Follow-up	BMD L1-L4 g/cm2	Ref. value*
basis	0.814	1.277+/-0.180
1 year	0.854	idem
2 years	0.920	idem

IM van der Sluis et al Arch Dis Child 2002;87:341-347

Conclusion. Consider the Hypotonia-Cystinuria Syndrome in patients with cystinuria plus and Prader-Willi-like syndromes. These patients have a need for growth hormone therapy at all ages.

PO2-135 GH and IGF Use II

Long-term multi-endocrine sequelae after childhood medulloblastoma: results from a follow-up of 30 survivors, treated at the Royal Marsden Hospital of London

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Thirty children (23 males and 7 females) long-term survivors from medulloblastoma were retrospectively studied to analyze their long-term neuro-endocrine sequelae. All the children included in the study were treated with surgery and radiotherapy; the radiation dose to the whole cranium and spinal axis was 32,3 ±6,3Gy (mean ±standard deviation,SDS) with a posterior fossa boost of 23,6 ±5,1 Gy; 27 of 30 children were treated with additional chemotherapy. All the children included in the present study received an endocrinological evaluation each 4-6 months after the end of any treatment for medulloblastoma. For each subject, The data collected included more than 200 variables, as well as the results of the endocrinological tests and body measurement made on every subsequent follow-up visit. Patients who discontinued the therapy or the follow-up visits, and those who died shortly after diagnosis were excluded. All the children were clearly pre-pubertal at the time of diagnosis. The mean age at the diagnosis was 6,3 years (range 1,8-11,4). The mean follow-up time was of 8,1 years from the diagnosis and of 7,2 years from the end of any cancer treatment. **Results:** in 27 of 30 (90%) subjects, endocrine abnormalities were observed. In 25 of 30 (83%) subjects, growth hormone (GH) secretion was impaired; within this group, all the patients received recombinant human GH (rhGH) therapy, with a mean age at rhGH treatment start of 8,9 years; at the time of the closure of the present study, 7 of 25 patients treated with rhGH reached the final height that resulted of a mean -1,7 ±0,7 SDS against target-height expected from biological potential (midparental height). 17 of 30 (53%) showed impairment of hypothalamus-pituitary-thyroid (HPT) axis: 2 of 30 (7%) showed primary thyroid disfunction, 5 of 30 (17%) central hypothyroidism and 10 of 30 (33%) subclinical hypothyroidism. Interestingly the mean time elapsed by the discover of an HPT abnormality and the end of any cancer treatment was 3,8 years (range 2-15,7). 4 of 7 (57%) girls included in the study showed early (3) or precocious (1) puberty. 1 of 23 (4%) males showed delayed puberty. Central adrenal insufficiency was not observed. **Conclusions**: this study shows the high incidence of various neuro-endocrine sequelae after treatment for Medulloblastoma. Since the time between the discover of an endocrine impairment and the end of cancer treatment can be very long, a long-term endocrine surveillance is highly recommendable.

PO2-136 GH and IGF Use II

A 15 year follow up analysis of parental attitudes toward short stature

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Background:

Patient-centered medicine requires an understanding of family attitudes. However, attitudes about short stature, their relationship to referral/treatment, and their stability over time are unknown.

Aims:

1) Compare attitudes towards stature among parents of short children referred to endocrinologists (referred) and parents of children with heights <10th% seen by pediatricians (controls), 2) conduct a 15y follow up (FU) to define the children's long-term outcomes (treatment, height) and current parent attitudes, 3) determine factors that predict changes in parent attitudes from baseline (BL) to FU.

Methods:

At BL (1993-4), 154 referred and 240 control parents completed a survey about stature. At FU (2008-9), 308 (76%) parents were identified and 202 (66%; 98 referred, 103 controls) completed an identical phone survey.

Results:

At BL, heights (SD) of referred and control children were -2.1±0.6 and -1.9±0.9 respectively. Referred parents perceived their children to suffer due to height significantly more than controls. FU indicated more referred than control children had received growth-promoting therapy (23.5% v 7.8%, P=.002). Among *un*treated children, adult heights (SD) were -0.47±0.49 in referred and -0.35±0.41 in controls (NS); the change (Δ) in height SD from BL was 1.6±0.6 and 1.5±1.1. Compared to BL, more referred than control parents perceived improvements in their children's well-being including self-esteem (47 v 20%), treatment by peers (28 v 14%) and ability to cope with height (71 v 32%) (each P<.02). Logistic regression indicated that improved self-esteem was significantly related to being referred (OR 3.8; CI 2.0, 7.4) and male (OR 2.2; CI 1.1, 4.4) but was not related to adult height SD, Δ height SD, or receipt of therapy. Predictors of improved treatment by peers were similar. Improved ability to cope was related to being referred and height attained, but not to gender or

receipt of therapy.
Conclusions:
1) At BL, referred parents viewed the impact of short stature more negatively than controls. However, 15y later, referred parents were more likely to perceive improved well-being in their children regardless of whether the child received growth therapy.
2) Untreated control and referred children gained considerably in height SDs over 15y, reaching similar adult heights.
3) Parent attitudes about the impact of short stature may change substantially with time raising questions about how to incorporate parent attitudes into management decisions.

PO2-137 GH and IGF Use II

Selective eating disorder masquerading as primary growth hormone insensitivity syndrome

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Selective Eating Disorder Masquerading as Primary Growth Hormone Insensitivity Syndrome
Background: Primary Growth Hormone Insensitivity Syndrome (PGHIS) is caused by IGF-1 deficiency due to intrinsic tissue resistance to growth hormone (GH). It is characterized by short stature, poor linear growth with IGF-1 deficiency despite high levels of GH. Treatment with biosynthetic IGF-1 can restore linear growth. Nutritional deficiency can mimic the endocrine features of PGHIS but fails to respond to IGF-1 therapy.
Objective: We want to report two patients initially considered to have PGHIS who were ultimately diagnosed with malnutrition due to a bizarre selective eating disorder.
Methods/Results: Two unrelated African-American males (ages 9 and 14 years old) were referred for endocrine evaluation of short stature and poor linear growth of many years duration with delayed bone age. Despite having deficient IGF-1 levels of 49 and 60 ng/ml, peak stimulated GH was 51.8 and 68 ng/ml, respectively. IGFBP-3 was between low and normal range (2.4-3.6 mg/L). One patient received a 6-month course of mecasermin (biosynthetic IGF-1) without improvement. Repeat chemistries revealed hypocalcemia in one patient and hypoproteinemia in both. With careful dietary history, it was found that both patients' major source of calories was French fries and mashed potatoes with infrequent intake of protein or dairy products. One patient opted for enteral alimentation by gastrostomy tube rather than to change his eating habits. After psychiatric counseling, the second patient increased the diversity and quantity of protein/calorie intake. Both subjects had subsequent improvement in growth and metabolic status.
Conclusions: Selective eating disorder may be difficult to diagnose and clinically resembles PGHIS. A careful and aggressive dietary history is recommended for children thought to have PGHIS before treatment with biosynthetic IGF-1. Long term psychological and nutritional support is necessary to overcome metabolic derangements and to restore and maintain normal growth in such patients with bizarre eating disorders.

PO2-138 GH and IGF Use II

Bone turnover biochemical markers during GH replacement therapy in prepubertal children

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GH deficiency (GHD) severely limits bone growth resulting in reduced rate of bone remodeling. In order to assess bone turnover during GH replacement therapy in prepubertal children with GHD we compared serum bone turnover biochemical markers concentrations in a control group of 30 healthy prepubertal children (8-14 years old) with those of 27 paired-aged children with documented isolated GHD. All the children of the GHD group were treated with a standard dose of human recombinant GH (hrGH) (0,5 IU/Kgr/week). Clinical evaluation (height, weight, BMI, HV) and bone turnover serum biochemical markers [alkaline phosphatase, bone TRAP, serum C-telopeptide of type I collagen (s-CTX), osteocalcin, osteoprotegerin] were measured before and in the 1st, 3rd, 6th and 12th month of therapy in the GHD group, while the same biochemical markers were measured once in the control group. Bone mineral density (BMD) and bone age were assessed at the beginning and at 12th month of therapy in treated children. Statistically significant difference was detected between the control group and GHD group before treatment initiation in s-CTX (median 258 ng/ml in the control group versus 112 ng/ml in GHD group, p< 0,01) and in bone alkaline phosphatase (ALP) (median 166 IU/l in the control group versus 110 IU/l in GHD group, p=0,02). These differences were gradually eliminated at the end of the first year of therapy for s- CTX (258 vs 243 ng/ml respectively) and at the 3rd month of therapy for bone ALP (166 vs 146 IU/l respectively). Osteoprotegerin concentrations show no significant difference between the two groups during the first year of therapy (median 4,7 pmol/l versus 5,2 pmol/l for control and GHD group respectively, p> 0,05). These results underline the significant changes in bone metabolism during the first year of therapy with hrGH in GHD children.

PO2-139 GH and IGF Use II

Predictive factors to response to GH treatment and final height in children with GH deficiency

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Aim: To identify predictive factors for good response to treatment with recombinant growth hormone (rGH), through a retrospective study of a large cohort of patients from the same North Italian Centre of Paediatric Endocrinology.
Subjects and methods: we analysed children treated with rGH for more than three years and almost reached their near final height. Patients were grouped according to the ethiology of GHD. Children with idiopathic GHD were grouped according to the gain mid-parental height (g-MPH): difference between near final height standard deviation score (SDS) and mid-parental height SDS. Good responders if g-MPH was >0,5 SDS, bad responders if it was <-0,5 SDS
Results: we studied 256 patients: 71% had IGHD, 18% had organic GHD, and 11% had other disorders which needed GH treatment.
A significant correlation between near final height SDS and mid-parental height SDS was found for children with IGHD (r=0,268 p<0,01) but anything for these with OGHD (r=0,258 p=0,286).
Among IGHD 21% were good responders (GR) and 34% bad responders (BR). Median height SDS at the start of treatment was similar between the two groups: –2.3 for GR and –2.4 for BR respectively. At this time, their stimulated peak GH levels in response to stimulus tests and their values of IGF1 were similar. Moreover GR were much younger at start of treatment (7,49±2,28 vs 9,73±3,17 years p<0,002), had a gain in height SDS after the first year of treatment and at the onset of puberty, double than BR (first year: 0,78±0,32 vs 0,38±0,6 p<0,005; puberty: 1,24±0,58 vs 0,70±0,83 p<0,01). Interestingly GR were younger at the onset of puberty than BR both for chronological (11,05±1,78 vs 13,1±1,48 p<0,001) and bone age (9,86±2,61 vs 14,18±1,22 p<0,032). GR reached a better near final height SDS than BR (-0,78±0,51 vs -1,58±0,81 p<0,001) but genetic height potential (cm and SDS) for GR was lower than BR (cm: 160,35±7,27 vs 169,15±7,72 p<0,001; SDS:-1,89±0,5 vs -0,38±0,75 p<0,001).
Conclusion: Earlier age at the start of GH treatment and better gain in height after the first year of treatment and at the onset of puberty are the principal factors influencing the response to GH treatment with conventional doses and the final height. Given the high percentage of bad responders, further clinical and genetics studies are required to evaluate the individualised optimal treatment regimen if adverse characteristics (as older age at start, puberty, very low MPH) are present.

PO2-140 GH and IGF Use II

Near final and final adult height in Egyptian children with isolated idiopathic growth hormone deficiency treated with recombinant growth hormone

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***Background*:** Despite the availability of growth hormone (GH) therapy that is unlimited by production capacity, many studies show that most patients still fail to reach their genetic target heights.
Objectives: to evaluate the final and near final adult height in Egyptian children with isolated idiopathic growth hormone deficiency and to determine factors affecting adult height.
***Subjects and methods*:** this is a retrospective study including patients diagnosed with isolated idiopathic growth hormone deficiency and on GH therapy who reached their near final or final adult height. All these data are obtained: onset of the condition, age at diagnosis and start of therapy, duration of delay of therapy, duration of treatment, and full anthropometric data including: target height, estimated mature height, serial height, weight, growth velocity measurements and their SDS, bone age, and stage of puberty at start of therapy and age of spontaneous puberty.
***Results*:** Eighty-seven out of 374 patients with isolated idiopathic growth hormone deficiency reached final and near final adult height. They are 53 males and 34 females. The mean near final adult height (NFAH) SDS in boys is -2.34 ± 2.46 and their mean final adult height (FAH) SDS is -2.31 ± 0.34. While the mean NFAH SDS in girls is – 2.25 ± 1.89 and their FAH SDS is -2.38 ± 1.74. Their height gain SDS was 1.46 ± 1.1 SDS for males and 1.48 ± 0.62 SDS for females.
The height gain after reaching near final height was 2.2±1.3 cm in males and 1.97±1.1 cm in females. FAH SDS was positively correlated to basal height SDS (p = 0.0001) and age at onset of puberty (0.02), age at start of therapy (p=0.02), degree of growth hormone deficiency (p=0.02), and duration of GH treatment (p=0.03).
Conclusions: FAH of GH deficient children is below their target height by1.0 ± 0.9 SDS with 88% within their target range. Early diagnosis, intensifying therapy before puberty, compliance and continuation of therapy will help to improve their FAH.

PO2-141 GH and IGF Use II

A comparison of referral patterns to the pediatric endocrine clinic before and after FDA approval of growth hormone for idiopathic short stature

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Background: Concern for short stature (SS) is a common reason for referral to the pediatric endocrine clinic. In 2003, the FDA approved the use of growth hormone (GH) for the treatment of children with idiopathic short stature (ISS). Whether this new indication for GH therapy has altered referrals for SS is unknown.
Objective: To analyze referral patterns for SS in 2 one-year time periods preceding and subsequent to FDA approval of GH for ISS.
Design/Methods: A retrospective chart review of children referred for SS between July 1998 and June 1999 (interval one) and between July 2005 and June 2006 (interval two) was performed. Variables collected included age, gender, height (ht), and parental hts. Children less than two years of age were excluded.
Results: In interval one, 138 subjects (90 boys) aged 9.15 ± 4.48 years were identified. In interval two, 268 subjects (171 boys) aged 9.51± 4.45 years were identified. No differences in age or gender distribution between intervals were found. Average ht SDS in the recent time period was -2.14 ± 0.83, which was unchanged from the earlier interval, in which it was -2.11± 0.9 (p=0.74). The percentage of patients who did not meet the definition of SS (ht<-2SD) was 43.1% in interval one and 43.8% in interval two. Similarly, no differences between intervals were found in either target height (Tht) SDS (p=0.49) or Child ht SDS-Tht SDS (p=0.35).
Conclusion: Although GH was approved for ISS in 2003, no difference in referral patterns for SS following this new indication in our area were identified. In both time periods, nearly half of all children referred did not meet technical criteria for SS. Continued education directed at primary care providers regarding definition of SS and eligibility for GH therapy should be pursued.

PO2-142 GH and IGF Use II

Childhood craniopharyngioma in Macedonia: incidence and outcome

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Objectives: To assess the incidence and outcome of treatment of childhood cranyopharyngioma in Macedonia. Methods, patients, results: Thirteen cases of cranyopharyngioma in children (aged 8.43+/-3.61 years; range 2.90-13.85; M:F=9:4) treated between 1989-2008 in Macedonia were reviewed. Results: Initial signs were vision disturbances (7/13, 53.84%), seizures (1/13, 7.69%), growth retardation (8/13, 61.53%), diabetes insipidus (2/13, 15.38%). Suprasselar/intrasellar extraventricular localization was noted in 11 patients (84.61%%), and two patients (15.38%) had a tumor extension in the third ventricle. Cranial irradiation was performed in 12/13 children (92.3%), intracystic bleomycin was applied in one child (7.69%). Follow-up time ranged from 6 to 229 months (mean 104.92+/-76.11 months). All children had multiple pituitary deficiencies after surgical removal of the tumor (100%). Obesity was not found before treatment. Body mass index (BMI) however increased from 16.93+/-6.34 SDS at the time of diagnosis to 26.33 +/-5.91 SDS (p>0.005) at the last follow-up. Growth hormone treatment resulted in normalization of the adult height: from -1.27 +/-1.52 SDS at the start of the treatment to -0.13 +/-1.39 SDS at the end. The final height was not significantly bellow the genetic target height (p>0.005). All children had multiple pituitary deficiencies, DI was permanent in 9/13 children (69.23%). Main permanent deficit was vision impairment: blindness on one or both eyes was found in 4 children (30.72%), bitemporal hemianopsia in 4 (30.72%), other visual defects in 2 (15.38%). Recurrence was removed in one case, after 31 months. Conclusions: It is remarkable that no mortality was observed for the whole observation period of 104.92+/-76.11 months. Overall incidence for the period 1989-2008 in Macedonia was estimated to 1,43 per 1.000.000 person-years.

PO2-143 GH and IGF Use II

Two-years GH therapy with intermittent discontinuation: a cost effective therapy in SGA

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The potential benefits of GH treatment, resulting in a significant Ht gain in children born SGA, are well demonstrated. However, no consensus on the best treatment regime to attain the highest possible adult Ht. Opinions are divided between a relatively short treatment course of 3-4 years (y) or up to the end of growth.The shorter treatment regimens, while registering impressive growth during the first 2 y of treatment, are followed by a dramatic decline in growth velocity upon cessation. Previous studies in patients with ISS demonstrated good response to GH in the first 2y and no increase in Ht SDS thereafter. Yet, treatment interruption results in catch down growth. we have previously shown that **alternate day therapy (ADT**) prevents catch down.**Hypothesis:** By following a 2 y treatment regime with a 4 month period of ADT, catch down can be avoided.**Patients and Methods:** 29 SGA children were enrolled. The study group was divided into 2 groups. Group A (4F/11M, gestational age (GA) 33±4.2 w, birth weight (BW) 1359±100gr, Target Ht SDS 0.01±1.19, age of starting GH treatment 6.15 ±1.6 y) were treated for 4 y with daily rGH 33mg/kg/ day. Group B(6F/8M, GA 36.5±3.2 w, BW1895±529 gr. Target Ht SDS-0.13±12, age GH treatment 6.3±1.9y) were treated for 2y with daily rGH 33mg/kg/day followed by 4 months of ADT at 66mg/kg/day. There was a significant differences in between the groups only in GA p<0.01 and BW p<0.02. Patients were followed up to 4y from the date of starting treatment. **Results:**Cassation treatment after 2y of daily rGH treatment followed by 4

months of ADT prevented catch down, as measured by delta Ht SDS. The total delta Ht SDS 4y was in groupA 1.35 ± 0.55 vs 1.23± 0.4 Ht SDS in B, **p<0.53**. The main gain in Ht SDS was during the first 2y of reatment 1.14±0.5 in A vs 1.35±0.3 in group B, p≤0.18. During the 1st y the gain was 0.88±0.6 in A vs B 1.10±0.4 Ht SDS, p<0.5 and during the 2nd y it was 0.2±0.6 in A vs 0.34±0.3 Ht SDS in B, p≤0.5. During the 3rd y A gained 0.2 ±0.15 Ht SDS while B -0.05±0.02 p<0.009. During the 4th y A gained 0.006±0.68 Ht SDS while B -0.11±0.17 p≤0.03. No correlations was found in between delta Ht SDS in the first two y of treatment and GA, BW, target Ht, the age or the Ht at the beginning of treatment.**Conclusions:**SGA children can benefit a 2y treatment regime of GH with a 4 month period of ADT without a decline in their gained Ht SDS after 4y. The 4 month of ADT provides a promising approach to avoid catch down growth.

PO2-144 GH and IGF Use II

Effect of growth hormone treatment in a girl with mono-allelic deletion of the IGF-I receptor gene

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IGF-I receptor signalling is crucial for normal pre- and postnatal growth. Children with heterozygous deletions or mutations of the IGF-I receptor gene are rare and very few reports exist about the therapeutical approach with GH in these very short children. We have earlier reported on in vitro experiments based on cultured fibroblasts of a girl with severe growth failure and heterozygosity of the IGF-I receptor gene, which was confirmed by fluorescence in situ hybridization. IGF-I receptor protein level was significantly reduced, but response of the girls fibroblasts following stimulation with IGF-I did not differ from control fibroblasts. There is still controversity in the literature about the functional significance of loss in IGF-I receptor expression.
We now present our patients growth response to GH treatment and give an interpretation with reference to our former in vitro tests. The girl was born after 39 weeks of gestation with a weight of 2,13 kg and a height of 45 cm. At the age of 8 years and 9 months her height was 97,6 cm (-7,4 SDS), her height velocity was 2,3 cm per year (-4,6 SDS), serum IGF-I was +1,6 SDS. GH therapy was initiated (0,03 mg/kg/day), which improved height velocity to 6,6 cm per year (+1,4 SDS), but catch up growth was not sustained. GH dosage was then gradually increased: the girl is presently treated with 0,05 mg/kg/day, IGF-I is in the range between +2,8 and +3,2 SDS. She is now 11 years old, her height is 110,1 cm (-6,0 SDS), height velocity is 4,8 cm per year (-0,26 SDS). GH does improve height velocity in our patient with mono-allelic deletion of the IGF-I receptor, she gained 1,4 height SDS during 27 months of GH treatment. However, catch up growth was not sustained after the first treatment year, although she receives a supraphysiologic GH dosage.
The treatment effect in our patient is similar to that seen in other reported patients with IGF-I haploinsufficiency. The reduced responsiveness to GH in these children supports the assumption that haploinsufficiency of the IGF-I receptor gene leads to IGF-I insensitivity. This is in contrast to the results of our in vitro experiments and therefore a hint that in this case, the fibroblast culture does not reflect the in vivo situation properly.

PO2-145 GH and IGF Use II

International meta-analysis of GH in cystic fibrosis

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Background: Poor growth occurs in 25% of CF children. Studies document improvement with growth hormone (GH). Our purpose was to perform a meta-analysis of studies conducted in prepubertal and pubertal children with CF from Germany and the U.S.
Methods: We created a combined database using raw data from 6 previously published studies, with separate reporting for teens and children. Subjects categorized as controls (CONT) were enrolled in study but not treated with GH. GHTX were treated with GH (0.11 – 0.21 IU/kg/day) for 6 - 12 months. Subjects were randomized, except for nine. *Results:* There were 60 CONT + 87 GHTX perpubertal subjects (age 9.9 ± 1.7, 54% male) and 22 CONT + 54 GHTX pubertal subjects (age-13.4, males-64%). Baseline characteristics were similar for GHTX and CONT in the pubertal groups. Data from one year of study is presented in **Table 1** for prepubertal and **Table 2** for pubertal. GH resulted in significantly better height (Ht), weight (Wt), lean mass (LTM) and hospitalizations in both pubertal groups. However, there was no difference in FEV1 in prepubertal children and FEV1 % predicted was lower in GHTX. In teens, GHTX had significantly better FEV1 and FEV1 % predicted.
Summary: The results confirm significantly higher HT, WT, and LTM and lower hospitalizations in subjects treated with GH. Results of pulmonary function suggest that absolute FEV1 is not lowered by GH treatment. The decrease in % predicted FEV1 prepubertal with an increase in teens, suggests improvement FEV1% predicted is dependent on the Knudson score target, which increases to a greater degree with height change in younger children than in teens. *Conclusion:*Our study represents the largest data set for both prepubertal and pubertal subjects and support the anabolic use of GH in CF. Our study also suggests the need for a better surrogate than FEV1% predicted to define clinical improvement in rapidly growing children with CF.

TABLE 1. Results for 12 months in Prepubertal Children

	Height (cm)	Weight (kg)	LTM (kg)	FEV1	FEV1 %prd	Hosp
GHTX	138.7 ± 11.7*	30.3 ± 5.9*	25.8 ± 5.7*	1.43 ± 1.5	73.6 ± 24.8*	0.55 ± 1.1*
Cont	130.1 ± 14.9	26.2 ± 6.8	20.4 ± 3.4	1.74 ± 2.7	77.0 ± 26.6	1.2 ± 0.9

TABLE 2. Results for 12 months in Adolescents

	Height	Weight (kg)	LTM (kg)	FEV1	FEV1 %prd	Hosp
GH TX	158.2 ± 8.8*	42.4 ± 7.9*	36.9 ± 6.6*	2.63 ± 1.27*	80.4 ± 23.8*	0.84 ± 0.84*
Cont	153.2 ± 7.7	39.1 + 6.5	33.1 ± 5.9	1.99 ± 0.77	61.7 ± 26.9	1.9 ± 1.4

PO2-146 GH and IGF Use II

Anabolic use of growth hormone in pediatric Crohn's disease

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Background: Crohn's disease is a condition of chronic relapsing and remitting inflammation involving the gastrointestinal tract. Many patients also have poor weight gain and short stature, which can occur despite adequate nutrition. The purpose of this on-going study is to test the hypothesis that poor growth in Crohn's disease is associated with protein catabolism, secondary to inflammation, and that these underlying mechanisms of poor growth are improved by GH. *Methods:* We plan to recruited 15 previously diagnosed and 10 newly diagnosed children with Crohn disease; none treated with Remicaid™. Height and weight is measured every 3 months; lean mass and bone density is measured by DXA every 6 months. Evaluation of whole body protein turnover is measured using the stable isotope [1-^{13}C]leucine and GC mass spectrometry. Nutritional analysis, cytokines and measures of disease severity are conducted every six months. After six months of study, patients are treated with Genotropin 0.30 – 0.40 mg/kg/week. *Results:* To date we have recruited 15 patients (6 newly diagnosed, 9 previously dx). Nine have been treated with GH. As demonstrated by Table 1, GH has resulted in improvement in height, weight, lean mass and protein catabolism. No adverse events have occurred. *Conclusion:* GH is affective adjunct therapy for improving growth and nutrition in children with Crohn disease. We will have more data at the time of the meeting.

	Height Velocity (cm/yr)	Weight Velocity (kg/yr)	LTM	IGFSD
Control	2.8 ± 4.3	3.4 ± 5.3	25.5 ± 6.1	-2.42 ± 0.9
GHTX	14.2 ± 5.1*	12.5 ± 5.8*	34.4 ± 5.9*	+0.9 ± 0.8*

*P< 0.05 as compared to control

PO2-147 GH and IGF Use II

Efficiency of growth hormone therapy in children treated for medulloblastoma or ependymoma: effects on height, body proportions and IGF-I

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Background: Most children treated for brain tumors with craniospinal irradiation and chemotherapy develop growth hormone deficiency (GHD). Although final heights achieved by children receiving GH-replacement have improved, most patients fail to reach their genetically determined length. Possible explanations are impairment in responsiveness to GH or tissue damage. We report on the auxological and endocrinological parameters of 22 patients receiving growth hormone treatment for 14 months to 9 years.

Patients and methods: Between 1991 and 2005 54 patients were treated for medulloblastoma or ependymoma including craniospinal irradiation at the Medical University of Vienna. 22 patients (m : f = 10:12) who developed GHD received continuous GH-replacement therapy. Mean age at diagnosis of these 22 patients was 6.75 (±3.5) years and at start of GH-treatment 11.27 (±3.1) years.

Results: Mean height at diagnosis was -0.14 SDS. During the first two years after irradiation and chemotherapy growth was significantly reduced leading to a decrease of height SDS of -1.95 at time of diagnosis of GHD. At this time sitting height was already significantly decreased (-2.67 SDS ±1.17). During GH-therapy (25µg/kg/d) height (p=0.007), IGF1 (p=0.001), and leg length (p=0.000) increased significantly. As expected there was only a small increase in mean sitting height during therapy and therefore the disproportion increased with a difference of up to 3.1 SDS between sitting height and leg length.

Conclusions: Growth hormone therapy using a standard substitution dose is efficient with respect to normalization of IGF1 and long bone growth. Failure to achieve genetically determined height despite GH-therapy is most likely due to tissue damage to the spine by the cytotoxic effect of irradiation. We do not recommend supraphysiological GH-doses in this cohort of patients. Further studies will show whether newer treatment protocols using a lower craniospinal dose or different fractionation schedules such as hyperfractionation will improve final height by reducing the disadvantageous impact of irradiation on the spine.

PO2-148 GH and IGF Use II

Gender bias in children receiving growth hormone treatment in Australia: no evidence of an ascertainment bias

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Introduction

It is well documented, across many different countries and health systems, that about twice as many boys than girls are treated with growth hormone (GH) for conditions resulting in short stature. Anecdotally it has been claimed that the "more likely" explanation is that boys are more frequently referred to growth clinics as it is socially more acceptable for girls to be short than boys. Studies by August *et al.* (J Pediatr 1990;116(6):899-903) and Grimberg *et al.* (J Pediatr 2005;146(2):212-6) suggested that ascertainment bias was likely to be occurring as they noted that, in terms of height standard deviation scores (SDS), girls were significantly shorter on average for their age and sex than boys on referral, at least for some diagnostic categories.

Subjects and Methods

A total of 1462 children were selected from the OZGROW database who were currently receiving GH (as of 3rd December 2007). Height data was also analysed from two unbiased surveys of Australian children. Height measurements were converted to SDS values according to sex and age at measurement using the LMS procedure and the CDC 2000 charts. Patients were categorized by "Indication", one of seven criteria for recieving GH as a Pharmaceutical Benefit, and by "Diagnosis" as described by the treating paediatric endocrinologist. Frequency differences between girls and boys were assessed by goodness-of-fit Chi square analyses. Differences between girls and boys in Height SDS, Target Heights and Height Deficit, at first visit to a growth centre were assessed by t tests and Mann-Whitney U tests.

Results

Total boys, 812, out numberd Total girls other than Turner, 453 ($P=3.68\times10^{-20}$). This was seen to be the case accross most Indications and Diagnoses with the exception of Hypoglycaemia and Familail Short Stature, Eg. GH Deficiency ($P=1.09\times10^{-7}$) and Idiopathic Short Stature ($P=4.69\times10^{-10}$). Height SDSs at first presentation were not significanly different between boys and girls with the exception of GH Deficiency (P=0.015) and Cranial Irradiation (P=0.004) in which boys were shorter. No differences were detected for Target Height or Height Deficit. In the two unbiased samples, the numbers of boys and girls shorter than the 1st CDC centile were 11 and 6, and 16 and 8.

Conclusion

A gender bias exists in this Australian population recieving GH. The bias does not appear to be an ascertainment bias as girls are not significantly shorter and boys are still over represented in unbiased samples.

PO2-149 GH and IGF Use II

GH treated children: difference in age at first presentation between boys and girls

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Introduction

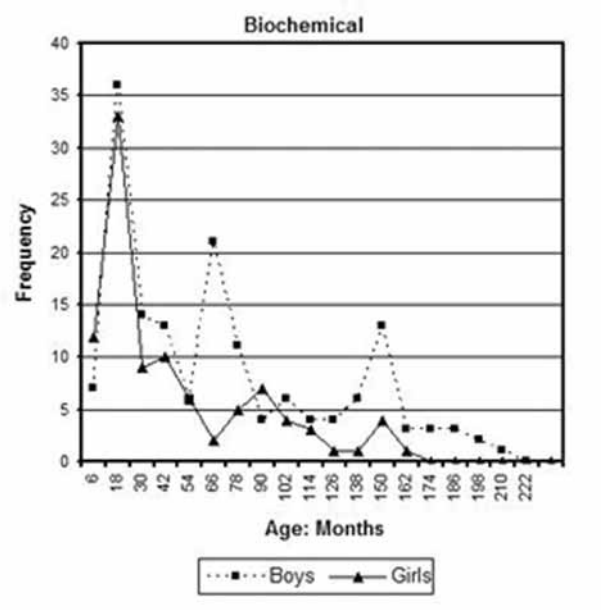
It is well documented that about twice as many boys than girls are treated with growth hormone (GH) for conditions resulting in short stature. This difference was again seen, across most indications for GH therapy, in our survey of patients currently (to the 3rd December 2007) receiving GH in Australia. There are a number of possible explanations for this observed gender bias some of which relate to differences in ages of puberty or other periods of significant sex hormone secretion between boys and girls. Here we further characterize the nature of the observed frequency difference between boys and girls in terms of diagnosis and age at first presentation.

Subjects and Methods

In this study patients were selected from the OZGROW database who were currently recorded as receiving GH (as of 3rd December 2007) and had made at least one visit to a growth clinic in 2007. A total of 1462 children fulfilled these criteria and were included in the study. Patients were categorized by "indication", one of 7 reasons for which GH is prescribed on the pharmaceutical benefits scheme, and by the diagnosis given by the treating paediatric endocrinologist. Age at first presentation was recorded in Months. The difference in ages between boys and girls was tested by t tests and Mann-Whitney U tests.

Results

Girls are significantly younger ($P=2.25\times10^{-5}$) at first presentation than boys. Much of this difference is due to the relatively young age of diagnosis of Turner Syndrome in girls. With the Turner girls removed, the remaining girls are still significantly younger ($P=1.65\times10^{-4}$). Specifically, girls show a significantly earlier first presentation for the "Biochemical" indication ($P=2.49\times10^{-4}$) and the combination of all diagnoses of GH deficiency ($P=3.15\times10^{-4}$). The nature of these age differences can be appreciated from Figure 1.



Boys display a unique spike at 66 months and consistently more boys than girls presenting from at least 126 months including a period from 186-210 months in which essentially no girls were presented.

Discussion

The significance of the timing of the observed boy spikes of GH deficiency diagnosis remains to be elucidated.

PO2-150 GH and IGF Use II

Short term growth response and final height in children treated with growth hormone for unlicensed indications: experience of a single centre

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Background: Unlicensed indications account for up to a quarter of the total growth hormone (GH) prescriptions in children in Scotland. The efficacy of this expensive drug in this area is still under debate.

Objectives: To retrospectively evaluate the short term height response and final/near-final height in children treated with GH for three unlicensed indications: idiopathic short stature, dysmorphic syndromes and skeletal dysplasia.

Patients and Methods: Data on patients from our tertiary centre who commenced GH therapy between 1989-93 and were enrolled in the Pfizer International Growth Database (KIGS) were analysed. We selected 153 patients from three categories for retrospective analysis: an unclassified group including familial and idiopathic short stature (ISS n=62), dysmorphic syndromes (DyS n=49) and skeletal dysplasia (SkD n=42).

Results: GH treatment was commenced at a mean age of 10.05 years**.** At the start of GH therapy median height SDS were -2.98 (ISS), -3.61 (DyS) and -4.44 (SkD); cumulative change in height SDS at the end of years 1 and 2 for these groups were 0.66 & 0.8; 0.38 & 0.92; and 0.80 & 1.2, respectively. Seven patients dropped out before completing the first year of GH treatment and were excluded from the analysis. Near final height data (defined as height velocity <2cm/year in girls ≥ 15 yr and boys ≥ 17 yr) in subjects treated with GH for ≥ 1 yr were available for 40 patients in this cohort (ISS n=13, DyS n=20, SkD n=7). Median height SDS increased from -3.56 at baseline to -2.55 at near final height. Four of these patients showed an increase in median height SDS of less than 0.2 (ISS n=1, DyS n=1, SkD n=2).

Conclusion: Our data show that the *ad hoc* use of GH in unlicensed conditions can produce favourable results but that patient selection is important. In this heterogeneous cohort, median increase in final height was 1.01 SDS.

PO2-151 GH and IGF Use II

Safety of two doses of growth hormone in Japanese short children born small for gestational age

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In short children born small for gestational age (SGA) GH therapy improves childhood growth and increases adult height. The safety and effectiveness of GH in short Japanese SGA children is not well established.

This report evaluates IGF-1 and IGFBP-3 levels and glucose metabolism following 2-yr GH treatment in short Japanese SGA children. Results after 1 yr were compared with an untreated group. Prepubertal Japanese nonGH-deficient SGA children (mean)(age 5.2 yr; 60% male; HSDS, -2.9; HVSDS, -1.8) were randomised to 2-yr treatment with 0.033mg/kg/d (n=33) or 0.067mg/kg/d (n=34) Norditropin® SimpleXx® (Novo Nordisk A/S, Denmark); or no treatment (n=15) for 1 yr.

Baseline IGF-I SDS was below the average of the reference population (-1.0 to -0.6). After 1 yr, IGF-1 SDS was -0.5 in untreated patients and 0.9 and 1.5 in the 0.033 and 0.067mg/kg/d groups, respectively. Mean (SD) values at 2 yr in GH-treated groups were within the normal range (0.4 [1.1] and 1.6 [1.0] in the 0.033 and 0.067mg/kg/d groups, respectively). Baseline IGFBP-3 SDS levels were above the reference population (0.8 to 0.9). At 1 yr mean (SD) values were 1.6 (1.1), 2.4 (0.8), and 3.2 (1.1) in the no treatment, 0.033 and 0.067mg/kg/d groups, respectively. At 2 yr IGFBP-3 SDS values had decreased (2.0 [0.9] and 2.6 [0.7], in the 0.033 and 0.067mg/kg/d groups, respectively). After an OGTT, $AUC_{glucose}$ was not different between GH-treated and untreated groups at 1 yr (Table 1). In the 0.033mg/kg/d group, $AUC_{glucose}$ was significantly increased from baseline at 1 (treatment ratio [95% CI]; 1.05 [1.00, 1.10]) and 2 yr (1.08 [1.03, 1.13]). There was no difference in $AUC_{glucose}$ between GH dose groups. A significant increase in HbA1c from baseline was observed in both GH groups at 2 yr (mean [95% CI); 0.19 [0.11, 0.27] and 0.28 [0.21, 0.34] %, respectively) (Table). There was no significant difference between the two dose groups.

In summary, GH treatment was safe in short Japanese children born SGA. No adverse effects on IGF-1, IGFBP-3 or parameters of glucose metabolism were reported.

AUCglucose and HbA1c (%) by treatment group			
	No treatment	0.033mg/kg/d	0.067mg/kg/d
	(n=15)	(n=32)	(n=34)
AUCglucose (hr*mg/dL), geometric mean			
Baseline	224.1	224.8	238.3
Yr 1	233.4	236.2	244.5
Yr 2		242.4	243.9
HbA1c (%), mean (SD)			
Baseline	4.73 (0.31)	4.79 (0.30)	4.66 (0.19)
Yr 1	4.77 (0.17)	4.81 (0.33)	4.81 (0.17)
Yr 2		4.97 (0.26)	4.94 (0.20)

PO2-152 GH and IGF Use II

Adult height and quality of life after GH therapy in Turner syndrome patients

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Objectives: To evaluate health-related quality of life (HRQOL) in girls with Turner syndrome before and after GH treatment to adult height (AH).

Patients and Methods: 71 Turner syndrome girls were randomised into an

open, parallel group, multi-centre trial comparing three dose regiments of Norditropin® administered as once daily subcutaneous injections (group 1: 4 IU (0.045 mg/kg/d): 4 IU/m^2/day for four years; group 2: 4-6 IU: 4 IU/m^2/day the first year and 6 IU/m^2/day (0.067 mg/kg/d) the second, third and fourth year; group 3: 4-6-8 IU: 4 IU/m^2/day the first year, 6 IU/m^2/day the second year, 8 IU/m^2/day (0.089 mg/kg/d) the third and fourth year. After the first four years all patients continued receiving the same dose as in year 4; treatment continued til adult height. Height was assessed at baseline and at 3-monthly intervals to AH (HV < 2 cm/year). Height SDS was mapped onto estimated HRQOL scores up to AH (Christensen, 2007).

Results: Estimated HRQOL scores increased from baseline at AH in all three treatment groups. HRQOL scores in treatment group 2 and 3 were significant increased compared to group 1 ($p < 0.017$ and $p < 0.012$, respectively). The difference in HRQOL scores between group 2 and 3 was not significant. Table 1 shows the derived EQ-5D mean (SD) for the three treatment groups.

A greater height gain and thereby adult height was observed in group 2 and 3 than in group 1. The estimated mean AHSDS (Turner reference) was considerably greater than the mean of untreated girls with Turner syndrome (1.7, 2.5 and 2.6 in group 1, 2 and 3, respectively). The girls also approached the height of normal children and were in the lower end of the national height range at adult height (-1.9, -1.2 and -1.1 in group 1, 2 and 3, respectively).

Derived EQ-5D results

Parameter	Treatment group 1: 4IU	Treatment group 2: 4-6IU	Treatment group 3: 4-6-8IU
Baseline EQ-5D	0.73 (0.09)	0.74 (0.08)	0.74 (0.07)
Final EQ-5D	0.78 (0.07)	0.83 (0.03)	0.83 (0.02)
Change in EQ-5D	0.05 (0.07)	0.09 (0.06)	0.10 (0.06)

Conclusions: GH treatment in Turner syndrome girls was associated with significant improvement in HRQOL and normalization of AH. The applied approach is quite conservative since the HRQOL benefit only focused on height improvements and not on any other clinical improvement related to the growth hormone treatment.

PO2-153 GH and IGF Use II

Effects of growth hormone (rhGH) replacement on growth, cardiac structure and function in children with GH deficiency (GHD)

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Patients with GHD have an increased cardiovascular risk. GH appears to have a trophic effect on cardiac growth, and its deficiency has been associated with low cardiac mass and function. Improvement of cardiac mass and performance has been demonstrated in GHD adults receiving GH, this is unclear in children. To clarify whether GH will affect cardiac size and function we prospectively evaluated the cardiac mass and its performance in a group of GHD children before and during GH treatment. **Patients and methods:** 12 prepubertal GHD males (10 idiopathic) (Mean age: 8.5 ± 4.3 years) were evaluated. Anthropometry, echocardiograms- Doppler and the Bruce Treadmill test were performed basally and at 12 and 24 months of rhGH treatment (0.33 mg/Kg/week). Left Ventricular Mass (LVM) was compared with the percentiles calculated for normal male children. Lipid profile was also measured. **Results:** Growth velocity (cm/year): 2.8 ± 1.8 vs. 12.6 ± 1.9 vs. 8.0 ± 1.5 and height (SDS): -4.2 ± 1.4 vs. 1º st year: -2.7 ± 1.6, 2 vs. 2º nd year: -2.2 ± 1.6 improved significantly during treatment. Blood pressure remained normal and no arrhythmias were detected. Cardiac performance showed a significant improvement of exercise capacity during the first and second year of treatment ($p< 0.01$). (8.66 ± 2.4 vs. 11.6 ± 2 vs. 13.06 ± 2.57 min). Left ventricular mass was in the lower normal percentile at baseline and increased significantly ($p< 0,01$) to the higher percentiles after 2 years of treatment (50 ±15 vs 69 ± 14 g/m^2 body surface.) No cardiac hypertrophy was found. Systolic and diastolic function remained in normal levels Lipid profile was always within the normal range **Conclusion:** GHD patients showed reduced left ventricular mass which increased significantly during two years of rhGH therapy. Endurance time improved significantly during treatment. No adverse effects were detected.

PO2-154 GH and IGF Use II

Hypopituitarism: a consequence of childhood traumatic brain injury?

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Objectives: To investigate the prevalence/aetiology/clinical significance of pituitary dysfunction after moderate/severe childhood traumatic brain injury (TBI).

Subjects/methods: Retrospective observational study involving 33 survivors of childhood TBI (25 males). Mean (SD) age at study: 13.4y (3.7y) and time since injury: 4.1y (1.6y). King's Outcome Scale for Childhood Head Injury (KOSCHI) rating: 15 good recovery, 16 moderate disability, 2 severe disability. Early morning urine samples were obtained for osmolality. Basal hormone evaluation was performed at 0800-1000h, followed by a GnRH test and insulin tolerance test (ITT, n=25) or glucagon test (if previous seizures, n=8). Subjects were not primed. Standardised age-appropriate quality of life (QoL) questionnaires were completed.

Results: No subject had clinical evidence of impaired growth: mean (SD) height SDS +0.5 (1.2), range -1.6 to +3 SD. Median (inter-quartile [I-Q] range) peak GH response to stimulation was 7.9 (5.2-13.6) µg/L. Sub-optimal responses (<5 µg/L) were observed in 6 peri-pubertal males, all with height SDS within ± 2 (range -1.5 to +1.4) SD and normal growth velocities. GH response was borderline low in one post-pubertal male (3.2 µg/L).

Median (I-Q range) peak cortisol response following ITT was 538 (462-598) nmol/L. 8/25 had sub-optimal responses based on local age-related cut-offs, none requiring routine glucocorticoid replacement. In 2/8, steroid cover was recommended for moderate/severe illness/injury. In 5/8, repeat testing was advised; the eighth had a high basal cortisol (624 nmol/L). Sub-optimal cortisol responses to glucagon were seen in 2/8 subjects, one with a high basal level (722 nmol/L).

No subject had diabetes insipidus. Thyroid function, IGF-I, oestradiol/testosterone, and baseline/GnRH-stimulated LH/FSH concentrations were appropriate for age/sex/pubertal stage. One male was prolactin deficient (<50 mU/L). Abnormal endocrine findings were unrelated to degree of primary/secondary brain injury or KOSCHI rating. No significant difference in QoL was observed between those with normal/abnormal pituitary function <16y. QoL was poorer in the post-pubertal male with GH deficiency than in other subjects aged ≥16y.

Conclusions: Whilst mild pituitary 'dysfunction' was common (39%), no unequivocal clinically significant endocrinopathies were found, although the GH/pituitary-adrenal axes may be vulnerable. A two-centre prospective study is underway.

PO2-155 GH and IGF Use II

National audit of patient choice in GH therapy

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There is now evidence that patient choice in growth hormone (GH) therapy not only improves adherence with GH therapy, but in addition is associated with improved height velocity *(Kapoor et al., 2008).* In order to find out how common free patient choice is for those commencing GH therapy, a questionnaire was sent to all members of our national paediatric endocrine society.

A total of 49 questionnaires were received, including 21 from (all) historic growth centres (Group 1), 13 other tertiary centres (Group 2), and 15 District General Hospitals (Group 3). They currently commence a total of 810 patients/year on GH: 642 (79%) in Group 1.

Of the 49 units all but 6: 3 in Group 1, 2 in Group 2 & 1 in Group 3 stated

that they offer free patient choice (88% of total). The patient choice is done with the nursing staff in 40/43 units, using: demonstration of devices (N=14), showing/giving the patients instructional DVDs (5), both (20), or other (3). The median(range) time spent choosing the device was 60(25-150)minutes for all units, and 75(30-150)minutes for Group 1.
Demonstration of devices involved: assembling/dissembling devices (34 units), dialling up doses (33), reconstitution of GH (29), considering home nursing & other facilities provided by GH manufacturers (18), injecting patient (17), injecting parent (16), showing additional material (eg. promotional, stickers etc. (13), consideration of cost (7). Median(range) number of aspects shown/ unit was 5(2-8) overall but 6(3-8) in Group 1.
For those units offering patient choice, devices were offered from a median of 5 manufacturers, with correlation between new patients commenced on GH/ unit and both numbers of manufacturers R=0.58 and number of devices offered R=0.57 (both p<0.01).
In conclusion the vast majority of units now offer some form of patient choice for new patients commencing GH therapy, although this can involve a number of different methods. There is evidence that larger units spend more time on patient choice, offering more aspects, more manufacturers and more devices.

PO2-156 GH and IGF Use II

Growth hormone therapy in CHARGE syndrome

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Although short stature is well recognised in CHARGE Syndrome, with the R in the acronym representing Retarded growth and development, there are few data on spontaneous growth, growth hormone (GH) secretion, or response to GH in this condition. Data on 32 patients (20 boys (63%)) with CHARGE Syndrome treated with GH therapy was obtained from the KIGS database. 26 patients had GH stimulation testing, with a mean(SD) peak GH of 9.38(6.78) µg/l. At the start of GH therapy patients were a median of 6.86 years, with all but 3 patients being pre-pubertal. Mean(SD) height SDS at the start of GH therapy was -3.26 (1.33), compared to a mean(SD) MPH of -0.16(0.91)SDS, and 29 patients (91%) had heights ≤-2 SDS. Treatment with GH at a mean(SD) dose of 0.26(0.09) mg/kg/wk led to an increase in height velocity from a mean(SD) of 4.95(2.85) cm/year at the start of therapy to 8.59 (2.48) cm/year after 1 year, and 6.82 (1.61) cm/year after 2 years in prepubertal children.
In summary, these children with CHARGE syndrome were short, with a number having biochemical GH deficiency. GH therapy produced an increase in height velocity over the initial 2 years. Further studies are required to determine which patients within this group are most likely to benefit from GH therapy, and whether this benefit is maintained long-term, with increased final height.

PO2-157 GH and IGF Use II

Insulin and IGF-I levels during 2 yrs of treatment with individual GH doses (17-100 µg/kg/d) do not differ to standard dose (43 µg/kg/d) in prepubertal GHD and ISS children

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In a randomized prospective study, the set target: height SDS close to MPH SDS after 2 years was more successful when GH dosing was guided by GH responsiveness estimated by our model predicting growth response, compared to standard treatment, 43 µg/kg/d (JCEM febr 2009). Individual dose ranged 17-100 µg/kg/d, estimated from GH growth responsiveness and distance to MPH SDS.
Aim: insulin and IGF-I levels during treatment with individualized GH dosing up to 100 µg/kg/d were hypothesized not to induce higher levels than during standard dosing.
Studygroup: 128 prepubertal children (3-11yrs); height SDS <-2 at start of treatment, IGHD (75%) or ISS, were randomized to either individualized dose (2/3) or to standard dose.
Methods: IGF-I levels were transformed into SDS, insulin was measured in mU/L.
Results: For the total group insulin levels increased from start to 2 yrs, r=0.20, p=0.027, as IGF-I SDS, r=0.56, p<0.0001.
In *the individualized dose group*, comparing the six dose steps after 2 yrs, the maximum levels in insulin and IGF-I did not differ.
Comparing *the randomization groups* after 2 yrs: in IGF-I SDS there were no differences in mean (SD) or median (range), nor in $_{\Delta}$ IGF-I 2-0 yrs. Insulin range in the individualised GH dose group was 22% reduced compared to the range for the standard dose group but there were no differences in mean or median levels, see Table.

		Individualized doses, n=87	Standard dose, n=41
		17 to 100 ug/kg/d	43 ug/kg/d
IGF-I SDS at 2 yrs	Mean (SD)	1.55 (1.25)	1.54 (1.09)
	Median (range)	1.59 (-1.48 to 3.82)	1.44 (-0.65 to 4.21)
ΔIGF-I 2-0 yrs	Mean (SD)	2.65 (1.26)	2.63 (1.11)
	Median (range)	2.50 (-0.21 to 6.74)	2.69 (0.14 to 5.40)
Insulin at 2 yrs	Mean (SD)	10.80 (5.68)	11.18 (8.68)
	Median (range)	9.80 (2.0 to 30.0)	9.30 (3.0 to 39.0)
ΔInsulin 2-0 yrs	Mean (SD)	6.19 (5.85)	6.66 (8.01)
	Median (range)	4.80 (-9.10 to 25.0)	4.90 (-3.10 to 30.9)

Conclusion: Considering individual responsiveness for GH dosing, no differences in serum levels of IGF-I and insulin were seen between the randomization groups, despite use of higher than standard dose in 50 out of 87 children. Actually, the range for insulin was reduced in the individualized dose group. Thus, individualizing GH dose according to estimated growth responsiveness also works for IGF-I and insulin.

PO2-158 GH and IGF Use II

Common genetic variants of growth hormone receptor (GHR) and vitamin D receptor (VDR) genes do not contribute to the response to growth hormone of patients with growth hormone deficiency (GH) and Turner syndrome (TS)

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The response to human recombinant growth hormone (rhGH) therapy exhibits considerable interindividual variability and might be explained by the interaction between several low penetrance genetic factors. However, only the GHR gene has been investigated.
Objective: To assess prospectively the influence of GHR and VDR gene polymorphisms on the 2-year response to rhGH therapy in children with GHD and TS.
Setting: Referral university hospitals.
Design: Prospective, multicenter observational 2-year study.
Patients and measurements: Venezuelan prepubertal subjects with GHD (n=28) or TS (n= 25) were recruited. Molecular analysis of the GHR and VDR genes was performed and completed at the same hospital. Clinical data at baseline and Δ height velocity (HV) and height-SDS (H-SDS) during the first and second years of rhGH treatment were compared in GHD and TS patients with different genotypes.
Results: Clinical data at the start of treatment and rhGH doses were indistinguishable among patients with GHD or TS with different GHR or VDR genotypes. Although, rhGH therapy significantly (P<0.0001) increased GV (cm/yr)

and standing height (SDS and cm) of GHD and TS patients during the first and second years, these changes were not significantly different among the various genotype groups (GHR genotypes: fl/fl vs fl/d3+d3/d3; p=0.565 or p=0.462 or VDR genotypes: Bb+BB vs Bb+bb; p= 0.632 or p=0.645). In addition, there was no significant difference in the response to rhGH among subjects when both these genotypes were combined (fl/fl+Bb+BB vs fl/d3+d3/d3+Bb+bb, p=0.325 or p=0.345).
Conclusions: Gene polymorphisms in low penetrance genes do not contribute to the rhGH response of patients with GHD and TS.

PO2-159 GH and IGF Use II

Magnetic resonance imaging (MRI) and multiple hormone evaluation in children with panhypopituitarism

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Background to explore the relationship between MRI findings and multiple pituitary-target hormones in patients with multiple pituitary hormone deficiency(MPHD) or panhypopituitarism (PHP)
Methods 125 patients with MPHD (102 boys) (Group A) and 90 age-, sex--matched normal children (Control Group A) were enrolled. Of the patients in Group A, 75 male patients aged ≥11 years (Group B) and 44 male older children ≥11 years (Control Group B) were also identified. Ninety-six of the patients with MPHD underwent MRI scans of the hypothalamic-pituitary area. The patients were subdivided into five grades according to their MRI findings. The serum concentrations of GH, IGF-1, FT4, TSH, ACTH, cortisol (COR), FSH, LH, prolactin (PRL), testosterone (T) and estradiol (E2) were measured in patients and in controls.
Results MRI grade was significantly positively correlated with the number of pituitary hormone deficiencies (r=0.9, P<0.001). MRI grade was negatively correlated with peak GH, IGF-1, FT4, COR and anterior pituitary height (r= –0.43, –0.47, –0.67, –0.54, and –0.49, respectively, P<0.01). All of the patients had a GH response of less than 7 μg/L. All 40 patients in Group B who underwent the three GH stimulation tests had a GH response of less than 5 μg/L in all three tests. The peak GH appeared within 90 min in all 40 patients (100%) when using RI, in 35 cases (87.5%) when using Arg and in 36 patients (90%) when using L-DOPA. There were no significant differences in mean peak GH levels from ten serum samples obtained over 120 minutes with the Arg+RI test or from three samples obtained over 90 minutes with the individual Arg, RI and L-DOPA tests. A positive linear correlation (r=0.69, *P*<0.01) was found between the peak GH and IGF-1.The serum IGF-1, FT4, ACTH and COR levels in Group A were significantly lower than those in the Control Group A. The serum FSH, LH, and T levels were significantly decreased (P<0.001); however, E2 and PRL were significantly increased (*P*<0.001) in Group B compared with the Control Group B; 34 cases were found to have CDI.
Conclusion Abnormal MRI are markers of MPHD.There is a close correlation between MRI and pituitary function.The GH secretory function in patients with MPHD can be determined accurately by one GH stimulating test lasting 90 min and combined with serum IGF1 levels

PO2-160 GH and IGF Use II

Magnetic resonance imaging (MRI) and pituitary function in children with pituitary stalk interruption syndrome

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Objective: To explore the relationship between MRI and pituitary-target hormones and to investigate the dynamic changes in hormone levels after hormone replacement therapy (HRT) in patients with pituitary stalk interruption (PSI) syndrome (PSIS).
Method: 59 patients (male 50)with PSIS (Group A) were included in this study. Forty male patients aged ≥11 years from Group A were also evaluated separately (Group B). The control groups comprised 90 and 44 age- and sex-matched healthy children, respectively. The 59 patients in Group A were subdivided into two groups according to the appearance of the pituitary stalk on MRI: Grade I: partial PSIS, and Grade II: complete PSIS. GH, IGF-1, FT4, TSH, ACTH, cortisol (COR), FSH, LH, PRL, testosterone (T),and estradiol (E2) were measured in patients and in controls
Results: There were significant difference between the two grades in the number and severity of pituitary hormone deficiencies (P<0.001–0.05). The peak GH level was less than 5 mg/L in all 59 patients with PSIS. The serum IGF-1, FT4, ACTH and COR levels in Group A were significantly lower than those in Control A before treatment and TSH, ACTH and COR were lower after HRT. In Group B, the serum FSH, LH, and T levels were significantly decreased in all patients (100%) (*P*<0.001); however, E2 and PRL were significantly increased (*P*<0.001) compared with the Control Group B. E2 and PRL levels were increased in 25 (55%) and 20 (50%) cases, respectively.
Conclusion: PSIS and its severity can be confirmed by MRI. PSIS can result in multiple pituitary hormone deficiency, which requires total HRT, which should be started at appropriate times and dosages to improve the quality of life and normal growth of the patient and to avoid adverse effects

PO2-161 GH and IGF Use II

Effect of PEGylated recombinant human growth hormone on action potential in guinea pig papillary muscles

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Objective To investigate the effect of PEGylated recombinant human growth hormone (PEG-rhGH) on the action potential in guinea pig papillary muscles, and compare the bioactivity in vitro of PEG-rhGH with that of common rhGH.
Methods Twelve healthy male guinea pigs were randomly divided into PEG-rhGH treated group and common rhGH treated group according to the types of rhGH (Jin Sai Company, Chang Chun, China). The effect of different concentrations (1 - 1000 μg/L) of PEG-rhGH and common rhGH on action potential (AP) in guinea pig papillary muscles was observed by the cumulative dose-response technique, and compared the AP before and after treatment. Intracellular microelectrode technique was used to record parameters of action potential, which including resting potential (RP), action potential amplitude (APA), and duration of action potentials at 50, 90% level of repolarization (APD_{50} and APD_{90}).
Results Both PEG-rhGH and rhGH groups had significant longer APD_{50} and APD_{90} with concentration dependent (*P*<0.05), as showed in Table 1. However, RP and APA did not change in both PEG-rhGH and rhGH groups (*P*>0.05). There were not significantly different of APD_{50} and APD_{90} between PEG-rhGH and rhGH group (*P*>0.05).

Table 1. The effect of PEG-rhGH and the common rhGH on APD50 and APD90 in papillary muscles

Concentration	PEG-rhGH		Common rhGH	
	APD50 (ms)	APD90 (ms)	APD50 (ms)	APD90 (ms)
0 (control)	205.7±18.9	240.0±21.0	206.8±45.8	241.7±43.0
1 μg/L	207.8±19.6*	241.4±21.3*	210.4±44.9*	245.2±42.2*
5 μg/L	213.4±19.4**	247.0±21.6*	212.5±46.6*	246.9±43.6*
10 μg/L	215.9±18.9**	249.4±20.9**	215.0±45.4**	249.2±42.8*
50 μg/L	217.6±14.3**	251.4±15.9**	216.8±46.6**	250.9±43.9**
100 μg/L	219.7±15.0**	253.4±16.9**	218.5±44.5**	252.6±41.9**
500 μg/L	222.4±13.6**	256.0±15.7**	224.2±44.5**	258.4±42.0**
1000 μg/L	223.1±13.0**	256.6±14.6**	224.8±43.9**	258.8±41.5**

P<0.05, **P<0.01 compared with the controls

Conclusion Our data suggest that PEG-rhGH, as well as common rhGH, prolonged the action potential duration of guinea pig cardiocytes by increasing calcium influx, and PEG-rhGH has similar biological activity on the action potential as rhGH in vitro.

PO2-162 GH and IGF Use II

Beneficial effects of growth hormone treatment in boys with Aarskog syndrome

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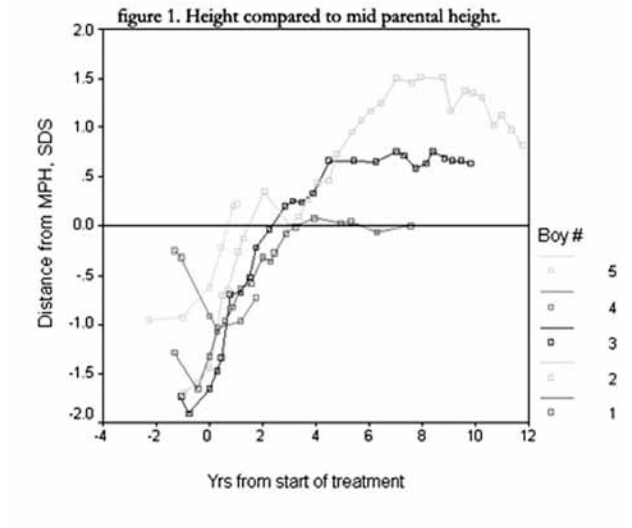
Background: Since Aarskog described the syndrome in 1970 there has been an increasing recognition of the disorder but the true prevalence of the syndrome has not been estimated. Aarskog syndrome is caused by mutations in the FGD1 gene, located on the short arm of the X chromosome (Xp11.2) and full expression of the syndrome only occurs in males. The clinical picture is distinctive patterns of physical findings with hypertelorism, bradydachtyli, short stature and shawl scrotum as common signs.
Patients: We describe 5 boys primarily investigated due to short stature. Each of them had at least 5 clinical features of Aarskog syndrome and a pronounced short stature (-3.8 to -2.2 SDS). They had normal IGF-1 and IGFBP3 values. The patients were treated with human Growth Hormone (GH)(0.03mg/kg daily). Three of the boys were diagnosed and treated early (4.4-7.5 years of age) and allowed evaluation of treatment effects during 7 years of treatment (Boy 1-3) while the other 2 boys were 12 and 15 years at the start of treatment and only allowing assessment the first 1-2 years of treatment (Boy 4-5).
Results:
Height is expressed as standard deviation scores (SDS) and treatment effects are evaluated compared to the Swedish reference data. During the first 2 years of treatment the 3 children treated at young age increased their height from -2.5 (range -2.7 to -2.2) to -1.0 SDS (range -1.7 to -0.42 SDS) with further improvements up to 7 years of treatment.

Table 1. Mean Standard Deviation Score (SDS) of Height compared to Swedish normal values

	Boy 1-3	Boy 1-5
Height at start of GH treatment	-2.5	-2.7
GH treatment 1 year	-1,6	-2.0
GH 2 years	-1.0	
GH 7 years	-0.23	

During the first year of treatment, a clear increase in growth was apparent for all 5 patients.
Growth was also analyzed compared with mid parental height (MPH). A clear increase in growth was apparent during the first year of treatment for all 5 patients with further improvements during longer treatment in the patients treated early (Boy 1-3).



Conclusion:
Our data indicate that early GH treatment in boys with Aarskog syndrome has both short and long-term beneficial effect on growth.

PO2-163 GH and IGF Use II

IGHD type II: knock down of the harmful Δ3GH isoform by expression of shRNA microRNAs (shRNAmirs) rescues *wt*-GH secretion in pituitary cell lines

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Isolated Growth Hormone Deficiency Type II (IGHD II) is the autosomal dominant form of Growth Hormone (GH) deficiency. In the majority of the cases, this disease results from specific *GH-1* gene mutations that lead to mRNA missplicing and subsequently to a loss of exon 3 sequences. When such misspliced RNA is translated, it produces a dominant negative isoform of GH (Δ3GH) that blocks trafficking and secretion of *wild type-GH (wt*-GH). Currently, patients suffering from this form of GHD are treated with daily injections of recombinant human GH in order to restore normal growth. However, this type of replacement therapy may not prevent harmful effects of the Δ3GH mutant isoform on the pituitary gland leading to other hormonal deficiencies.
We developed a strategy involving Δ3GH isoform knock down mediated through expression of microRNA-30-adapted-shRNA (shRNAmirs) targeting the novel junction sequences of exon 2 and exon 4 arising from mis-splicing of the GH transcript using lentiviral vectors. Silencing efficiency was determined by western blot analysis using α-human GH antibody on Human Embryonic Kidney cells transiently transfected with a vector expressing human Δ3GH and anti-Δ3GH shRNAmir from lentiviral vector. Further, the effect on GH secretion was assayed after transient expression of human *wt*-GH, Δ3GH and shRNAmirs in mouse neuroendocrine tumour AtT20 cells. Finally rat neuroendocrine tumour GC cells endogenously expressing rGH, and expressing Δ3GH transiently or after doxycyline induction were transduced with lentivirus in order to determine knock down efficacy from microRNAs proviral expression. Western blot analysis and secretion measurements showed that shRNAmirs expression either by transient transfection or by transduction, lead to a large decrease in Δ3GH content and an overall increase in *wt*-GH secretion of up to 25%. In addition, cell proliferation and apoptosis assay showed no evidence of cell toxicity in transduced cells expressing shRNAmirs.
Therefore, lentiviral vectors have shown their ability to infect, stably integrate and express microRNAs without affecting viability of various pituitary cell lines. This methodology should enable us to knock down the Δ3GH variant either in primary cell from IGHD II mice, which opens the prospect of treating affected mice with viral shRNAmirs to rescue their GH- expressing cells *in vivo*.

PO2-164 GH and IGF Use II

How to select short-statured children for growth hormone testing: external validation of a clinical decision rule

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Background: Numerous short-statured children are evaluated for growth hormone (GH) deficiency (GHD). GH provocative tests are often normal and a posteriori unnecessary in many patients. Subsequently, a clinical decision rule has been derived.
Objective: To perform external validation of a specific predictive tool in order to avoid unnecessary GH provocative tests.
Methods: A retrospective monocentric cohort study included short-statured children, aged 19-88 months in a Parisian teaching hospital from September 2007 to april 2008. GHD was defined by the presence of 2 GH peak concen-

trations < 10 ng/ml. Certain GHD was defined as GHD and a pituitary stalk interruption syndrome on MRI. Various clinical, biological and radiological potential predictors were compared between patients with and without GHD using uni-and multi-variate analyses. Independent predictors were combined in a decision rule using recursive partitioning with the aim to obtain a highly sensitive and quite specific predictive tool. The rule was then validated in one other population.
Results: 74 patients were included and 31 (41%) had GHD including 6 (8 %) with certain GHD. Independent predictors of GHD were: growth rate < -1 DS (AOR = 1.8, 95%CI [1.2-3.2]), IGF-I concentration < -2 DS (AOR = 2.4 [1.0-4.1]) and BMI ≥ 0 z-score (AOR = 1.4 [0.6-3.3]). A clinical decision rule suggesting to test only patients with a growth rate < -1 DS and a IGF-I concentration < -2 DS achieved 100% sensitivity [56-100] for certain GHD and 63% [38-71] for GHD, respectively, and a specificity of 84% [73-90]. When this rule was applied to another population (n = 29 patients with certain GHD), sensitivity was 91% [77-99] for certain GHD.
Conclusion: We performed an external validation of a highly sensitive decision rule that could help to avoid more than 3/4 of unnecessary GH testing. Multicentric validation of this rule is needed before application.

PO2-165 GH and IGF Use II

Growth hormone and the hand: growth dynamics, bone mass and maturity

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The aim of this study was to provide a comprehensive analysis of the effects of GH on bone geometry, mass and maturity in children with growth hormone deficiency (GHD). 602 non-dominant hand X-rays from 178 short prepubertal children (122 boys) with GHD undergoing GH treatment were analyzed for changes in metacarpal thickness (T), width (W), length (L), medullary diameter (M), area bone mineral density (BMD) and bone age (BA) in the time interval from one year preceding until four years after start of GH treatment using BoneXpert, a fully automatic image analysis software. The parameters were transformed into chronological age (CA), BA and height-related standard deviation scores (CASDS, BASDS and HSDS). GH had strong stimulatory effects on T, L and W, ranking in this order, and no effect on M. T also increased significantly in terms of height- and BA-related standard deviation scores (HSDS and BASDS), while L and W increased nonsignificantly in this regard. BMD showed a significant increase for CA, BA and height. Catch-up growth was strongest in the first year and, by BASDS, the more pronounced for a parameter the greater the baseline deficit was for that parameter. BA catch-up was slower than catch-up growth of T and of height during the first year and thereafter faster, showing the same gain in terms of CASDS as T after four years.
Conclusion: The strongest effect of GH treatment on metacarpal bone is not elongation but increase in cortical thickness as a result of subperiosteal bone deposition. GH treatment of short children with GHD already leads overall to a normalization of metacarpal measures within the first year. Skeletal maturation acceleration begins in the second year.

PO2-166 GH and IGF Use II

Effect of human recombinant growth hormone therapy on circulating levels of erythropoietin and granulocyte-colony stimulating factor in short children

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Several reports suggest a role of GH in the regulation of the haematopoietic system, as regards the normal differentiation and function of blood cells.
The aim of this pilot study was to evaluate the influence of rhGH therapy on erythropoietin (Epo) and granulocyte-colony stimulating factor (G-CSF) levels in 18 prepubertal short children with idiopathic GHD or without GHD, during the first year of treatment.
Eight short children (5 M and 3 F) with idiopathic GHD and 10 short children (8 M and 2 F) with normal GH secretion entered the study. All patients were pre-pubertal and remained pre-pubertal during the follow-up. Short children with normal GH secretion (non-GHD) showed low GH bioactivity, as measured by Nb_2 bioassay. Recombinant hGH therapy was administered at the weekly dose of 0.25 mg/kg subcutaneously, divided into 6 daily doses in the evening, in both GHD and non-GHD children. Blood samples from each patient were collected before and 3, 6 and 12 months after starting rhGH therapy and used to determine haemachrome and circulating levels of Epo and G-CSF. Serum Epo and G-CSF were measured by commercially available enzyme-linked immunosorbent assays. The normal distribution of the variables was evaluated by Shapiro's test and the data are presented as median and interquartile range; non parametric tests were used for the analysis (Mann-Whitney U test and Friedman ANOVA test). A p value < 0.05 was considered to be statistically significant.
In non-GHD children Epo levels significantly decrease (basal: 6.2 mUI/ml, 5.3-8.1 mUI/ml; 12 months: 5.0 mUI/ml; 2.5-6.0 mUI/ml; p<0.05) and G-CSF levels increase (basal: 13.7 pg/ml; 10.8-18.6 pg/ml; 12 months: 34 pg/ml; 25-43 pg/ml; p=0.025) from basal to 12 months of therapy, whereas in GHD children they do not significantly change (Epo: basal: 5.9 mUI/ml, 4.4-10.1 mUI/ml; 12 months: 8.0 mUI/ml, 7.0-10.0 mUI/ml. G-CSF: basal: 10.0 pg/ml, 7.1-11.2 pg/ml; 12 months: 10.0 pg/ml, 10.0-15.0 pg/ml). Circulating levels of G-CSF were significantly lower (p<0.05) in GHD than in non-GHD children. In non-GHD children haemoglobin (basal: 13.2 g/dl, 12.7-14.1 g/dl; 12 months: 13.5 g/dl, 12.7-14.8 g/dl; p=0.038) and haematocrit (basal: 38.95%, 38.3-40.8%; 12 months: 40.05%, 38.7-43.5%; p=0.014) values significantly increase up to 1 year of rhGH treatment.
In conclusion, rhGH therapy influences Epo and G-CSF levels in short non-GHD children, but not in GHD children.

PO2-167 GH and IGF Use II

Clinical variations of hypopituitarism exhibiting an invisible pituitary stalk by MRI

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Objective: The purpose of this study was to investigate the clinical findings of eight patients with hypopituitarism exhibiting both an invisible pituitary stalk and a posterior pituitary lobe by magnetic resonance imaging (MRI). **Patients and Methods:** We investigated eight patients (four males and four females; aged 3-19 years) with invisible or thin pituitary stalks by MRI, along with an ectopic or invisible posterior lobe. We excluded patients with other brain abnormalities. **Results:** Two patients were born by breech delivery. Another patient had a history of brain trauma with monocular strabismus. The other five patients were born by cephalic or cesarean delivery and had no history of traumatic brain injury. We diagnosed the patients as having developed growth hormone deficiency (GHD) at the age of 2.6-11.0 years. Their height SD scores ranged from approximately 2.3 to 6.0 according to Japanese standards at diagnosis. Although four patients exhibited severe GHD, the other four patients had only moderate GHD. Four of the eight patients also underwent a test for growth-hormone-releasing factor, and three of these were determined to have a low response. Although only one patient exhibited combined pituitary hormone deficiency (CPHD), the other seven patients were shown to have isolate growth hormone deficiency (IGHD) at diagnosis. Four of the eight patients had an ectopic posterior pituitary lobe. While we did not detect a posterior pituitary lobe by MRI in four patients, we were able to confirm an ADH deficiency in one of these. One patient with IGHD developed CPHD due to central hypothyroidism and hypogonadism. **Conclusion:** It is possible for patients without history of traumatic delivery or brain injury to have an invisible or thin pituitary stalk by MRI. We believe this to be only one of the interesting aspects relating to the root of invisible stalk syndrome, and are actively considering genetic studies to expand on our clinical findings. GHD ranges from moderate to severe in patients with invisible stalk syndrome. We suggest that an MRI should be performed on patients with GHD, even if they exhibit IGHD at diagnosis. In

addition, we suggest that patients with invisible stalk syndrome combined with an abnormal posterior lobe might gradually develop CPHD including an ACTH deficiency over the long term.

PO2-168 GH and IGF Use II

A study of the metabolic effects of growth hormone replacement in patients with growth without growth hormone

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Background: Growth without growth hormone (GH) has occasionally been described in patients who have had tumors removed in the hypothalamic-pituitary area. Most of these patients have metabolic abnormalities such as obesity, dyslipidemia, fatty liver, and low bone mineral density. In Japan, GH replacement therapy for the purpose of the improvement of metabolic abnormalities has been approved for adult GH deficiency, but not for is childhood GH deficiency. Previous studies have not fully elucidated the long-term effects of GH replacement in pediatric patients with growth without GH who have metabolic abnormalities. This study describes the metabolic effects of GH replacement in pediatric patients with growth without GH.
Subjects: Two children in whom the growth without GH phenomenon occurred, after therapy for brain tumors participated in this study. Case 1 is a 15-year-old Japanese girl, diagnosed with intracranial Langerhans cell histiocytosis at the age of two. She showed a slight body fat increase, dyslipidemia and fatty liver. Case 2 is a 10-year-old Indonesian boy, diagnosed with craniopharyngioma at the age of three. He was obese and had low bone mineral density.
Method: GH therapy was started at 0.042 mg/kg/week (6.0 μg/kg/day) and then the dosage was increased to achieve serum IGF-I levels in the age-related reference range. Body composition, bone mineral density, and visceral abdominal area were measured every 3 months. Serum fasting blood glucose, insulin, aspartate aminotransferase (AST), triglyceride (TG), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), leptin, adiponectin levels were also determined every 3 months.
Results: Case 1 showed improvement of fatty liver (AST 64 IU/l ® 16 IU/l) and dyslipidemia (TG 239 mg/dl ® 129 mg/dl) though did not show a decrease in the visceral fat area or the body fat percentage. Case 2 showed a decrease in body fat (34.6 % ® 28.5 %) and the visceral fat area (41.1 cm2 ® 26.1 cm2), accompanied by elevated serum adiponectin and decreased leptin levels.
Conclusion: GH replacement therapy is effective for the improvement of metabolic abnormalities in pediatric growth without GH patients, as well as in adult GH deficiency.

PO2-169 GH and IGF Use II

Growth hormone deficiency is associated with selective deficits of memory and executive function in children

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Background:
Recent evidence suggests that Growth Hormone Deficiency (GHD) may be associated with cognitive impairment in adults, specifically in the domains of memory, attention and executive function. However to date no comprehensive investigation of the cognitive sequelae of growth hormone deficiency in children has been undertaken. We aimed to determine the effect of GHD on standardized measures of memory, attention, executive function, IQ, motor abilities, language, behaviour and communication.
Method:
Twelve children (8.4±1.2 years) with isolated growth hormone defiency (peak GH level < 6.7 μg/l on 2 tests of GH release, or one stimulation test with a peak GH < 6.7 μg/l plus a pathologically low IGF-I concentration for age) and nine short stature (normal peak GH in response to stimulation (>10μg/l), normal IGF-1 measurements and normal growth rate) controls (8.5±1.8 years) underwent a comprehensive neuropsychological assessment. This included the Cambridge Neuropsychological Test Automated Battery (CANTAB) and NEPSY batteries to assess memory, attention, executive function and language; the Wechsler Intelligence Scales for Children for IQ, the Movement-ABC for neuromotor function, and the Social Communication Questionnaire and Child Behavior Checklist to assess communication and language.
Results:
We observed significantly worse performance in GHD patients compared to short stature controls on the CANTAB Pattern Recognition Memory ($p<0.05$) and NEPSY Inhibition ($p<0.05$) tasks, suggesting poor visual memory and poor inhibitory (executive) control in the GHD relative to the control group. These group differences in memory and executive function task performance remained significant after controlling for the presence of autism features (according to the Social Communication Questionnaire) in a covariate analysis (all p values <0.05). Trends were observed towards lower scores on the neuromotor and other memory (narrative memory) and attention (rapid visual information processing) tasks in the GHD compared to the control group. No significant differences were found between the groups on the IQ, language, behaviour and communication measures (all p values >0.05).
Discussion:
These preliminary findings show subtle deficits in measures of executive function and memory in children with GHD compared to a short stature control group, and suggest a role for GH in specific neurocognitive functions.

PO2-170 GH and IGF Use II

Effects of interferon-α therapy on insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) levels in children with chronic hepatitis B

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Introduction: The liver is the major source of IGF-1 and its binding protein IGFBP-3. The concentrations of IGF-1 and IGFBP-3 are decreased in patients with chronic liver disease. There is no report investigating whether or not to affect serum levels of IGF-1 and IGFBP-3 connected with interferon therapy in chronic hepatitis B. The aim of this study was to evaluate the effects of interferon-α therapy on IGF-1 and IGFBP-3 levels in children.
Methods: Thirty-three children were included in this study. Serum IGF-1 and IGFBP-3 were measured in 19 patients before and in 14 patients after 6 months of interferon--α therapy by RIA (ng/ml). Liver biopsy was performed to 25 patients. The histological activity index (HAI) and fibrosis were evaluated according to Knodell's index. None of the patients had glucose intolerance and cirrhosis. Normalization of alanine aminotransferase (ALT), loss of HBV DNA and HBeAg, and development of antibody to HBeAg altogether was considered as the end of therapy responce (ETR)
Results: Baseline IGF-I and IGFBP3 levels were significantly lower pretreatment patients than treated with interferon α. (mean 237.6 ± 138 ng/ml versus 382.6 ± 154 ng/ml, $p<0.0001$, 3.9±0.89 ng/ml versus 4.6± 0.69 ng/ml, $p<0.0001$ respectively)., After 6 months of interferon therapy, of the 14 patients, four (28.6%) had ETR. We did not find any relationship between IGF-1 levels, IGFBP-3 levels and ETR ($P > 0.05$).
Conclusions: These results suggest that interferon-α may increase the release of IGF-I and IGFBP-3 in children with chronic hepatitis B without cirrhosis. Further studies required to detect the role of between ETR and IGF-1, IGFBP-3 levels.

PO2-171 GH and IGF Use II

Serum IGF-1 and IGFBP-3 levels in healthy Turkish children between 0-6 years of age

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The purpose of the present study was to establish the reference values of IGF-1 and IGFBP-3, which is used for diagnosis of growth hormone deficiency and its follow-up result on growth hormone therapy in healthy Turkish children younger than 6 years of age.

A total of 567 healthy children were included in the study; 257 were girls, 310 were boys. Serum IGF-1 and IGFBP-3 levels were measured in 3th, 6th, 12th, 24th, 48th, 60th, and 72th month.

Serum IGF-1 level decreased at the 6th month in both sexes which was not significant. These levels were found to increase in 12th month in girls and 24th month in boys as the child growth. However, serum IGFBP-3 level decreased at 6th month only in girls which was not statistically significant. No correlation was found between serum IGFBP-3 and BMI, weight and height SDS. On the other hand, a weak correlation was observed between serum IGF-1 level and BMI, weight and height SDS. Similarly, a weak correlation was observed between serum IGF-1 and IGFBP-3.

We believe that the reference level which was established according to age and gender in our country is beneficial in the diagnosis of growth hormone deficiency and its follow-up result on growth hormone therapy.

PO2-172 GH and IGF Use II

Growth hormone therapy associated with resolution of erythromelalgia symptoms

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Introduction

Erythromelalgia (EM) is a neuropathy characterized by pain and redness of the extremities that is triggered by warmth. EM has been associated with missense mutations of the voltage-gated sodium channel, $Na_v1.7$, which is preferentially expressed in most nociceptive dorsal root ganglia and sympathetic ganglion neurons. It is a rare disorder, with prevalence estimated at approximately 3 cases per 1 million persons a year. The pain is often refractory to treatment. The natural history of EM can be quite variable.

Case Presentation

We report a case of a 14 and 8/12 year old white male (NC), who presented with symptoms at age 3 and was diagnosed with EM at age 11, who was referred to Endocrinology for short stature, growth failure, and delayed puberty. NC has a known mutation of the $Na_v1.7$ gene. When referred, NC's height was -2.25 SD (mid-parental height 183 cm). He had not had linear growth for two years, and was Tanner 1 for pubic hair and genitalia. NC's EM was characterized by severe bilateral foot pain requiring multiple pain medications in addition to ice water soaks. Endocrine evaluation for growth failure and delayed puberty revealed normal thyroid function tests, prepubertal LH and FSH, a negative work-up for Cushing's disease, low IGF-1, normal IGFBP-3, and a bone age that was 2 years delayed. NC was started on growth hormone (GH) therapy (0.4 mg/kg/week) for idiopathic short stature. Within one month of initiation of GH therapy, NC had improvement of his EM symptoms. Within nine months of GH initiation, NC was off of all pain medications and was pain free. NC's growth velocity in the first year of GH therapy was 16.2 cm/year. After 2 years of continued GH therapy, NC is at the 50th percentile for height, Tanner 4 pubic hair, Tanner 4 genitalia, and remains pain free.

Discussion

This is the second case report of a child with EM who experienced resolution of EM symptoms after treatment with GH. The natural history of EM is quite variable, however, in primary cases, such as NC, it is unlikely that it would spontaneously resolve or improve with puberty. While the resolution of NCs symptoms may be unrelated to GH therapy, the timing of the resolution of the symptoms with the GH therapy is remarkable. The observed improvement may be a direct effect of GH, or possibly of IGF-1, on the mutant sodium channel. Further studies are needed to explore this relationship as GH therapy may prove to be a beneficial treatment for this debilitating condition.

PO2-173 GH and IGF Use II

Efficacy of growth hormone in the treatment of children with hypochondroplasia (HCP): comparison with a historical cohort of HCP non-treated children

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Background: Hypochondroplasia (HCP) is a skeletal dysplasia, mainly caused by mutations in the Fibroblast Growth Factor Receptor 3 (FGFR3) gene expressed in the growth plates of long bones during enchondral ossification. The importance of growth defect is variable and due in part to an inadequate pubertal growth spurt.

Objective: To determine the efficacy of growth hormone (GH) therapy in 19 children with HCP, compared to a historical cohort of 40 non-treated HCP subjects.

Methods: The HCP subjects were diagnosed on specific skeletal abnormalities and confirmed by 2 experienced physicians of the Bone Dysplasia Center at Necker Hospital. From the historical cohort data, growth charts have been derived and Height-Standard Deviation Score were calculated. The 19 studied patients (9 males, 10 females) with initial height ≤ -2 SDS were treated at a mean age of 9.3±3.2 yrs (range 3-14yrs) with GH (Saizen®, Merck Serono) dose of 0.057 mg/kg/day during 3 yrs. We present interim results after 2 yrs of treatment in 15 of them.

Results:

	Baseline	Gain during 1st yr treatment (p)	Gain during 2nd yr treatment (p)
Height velocity (cm)		8.1± 1.9	6.3 ± 2.1
Height (SDS)/ Sempe[1]	-3.10	0.6 (0.002)	-0.04 (ns)
BMI[2] (SDS)/ Sempe[1]	1.72	-0.34 (ns)	-0.19 (ns)
Height / HCP[3] (SDS)	-0.65	0.83 (0.003)	0.32 (ns)
BMI / HCP[3] (SDS)	-0.19	-0.08 (ns)	0.01 (ns)
Upper segment (SDS)	-1.25	0.45 (0.002)	0.2 (ns)
% Fat body mass[4]	1.16	-0.63 (0.002)	0.04 (ns)
BMD[4] (Zscore)	-2.00	0.15 (ns)	0.18 (ns)
IGF-1 (SDS)	-0.74	2.16 (0.001)	-0.28 (ns)

[1] Sempe table; [2] body mass index; [3] non-treated historical cohort with HCP; [4] Body composition and lumbar spine mineralometry evaluated by X-ray absorptiometry

The height gain was +0.59SDS, obtained essentially during the 1st year of treatment, but it was +1.1 SDS compared to a non-treated HCP patients. Upper

segment increased proportionally, % fat mass decreased during the 1st year. There was no significant change in either BMI, vertebral BMD or response between patients with FGFR3 mutation (n=11) and the not mutated patients.
Conclusion: These 2 years interim results suggest that GH is effective in improving growth of patients with HCP. The effect on pubertal growth spurt remains to be determined.
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PO2-174 GH and IGF Use II

Growth hormone therapy in Noonan and Silver Russell syndrome: relationships between genotype and growth in our experience

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Introduction: Growth hormone (GH) therapy has been used as part of clinical trials in a lot of syndromes presenting with short stature, especially in Noonan Syndrome (NS) and Silver Russell Syndrome (SRS). At present, the direct cause of short stature in these syndromes is not known. Usually there is not GH deficiency but pathology in the GH/IGF-I axis has been reported.
However, there's not a consensus on response to rhGH in these syndromes especially in correlation with different genotypes. Some data suggest that the absence of PTPN11 gene mutation in NS and the presence of uniparental disomy of chromosome 7 (UPD7) in SRS contribute to better response to rhGH.
Objective: we aimed to compare growth during rhGH therapy in patients with SRS and NS with or without identified genetic mutations.
Patients: 8 patients with NS and 5 patients with SRS treated with rhGH for at least 1 year (mean rhGH dose: 0.25 mg/kg/week).
Results: SRS: genotyping was performed in a subgroup of 3 individuals: 1 patient with UPD7 (33%) and 2 patients without it (67%). NS: 7 patients with PTPN11 mutation (87%), 1 patient without it (13%). The results are based on national standard.

	SRS UPD7 +	SRS UPD7 -	SRS genetic in progress	NS PTPN11+	NS PTPN11-
Nr	1	2	2	7	1
Age at start GH (yr)	3	3.5	5.4	11.8	13.9
Height at start GH (SDS)	-3	-3.6	-3	-4	-3.5
Height during therapy (SDS)	-1.4	-1.2	-1.8	-3.4	-3.7
Years of treatment	4.3	8.4	6.2	3.4	1
ΔHtSDS during therapy	+1.6	+2.4	+1.2	+0.6 (range -0.6; +2.6)	-0.2
Final height	/	/	-2.8 (n=1)	-2.2 (n=3)	/

Conclusion: SRS and NS are prototypical disorders for the study of the epigenetic regulation of prenatal and postnatal endocrine growth. A beneficial effect of GH treatment on growth in NS and SRS was reported. Our data suggest that the presence of UPD7 in SRS and the presence of PTPN11 mutations in NS doesn't impair response to rhGH treatment. Our study shows high variability to rhGH in patients with mutations in the same gene (PTPN11+: ΔHtSDS range: -0.6;+2.6).
These findings confirm different patterns of growth in relation to different genotypes in these syndromes, especially in patients with PTPN11 mutations, even if they need to be confirmed in additional studies involving a larger number of subjects treated with rhGH therapy.

PO2-175 GH and IGF Use II

Diagnosis of GH deficiency in children: can auxology, IGF-1, IGFBP-3, MRI and genetic tests replace GH stimulation tests?

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Objective Two different growth hormone stimulation tests (GHSTs) are traditionally used for the diagnosis of GH deficiency (GHD) in children. Since GHSTs are imprecise, time-consuming, costly and unsafe, it has been proposed to use auxology, IGF-1 and IGFBP-3 levels and then performing brain MRI and genetic tests to identify children with GHD. The aim of the current study was to compare between these two different approaches and to assess whether the new approach can replace GHSTs for diagnosis of GHD in children.
Design and Patients Fifty-three children (18F/35M), diagnosed as having GHD by two different GHSTs and under GH treatment, were enrolled (aged at presentation 0.2 to 12.7 years; mean 5.4 ± 2.6 SDS). They all underwent auxology, IGF-1 and IGFBP-3 measurements and brain MRI prior to GH treatment. Sequence analysis of *GH-1, ghrelin* (*GHRL*) and ghrelin-receptor (*GHSR*) genes was carried out. **Results** Prior to GH therapy, mean height (SDS) was -2.8 ± 0.9 (range, -5.1 to -1.3), IGF-1 (SDS) -1.3 ± 0.6 (-2.9 to 0.1), IGFBP-3 (SDS) -1.2 ± 1.6 (-4.0 to 3.0). Peak GH levels in response to clonidnie 4.2± 2.1 (0.7-8.7 ng/ml) and arginin 3.7± 1.9 (0.6-7.8 ng/ml). Pituitary abnormalities were demonstrated in 28 out of 48 children (58%): in 26 of them the adenohypophysis was hypoplastic and 13 had an ectopic posterior pituitary. Fourteen children (26%), belonging to two different core families, carried heterozygous G6664A mutation of *GH-1* and the others had no mutations of *GH-1, GHRL or GHSR*. Using cut-off levels of -1.5 SDS for each of the following parameters: height, IGF-I, IGFBP-3, excluded the diagnosis of GHD in 11%, 68%, 81% of the children (respectively), while a cut-off of -2 SDS excluded 23%, 89%, 96% (respectively). MRI and genetic tests disregarded 42% (28/48 with MRI) and 74% of the children with GHD (respectively). **Conclusions** Using the suggested new approach for the diagnosis of GHD in children will disregard 11 to100 % of the children who are currently being treated with GH. We recommend that until future helpful tools become available, the diagnosis of GHD in children should be judged on combined clinical, auxological, MRI, genetic and GHSTs.

PO2-176 Gonads and Puberty II

Reproductive hormones in girls with Prader-Willi syndrome (PWS) from infancy through adulthood

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Background: Hypogonadism is a cardinal feature of PWS, however age-related changes in the hypothalamic-pituitary-gonadal axis have not been well-documented in females. Recent findings in PWS boys and adult women suggest that primary gonadal dysfunction contributes to the hypogonadism.
Objectives: To characterize age-related changes in gonadotropins, sex-steroids, and gonadal peptides in a cross-section of PWS females from infancy through adulthood and to investigate the relative contributions of hypothalamic vs primary ovarian dysfunction in the etiology of hypogonadism.
Methods: Blood samples were taken from 42 females (ages 6 weeks to 32 yrs) with PWS confirmed by genetic analysis of 15q11-13 (paternal deletion in 24, uniparental disomy in 17, and imprinting center defect in one). None were treated with estrogens or progesterone at the time of the study. 14 girls (age <16 yrs) received growth hormone. Two women had diabetes mellitus treated with insulin.
Results: Menarche was reported in 7 females (ages 12 - 29 yrs) but all devel-

oped secondary oligo- or amenorrhea. BMI-sds (mean±SD) was -0.96±1.88 for girls <3 yrs and +1.75±0.86 for girls >3 yrs.

Hormone levels (mean ± SD) by age in PWS women				
Age groups	< 2 yrs	2 – 7 yrs	8 –15yrs	> 16 yrs
N:	5	13	9	15
LH (mIU/mL)	3.5±6.8	0.38±0.32	1.2±2.0	2.4±2.5
FSH(mIU/mL)	10.4±11.1	1.0±0.9	6.2±7.6	5.0±3.8
Estradiol (pg/ml)	4.3±5.8	6.6±6.6	20.4±19.6	39.0±36.9
AMH (ng/mL)	0.9±0.8	1.5±1.9	1.6±1.5	1.2±0.8
Inhibin B(pg/mL)	15.8±15.7	7.3±0.9	8.3±1.7	17.0±15.4
SHBG(nmol/L)	129.0±66.2	73.5±50.1	39.6±20.8	36.3±20.4
Testosterone(ng/mL)	0.12±0.05	0.16±0.09	0.30±0.13	0.41±0.20
Androstenedione(ng/mL)	0.42±0.27	0.35±0.12	0.86±0.41	1.41±0.70
DHEA-S(mcg/mL)	16.8±6.2	52.2±48.5	130.2±90.3	196.9±111.6

All had normal TSH levels; 5 girls had mild hyperprolactinemia (26 -30 ng/mL).

Conclusions: Hormone patterns vary among PWS females: LH and FSH were low in most, but some had normal to high gonadotropin levels. AMH was generally normal, but inhibin B levels were low or undetectable in all. Androgens were slightly elevated in young PWS girls, but normal in adolescents and adults. Our results suggest that a unique primary ovarian defect, in addition to variable degrees of hypothalamic dysfunction, contributes to hypogonadism in PWS females.

PO2-177 Gonads and Puberty II

Growth during puberty in cystic fibrosis patients. What can we learn from registries?

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The observation of a pubertal development delay in cystic fibrosis (CF) patients is commonly assumed but continuously questioned in the literature. The impact of growth during puberty on CF adult height has never been studied.

Material and method: To evaluate the contribution of this period on final height, we analyzed during 5 years in a cross-sectional and longitudinal study the height velocity during puberty of 725 CF patients (boys: 54.4 %) extracted from the French CF National Observatory (ONM). The patients were 8 to 15 years old in 1999 and their height was observed every year. Mean age and height at onset of puberty, pubertal height gain, peak height velocity and duration and contribution of the pubertal growth spurt as percent of total height were analyzed and correlated with body mass index and pulmonary function tests.

Results: Height started to accelerate at a normal mean age according to the French range for both sexes. Height velocity during puberty was low with a maximum peak of 5.6 +/- .7 cm/year for girls at 11 9/12 years of age (n: 7.8 +/- 1) and 6.4 +/- .6 cm/year for boys at 14 3/12 (n: 9 +/- 1.1). The contribution of the puberty on final height was rather low: 16.7 % for girls (n: 18.6 +/- .7) and 15.5 % for boys (n: 18.4 +/-.8) and explained the suboptimal adult height of the CF French cohort (z-score: -.4 SDS for women and -.6 SDS for men). No or minimal influence of nutritional status and respiratory functions were observed on the height gain during puberty.

Conclusion: The results confirmed that CF patients begin their puberty at normal age, but the amplitude of the peak height velocity is too low and the duration of this peak too short to assess a normal adult height. These data can be explained by sex hormone insufficiency, by GH and IGF1 resistance related to chronic inflammatory status, by a decline of the nutritional status during puberty and by an increase of the resting expenditure in CF during this period.

PO2-178 Gonads and Puberty II

Bone mineral density and body composition before and during treatment with gonadotropin-releasing hormone agonist in Korean girls with central precocious puberty

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Aims: Concern has been raised that children with central precocious puberty who were treated with gonadotropin-releasing hormone agonist(GnRH agonist) may develop obesity and/or loss of bone mineral density(BMD). We studied BMD and body composition as well as biochemical and hormonal profiles of Korean central precocious puberty girls before and after one year of GnRH agonist therapy.

Methods: This study included 88 girls with central precocious puberty who were treated with depot leuprolide acetate 3.75mg from 2007 to 2009 in the Ajou university hospital, Korea. Anthropometry, bone age, biochemical and hormonal profiles were assessed at baseline, after 6 months, and after one year. BMD and body composition were measured with dual energy x-ray absorptiometry at baseline and after one year.

Results: Before therapy, mean lumbar spine BMD SDS(standard deviation score), lumbar spine bone mineral apparent density(BMAD) SDS, femur neck BMD SDS and total body BMD SDS for chronological age were significantly higher than zero. However, SD scores of those for bone age were significantly lower than zero at baseline. Mean lumbar BMAD SDS for bone age was near zero at baseline. After one year of therapy, BMD and BMD SDS for chronological age and bone age were all significantly elevated. But, mean lumbar BMAD SDS for bone age was unchanged. Also, body mass index(BMI), BMI SDS for chronological age and BMI SDS for bone age were significantly elevated after one year. However, there were no SDS changes for chronological age and bone age in fat content and percent of body fat. SD scores for chronological age and bone age of lean body mass and bone mineral content were significantly elevated after one year GnRH agonist therapy.

Conclusion: Our analysis of one year follow up suggests that GnRH agonist therapy on central precocious puberty has no negative effect on bone mineral density and obesity.

PO2-179 Gonads and Puberty II

Pubertal metformin therapy to reduce total, visceral and hepatic adiposity beyond puberty

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Background & **Aim:** Early life is a critical window in which the environment partly dictates the subsequent gain of fat across childhood. We hypothesized that puberty is a second window in which adiposity and its correlates are fine-tuned towards reproduction. This concept implies that puberty provides a unique opportunity to reprogram a misprogramming that may have occurred in early life. We tested this hypothesis in low-birthweight (LBW) girls with precocious pubarche (PP), who are notoriously at risk for hyperinsulinemic body adiposity during and beyond puberty.

Study Population & **Design:** LBW-PP girls (N=38; mean age 8 yr) were randomized to remain untreated or to receive metformin across puberty (425 mg/d for 2 yr, then 850 mg/d for 2 yr; Ibanez et al, JCEM, May 2008); subsequently, all girls were followed for 1 yr without intervention. Here we report on the latter year of follow-up.

Results: The benefits of metformin were mostly maintained during the post-treatment year so that, after 5 yr, metformin therapy was associated with more lean mass; with less total, visceral and hepatic fat; with lower circulating levels of androgens and leptin; and with elevated levels of high-molecular-weight (HMW) adiponectin and undercarboxylated osteocalcin.

Conclusion: In LBW-PP girls, pubertal metformin therapy was followed by a favourable adipokine profile and by a reduction of total, visceral and hepatic adiposity beyond puberty. The concept that early misprogramming can be reprogrammed across puberty deserves further challenge.

PO2-180 Gonads and Puberty II

Sexual precocity. A review of 68 cases from a single centre

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Introduction
Sexual precocity is a frequent cause of consultation in paediatric endocrinology and can be physically and emotionally difficult for children and parents. Management is sometimes difficult because of the clinical polymorphism and the unpredictable evolution.
The aim of our study is to analyze etiology, management and follow up of cases seen in our centre.
Methods :
Retrospective study of patients with sexual precocity, defined as onset of sexual maturation in girls < 8 years and boys < 9 years, followed up from September 1999 to March 2007. We excluded children with advanced puberty. Clinical evaluation included measurement of height status and velocity, breast development in girls and increase of the testicular volume > 30 x 20 mm in boys, bone age, serum testosterone for boys, estradiol and pelvic ultrasound for girls. The GnRH test was used to distinguish between true central precocious puberty (CPP) and precocious pseudopuberty. Other laboratory investigations carried out if clinically indicated included: 17 hydroxyprogesterone, DHAS, cortisol, ACTH, DOC, 11-Desoxycortisol, ßHCG and alpha-fœto-proteins. Cranial MRI was carried out if hormonal studies indicated a diagnosis of CPP.
Results :
We had 68 cases: 10 boys/ 58 girls
Breast development was the most frequent presenting feature (65%) in girls - 23% unilateral and 42% bilateral. Next commonest was pubic hair (28%) followed by premature menarche, tumoral syndrome and tall stature (7%).

Aetiology and management	
Aetiology	management
CPP: 13 cases (13F: 0M)	GnRH analogue in 2 patients with poor adult height prognosis.
Premature thelarche 26 cases	No treatment
Premature pubarche 6 cases(3F: 3M)	No treatment
Premature menarche : 4 cases	No treatment
Adrenal enzyme disorders: 9 cases (5F : 4M)	Cortisone and genital surgery. NB : 2 genetic females underwent hysterectomy and masculinising surgery
Adrenal tumour 7 cases (4F: 3M)	Surgery (unsuccessful in 2)
McCune-Albright syndrome : 3 cases (3F)	Aromatase inhibitor (1) without good result

Discussion
Sexual precocity is a serious problem and should lead to a rigorous evaluation, distinguishing between true CPP and precocious pseudopuberty. Problems encountered in the context of our Moroccan centre include delay in seeking medical help, and the cost of GnRH analogue treatment when this is indicated.

PO2-181 Gonads and Puberty II

Etiology of thelarche in girls younger than 7 years: a study in Southern Thailand

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Background: Thelarche or breast development is the most common clinical presentation of precocious puberty in girls.
Objective: To determine the etiologies and characteristics of girls presenting with thelarche at age <7 years.
Subjects and method: Medical records of 115 girls with breast development before the age of 7 years who attended at Songklanagarind Hospital from 1995 - 2007 were retrospectively reviewed. All girls were initially evaluated for bone age. Girls with advanced bone age were further evaluated by LHRH stimulation test.
Results: The etiologies of thelarche in young girls were premature thelarche (76.5%), central precocious puberty (17.4%: idiopathic central precocious puberty 12.2% and hypothalamic hamartoma 5.2%), ovarian cyst (5.2%), and exogenous estrogen exposure (0.9%) The mean age at the time of initial presentation was 3.4 ± 1.3 years. The presence of pubic hair was found only in girls with central precocious puberty. Bleeding per vagina was found in girls with central precocious puberty and ovarian cyst. Height and weight in girls with central precocious puberty were significantly greater than those with premature thelarche and ovarian cyst ($p < 0.01$)
Conclusion:.Premature thelarche is the most common etiology of breast development in girls younger than 7 years of age. Girls with premature thelarche were at average weight and height and had average bone age The presence of pubic hair and bleeding per vagina are suggestive signs of central precocious puberty.

PO2-182 Gonads and Puberty II

Combined cortisol and growth hormone testing

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Background: Growth hormone (GH) stimulation tests are recommended for evaluation of GH deficiency. Glucagon has the advantage of providing assessment of both growth hormone and ACTH status. The impact of the test order on the individual result using glucagon and arginine stimulation is unknown.
Aim: To investigate the effect of the order of glucagon and arginine stimulation performed sequentially on the same day on GH and cortisol response.
Material and Methods: In this retrospective chart review we included 149 of 242 children referred for evaluation of short stature who underwent glucagon and arginine stimulation testing. 93 children were excluded: current glucocorticoid replacement, high likelihood of pituitary pathology, and incomplete data. We recorded auxological data, pubertal staging, and GH was measured during arginine stimulation (0.5g/kg: max 30g) and cortisol, and glucose levels were also measured during glucagon stimulation (15mcg/kg; max 1mg).
Results: 111 individuals were classified as being GH sufficient with a GH value > 15 mU/l in one of the two tests. Both test orders were similar in identifying GH deficiency (GHD): 14/85 and 16/56 for arginine-glucagon and glucagon-arginine, respectively ($p=0.10$). The number of second test GH peaks > 15mU/l with first test GH peak < 15mU/l were 14/30 for glucagon-arginine and 13/27 for arginine-glucagon ($p=0.47$). Test order arginine-glucagon gave higher GH peaks than glucagon-arginine ($p=0.003$). Peak cortisol level was associated with baseline cortisol level ($p<0.0001$) and nadir glucose level ($p=0.02$), but not test order. Only 35% with normal basal cortisol levels were verified as being cortisol sufficient (cortisol > 500nmol/l).
Conclusion: The combination of the two tests significantly improved the sensitivity of diagnosing GHD, however the cortisol response to the GST was poor in confirming an expected normal cortisol response within the non-GHD, otherwise normal short children.

PO2-183 Gonads and Puberty II

Hypogonadism and endocrine testing in infants with CHARGE syndrome

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Background: CHARGE syndrome is a multiple congenital anomaly disorder characterized by the acronym Coloboma, Heart defects, Atresia of the choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies. It is usually (~60%) due to alterations in transcription of tissue-specific genes normally regulated by the chromodomain gene CHD7 during development. Although genital hypoplasia is described within the acronym, there are few data on these patients.
Patients: A total of 6 patients (4 male and 2 female) with CHARGE syndrome from within our unit, diagnosed clinically using both the old and new diagnostic criteria, have been investigated at < 1 year of age. Confirmatory CHD7 gene mutation testing was carried out on all patients and showed a mutation in 5 (83%).
Clinical findings: Genital hypoplasia was seen in all patients, of the 4 males all had micropenis and also cryptorchidism (bilateral in 5, unilateral in 1), and both female patients had hypoplastic labia.
Results: Hypothalamo-pituitary-gonadal axis testing was performed at a median age of 0.22 years (range 0.12-0.71). Baseline gonadotrophins were measured in all: median(range) for LH was 0.15(0.1 to 0.5)U/l, and for FSH 0.4(0.1 to 5.7)U/l; only 1 patient (female) had a baseline level (in FSH) >1.0U/l. Baseline testosterone performed in all males was a median(range) of 0.15(0.1-0.6) nmol/l, with oestradiol levels in the two girls 31 & 36pmol/l respectively. Dynamic testing with LHRH was carried out in all 4 male patients: median(range) peak for LH was 1.8(0.1-8.6)U/l, and for FSH 5.5(0.4-8.7)U/l. Only 1 patient demonstrated a peak level of LH or FSH <5U/l.
Conclusion: The "mini-puberty" with gonadotrophin surge in the first months after birth offers an excellent opportunity to investigate the hypothalamo-pituitary-gonadal axis in hypogonadal patients. Although data are currently limited hypogonadism appears common in infants with CHARGE syndrome, with low baseline gonadotrophins and testosterone/oestrogen. LHRH testing in males, however, did produce a greater gonadotrophin response than that which we have previously described in this group peri-pubertally.

PO2-184 Gonads and Puberty II

Central precocious puberty in 5-year old boy with McCune-Albright syndrome

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Introduction: Boys with McCune-Albright syndrome (MAS) typically do not present precocious puberty (PP). Testicular abnormalities usually include testicular microlithiasis. Testicular enlargement and gonadal hyperfunction are very rare.
Clinical case: A boy was born to non-consanguineous parents with birth weight 3300 g, length 50 cm. He was noticed to have moon face and hypertrichosis shortly after birth. At his first examination in our clinic at 3 yrs of age the boy was severely growth retarded (height SD=-5.25) with leg deformities, testicular enlargement (4 and 5 ml), a large cafe-au-lait skin spot on the right femur and buttocks, pubic hair, hypertrichosis, moon face.
Laboratory evaluation is summarized in Table1 and Table2.

Table 2

Hormone	Time point 8.00	Time point 23.00	Liddle 's test
DHEA-S (nmol/l)	>27000	-	>27140
Cortisol (nmol/l)	552	556	688
ACTH (pg/ml)	12.0	14.0	-
Testosterone(nmol/l)	4.1	-	7.1

He was diagnosed with MAS presented with typical skin pigmentation, fibrous dysplasia of bones, ACTH- independent Cushing's syndrome, and testicular enlargement. Elevated testosterone could have either testicular or adrenal origin but extremely elevated DHEA-S reflects increased adrenal steroid synthesis. Unilateral adrenalectomy was performed at the age of 4. Next follow up a year after surgery showed increasing in height velocity, testicular volume and bone age significantly increased as well. Ultrasound showed normal testicular tissue. GH-RH test revealed central precocious puberty.

Table 1

Age (yrs)	3.0	5.0
Bone age (years)	1	6
Height velocity (SD)	-0.95	+4.97
Testis volume (right/ left) ml	4/6	5/15
LH(U/L) (basal level/peak)	0/1.6	0/16.7
FSH (U/L) (basal level/peak)	0/3.0	0/0.2
Testosterone(nmol/l)	4.1	3.9

Conclusion: We suppose that increased steroidogenesis in micronodular adrenal hyperplasia with high androgen production could lead to the activation of the hypothalamic-pituitary-gonadal axis and central precocious puberty in MAS without preliminary gonadal hyperfunction.

PO2-185 Gonads and Puberty II

Comparison of masculinity in patients with hypogonadotropic-hypogonadism in Kallmann syndrome and patients with pituitaray stalk transection

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Patients with Kallmann syndrome (KS) show a low marriage rate compared with the same aged young men, even those treated by gonadotropin therapy. In our cases, only one patient of six with KS had married, and the other 5 had no partner. On the other hand, patients with hypogonadism due to pituitary stalk transection (PST) showed that of 5 patients 4 had married and one patient had a partner. We treated these two groups with the same menu of gonadotropin therapy over 10 years and all patients developed to TannerV gonadal stage. Recent ages in the 6 patients with KS are 25 to 40 y.o. and in the 5 patients with PST are 22 to 45 y.o. From these data, the marriage rate in KS patients is lower than that in PST patients. In comparison of these two hypogonadotropic-hypogonadism groups, KS lacks the intrauterine testosterone shower (Ts) but PST dose have the Ts. To clarify the contribution of this Ts to the rate of marriage, we examined the masculinity scores in both groups. The average of masculinity scores in patients with KS was markedly low compared with patients with PTS (Perfect : KS : PST showed 25: 14: 24). In conclusion, intrauterine Ts may contribute to the development of masculinity and activity to get a partner. If we can diagnose early in the intrauterine period, the intrauterine exposure of testosterone will contribute to improving the quality of life of KS patients.

PO2-186 Gonads and Puberty II

Insulin-like growth factor-1 (IGF-1) deficiency prevents the appearance of pre-acne and acne

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Background: The interaction between IGF-1 and sex hormones plays an important role in the pubertal process and the development of the secondary signs such as acne.
Objective: Laron syndrome (LS) with congenital IGF-1 deficiency is a unique model to study the effect of IGF-1 lack and its pharmacologic replacement on the appearance of pre-acne (microcomedones) and acne.
Subject: Two groups of LS patients were studied: Group I: 14 untreated patients (8M, 6F) age 10-48 years and Group II: 9 LS patients, 4 young (2M, 4F) aged 10-14 years and 5 adults (1M – 28 years, 4F – aged 30-39 years) treated by IGF-1 (150 and 120 microgram/Kg/s.c. once daily, respectively), All had been followed regularly from infancy to adult age.

Results: The 14 untreated LS patients had delayed puberty. 13/14 patients did not develop acne; 5 of these 13 patients presented few pre-acne between ages 14-19 (normally 8-10 years). One male showed slight acne at age 22 while reaching full puberty. The 3 treated males (IGF-1 levels 4 hours after injection: 80-100 ng/ml) did not develop acne despite significant increase of LH (<0.3 to 1-6 mIU/L), FSH 0.8 to 1.7-7.3 mIU/L and testosterone (<0.7 -18 to 3.8 – 28 nM/L), but no change in Δ 4 androstendione (2.7 – 6.8 uM/L) and DHEA-S. Also the untreated females did not develop acne. Of the 6 treated female patients, 4 developed signs of hyperandrogenism (oligo/amenorrhea) and acne. Before IGF-1 therapy, Δ 4 androstendione was 2.2-7 nM/L, DHEA-S: 2.4-3.6 microM/L, basal IGF-1: 4-7.1 ng/ml, and 4 hours post injection: 35-57 ng/ml. During treatment the values were: Δ 4 androstendione: 8-10 nM/L; DHEA-S: 2.1-4.7 microM/L; IGF-1 69-274 ng/ml (basal) and 275-806 ng/ml (4 hours post daily injection).
The acne disappeared with reduction of the IGF-1 dose to 50 micrograms/Kg/day or stopping treatment; concomitantly the androgen concentrations decreased and the monthly cycle normalized.
Conclusion: Our study demonstrates for the first time the relationship between serum IGF-1 levels and the development of pre-acne and acne.

PO2-187 Gonads and Puberty II

The U.S. multicenter trial of monthly depot leuprolide for central precocious puberty (CPP): post-treatment outcomes

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Introduction: Leuprolide acetate for monthly depot suspension (Lupron Depot, LD) was approved by the FDA for treatment of CPP based in part upon results of a U.S. multicenter trial initiated in the 1990s but never reported. Here we summarize study results from the follow-up period.
Methods: A total of 55 naïve patients with CPP received 7.5 mg, 11.25 mg, or 15 mg of LD for a mean±SD of 3.9±2.0 years (y). Forty (35 girls, F) entered into long-term follow-up for an average of 3.5±2.2 y. Tanner staging, hormone analysis, height measurement and bone age radiographs were performed every 6 months (mo) until attaining pubertal hormone levels, then every 12 mo for 5 y; height was obtained at adulthood (≥21 y) when available. Predicted AH (PAH) was determined by the Bayley-Pinneau method. Adult height (AH) was obtained at adulthood or established at growth velocity <1 cm/y or bone age >14 y for F.
Results: At all treatment measurements, 90.9% of patients (50/55) had peak stimulated LH levels suppressed to <3 IU/L. All patients (32/32) had pubertal peak LH levels (>5 IU/L) within 12 mo after discontinuing treatment, and 74.2% of patients (23/31) reached Tanner stage 5 by 4 y post treatment. F (n=26) had menses at 1.5±0.5 y post treatment. The mean growth rate at the end of treatment for all F was 3.7±1.9 cm/y, increased to 4.4±2.2 cm/y in the first 6 mo post-treatment, and returned to 3.5±2.2 cm/y in the 2nd post-treatment y.

Height Outcomes for Females

	Baseline	End of Treatment	AH
TH	163.8±7.4 (N=29)		
Height (cm)	132.6±15.6 (N=29)	153.1±7.9 (N=28)	162.7±7.7 (N=29)
Height SDS	1.8±1.0 (N=29)	0.9±1.2 (N=28)	0.0±1.2 (N=29)
PAH	157.5±7.8 (N=26)	166.7±5.0 (N=18)	

Mean AH for F was significantly higher than mean baseline PAH (P<0.001), and was at the population average. Height outcomes are not presented for boys because of small sample size (n=4). Patients with more LH suppression (mean peak stimulated LH <0.5 IU/L during treatment) had AH SD scores similar to those of patients with less LH suppression (mean SD scores of -0.09 vs -0.11, respectively).
Conclusions: LD monthly injection was highly effective at suppressing puberty and was associated with gains in AH for F. All patients studied had resumption of a pubertal hypothalamic-pituitary-gonadal axis by 12 mo after therapy was discontinued. Approximately 86% of F attained AH within their target height (TH) range.

PO2-188 Gonads and Puberty II

Experience with extended use of the histrelin implant for the treatment of central precocious puberty

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BACKGROUND: Thus far, only relatively short term data regarding use of the histrelin implant for the treatment of central precocious puberty (CPP) are available. In addition, the timing of recovery of the hypothalamic-pituitary-gonadal (HPG) axis following explantation has not been well described.
OBJECTIVE: To evaluate the effects of >2 years of treatment with the histrelin implant in children with CPP and to investigate recovery of the HPG axis once therapy is discontinued.
DESIGN/METHODS: This was a multicenter, prospective extension study following an initial 12 month phase III trial of 36 patients (20 naïve, 16 previously treated) and a 2 year extension phase with 31 patients. Patients continuing therapy had their previous implant removed by a pediatric surgeon and a new implant placed during the same procedure. GnRHa stimulation tests and bone ages x-rays were performed annually. Data regarding timing of menarche and gonadotropin and sex steroid hormone levels were collected in subjects who discontinued therapy.
RESULTS: Twenty-two patients (1 boy) received a third implant and 14 received a fourth. Peak LH at 36 months was 0.28 ± 0.14 mIU/ml in the treatment naïve group and 0.44 ± 0.14 in the previously treated group (n=14). Change in the ratio of bone age to chronological age was -0.05 ± 0.06 (p = 0.0091). Growth velocity SDS from 24 to 36 months was -1.77 ± 2.46 (p = 0.0300). Predicted adult height increased during year 3 by 1.5 cm (p = 0.014) in the treatment naïve group but did not change in the previously treated group. HPG axis recovery data was available for 12 patients. Gonadotropin and/or sex steroid hormone levels returned to pubertal ranges in all subjects within 6 months of explantation. In subjects treated up to 3 years, menarche occurred 2-12 months after implant removal (n=5).
CONCLUSIONS: The histrelin subcutaneous implant continues to provide HPG axis suppression and a decreased rate of skeletal maturation in patients treated for more than two years with a new implant placed every 12 months. Predicted adult height continued to increase in patients who had been treatment naïve at baseline. Recovery of the HPG axis after discontinuation appears comparable to depot GnRHa. Long term follow-up is needed to further evaluate this therapeutic modality in children with CPP.

PO2-189 Gonads and Puberty II

Long term bicalutamide and 3rd generation aromatase inhibitor therapy in two boys with testotoxicosis

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Background: Testotoxicosis results from a constitutively activated LH receptor. The anti-androgen bicalutamide and a 3rd generation aromatase inhibitor have yielded encouraging short term results. We present long term data in 2 boys treated with this combination.

Case Presentations: Patient 1 presented at 3.1 years with genital enlargement and tall stature. Initial evaluation revealed bone age (BA) of 4 years, testosterone of 65 ng/dL, and stimulated LH of 2 IU/L. An LH receptor mutation was found in mother and child. Accelerated growth and BA advancement continued despite treatment with spironolactone and testolactone such that BA was 10 when the child was 5.4 years (BA/CA of 1.8). Therapy was changed to bicalutamide 50 mg and letrozole 2.5 mg PO daily. Depot leuprolide was added at 5.5 yrs when gonadotropins increased to pubertal levels. Treatment has been well tolerated for 4.5 years with recent BA/CA of 1.0 and little virilization (Tanner 2 pubic hair, phallic length 10 cm). Laboratory studies include undetectable estradiol levels, suppressed gonadotropins, and testosterone levels ~200 ng/dL. Patient 2 presented for evaluation at age 4.1 years after failing leuprolide treatment for presumed central precocious puberty. Two months after discontinuation of leuprolide, LH was <0.02 IU/L and testosterone was 434 ng/dl. BA was 9 years at CA of 4.25 years (BA/CA of 2.1). An LH receptor mutation was found in father and child. Bicalutamide 50 mg and anastrozole 1 mg daily were initiated. Depot leuprolide was restarted at age 5.5 years for central puberty. Results from the first 17 months of therapy have previously been reported. Over 5.25 years of treatment, BA/CA has declined to 1.5, testicular volume has stabilized at 8 ml, and pubic hair has stabilized at Tanner stage 2. Laboratory surveillance has revealed undetectable estradiol, suppressed gonadotropins, and testosterone levels of 125-378 ng/dl. Predicted adult height (PAH) has improved from 168.9 cm to 182.4 cm.
Conclusions: Long-term therapy with bicalutamide and a 3rd generation aromatase inhibitor in 2 boys with testotoxicosis has been well tolerated and successful. Benefits have included once daily dosing, attenuation of pubertal progression, and improved PAH. Although promising, prospective controlled studies utilizing this regimen with long term follow-up are needed.

PO2-190 Gonads and Puberty II

Comparison of weight gain during either buserelin or triptorelin treatment in children with pubertas praecox

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Aim: To evaluate the clinical praxis of GnRH analogue choice (either Triptorelin or Burserelin) during the past years and to evaluate the weight outcome during the first treatment year.
Background: A couple of reports have examined the effect of GnRH analogue treatment on weight gain. Recently a consensus document was published reviewing the subject and emphasized that no study has shown any long-term effects on weight gain or BMI (Pediatrics, 2009;123:e752-762).
Methods and population: Twenty-three young children (1 male, 22 females) were referred during the past seven years to the outpatient endocrine unit due to precocious or early puberty (Tanner stadium 2 or more: breast stage 2 or more for females and testes volumes more than 3 ml). Syndromes or malignancies were excluded. Examinations did not find any pathological ethiology behind the condition. All were found to have high estradiol levels at evaluation and accelerated bone maturation.
Results: Thirteen children were treated with Burserelin 9.45 mg every third month (B) and ten with Triptorelin 3,75 mg every month (T). Mean age, bone age and estradiol levels differed between groups. T-group were found to be younger compared to B-group (mean (SD) 7.46 (1.9) years versus 9.09 (1.3) years, P<0.01), larger difference between bone age and chronological age (mean 1.56 (1.6) years versus 0.27 (1.1) years, P<0.05) and higher estradiol levels (mean 33 (26) versus 15 (2.5), P<0.001). Height SDS and weight SDS did not differ significantly between groups at start of treatment (T-group 0.12 (1.5) and 0.65 (2.0), versus B-group -0.62 (2.1) and -0.55 (1.2), respectively). Delta weight SDS from start of treatment to 3 respective 12 months of treatment did not increase significantly and no difference was found between groups (T-group 0.16 (0.22) and -0.04 (0.34), versus B-group 0.10 (0.3) and -0.11 (0.4), respectively).
Conclusion: The data presented in this non-randomized study did not support the hypothesis that anyone of the used GnRH analogue accelerates weight gain. This is in accordance with previous non-randomized studies, but could also be due to small number of patients. If treatment evaluation is to be done, a prospective, randomized study design is crucial to obtain appropriate results.

PO2-191 Gonads and Puberty II

Central precocious puberty after pseudo-precocious puberty due to a Leydig cell tumor of the testis in a six year old boy

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Introduction: Precocious puberty can be classified in true (central or gonadotropin-dependent) or pseudo (peripheral of gonadotropin-independent) precocious puberty. In rare cases, secondary true precocious puberty develops after gonadotropin-independent precocious puberty.
Case report: A 7.5 years old boy was presented because of premature sexual development and an accelerated height velocity. At examination his height was above the 97th percentile, he had pubic hair, an enlarged penis, facial acne and a smooth tumor in the left testis. Laboratory investigations showed an elevated testosterone serum level (144 ng/dl), very low basal levels of LH and FSH and a blunted response to LHRH. The urinary steroid profile determined in a 24h-urinary sample showed elevated excretion rates (in microgram/day) of androsterone (662; P95=173), pregnanetriol (446; P95=147) and pregnanolone-5ß,3α (206; P95=24) but normal pregnanetriolone (14; P95=22). His bone age was 12.5 years. A Leydig cell tumor of the left testis was suggested by ultrasound. The tumor was removed surgically and the diagnosis confirmed by histology. After surgery the symptoms improved, serum testosterone levels declined and the urinary steroid profile normalized completely. Two months later the symptoms occurred again, the testosterone serum level increased (201 ng/dl) but ultrasound excluded a recurrence of the tumor. Since LH and FSH now showed a pubertal response to LHRH, secondary true precocious puberty was diagnosed and treatment with leuprolide was started. Clinical symptoms regressed and laboratory results returned to normal ranges for age.
Conclusion: Leydig cell tumors are a rare cause of pseudo-precocious puberty in males. The urinary steroid metabolome shows increased androgen production but also elevation of some markers of 21-hydroxylase deficiency. Development of a secondary true precocious puberty after a gonadotropin-independent precocious puberty may occur. Long-term follow-up investigations of patients with a Leydig cell tumor are needed.

PO2-192 Gonads and Puberty II

Phenotypic variability in patients carrying PROKR2 mutations

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Background:
Idiopathic Hypogonadotropic Hypogonadism (IHH) is characterized by a spectrum of sexual maturation disruption secondary to GnRH deficiency. To date, there are 8 known loci that underlie IHH that are identified in only 30% of cases. Recently, Prokineticin and Prokineticin Receptor (PROK2 and PROKR2) genes have emerged as putative IHH loci and the breadth of the associated phenotypic spectrum is evolving.
Objectives:
Herein, we present two subjects with uncommon presentations of pubertal disruption.
Results:
Case 1:
This male proband was 13-years-old boy when he presented with vertebral fracture. Both the proband's brother and father had delayed puberty and father had osteoporosis as well. At that time, he was overweight with 2cc (prepubertal) testes and Tanner 1 pubic hair; He was normosmic. Further investigation revealed prepubertal baseline FSH, LH and Testosterone levels with minimal response to Luprolide stimulation. An HCG stimulation test, inhibin B, and

antimullerian hormone levels suggested functional testicular tissue. At 14 6/12 years, Testosterone therapy was initiated and thereafter pubertal progression began. However, in mid-puberty, his maturation stalled. At 15 years, he incurred a second fracture (tibia), with minimal trauma and a DXA scan revealed osteoporosis (Z score -2.7 SDS). Mutation analysis in both the proband revealed a novel V297I PROKR2 mutation.
Case 2:
This proband was 16 years when she presented with secondary-amenorrhea. Her mother had delayed puberty and her maternal aunt also had an undefined menstrual problem. Compared to her target height percentile, the proband was tall with eunachoidal proportions. She had Tanner 2 breasts and pubic hair. At that time, she had prepubertal FSH, LH and E2 levels with a normal female karyotype. A pelvic sonogram revealed pre-pubertal uterus (3cm) and normal ovaries. In this case, the proband's mutation analysis revealed an L173R PROKR2 mutation.
Discussion:
In this report, we broaden the phenotypic scope of reproductive and skeletal disorder associated with PROKR2 mutations, including osteoporosis in a boy with partial puberty and secondary amenorrhea in teenage girl. The latter phenotype is reminiscent of an early onset form of Acquired IHH, a rare phenotype previously described in a subset of subjects with FGFR1 mutations.

PO2-193 Gonads and Puberty II

GnRH analog treatment in childhood: long-term efficacy and safety

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Objective. The efficacy and potential side-effects of GnRH analogs (GnRHa) are still under scrutiny. In this single-center study we evaluated the effect of GnRHa treatment on final height (FH), body mass index (BMI), body composition, bone mineral density (BMD) and ovarian function, as well as possible adverse events.
Subjects and methods. The study included 92 female patients, followed during childhood in our Center, and categorized as follows: Group A: Idiopathic Central Precocious Puberty (ICPP) (33 GnRHa-treated and 14 untreated subjects), Group B: Isolated Growth Hormone Deficiency (iGHD) (15 subjects treated with GnRHa and GH and 9 only with GH), and Group C: Idiopathic Short Stature (ISS), treated with GnRHa and GH (n:7), treated only with GnRHa (n:7), and not treated (n:7). All 92 patients were re-evaluated in adulthood (age 16-32.3 years) with an interim history, clinical examination, pelvic ultrasound, DXA scan, and blood collection for the determination of biochemical and hormonal profile.
Results. The characteristics of GnRHa-treated and untreated patients of all three groups (A,B,C) were comparable at initial evaluation. The FH, BMD and percent fat mass of GnRHa-treated patients of all 3 groups were comparable to those of the respective untreated subjects. The BMI values of GnRHa-treated and untreated patients with ICPP and of the 3 subgroups of patients with ISS were comparable. In patients with iGHD, a higher BMI was found in subjects treated only with GH than those treated with GnRHa and GH. Menarche occurred about 1 year after discontinuation of GnRHa treatment, and the characteristics of menstrual cycle and acne were comparable among the subgroups of each category. Untreated patients with ICPP had greater maximal ovarian volumes and higher Ferriman scores than GnRHa-treated patients (p:0.02). The prevalence of polycystic ovary syndrome (PCOS) was 11.3% (7/62) in GnRHa-treated patients and 27.6% (8/30) in patients without GnRHa treatment (p:0.06).
Conclusions. In this long-term follow-up study, children and adolescents treated with GnRHa for various indications attained a normal FH, BMI, BMD, body composition and ovarian function and had a lower prevalence of PCOS in early adulthood than untreated subjects.

PO2-194 Gonads and Puberty II

The androgen status of young women with premature ovarian failure depends on the female sex steroid replacement regimen

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Aims: To compare the effect of a standard Sex Steroid Regimen (sSSR) with a physiological SSR (pSSR) on androgen status in young women with premature ovarian failure (POF).
Patient Population: Seven women with POF were evaluated for the study; 5 had idiopathic POF and 2 had iatrogenic POF, secondary to hysterectomy or treatment of malignancy. The median age of the 7 women was 28yrs (range 21-36) and the
median duration of ovarian failure was 14yrs (range 4-25).
Methods: An open label randomised, controlled, crossover study over 28 months comparing the effect of sSSR and pSSR on androgen status. Treatment consisted of a 12 month period of 4-week cycles of pSSR (transdermal estradiol 100mcg daily for week 1 and 150mcg for weeks 2-4 and either 200mg progesterone vaginal pessaries or progesterone 10mg orally twice daily in weeks 3-4), or sSSR (Loestrin 30, Galen Ltd; ethinylestradiol 30mcg and norethisterone 1.5mg daily for weeks 1-3, followed by 7 "pill-free" days), separated by run-in and wash-out periods. Serum Testosterone (T), Androstenedione (A4), SHBG were measured and the Free Andogen Index (FAI={serum T/SHBG} x 100) calculated at 0, 6 and 12 months during treatment periods.
Results: At baseline, in the sSSR group, median (range, ie min and max) T, A4 and SHBG were 1.7nmol/l(1.5;10.6), 5nmol/l(3.3;8.1) and 71nmol/l(26;78) respectively and not significantly different from those in the pSSR group: 1.3nmol/l(0.5;2.7), 4.6nmol/l(2.9;5.2) and 89nmol/l(32;130), respectively. In comparison, age matched women without ovarian failure would be expected to have a median T, A4 and SHBG of 1-3.2nmol/l, 0.6-8.8nmol/l and 30-120nmol/l. Median T at 6 and 12 months were 1.1 and 1nmol/l (sSSR) and 1.6 and 1.8nmol/l (pSSR). Median A4 at 6 and 12 months were 5.6 and 4.7nmol/l (sSSR) and 5.5 and 6.4nmol/l (pSSR). Median SHBG at 6 and 12 months were 105 and 110nmol/l (sSSR) and 62 and 66nmol/l (pSSR). Median SHBG was significantly higher in the sSSR group at 6 and 12 months ($p<0.02$). Median FAI fell in the sSSR group from 2.4(2.2;7.4) at 0 months to 0.7(0.5;3.1) at 12 months ($p=0.02$); this fall was not seen in the pSSR treatment arm.
Conclusion: Young women with POF have relatively low levels of free androgens, which are further reduced following the introduction of a sSSR. The pSSR used in this study, not associated with any further decline in free androgen levels, is an attractive treatment option for long-term replacement in young women with POF.

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Growth and pubertal status following testosterone therapy for pubertal induction in boys with IBD

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Background: Children with inflammatory bowel disease (IBD) may suffer from

growth and pubertal delay.
Objective: To assess pubertal status and growth in a group of boys with IBD before (T+0) and 6 months after (T+6) initiation of testosterone therapy.
Methods: Retrospective study of boys with IBD undergoing pubertal induction. Height (Ht) and pubertal status were obtained at T+0 and T+6. Markers of disease activity and data on concomitant medication were also collected. Response to testosterone was based on advance of pubertal status and a greater than 50% increase in height velocity (HV).
Results: Eight boys with IBD (7 with Crohn's disease and 1 with Ulcerative colitis) and median age of 14.8 years (range, 13.6, 15.6), median TS of 1 (1, 2) and a median bone age delay of 2.9 years (0.8, 3.5) had pubertal induction using either Sustanon 50 mg i.m. monthly or Andropatch 2.5 mg or 5 mg daily applied for 12 h each day. Seven boys showed an advance of pubertal status to a median TS of 3 (2, 4) and 6 boys had a greater than 50% increase in HV following Testosterone. Median HV at T+0 and T+6 was 1.6 and 6.9 cm/year, respectively (P=0.005). Median Ht SDS at T-6, T+0 and T+6 was -1.4(-3.0, -0.8), -1.6 (-3.4, -1.1) and -1.4 (-3.3, -1.1), respectively. Median HV SDS at T+0 and T+6 was -4.2 (-7.6, +2) and +1.2 (-2.7, +8.5) respectively. HV SDS was significantly different following treatment when corrected for both age and pubertal stage (P=0.005 and P=0.01). Median albumin, CRP, ESR and platelets were similar at T+0 and T+6. Although median CRP showed a significant correlation with HV at T+6 (r=-0.786; P=0.021), there were no clear associations of growth response to concomitant therapy.
Conclusion: In this group of boys with IBD Testosterone therapy improves growth and pubertal status to a variable extent and needs further systematic study.

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Gonadotropin-independent precocious puberty with acute progression in a 5-year-old girl; a case report

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(Introduction)Sexual precocity is unusual. We report who presented acute progressive incomplete sexual precocity, and the successful use of tumor markers for differential diagnosis.
(Case report)A 5 5/12-old girl was referred to our hospital further exam: nation of acute breast development. It occurred before only 6 days. At first medical evaluation, the breast was Tanner stageII and pubic hair was Tanner stageI. She had no café-au-lait spots, had no linear growth(±0SDS) and bone age acceleration. There are no mass in her lower abdomen. Laboratory examination revealed serum estradiol was 51pg/ml, LH/FSH levels were <0.5/<0.5 mIU/L. Peak LH level was <0.5mIU/ml, and peak FSH level was 0.9mIU/ml after GnRH test. Abdominal MRI showed ovarian cyst in her left ovary. It was look like juvenile granulose cell tumor (JGCT) or benign ovarian cyst. Serum LDH, hCG, CA125, AFP elevation were not detected. We also obtained serum inhibin values are as reasonable concentration for her age, so that keep to observe. Her ovarian cyst was decreased after 30 days, and then breast budding also disappeared. Serum inhibin has not been elevated finally. So we diagnosed that she was precocious pseudo puberty associated with isolated ovarian follicular cysts.
(Discussion)JGCT convalesce satisfactorily, but the cases more than Stage II is poor. At first, we couldn't denied JGCT, even our case is diagnosed as JGCT, that classification is Stage I of FIGO (Federation of gynecology and Obstetrics) stage.
(Conclusion)We waited and succeeded to avoid surgery. To exam inhibin and careful observation were important when doubted JGCT.

PO2-197 Gonads and Puberty II

Pubertal development in congenital disorder of glycosylation type Ic (ALG6 deficiency)

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Background: Congenital Disorders of Glycosylation (CDG) are a group of rare disorders due to enzyme defects necessary for protein glycosylation and for processing of protein-linked oligosaccharides. Glycoproteins are important components of the hormone cascades regulating growth, metabolism and sexual development. Gonadotropin glycosylation changes at different stages of the menstrual cycle and at different ages. Most females with Congenital Disorders of Glycosylation (CDG) of pubertal age have been described as having an absence of secondary sexual characteristics. Less than 50 cases of CDG Ic (hALG6 deficiency, OMIM #603147) have been described worldwide, none of whom have been reported to have normal pubertal development.
Objective: To report the pubertal development of a female with CDG Ic (ALG6D).
Design/Methods: The medical records and laboratory data for a female with CDG Ic were reviewed. The medical literature was reviewed for the description of pubertal development in other forms of CDG.
Results: An 18y11m Caucasian female with CDG Ic was referred for evaluation of severe premenstrual symptoms. Her pubertal development was delayed with the onset of breasts at about 13 years. Menarche occurred at about 15 years. Menstrual periods last 5-6 days without dysmenorrhea or menorrhagia. Premenstrual symptoms include fatigue, emotional lability, and aggression, which are atypical for her at other times of the month. There was no hirsutism or menstrual irregularity. On examination Breasts were Tanner stage V. Axillary hair was present Pubic hair was Tanner stage V. There was no evidence of clitoromegaly or hirsutism. Laboratory evaluation was normal for the follicular phase of the menstrual cycle: Androstenedione 1.8 ng/mL (0.5-4.3). DHEA-S 81 mcg/dL (35 to 430). Estradiol was 26 pcg/mL (11 to 165 follicular). LH 2.5 IU/L (1.9 to 12.5) FSH 4.1 IU/L (2.5 to 10.2) 17-hydroxyprogesterone 39 ng/mL (20-170). Progesterone 0.8 ng/mL (0.15 to 1.4). Testosterone 36 ng/dL (14 to 75).
A transabdominal pelvic ultrasound showed a normal uterus with a 5 mm endometrial stripe. The ovaries were normal in size with a dominant follicle in the right ovary and no evidence of polycystic ovaries.
Conclusions: A case of normal pubertal development in CDG Ic is presented. The understanding of a defect in glycosylation of gonadotropins and their receptors may help delineate the importance of different glycosylation patterns in normal pubertal development and fertility.

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Klinefelter syndrome – puberty: experience of a paediatric hospital

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BACKGROUND: Boys with Klinefelter Syndrome (KS), begin puberty at the expected age but soon, testicular growth is stopped, simultaneously with the development of hipergonadotrophism.
OBJECTIVE: To review the current knowledge about KS during and after puberty and examine a paediatric population with KS in terms of: age, weight and height at onset of puberty, clinical and analytical evolution of pubertal development, need for testosterone therapy and learning and behaviour difficulties.
MATERIAL AND METHODS: Population: 19boys with KS and more than 9 years of age, followed in a Paediatric Endocrinology Unity. Statistical analysis used descriptive methods and the results were presented in absolute and relative frequency, mean, standard deviation, maximum and minimum.
RESULTS: 19 boys aged more than 9 years old. Karyotype: 15 (79%) were 47, XXY, 2 (11%) were mosaics and 2 (11%) had other variants. Four boys had not yet started puberty, 2 failed the onset of puberty and in 13 puberty began spontaneously. Spontaneous puberty began at an average age of 12.80 ± 1.32 years. The mean SDS for weight was 0.05 ± 1.31 and for height - 0.45 ± 1.26.
10 stopped the pubertal development and the rise of gonadotrophins occurred at 13.47 ± 1.97 years, with FSH: 31.29 ± 7.71 mUI/ml, LH: 8.63 ± 3.40 mUI/ml and Total Testosterone: 184.98 ± 136.19 ng/dl. The testicular volume was

4.73 ± 1.74 mL.
Nine patients started testosterone therapy after the rise of gonadotrophins. In the last visit, the mean age was 16.60 ± 1.15 years. Reached the target height 3 boys, and only 2 were above. Of the 19 boys examined in the last appointment, 15 (79%) had learning difficulties and 10 (53%) behaviour problems. Looking at the pubertal stage and testicular volume before and after the therapy.

Pubertal stage and testicular volume before and after therapy				
	Mean	SD	Maximun	Minimun
Testicular volume (before therapy)	5,13	1,46	8	4
Testicular volume (last appointment)	6,75	1,04	8	6
Pubertal stage (before therapy)	3	0,7	4	2
Pubertal Stage (last appointment)	4	0,6	5	3

CONCLUSION: Most boys with KS started puberty at the expected age, but pubertal delay also occurred. This syndrome should be considered in cases of delay or interruption of pubertal development, associated to learning and behaviour difficulties.
Supplementation with testosterone can enhance the development of secondary sexual characteristics and testicular volume, but the results fell short of normal.

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Gonadal dysfunction in PHP Ia

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Pseudohypoparathyroidism Ia (PHP Ia) is caused by loss-of-function mutations in *GNAS,* an imprinted gene coding Gs{alpha}, the alpha-subunit of G stimulatory protein coupled to GPCRs. The expression of *GNAS* is monoallelic and predominantly maternal in the pituitary, ovaries and testes. Many developmental and functional pathways in ovaries and testes are regulated by GPCRs, including LHR, FSHR, LGR8, whose signalling is likely to be affected by Gs{alpha} haploinsufficiency.
We reviewed the medical records of 25 PHP Ia patients aged 19.3 years [1-72] (12 F, 13 M) carrying a loss-of-function *GNAS* mutation. 7/13 boys had 1 (2) or 2 (5) undescended testes, 2 had micropenis, none had ambiguous genitalia. Girls had age of menarche 14 +/-1.7 years and 5/9 had oligo-amenorrhea. None of the 5 adult men had children. 2/5 adult women gave birth to 1 and 3 children and 2 others had 1 and 3 miscarriages. Neither pelvic ultrasound nor spermogram were performed. FSH and LH levels were 3 fold about normal in 6/7 F, 2/6 M and 1/7 F, 2/6 M respectively. Sex steroids levels were within normal limits.
These retrospective data indicate a gonadotropic and/or gonadal dysfunction in many PHP Ia patients suggesting the impact of Gs{alpha} haploinsufficiency on signalling through LHR, FSHR and LGR8 or other GPCRs. Resistance to gonadotrophins, especially FSH, is present in most of the girls and is characterized by mild clinical gonadal insufficiency. In boys, the high prevalence of cryptorchidism without sexual differentiation disorders suggests that Gs{alpha} haploinsufficiency does not impair testosterone secretion by Leydig cells in early pregnancy, but alters testes descent in late pregnancy possibly through mild Leydig deficiency or dysfunction of LGR8 in gubernaculum testis. The gonadotropic and gonadal functions should be further scrutinized in PHP Ia in the first 6 months of life and at puberty in order to characterize the mechanisms.

PO2-200 Gonads and Puberty II

Combined central precocious puberty and primary gonadal failure after treatment of childhood malignancy: a report of 2 boys

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Background: Both central precocious puberty (CPP) and primary gonadal failure (PGF) are known sequelae of childhood cancer treatment. However, the coexistence of these disorders in the same patient following cancer therapy has never been described.
Objective: To report 2 boys with CPP and PGF following treatment for childhood malignancies.
Methods: Medical records of the 2 identified patients were reviewed.
Results: Case 1 is an African-American male who was diagnosed with pilocytic astrocytoma at age 5. He received 8 cycles of chemotherapy. At 8.2 years, he presented with a one year history of pubic hair and body odor. He had Tanner IV pubic hair and 2 cc testes. Adrenal androgens were normal and testosterone was 48 ng/dl. Bone age x-ray was 10 years. He presented again at 10.25 years with an unchanged physical exam and a growth velocity of 7.4 cm/yr. However, bone age xray was 16 years. Laboratory studies revealed: testosterone - 251 ng/dl, FSH- 24.03 mIU/ml, LH - 6.10 mIU/ml, Inhibin B - < 10 pg/ml and IGF-1 - 86 ng/ml. Peak FSH and LH post Leuprolide stimulation were 59.2 and 73.2 mIU/ml respectively. Growth hormone stimulation testing confirmed growth hormone deficiency. **Case 2** is a Hispanic male who was diagnosed with ALL at age 3. After multiple cycles of chemotherapy, he relapsed and received testicular and total body irradiation as well as stem cell transplantation. At 7.3 years old, he presented with 4 cc testes and Tanner I pubic hair. Laboratory studies revealed a testosterone of 273 ng/ml, FSH of 20.3 and LH of 7.5 mIU/ml. Tumor markers, adrenal androgens, and IGF-1 were normal. He received 3 doses of a GnRH analog and was lost to f/u. He presented again at 9 years and was found to have 2 cc testes and Tanner II pubic hair. Bone age was 11 years and growth velocity was 3.8 cm /yr. Testosterone was 172 ng/ml. FSH- 254 mIU/ml and LH-72 mIU/ml.
Conclusion: Despite a small testicular volume and elevated gonadotropins indicating primary hypogonadism, both patients had evidence of precocious puberty. In case 1, this was associated with an exaggerated tempo, masking the presence of underlying GHD. These cases underscore the potential for unusual and atypical combinations of endocrinopathies in survivors of childhood cancer, and the need for careful and continual surveillance in this patient population.

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The U.S. multicenter trial of monthly depot leuprolide for central precocious puberty (CPP): results during a 4 year treatment period

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Introduction: Leuprolide acetate for monthly depot suspension (Lupron Depot, LD) was approved by the FDA for treatment of CPP based in part upon results of a U.S. multicenter trial initiated in the 1990s but never reported. Here we summarize study results from the treatment period.
Methods: Girls (F) with Tanner breast stage ≥2 reported before age 8 and boys (M) with genitalia ≥2 before age 9 were screened for CPP and enrolled for GnRH-stimulated LH >10 IU/L and bone age (BA) advance >1 year (y). LD was dosed at 300 μg/kg every month (mo; 7.5, 11.25 or 15 mg). GnRH stimulation tests were repeated at 0, 1, 3, 6, 9 and 12 mo and every 6 mo thereafter. LD dose was increased for peak LH >1.75 IU/L, detectable sex steroid levels, menses or advance ≥2 stages. BA was obtained at 0, 6, and 12 mo then annually. Adverse events (AE) were assessed at every visit and safety labs annually. Treatment discontinuation was at appropriate age for puberty per investigator.
Results: 55 naïve patients (49 F, 6 M) were treated for 3.9±2.0 y (mean±SD). Mean age at start of treatment was 6.8±1.9 y (F) and 7.5±1.9 y (M); mean BA was 10.3±2.1 y. At baseline, 78% of patients were Tanner stage ≥3. LD therapy resulted in lasting suppression of pubertal stage advance, with 82% (40/49) ≤ initial Tanner stage at 1 mo and 78% (42/54) at final visit. Mean peak LH, ΔBA/ΔCA and growth velocity declined during year 1 and then stabilized.

Treatment Data (mean±SD)							
Month	0	1	6	12	24	36	48
N	55	55	53	54	46	36	20
Peak LH (IU/L) N>1.75	35.0 ±21.355	0.8±0.6* 2	0.8±0.8* 5	0.6±0.5* 3	0.4±0.3* 0	0.4±0.2* 0	0.4±0.2* 0
Basal LH (IU/L)	2.0±2.0	0.5±0.3*	0.3±0.2*	0.3±0.2*	0.3±0.2*	0.3±0.2*	0.2±0.2*
ΔBA/ ΔCA	na	na	1.3±0.8*	0.8±0.5*	0.6±0.3*	0.6±0.2*	0.6±0.2*
Growth Velocity (cm/y)	10.5±3.5	11.0±7.4	6.7±2.5*	5.4±2.3*	4.6±2.4*	4.5±1.9*	4.3±1.9*

*P<.05 vs baseline using paired t-tests

Dose was increased in 7 patients in the first 6 mo, and all suppressed to peak LH <1.75 IU/L. Rash, emotional lability, acne, injection site pain and vasodilatation were the most frequently (>10%) occurring AEs. Mean age at discontinuation (F) was 11.1±1.1 y.

Conclusion: Monthly LD rapidly and consistently suppressed clinical and hormonal parameters of puberty to prepubertal levels and maintained suppression during treatment.

PO2-202 Gonads and Puberty II

Transdermal estrogen patches are easy to dose and administer for pubertal induction

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Background: Transdermal matrix patches of 17β-estradiol is used off-label for induction of puberty in girls. The matrix formulation of Evorel® 25 µg/24 hours Janssen-Cilag) makes it possible to cut the patch into suitable size and thereby individualise the dose and to mimic the spontaneous levels as well as the diurnal pattern of serum 17β-estradiol in early puberty (JCEM 2001: 86:3039). The aim of the study was to study how the recommended starting dose (0.08-0.12 ug/kg), worked in out patients on serum 17β-estradiol levels.

Methods: The patch is placed on the skin (glutea, superior lateral) in the evening at bedtime and removed the following morning when the girl has wakened. At the blood sampling, the patch was removed after blood sampling. All samples used in this study are serum samples submitted for 17β-estradiol analysis at the Tillväxtlab, The Queen Silvia Children's Hospital, Göteborg, Sweden as part of controll of the start of pubertal induction therapy. After 17β-estradiol determinations, identification markings were removed from all samples.

Results: The study population consist of 30 girls dosed with 0.05-0.14 ug/kg. 20 girls dosed with the recommended starting dose 0.08-0.12 ug/kg, 3 had to high serum levels of 17β-estradiol levels (above the early pubertal range) and 2 girls to low levels (in the prepubertal range). Of the 8 girls who were started with a lower dose 0.05-0.07 ug/kg one girl were in the prepubertal range of 17β-estradio. 2 of 3 girls who started with a higher dose 0.13-0.14 ug/kg had 17β-estradiol higher than early pubertal range. For all girls (n=30) a linear correlation was found between the dose and morning serum 17β-estradiol (R=0.38, P=0.04).

Conclusion: The starting dose recommendation will be changed to 0.06-0.10 ug/kg to reduce the risk to have to high 17β-estradiol levels.

PO2-203 Gonads and Puberty II

Frequency of different types of pathologic delayed puberty and height alteration after treatment

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Introduction: Delayed puberty (DP) is defined as lack of secondary sexual characteristics at the age that is >2.5 SD of the mean age of pubertal onset. The aim was determining the frequency of different pathologic types of DP and their height changes before and after treatment. **Methods:** The medical records of 72 patients, (genetically, 26 males, and 46 females) were reviewed. Height standard deviation score (HSDS) was calculated according to gender and age by using CDC2000 tables. Paired T test was used by SPSS version 13. Duration of follow up was 1-17 yr (mean ± SD) 6 ± 4 yr. Therapy with low dose sex hormones commenced at the mean age of 16 ± 2.7 yr in males and 15.8 ± 2 yr in females, in addition, growth hormone was administered for growth hormone deficiency (GHD) and hydrocortisone for 17-hydroxylase deficiency. Sex hormone therapy was discontinued periodically for hypogonadotropic hypogonadism (HypoH) to evaluate spontaneous pubertal progress during first years of treatment. **Results:** The frequency of different disorders and HSDS before and after treatment, are shown in the table 1.

Diagnosis	Type	N (%)	HSDS-Entry	HSDS-End	P-value
HyperH N=31	17-OHD	6 (19.4)	-0.70 ± 1.00	0.14 ± 0.88	0.06
	Klinefelter	2(6.5)	0.88 ± 1.03	0.84 ± 1.09	0.50
	MGD	2(6.5)			
	Ovariectomy	2(6.5)	-0.17 ± 0.55	0.24 ± 0.99	0.76
	Partial AIS	2(6.5)	1.29 ± 0.20	0.06 ± 0.05	0.09
	complete AIS	2(6.5)	-0.01 ± 0.86	0.71 ± 0.32	0.54
	ovotesticular DSD	1(3.2)			
	XX,PGD	14(45.2)	-1.68 ± 1.57	-0.83 ± 1.52	<0.001
HypoH N=41	Brain tumor	7(17.1)	-2.86 ± 1.72	-0.96 ± 1.03	0.09
	GHD	5(12.2)	-4.28 ± 1.63	-1.09 ± 1.06	0.01
	Isolated, idiopathic	22(53.7)	-1.10 ± 1.10	-0.50 ± 1.10	0.002
	Kallmann syndrome	2(4.9)	-2.18 ± 1.34	-1.43 ± 0.28	0.50
	PHP	5(12.2)	-4.45 ± 1.64	-1.34 ± 2.53	<0.001

HyperH, hypergonadotropic hypogonadism; HypoH, hypogonadotropic hypogonadism; 17-OHD, 17 hydroxylase deficiency; MGD, mixed gonadal dysgenesis; AIS, androgen insensitivity syndrome; DSD, disorder of sex development; PGD, pure gonadal dysgenesis; PHP, panhypopituitarism

Conclusion: Height SDS significantly increased in patients with GHD, Panhypopituitarism, pure gonadal dysgenesis and isolated idiopathic hypogonadotric hypogonadism.

PO2-204 Gonads and Puberty II

Contrasting hormonal phenotypes of Leydig cell tumors in a 9-years-old boy and in a 12-years-old girl

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BACKGROUND: Leydig cell tumors are rare sex cord-stromal gonadal tumors, comprising 1-3% of all testicular neoplasms and <1% of all ovarian tumors in adults and even less in the pediatric age group. These tumors can be associated with somatic mutations activating the LH-hCG receptor or the alpha subunit of G proteins. We report two cases of Leydig cell tumors in children, presenting with contrasting hormonal phenotypes. **CASES:** Patient 1 is a boy who presented at 9 years of age for precocious puberty: severe acne, pubic hair, increased growth velocity, deepening of the voice and muscle development. Physical examination revealed a boy with increased muscle mass, severe acne with pubertal stage P4G4 but discordant testes volume (3 ml and 6 ml on the L and R, respectively) and painful right gynecomastia (1.5x1.5 cm). Patient 2 is a girl who presented at 12 years-of age with a 6-month history of virilisation (mild acne, increased hair growth and deepening of the voice). On physical examination, she had mild acne, moderate hirsutism and increased muscle mass. Tanner stage was P5B2 and there was clitoromegaly. Endocrine investigations revealed:

	Patient 1 (9-y-old boy)	Patient 2 (12-y-old girl)
Testosterone	9.3 nmol/L	30.6 nmol/L
DHEAS	2.9 µmol/L	2.1 µmol/L
Androstenedione	12.3 nmol/L	11.1 nmol/L
LH	<0.1 UI/L	5.1 UI/L
FSH	< 0.3 UI/L	4.8 UI/L
Estradiol	218 pmol/L	117 pmol/L
Ultrasound	Tumor within right testis	Tumor within left ovary

The tumors were resected, pathological analysis showed pure Leydig cell tumors and hormone values normalized quickly after surgery. Sequencing of LHCGR and GNAS in DNA extracted from tumor tissue was normal.
DISCUSSION: More efficient aromatization of testosterone to estradiol in patient 1 than in patient 2 may account for the complete suppression of plasma gonadotrophins in the former and not in the latter. The literature suggests that complete suppression of LH/FSH in patients with germ cell tumors requires an increase in both testosterone and estradiol. The absence of somatic mutations in LHCGR and in GNAS indicates molecular heterogeneity among Leydig cell tumors in children.

PO2-205 Gonads and Puberty II

Anti-Müllerian hormone as a marker of ovarian function in girls who underwent allogeneic heamatopoietic stem cell transplantation

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Conditioning regimens in HSCT are considered as a high risk factor for developing of ovarian failure.
The aim of this study was to evaluate the long-term effects of different conditioning regimens applied in HSCT on ovarian function using anti-Mullerian hormone (AMH) as ovarian reserve marker.
Patients and methods: LH, FSH, estradiol and AMH were measured in 25 girls (median age 16,5 y (range 14-25 y), who received allo HSCT. All patients were divided according to receiving either nonmyeloablative (group 1) or myeloablative (group 2) conditioning. Group 1 included 12 girls with severe aplastic anemia who received cyclophosphamide 100 mg/kg, group 2 consisted of 13 girls with acute myeloid leukemia who got busulfan 16 mg/kg or melphalan 180 mg/m². None of patients received total body irradiation.
Results: Patients of group 1 had normal ovarian function (mean levels of LH 5,1 IU/l, FSH 4,8 IU/l, AMH 2,7 ng/ml). All girls in group 2 who received busulfan revealed ovarian failure (LH 35 UI/l, FSH 70UI/l, estradiol 50 pmol/l, AMH 0,03 ng/ml), whereas all girls who received melphalan demonstrated normal levels of LH, FSH and estradiol (LH 6 IU/l, FSH 5 IU/l, estradiol 100 pmol/l) and had regular menstrual cycle, but serum AMH levels were significantly decreased (0,6 ng/ml) in this subgroup.
Conclusions: The ovarian damage after HSCT depends on the type of conditioning regimen. Myeloablative conditioning regimens produce gonadal damage in vast majority of girls - recipients of HSCT.
The detecting of AMH level in patients after HSCT is a usefull diagnostic tool, as it helps to reveal the ovarian impairment even in girls with regular menstrual cycle and normal gonadotropins levels.

PO2-206 Gonads and Puberty II

Functional ovarian hyperandrogenism in 15 elite adolescent swimmers

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Growth and pubertal retardation in children, as well as menstrual disorders in adolescents, have been well documented in sports requiring very low body weight (distance running, gymnastics, dance). However, little is known about menstrual disorders in female athletes in sports in which control of body weight is not necessary, particularly swimming.
We managed 15 elite peripubertal swimmers between 12 and 18 years old with a training volume of about 15 hours per week. All were girls with a Tanner pubertal stage of 4-5. Menarche appeared at a normal mean age of 12.7 ± 1 years vs. N: 12.5 ± 1.1 years. Oligomenorrhea was noted in 5 girls (33%), whereas primary amenorrhea was noted in 2 of the 9 girls older than 15 years (22%). All had a normal body mass index (BMI) with a mean of 20 ± 1.7 kg/m² without restrictive daily caloric intake or fat restriction. Clinical hyperandrogenism i.e., acne and/or hirsutism, was found in 23% of these girls. Their hormonal pattern revealed a clear hyperandrogenism with a high mean basal plasma testosterone (T) level of 0.6 ± 0.04 ng/ml (N < 0.45 ng/ml) and a D4androstenedione (D4) level of 2 ± 0.7 ng/ml (N < 2 ng/ml), whereas the mean basal plasma dehydroepiandrosterone sulfate (SDHEA) level was normal: 1.4 ± 0.6 mg/ml(N < 1.5 mg/ml). In addition, the mean basal LH/FSH ratio was high: LH/FSH=2 (N<1). Pelvic ultrasonographies are in progress.
In conclusion, we report a high prevalence of functional ovarian hyperandrogenism (FOH) and menstrual disorders in elite adolescent swimmers. This FOH may be due to a PCO-like syndrome, which has to be confirmed by ultrasonography. In addition, because of the severe metabolic and cardiovascular consequences of hyperandrogenism, we propose the long-term follow-up of girls practicing non-weight bearing exercise like swimming at an intensive level.

PO2-207 Gonads and Puberty II

Di-(2-ethylhexyl) phthalate and bisphenol-A levels in Korean girls with idiopathic precocious puberty

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Backgound: Exposure to di-(2-ethylhexyl) phthalate (DEHP), a component of polyvinylchloride (PVC) used in plastic toys or food packaging material, has been implicated in premature thelarche. Exposure to bisphenol-A (BPA) used for food packaging materials or rubber products may also affect sexual maturation by weak estrogenic activity. Studies regarding the effect of DEHP or BPA exposure on the precocious puberty are limited.
Objective: This study aimed to assess the levels of DEHP, monoethylhexyl phthalate (MEHP, one of the major metabolite of DEHP), and BPA in Korean girls with idiopathic central precocious puberty (ICPP) and premature thelarche (PT).
Method: Anthropometry, bone age x-ray and GnRH-stimulation test were conducted in 19 girls with ICPP and 12 girls with PT. Urinary DEHP, MEHP and BPA levels were also analyzed by gas chromatography/mass spectrometry method.
Result: The basal plasma estradiol level (27.0±18.7 (ICPP) vs. <10 pg/ml (PT), p < 0.001) and the peak LH response to LHRH stimulation (19.0±15.6 (ICPP) vs. 3.5±0.9 mIU/ml (PT), p<0.001) were significantly higher in girls with ICPP than PT. Urinary BPA levels did not show a significant difference between ICPP and PT groups (0.8±0.8 vs. 0.9±0.9 ng/ml). Urinary MEHP levels were not significantly different between the two groups (6.1±5.0 vs. 6.4±4.5 ng/ml), whereas urinary DEHP levels were significantly higher in ICPP than in PT groups (29.2±28.2. vs. 10.9±10.0, *P*=0.05). DEHP level was correlated with BPA (r=0.39, P<0.05) but not with serum estradiol or stimulated LH levels. MEHP level was correlated with BMI (r=0.63, P=0.005) and BPA (r=0.52, p<0.05).

	Precocious Puberty(n=19)	Premature Thelarche(n=12)	p-value
Chronological age (yr)	8.6±0.9	8.4±0.5	0.4587
Bone age (yr)	10.1 ±1.4	9.9±1.0	0.7676
Body mass index (kg/m2)	17.3±1.9	18.1±1.9	0.1335
Estradiol (pg/ml)	27.0±18.7	<10.0	<.0001
Peak LH (mIU/ml)	19.0±15.6	3.5±0.9	<.0001
Peak FSH (mIU/ml)	11.7±3.6	13.7±3.1	0.1558
BPA (ng/ml)	0.8±0.8	0.9±0.9	0.7831
MEHP (ng/ml)	6.1±5.0	6.4±4.5	0.7925
DEHP (ng/ml)	29.2±28.2	10.9±10.0	0.0582

Conclusion; DEHP, a ubiquitous endocrine disrupting chemical may be associated with the development of ICPP.

PO2-208 Growth Plate

Temporal and spatial expression of a growth-regulated network of imprinted genes in growth plate cartilage

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Genomic imprinting is an epigenetic phenomenon restricting expression of a subset of mammalian genes to one parental allele. A network of imprinted genes has recently been identified based on coordinated decline in expression in several tissues during postnatal growth. This finding, taken together with previous findings implicating these genes in regulation of somatic growth, suggests that the network contribute to the fundamental biological mechanism that causes the coordinated growth deceleration of multiple tissues that occurs during development. However, little is known about their role in longitudinal bone growth. In order to start to understand this possible role, we characterized changes in expression of the network during spatially-associated differentiation and temporally-associated senescence. Resting, proliferative and hypertrophic zone (RZ, PZ, and HZ, respectively) were microdissected from 1-week-old rats (n = 5) and PZ from 1, 3, and 9-week-old rats (n = 5 for all ages). Expression of Mest, Dlk1, Ndn, Igf2, Peg3, Plagl1, Slc38a4, Grb10, Cdkn1c, and Gtl2 were quantified by real-time PCR and expression levels were calculated relative to 18S rRNA. Similar to previous findings of developmental decline of expression in kidney, lung and liver, Mest, Dlk1, Igf2, and Gtl2 decreased with age in PZ ($P < 0.05$). In contrast, Cdkn1c, Grb10, and Slc38a4 increased with age ($P < 0.001$), whereas Peg3, Ndn, and Plagl1 did not change with age. During spatially-associated differentiation, Mest, Dlk1, Igf2, Peg3, Grb10 and Gtl2 decreased ($P < 0.05$) from RZ to HZ. In contrast, Slc38a4 increased from RZ to HZ ($P = 0.004$), whereas Ndn, Plagl1, and Cdkn1c did not change significantly with zone. Our findings suggest that the identified network of imprinted genes is only partly conserved in the growth plate and that the genes that show the expected decline with age tend to be higher expressed in RZ and PZ than in HZ. This finding taken together with previous functional studies suggest that these genes are involved in regulation of chondrocyte proliferation. The developmental decline in expression of these genes may thus contribute to the decline in chondrocyte proliferation that causes longitudinal bone growth to decelerate during development. Moreover, our findings do not support an imprinting-specific pattern of expression during temporally-associated senescence or spatially-associated differentiation.

PO2-209 Growth Plate

Expression of microRNAs in growth plate and metaphyseal bone during postnatal growth

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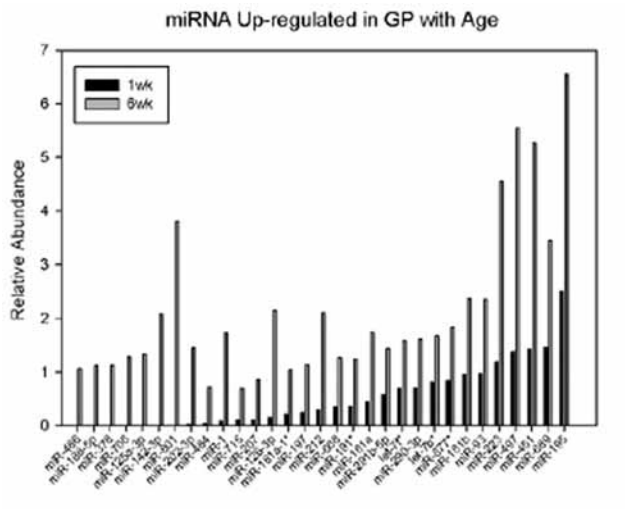
Longitudinal growth of bones occurs at the growth plate through endochondral ossification. In the growth plate, coordination of differentiation and proliferation is required for normal growth. Recently, cartilage specific knock-out of Dicer demonstrated that microRNAs (miRNAs), which are small non-coding RNAs that suppress gene expression at post-transcriptional level, are critical for normal growth plate chondrogenesis. In order to understand the role of miRNAs in longitudinal bone growth, we characterized miRNA expression in growth plate (GP; n = 4 both age groups) cartilage and metaphyseal bone (MB; n = 3) collected from proximal tibiae of 1- and 6-week-old castrated mice using microdissection and miRNA microarray. We found that a large group of miRNAs, including the cartilage specific miR-140 and miR-140*, the let-7 family members, let-7a, -b, -c, -d, and -f, miR-720, miR-199b, and -26a were highly expressed in growth plate of 1- and 6-weeks-old mice. In MB, the top ten expressed miRNAs include Let-7 family, miR-21, -199b, -451, -16, and -1224 throughout life. All of the 30 highest expressed miRNA in GP or MB at 1wk were also expressed at high levels later at 6-week-old. With age, as the growth velocity decreases, 32 miRNAs were up-regulated (figure 1), 12 were significantly down-regulated more than 2-fold in growth plate.



Target analysis of up-regulated miRNAs revealed that several of the up-regulated miRNAs specifically targets mRNAs within the IGF- FGF- and Wnt/β-catenin signaling families. In conclusion, we identified a group of miRNAs that are ubiquitously expressed in growth plate cartilage and metaphyseal bone of 1- and 6-week old mice, suggesting that they are needed in these tissues throughout development. In addition, we found that the 32 miRNAs that are significantly up-regulated with age includes miRNAs that target members of the IGF- FGF- and Wnt/β-catenin signaling families. These findings may thus help explain the fundamental biological mechanism that causes longitudinal bone growth to decline and eventually cease.

PO2-210 Growth Plate

Serum concentrations of amino-terminal propeptide of C-type natriuretic peptide in children with idiopathic short stature do not correlate with age or growth parameters

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C-type natriuretic peptide (CNP), a member of the natriuretic peptide family, is known as a small paracrine growth factor playing a role in regulating linear growth. CNP is expressed in the growth plate among other tissues. Synthesized as a precursor protein CNP undergoes a cleavage process. A stable and hence for measurement easily accessible cleavage product is the amino-terminal propeptide of CNP (NTproCNP) presenting in equimolar amounts with CNP. In recent literature it was speculated that NTproCNP could be used as a marker for growth plate activity. Therefore, we measured NTproCNP levels by a commercially available enzyme immunoassay in blood samples from children with idiopathic short stature (ISS) including patients with constitutional delay of growth and puberty and familial short stature. We compared two subgroups ("poor" [PE] and "good" [GE] eaters) according to the Child Eating Behavior Questionnaire (table). NTproCNP levels were 4.99 ±3.44 pmol/L (mean ±SD) in the total sample, 5.42 ±3.75 and 3.65 ±1.80 pmol/L in the PE and GE subgroup respectively with no significant difference between the two groups despite significantly differently height velocities (p=0.01). NTproCNP concentrations did not correlate with age, height SD score (SDS), height velocity or BMI SDS. Multiple linear regression analysis revealed no significant association of age and height velocity with NTproCNP levels. With PE and GE as categorical variables there was no association of age and height velocity with NTproCNP levels either.

Table. Clinical characteristics of the population. Means ±SD.

	Total sample (n=37)	Poor eaters (n=28)	Good eaters (n=9)
Girls: boys	11:26	9:19	2:7
Age (yr)	10.9 ±2.9	10.7 ±3.1	11.5 ±2.2
Height SDS	-2.34 ±0.59	-2.25 ±0.60	-2.60 ±0.51
Height velocity (cm/yr)	5.1 ±1.4	5.4 ±1.2	4.1 ±1.4
BMI SDS	-0.69 ±0.83	-0.78 ±0.86	-0.41 ±0.70

In summary, we did not find significant associations of NTproCNP levels with height velocity and age. Our data do not corroborate the hypothesis that NTproCNP reflects growth plate activity.

PO2-211 Growth Plate

Pituitary function after traumatic brain injury in children: a prospective study

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Introduction and aim:
In recent years, traumatic brain injury (TBI) has been identified as a significant cause of pituitary dysfunction in adulthood. Pediatric prospective studies are rare. The aim of this present study was to evaluate the frequency of hypopituitarism after mild, moderate and severe TBI in hospitalized children, and determining factors of this deficiency.
Design:
A prospective study was conducted in children from 2 to 16 years, hospitalized for 24 hours minimum after TBI. Clinical growth parameters, pubertal development, basal pituitary hormone concentrations and a dynamic test were performed at 6 and 12 months after TBI (insulin tolerance test or glucagon test if contra indicates for assessment of somatotropic and corticotropic axes).
Results:
24 patients, sex ratio H/F 18/6, median age 7.4 years (range 2.4 to 16 years). Distribution plot showed 20/24 Mild, 1/24 moderate and 3/24 severe TBI, according to Glasgow Coma Scale. Clinical parameters showed few growth abnormalities: 4 children had a low height velocity (<-2 SD) 3 months after TBI, 2 of them had a partial growth hormone deficiency (pGHD) and 1 a complete GHD (cGHD). 12 Months after TBI, 1 children had a low height velocity with normal GH function. Children with GH deficiency had normal growth 12 months after TBI. Pituitary insufficiency (PI) were present in 13/24 and in 6/24 patients at 3 and 12 months after TBI, respectively. Three of them had two hormonal deficits at 3 months (cGHD and hypocortisolism), and one of them at 12 months. GHD were present in 100% of PI (7 partial and 6 complete at 3 months, and 3 partial and 5 complete at 12 months). There was no correlation between GH response and trauma severity.

Pituitary function according to Glasgow scale after TBI						
Glasgow scale	0-8		9-12		13-15	
Number of children	n=3		n=1		n=20	
Times after TBI (months)	3	12	3	12	3	12
No deficit	1	3	1	1	9	14
pGHD	2	0	0	0	5	1
cGHD	0	0	0	0	6	5
ACTH deficiency	1	0	0	0	1	1
Elevated TSH, normal FT4	0	0	0	0	2	0
Elevated Prl	0	1	0	0	2	0
Insipid diabetes	0	0	0	0	0	0
Cognitive deficiency	3	2	0	0	3	1

Conclusion:
TBI is associated with PI, with high frequency even if a majority of patients has mild TBI. Further studies should be conducted in children to confirm these data, and children growth should be carefully followed up after TBI.

PO2-212 Growth Plate

Growth deceleration in males treated with aromatase inhibitors for premature adrenarche & short stature

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Background
Aromatase inhibitors have been reported to improve predicted and final height in short males by preventing progression of bone age. All published reports to date show that such treatment does not adversely affect growth velocity. We report here 3 cases of boys who sustained a growth deceleration while being treated with an aromatase inhibitor. The effect was reversed with discontinuation of therapy.
Case 1. An 8.6 yo male with history of idiopathic premature adrenarche was treated with anastrazole 1mg daily for advanced bone age and poor height prediction. He grew at a stable growth rate of 4.2cm/year on average for approximately 3.5 years, when he experienced a growth deceleration to 0.2cm/year annualized. Anastrazole was discontinued at that time. 6 months later, his growth velocity had improved at 6.0cm / year. Bone age stabilized within 6 months after starting anastrazole, and did not progress further during therapy. He was in early central puberty when growth deceleration occurred.
Case 2. A 9.4 yo male with a history of idiopathic premature adrenarche was treated with letrozole 2.5mg daily for advanced bone age and poor height prediction. He experienced a growth deceleration during the second year of treatment. Growth rate was 6.8cm/year pre-treatment, 5.5cm/year during the 1st year of therapy, down to 1.6c/year during the second year, and up to 4.7cm/year six months after discontinuing therapy. Similar to the first case, bone age stabilized within 6 months of letrozole therapy, and did not progress further. He was not in central puberty when growth deceleration occurred.
Case 3. A 13.2 yo male with a history of attention deficit disorder (treated with Lithium and Metadate) was started on letrozole 2.5mg daily for short stature. His pre-treatment growth rate was 10.8cm/year, and decreased immediately to 4.4cm/year (annualized) the first six months after starting therapy, at which point letrozole was stopped. 3 months later, his growth velocity had improved to 8.8cm/year (annualized). He was in mid-puberty when letrozole therapy was started.
Conclusion
To our knowledge, this is the first report of growth deceleration in boys

treated with aromatase inhibitors. In all cases, growth velocity improved with discontinuation of treatment. Our experience calls for larger studies and careful monitoring of growth in these patients.

PO2-213 Growth Plate

Effect on final height of subcutaneous injection of a somatostatin analogue in Marfan boys and girls

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Objectives : To assess the growth-reductive effect and side effects of somatostatin analogue (SMS) treatment in Marfan subjects.
Study-design : We have studied the effect of SMS given as a subcutaneous injection on the growth of nine tall children (three boys, six girls) with Marfan syndrome and with a predictive final height above 185 cm in girls and 200 cm in boys. SMS was given in doses of 30 mg twice a month until final height was reached. IGF1 level was measured every six months.
Results. Mean age at the start of the treatment was 10 years (range 9,5-11,5) in girls and 12,8 years (11,8-13,8) in boys. Growth velocity decreased from a median of 8.9 cm/year (range 6.1-11.9) to 3.2 cm/year (range 1.7-4.2) after one year of treatment. IGF1 level decrease was of 0.8 DS (0.2-1.7) Doses (45 mg) were increased to obtain low IGF1 levels in one girl and one boy. Mean duration of treatment was 27 months (22-32). Mean reduction in final height (compared with predicted adult height) was 4.5 cm (1.5-10,5 cm). In one boy, asymptomatic gall bladder microlithiasis was diagnosed.
Conclusions : These results suggest that SMS may have a role in the management of the growth of tall children with Marfan syndrome. The optimum mode of administration remains to be established.

PO2-214 Growth Plate

Effects of insulin resistance on longitudinal bone growth

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BACKGROUND
It is well known that overweight children are often taller than age-matched, normal-weight children. The mechanisms whereby obese children grow faster since early childhood are not known. Several explanations have been postulated, such as increased leptin concentrations, hyperprolactinemia, increased levels of IGF-1, and increased peripheral estrogen secretion. Overweight children are often hyperinsulinemic and insulin resistant: thus, we hypothesized that insulin resistance is causally related to the obesity-related tall stature and accelerated growth rate.
METHODS
To determine whether obesity and insulin resistance induce accelerated linear growth rate, 3- week-old mice were distributed in 4 groups: 1) CONTROL (standard chow diet, 4 % of energy as fat); 2) "HIGH-FAT" (diet containing 60 % of energy as fat); 3) "PIO", (standard chow plus 10 mg/kg wt of pioglitazone, an insulin sensitizer); 4) "HIGH-FAT+ PIO", (high-fat diet + 10 mg/kg wt of pioglitazone). Body length and tibiae's x-rays were obtained biweekly. After a 6-week period, a blood sample was collected for insulin determination.
RESULTS
At the end of the 6-week period, "HIGH-FAT" mice' body weight gain, body growth, tibial growth, and serum insulin were significantly greater than those of CONTROL mice (table).

	body weight gain (gm)	body linear growth (cm)	tibial growth (mm)	serum insulin (ng/dl)
CONTROL	12.9 ± 0.7	2.2 ± 0.1	4.0 ± 0.1	6.9 ± 0.1
HIGH-FAT	18.1 ± 0.9***	2.8 ± 0.1**	4.7 ± 0.1**	20 ± 0.5*
HIGH-FAT + PIO	19.4 ± 1.0***	2.4 ± 0.1#	4.2 ± 0.2#	5.9 ± 0.1#

* p<0.05 vs. control; ** p<0.01 vs. control; *** p<0.001 vs. control; # p<0.05 vs. high-fat

"HIGH-FAT+PIO" mice's were shorter, their tibial growth reduced, and their serum insulin lower than those of HIGH-FAT mice (table).
CONCLUSIONS
Our findings indicate that increased caloric intake accelerates body linear growth and longitudinal bone growth. The reversal of such effects secondary to the administration of an insulin sensitizer suggests that tall stature associated with obesity is due to insulin resistance. Future studies are needed to determine whether the insulin resistance-related growth acceleration is due to the direct effects of insulin on longitudinal bone growth.

PO2-215 Growth Plate

Genome wide screening and morphological analysis of the human growth plate during pubertal development

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Background: In humans, the mechanisms underlying the process of epiphyseal growth plate maturation and closure are largely unknown.
Aim: To gain insight into the morphological and molecular processes occurring during human growth plate maturation.
Material and Methods: We performed a longitudinal detailed histomorphometrical analysis on parafinn sections of two epiphyseal growth plate samples obtained from the same girl with one year interval; at age 12.5 yrs (Tanner pubertal stage B2, left proximal femur) and 13,5 yrs (Tanner B3, right proximal femur). To perform a cross-sectional study of gene expression patterns, tibial growth plate tissue samples were obtained from six female patients; two prepubertal (stage B1), 2 early pubertal (stage B2) and 2 late pubertal (stage B3,B4). High quality RNA was isolated from all samples. RNA was amplified, labelled and subjected to Affymetrix microarray analysis (HG-U133 Plus 2). A genewise linear model was fitted to the six arrays using the R package Limma to test differences between the three stages of puberty. Because of the limited power due to the small sample size, no adjustment was made for multiple testing, but attention was focused on the patterns in the uncorrected P values
Results: Longitudinal analysis of the two proximal femur growth plate samples obtained in the same pubertal girl showed changes of several morphological parameters. These included a clear decrease in overall width of the growth plate, an increased intercolumnar space and a decrease in height of each individual.
In the cross-sectional analysis, microarray results suggested 605 genes changing from prepuberty to early puberty, while 1275 genes changed from early to late puberty and as many as 2362 genes changed from prepuberty to late puberty. A pathway-based analysis with a global test for all genes revealed 11 pathways changing significant from early to late puberty. Affected pathways are associated with the extracellular matrix homeostasis, hormonal pathways and programmed cell death. These results are in agreement with the observed structural changes in morphology.
Conclusion: For the first time we show a developmental regulation of the gene expression pattern in the human epiphyseal growth plate. Most alterations

occurred during late puberty as the growth plate undergoes morphological changes suggesting that the affected pathways may be involved in the regulation of human epiphyseal growth plate maturation and fusion.

PO2-216 Growth Plate

The proteasome inhibitor, bortezomib induces growth retardation and delays tumor growth in young mice

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Background: The proteasome inhibitor, bortezomib (Velcade™, formerly known as PS-341), has recently been approved for the treatment of multiple myeloma in adults, and is under investigation in phase-II clinical trials in pediatric cancers. However, any secondary side effects on normal bystander tissues of previously administered life saving treatment are so far unknown.
Aim: To study if bortezomib treatment has any side effects on growth plate cartilage and longitudinal bone growth.
Methods: To address these questions, young growing male NMRI nu/nu mice (5-wk old) were used for establishment of neuroblastoma xenografts. When tumors had reached 0.1 cm^3, mice were treated with one cycle of a clinically relevant dose of bortezomib (1 mg/kg; 2 injections/wk; 2-wks treatment). Femur bone lengths were measured by X-ray analyses at start of treatment and at time of killing, 48 hrs after last injection. The effect of bortezomib was also studied in slices of human growth plate cartilage cultured for 24 hrs, where apoptosis was assessed by TUNEL-analyses.
Results: One 2-week cycle of bortezomib effectively impaired the ubiquitin/proteasome system (data not shown) and in parallel caused a significant growth retardation in treated mice. In bortezomib treated mice, femur lengths were 3.5±0.1mm while in controls 4.6±0.1mm; $p<0.001$).
In addition, we confirmed that human growth plate cartilage is also sensitive to bortezomib. After being cultured for 24 hrs with 1.0μM bortezomib, we found that chondrocyte apoptosis was significantly increased in the exposed human growth plate cartilage when compared to control (median 22.3% (interquartile range 11.5 to 38.6) vs. 5.8% (interquartile range 2.5 to 9.4), respectively; $p<0.001$).
We also confirmed that bortezomib reduced tumor growth in treated mice. The time for tumors to double in size from the start of treatment (day 0) was delayed in bortezomib-treated animals to 22.3 days (interquartile range 11.5 to 38.6) vs. 5.8 days in vehicle-treated animals, (interquartile range 2.5 to 9.4).
Conclusions: We conclude that bortezomib impairs longitudinal bone growth and induces chondrocyte apoptosis while as intended effectively reduces tumor growth in an experimental model of neuroblastoma, a malignant tumor mainly affecting young children. Our findings could have important implications for the use of proteasome inhibitors in the treatment of childhood cancers.

PO2-217 Growth Plate

Unexpected high frequency of bone dysplasia (BD) and low number of cases with severe primary IGF-I deficiency (SP-IGFD) in a single center large cohort of patients with growth retardation

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Background: Growth retardation in children remains often unexplained and characterized as "idiopathic" short stature or in more recent years in rare cases, referred as SP-IGFD.
Aims: The aims of our study was to characterize every patient referred to our specialized pediatric endocrinology unit for a growth disorder to specifically define the frequency of BD and of SP-IGFD.
Methods: During a four years period (2004-2007), using a standardized work-up, we classified the patients into groups corresponding to the well established categories of diagnosis. Patients were classified "normal" when having a normal growth velocity and height between –2SD and +2SD. Fifty-three percent of the 509 patients with idiopathic short stature (ISS, n=313) or short for gestational age (SGA, n=196) had so far bone x-ray survey (with expert reading) in order to search for BD. We also looked for patients with possible SP-IGFD to discuss the indication of recombinant human IGF-I, recently available in France.
Results: The cohort included 1570 patients (54% boys) with endocrine dysfunction (11.2%), of which 96% with growth hormone deficiency), normal growth (23.8%), transient slowing of growth rate (14.2%), SGA (13.9%), genetic syndrome (10%), chronic disease (4.7%), known BD (2%), ISS (20%) and 1 patient with SP-IGFD. Nearly 40% of the SGA (37.5%) and ISS (39.3%) had BD: 63.2% with a typical bone dysplasia (hypochondroplasia, dyschondrosteosis, epiphysal or metaphysal dysplasia) and 36.8% with mild skeletal anomalies. The incidence of these BD was significantly higher for SGA patients with at least one parent (or grand parent) with a final height < -2 SD as compared to other SGA patients (62.5%, versus 38.7% p=0.03). Only one patient has SP-IGFD. Nine ISS and 10 SGA patients (1.2% of the whole cohort) fulfilled the French requirements for recombinant IGF-I treatment : 11 normalized their IGF-I blood levels with better nutritional status or had a satisfactory growth with growth hormone treatment, 1 had a normal IGF-I generation test and the 7 left must still undergo an IGF-I generation test. **Conclusion:** Bone dysplasia can explain nearly 40% of the growth retardation of ISS or SGA patients, and more than 60% of SGA patients with one parent with height <-2 SD. It is advisable to systematically conduct a bone x-ray survey with a specialized reading in ISS and SGA patients with growth delay. SP-IGFD was a very rare cause of growth retardation in this cohort.
Supported by Ipsen-Pharma

PO2-218 Growth Plate

Growth hormone (GH) signaling in early life in rodents

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Background: GH concentrations are high at birth and decrease slowly but it is not clear when and where GH-signaling takes place or what the cellular consequence of GH signaling is. GH-dependent statural growth is thought not to occur until later and growth failure occurs at the earliest at 3-6 mo of age in severely GH deficient (GHD) babies. The activation of the JAK2-Stat5 signal transduction pathway by GH is necessary for the stimulation of growth and we recently showed direct action of GH on resting chondrocytes in the growth plate by visualizing Stat5 phosphorylation (pY-Stat5) after a single GH injection in post-pubertal GHD mice. We have now studied GH signaling in early postnatal life in vivo and in a GH-responsive pre-chondrocyte tumor cell line (ATDC5).
Methods/results: Immunochemistry (IHC) in 1 wk old murine tibial growth plates confirmed widespread staining for GHR/GHBP. GHD mice (1 wk old) were injected with mouse GH (1μg/g bodyweight) or vehicle and killed 25 min later. IHC showed pY-Stat5 in the immature chondrocytes at the periphery of the growth plate, including the Groove of Ranvier, in GH-treated mice of both sexes but not in controls. pY-Stat5 was also found in muscle, liver and bone marrow of GH-treated mice.
Immunofluorescence showed the presence of GHR in ATDC5 cells, and stimulation with hGH (200ng/ml), but not hIGF1, results in pY-Stat5, even in undifferentiated cells. To assess the effect of GH on these cells, proliferation was studied. Undifferentiated cells were incubated in serum free medium for 12 hr and then treated with hGH for 3 hr or 15 hr (5-200ng/ml), or with hIGF1 (100ng/ml) or vehicle for 15 hr. hIGF1 increased the proliferation index (32±1% vs 18±3%, $p<0.01$) but hGH had no effect.
To assess the effect of congenital absence of GH signaling, growth plates of 4-5 wk old knock-in mice that have partial (m569) or complete (m391) absence

of GH-induced Stat5 signaling were examined. Growth plate width was reduced in m391 and further reduced in GHR -/- mice but resting zone width was significantly increased in m391 mice. Proliferative zones were slightly reduced in m569 mice and further reduced in m391 mice. This suggests that besides decreasing IGF1 induced proliferation, STAT5 may affect transition of cells into the proliferative zone.
Conclusion: GH induces JAK-Stat5 signaling in very young mice in several tissues, and immature chondrocytes are a target for GH, maybe even before GH mediates a statural growth response.

PO2-219 Growth Plate

Difference between the postnatal growth of preterm infants born weighing less than 1500 grams and the population reference

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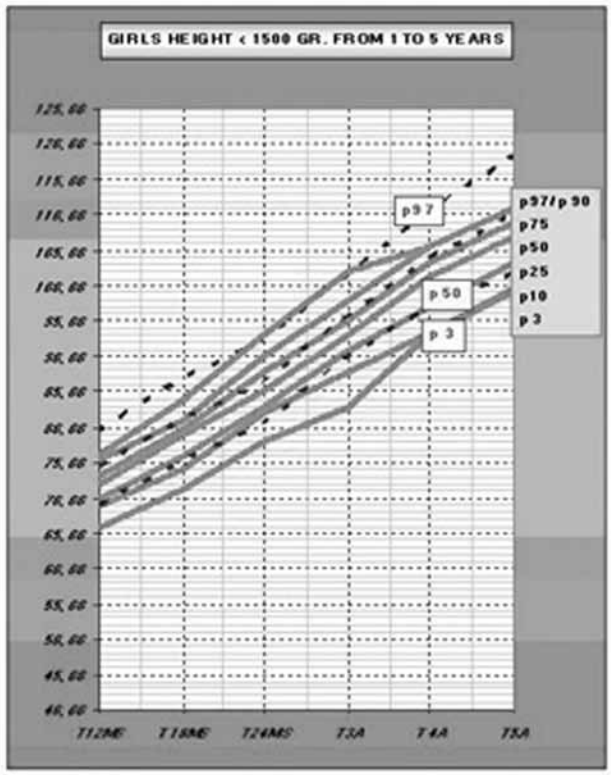
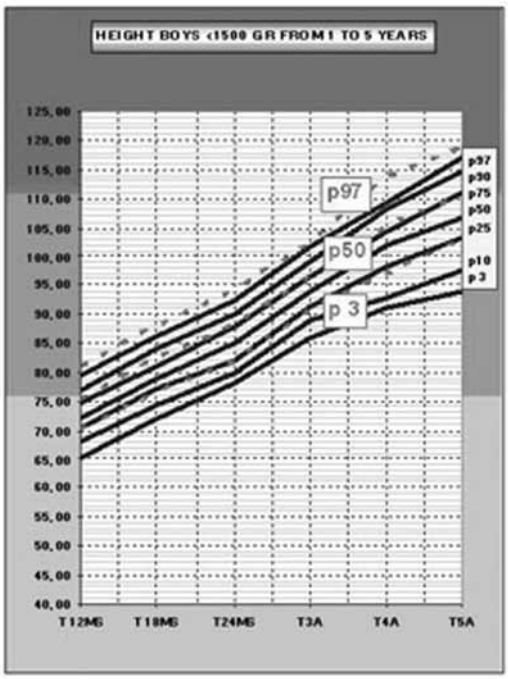
Introduction: between 0.5 and 1% of preterm have a weigh at born less than 1500 gr. These preterms required an increase in growth velocity during the first year to achieve normal growth.
Objective: Obtain growth percentiles of weight / height up to 5 years, in newborns birth weight <1500gr. Compare with the reference standards. Analyze how many children fall below the third percentile (p3) at 5 years and which are small for gestational age (SGA) or adequate for gestational age (AGA).
Material and Methods: Retrospective data from medical history in Spanish children born between 2001-2005. Comparison with Spanish population (Orbegozo 2004). SGA classified by Spanish Population (Delgado 1997).
Results: Followed 124 kids. Information: length / weight: 12 months: 121 cases (57 girls and 64 boys); 18 months: 109 (50 girls and 59 boys); 24 months: 113 (52 girls and 61 boys). Height / weight: 3 years old: 71 (27 girls and 54 boys); 4 years old: 28 (10 girls and 18 boys); 5 years old: 41 (17 girls and 24 boys). Difference between our own percentils and standard population. There are differences between preterm growth percentils and standard population both p3 as P97 and increased in both sexes at 3, 4 and 5 years. At 5 years old, there are 6 patients whose size is below -2 SDS: 1 child SGA and 5 AGA; 5 boys (20.83%) and 1 girl (5.88%).



Conclusion: 1.- There are more frequent males preterm <1500 gr., they grow less than females and their percentiles (P3, P50, P97) are always below the normal standards. 2.- Boys perform worse growth recovery, but it is not attributable to SGA. 3.- Children with adequate catch up growth are above the P25 of our population of preterm. 4.- We must take an interventionist attitude in those children who do not recover growth at 4 years, like children small gestational aged.



PO2-220 Growth Plate

Resveratrol modulates longitudinal bone growth of cultured fetal rat metatarsal bones in a biphasic manner

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High dose estrogen therapy has been used to promote growth plate fusion thereby limiting further growth in girls with predicted extreme tall stature. Conversely, in short boys treatment with an aromatase inhibitor was shown to suppress estrogen production and thereby growth plate fusion was delayed and adult height increased. Although being effective, both these approaches have several undesired side effects. Resveratrol (Res) is a phytoestrogen which has been reported, even at high concentrations, to be well tolerated in humans. We hypothesized that Res could be used to modulate longitudinal bone growth and tested this experimentally in fetal rat metatarsal bones that were cultured for 19 days while exposed to Res concentrations ranging from 30 nM to 50 µM. Over the 19-day culture period, bones exposed to a relatively low concentration of Res (300 nM) grow better than control bones (132±2.2% vs. 122±2.7% increase in bone length, respectively; $p<0.05$). However, when metatarsal bones were treated with relatively high concentrations of Res (10 or 50 µM), bone growth was suppressed. Over the 19-day culture period, bones exposed to Res at 10 µM grow 98.5±4.1% ($p<0.001$ vs. control) and at 50 µM growth was 25.5±1.5% ($p<0.001$ vs. control) while control bones grow (122±2.7%). To delineate the underlying mechanisms for this biphasic effect of Res on bone growth, chondrocyte proliferation (BrdU cell proliferation assays), apoptosis (TUNEL) and collagen type X production (immunohistochemistry) were studied. At high concentrations, Res induced massive apoptosis (17.6±2.9% apoptotic cells at 10 µM and 90.0±2.1% at 50 µM vs. only 1.5±0.2% in control

bones; p<0.001), an effect which was most obvious in resting and proliferative zone chondrocytes. The highest concentration of Res tested (50 µM) also suppressed chondrocyte proliferation (0.07±0.02% BrdU positive cells vs. 4.36±0.16% in control; p<0.001) and the length of the zone staining positive for collagen type X, a known differentiation marker (662±76 µm vs 1852±63 µm in control; p<0.001). In contrast, at lower concentrations (100 and 300 nM) Res had a tendency to increase type X collagen expression while cell proliferation was unaffected (data not shown). We conclude based on our in vitro data that Res has the capacity to modulate bone growth in a biphasic manner where growth is stimulated at low and inhibited at high concentrations.

PO2-221 Growth Plate

Prospective monocentric study in short stature children: prevalence in severe primary IGFD patients

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Recent study confirmed efficacy of rhIGF1 in children with severe IGF1 deficiency due to GH insensitivity but in our experience few patients seems to be available for the prescription.
Objectives: To find out the incidence of different etiologies causing short stature in our population and evaluate the incidence of severe primary IGFD patients, a monocentric prospective study was undertaken.
Methods: Hospital data based cohort, referred for evaluation of short stature to our paediatric endocrinology clinic over a period of one year was studied. Clinical, biochemical, endocrinological test results and radiological finding were analysed. Standards of height, weight and BMI were compared with standard chart valid for French patients. We select patients with height < -2SDS and idiopathic short stature and analysed IGF1 in these patients
Results: 131 children were referred to our paediatric endocrinology unit. 65 were explored because short stature below – 2 SDS and 66 because low height velocity. 27 patients (20.61%) had syndromic or non endocrinological causes, 29(22.13%) had GH deficiency, 25 (19.08%) had constitutional delay of growth and development, 18 (13.74%) were born small for gestational age and one patient (0.76%) had hypothyroism. The most common cause was the idiopathic short stature (25.95%).
Out of 65 patients with short stature < - 2 SDS: 7 (10.76%) had syndromic or non endocrinological causes, 16 (24.61%) had GH deficiency, 15 (23.07%) had constitutional delay of growth and development, 15(23.07%) were born small for gestational age, 1(1.53%) had hypothyroidism and 11(16.92%) had idiopathic short stature. Out of 12 patients with short stature < - 3 SDS: 1 had hypothyroidism, 2 had GH deficiency, 3 had constitutional delay of growth and development and 4 were born small for gestational age and 2 had non endocrinological causes.
The group of patients with height < - 2SDS and idiopathic short stature included 7 girls and 4 boys aged from 3.16 to 13.75 years. Their height was at – 2.33 +/- 0.15 and the BMI at -1.06 +/- 0.92. The target height was at 157.21+/- 7.22 in girls and 169.50+/- 3.46 in boys. IGF1 was normal for 2 patients, in low values for 3 patients and less than normal values for 6 patients. The prevalence of severe primary IGFD patients is 9.23% from patients referred for short stature below – 2SDS
Conclusion: From a monocentric prospective study in a paediatric endocrinology unit, only 4.58% of children may be treated with rhIGF1.

PO2-222 Growth Plate

Effects of tamoxifen on predicted adult height in pubertal, growth hormone deficient boys: a pilot study

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Background: Children with growth hormone deficiency (GHD) may not achieve their genetic potential for height. Delaying epiphyseal closure by decreasing estrogen production, as occurs with GnRH agonist or aromatase inhibitor therapy, has been utilized in patients who have sub-optimal predicted adult height (PAH). An alternate strategy to delay bone age (BA) maturation and increase PAH while allowing puberty to ensue might be to block estrogen effect by using tamoxifen, an estrogen receptor modulator.
Objective: To evaluate whether tamoxifen can delay BA maturation in pubertal boys with GHD, leading to an increase in PAH.
Methods: This was a randomized, prospective one-year study. Pubertal boys with GHD and a PAH >1 inch below target height were eligible. Patients were randomly assigned to tamoxifen, 20 mg PO qd or control. GH therapy was adjusted based upon standard clinical practice. Visits were every 3 months. BA radiographs and bone mineral densitometry (BMD) were conducted at baseline and one year. Testosterone, estradiol, FSH, LH, IGF-1, IGFBP-3, ALT and lipid panels were assessed at visits 1, 2 and 4. Radiographs were read by a pediatric endocrinologist blinded to patient treatment status. PAH was calculated using the Bayley-Pinneau tables.
Results: Five patients aged 13.9 ± .78 years were randomized to the treatment group and 4 patients aged 14.9 ± .94 years to the control group. Mean baseline height was 150 cm in the treatment group and 152 cm in controls (p=NS), with mean PAH of 165.8 ± 3.1 cm and 168.5 ± 2.5 cm, respectively. Mean baseline BA was 13.5 years in both groups. At one year, mean PAH had increased by 1.4 cm in tamoxifen treated subjects, and by 4.2 cm in controls (p=NS). Laboratory data did not suggest any adverse effects of tamoxifen on the hypothalamic-pituitary-gonadal axis. Mean BMD z-scores were similar and within normal ranges in both groups throughout the study.
Conclusion: Although well tolerated, tamoxifen treatment for one year did not increase mean PAH in pubertal boys with GHD. A larger sample size and longer duration of treatment would be needed to confirm lack of efficacy of this therapeutic approach.

PO2-223 Growth Plate

Influence of nutrition in the first 2 months of life in preterm infants weighing less than 1500 grams in postnatal growth

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Introduction: Nutritional guidelines for neonatal units have evolved with the use of artificial feeding formulas and / or enriched lactation in order to increase energy intake, without increasing the volume received. Is this indispensable to improve postnatal growth?
Objectives: Analyze postnatal growth in children whose birth weight <1500gr classified as SGA (small for gestational age) or AGA (appropriate age) in the first 5 years, and if it is related to caloric intake and weight increase achieved given in the first 2 months of life.
Material and methods: Retrospective study. Data taken at 7-15-days,1 and 2 months of calories and weight during hospitalization. Analysis of weight increase in these periods: (7-15days), (7d-1month), (7d-2m), (15d-1m), (15d-2m), (1m-2m). Spanish Populattion height (Orbegozo 2004) expressed in SDS, and SGA analyzed according to Spanish Populattion (Delgado 1997). Statistical analysis by SPSS v.11.
Results: Weight and energy data obtained at: 7 days (105 cases, 24/81 SGA / AGA), 15 days (114 cases, 27/87 SGA / AGA), 1 month (115 cases, 26/89 SGA / AGA) and 2 months (71 cases, 15/56 SGA / AGA).
Boys received lower energy intake than girls (significant at 1 month: 63 ♂52 ♂119.14 vs 126.81 cal / kg). Children whose height SDS is ≤ -2 (6 cases, 1 SGA and 5 AGA) at 5 years old vs height SDS> -2, had lower weight increase, significant at 7d-1 month (0305 vs 0437 kg) and 15d-1 month (0214 vs 0312 kg); and received less energy / kg / day until the first month of life (112.86 and 120.96 respectively). There is a significant correlation between weight increase (7d-2m) and height reached before 2 years old. In SGA children, index cal / kg was lower than AGA for 1 month without statistical significance;(7d: 77.18 vs 82.16; 15d: 106.71 vs 115.86; 1m: 121.65 vs 122.88). There was no statistical significance in weight increase between 7d-2m: SGA vs AGA: 1.04 and 1.05 kg.
Conclusions: 1.- Children with height <-2SDS had lower weight increase and energy intake. 2.- The caloric intake received during the first two months of postnatal life affects their posterior development. 3.- Special attention is

needed in the nutrition of SGA to improve weight gain and catch up growth.

PO2-224 Growth Plate

Short stature in children: the importance of a proper evaluation

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Pathologic short stature can be the first clinical manifestation of undetected diseases in childhood, for all of which early diagnosis and treatment are important. A total of 800 children (02-16 years) with short stature referred to our clinic (referral endocrine center) for evaluation during a ten year period, were studied. The inclusion criteria were height more than 2SDS below the mean for age and gender (<3rd percentile), growth failure (H.V <5 cm/year) and adequate follow-up (at least six months). Children with known severe somatic disease were excluded. Evaluation included: Detailed medical history, physical examination, appropriate weight/height plotting, relevant hematological/biochemical/hormone investigations, bone age and brain MRI, karyotype (in selected cases). 39.5% of children had pathological causes of short stature, the majority (52%) normal variants, such as constitutional growth delay 34%, familial short stature 18% and the rest idiopathic short stature 8.5%. The group with the pathologic etiology consists of: growth hormone deficiency (GHD) 25.75%, intra-uterine growth retardation 5%, hypothyroidism 2.5%, skeletal dysplasia (achondroplasia) 2.5%, Turner syndrome 2% and other 1.8% (rickets, Down/Noonan/Morquio syndrome, Chron's disease, and renal failure). The exclusive majority of children with GHD had idiopathic GHD, 68% were male (male/female ratio=2.1), 8% had hypothyroidism and 5% euthyroidic Hashimoto's thyroiditis before rhGH therapy was started, one had congenital panhypopituitarism secondary to PSIS, one diabetes insipidus and one had received surgery for craniopharyngioma. 27% of the girls with Turner Syndrome had autoimmune thyroiditis. Children with height scores more than 3SDS below the mean have severely short stature and the majority belongs in the group of pathological etiologies, with the least SDS and slowest H.V. related to hypothyroidism, GHD and skeletal dysplasia. It appears that GHD is more common in boys, while the percentage of other diagnosis is remarkably similar for both sexes. In this study 30.25% of the children with short stature had an endocrine disorder which when treated at an early stage has an optimal effect on adult/final height with a presumably better quality of life. A short child must be properly evaluated in order to identify the normal variants of his short stature or as early as possible a potentially treatable etiology.

PO2-225 Growth Plate

The lipid profile of pathologic short children

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Dyslipidemias in children are due to: primary dyslipidemias genetically transmitted from parents to their offspring and secondary caused by endocrine disorders, renal and liver diseases, obesity and drugs (steroids). In order to determine the incidence of lipid abnormalities in children evaluated in our clinic, we selected to study those having a pathological cause of short stature. The study population consisted of 290 children randomly selected with age range: 03-16 years. The values of total cholesterol (t-C) and LDL-C in at least two different periods of time were recorded as measured in the initial evaluation and follow-up and regardless of a family history of high risk factors for cardiovascular diseases. T-C and LDL-C were measured after a 12 hour starving and none of the children were obese, had fever, recent drug therapy or surgery. Normal considered values for children are: t-C< 170mg/dl and LDL-C< 110mg/dl. i) 36% of the girls with Turner syndrome had increased t-C/LDL-C levels, from which 25% had H.thyroiditis with hypothyroidism ii) 18 children had achondroplasia and 55.5% (6/9 boys and 4/9 girls) had increased levels of t-C/LDL-C iii) in the intra-uterine growth retardation group (IUGR) 35.5% had a significant elevation of t-C /LDL-C values (5/15 boys and 6/16 girls) from which 18% had hypothyroidism and 18% insulin resistance iv) Hyper-LDL-cholesterolemia was found in 22% of children with hypothyroidism (4/18) v) 206 children (140 boys and 66 girls) suffered from GHD. Before rhGH therapy was initiated 18.45% of all (18.6% of boys and 18.2% of girls) had increased t-C/LDL-C from which 23% had also hypothyroidism, 2.6% congenital panhypopituitarism and the rest isolated GHD. In conclusion 23% of the children evaluated for short stature and diagnosed with a pathologic cause had increased values of t-C/LDL-C with a similar incidence for both sexes. Hypothyroidism and IGHD are causes of hyper-t-C/LDL-C and in these children a lipid profile should always be obtained. The most common affected children appear to be those with a history of IUGR and achondroplasia. The association of dyslipidemias and IUGR children is well known and this group is at an increased risk for developing a metabolic syndrome. It is very important in children/adolescents with growth failure to screen for lipid abnormalities and identify those with the presence of multiple risk factors that may require behavioral/dietary and/or pharmacologic intervention.

PO2-226 Growth Plate

Is C-type natriuretic peptide (CNP) production elevated in Marfan syndrome?

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Marfan syndrome has features involving the skeletal, cardiovascular, muscular, pulmonary, and ocular systems. The skeletal features are due to overgrowth causing tall stature, chest deformities, and arachnodactyly. Marfan syndrome is caused by mutations in fibrillin-1, an extracellular matrix protein that binds the latent transforming growth factor-beta (TGF-β) complex. Recent data have shown that the cardiovascular, pulmonary, and muscular features of Marfan syndrome result from excessive tissue levels of active TGF-β. Three case studies of children with "Marfanoid" skeletal features and chromosomal translocations near the CNP gene have been reported. These children had elevated blood levels of CNP and its amino-terminal propeptide (NTproCNP). CNP is an important paracrine regulator of skeletal growth and levels in plasma correlate with linear growth velocity. CNP is also potently up-regulated by TGF-β *in vitro*. We hypothesized that the skeletal overgrowth in Marfan syndrome is due to up-regulation of CNP from excessive levels of TGF-β in the growth plate. Thirty-nine adults with Marfan syndrome were studied and compared with 103 healthy controls sampled as part of a separate study. For the subjects 18 to <41 years of age, NTproCNP levels were 18.3±5.1 pmol/L (mean±SD, *n*=26) compared to 16.5±4.2 (*n*=35) for healthy controls (*P*=0.14, ns). For adults 41 years and older, levels were 12.1±4.9 pmol/l (*n*=13) compared to 14.9±2.9 (*n=68) for controls (P*<0.01). There was no correlation between height SD score and NTproCNP SD score (*r=0.24, P*=0.14, ns).
Four children (6, 12, 17, and 17 years of age) were also studied. NTproCNP level SD scores were -1.1±1.4 and were not statistically different from the reference range.
These data demonstrate that in adults, plasma NTproCNP levels are not elevated. However, since it is the CNP produced in the growth plate that regulates skeletal growth, it is possible that elevated plasma levels would only be seen in children. Our data are insufficient to exclude this possibility but the trend for lower than normal values in affected children makes our hypothesis unlikely.

PO2-227 Growth Plate

Amino-terminal propeptide of C-type natriuretic peptide (NTproCNP): levels in healthy children and relation to growth velocity

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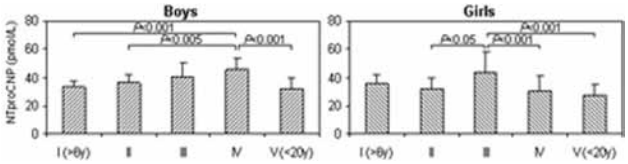
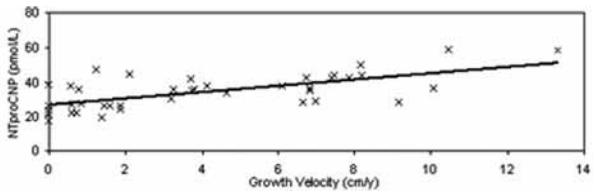
C-type natriuretic peptide (CNP) is a paracrine growth factor acting in the growth plate. CNP is rapidly cleared; however, its amino-terminal propeptide

(NTproCNP) is not subject to these clearance mechanisms and is easily quantified in plasma. We have previously shown that levels of NTproCNP correlate with growth velocity in healthy lambs and in children with non-growth related problems. The goal of this study is to determine if this holds true in healthy children.
124 children have been studied to date (70 boys, 54 girls). Median age was 12.7 years (range 0.2–20.4). Height velocity was available for 72 subjects (median interval between visits 0.6 years, range 0.4–1.0).
NTproCNP levels are high during infancy, decreased during school age years, rise again during puberty, and finally drop to low levels in adulthood. During puberty, levels peaked at 13.8 years for boys and 11.3 years for girls. These are very close to the published ages of peak growth velocity in American children (13.6±1.1 for boys, 11.5±1.2 for girls).
NTproCNP levels varied with Tanner stage, peaking at genitalia Tanner stage IV for boys and breast Tanner stage III for girls (Figure 1).



Linear regression analysis showed a positive correlation between NTproCNP levels and succeeding growth velocity (growth velocity *after* level was drawn) (R^2=*0.30, P<0.001, n*=72) and for preceding growth velocity (growth velocity *before* level was drawn)(R^2=*0.44, P<0.001, n*=40, Figure 2).



Growth may be the single most important index of a child's health, yet we have no means of measuring it at a single point in time. Markers of bone turnover have been examined for this role, but are not specific to the growth plate. NT-proCNP, viewed in the light of a validated reference range, may prove to have clinical utility in the evaluation of growth in children.

PO2-228 Growth Plate

Impact of IGF-I splice variants on growth spurt

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Introduction: The molecular mechanisms and the impact of autocrine and paracrine synthesized members of the IGF family on longitudinal bone growth in vertebrate development are complex and delicately regulated. We found a new player which should be considered with respect to the versatile orchestration of proliferation and differentiation. Mechano Growth factor (MGF) emerges from the IGF-I gene, but displays a C-terminus completely different to IGF-I Ea and IGF-I Eb. The aim of our work is to characterise the occurrence of mgf within the growth plate. We wanted to explore the influence of the MGF peptide and we tested the effect of exogenously added MGF-peptide to different cell culture systems in comparison to IGF-I on proliferation.
Material and Methods: Porcine cells from the resting zone, proliferative zone and hypertrophic zone were separated by laser microdissection. For the quantification of mRNA of IGF-I and MGF, real-time PCR was performed. On protein level MGF expression was detected by immuno histochemistry. Chondrocytes were stimulated with IGF-I and MGF and a combination of both. Effects on proliferation and metabolism were investigated with BrdU and EZ4U assays.
Results: We identified MGF in porcine growth plate chondrocytes and we discriminated this splice variant from the IGF-I core protein. Immuno histochemistry revealed that MGF is abundant in growth plates, namely in the resting zone and the prehypertophic zone. We found that in contrary to muscle tissue in vivo exogenous MGF peptide on its own has no impact on proliferation of growth plate chondrocytes, but there is good evidence that a combination of mgf and igf-I accerlerates growth even better than igf-I alone in vitro.
Dicussion: MGF is one of the autocrine and paracrine factors in muscle, which increases power output by hypertrophy and augmentation of tissue mass. We detected MGF in considerable amounts in the growth plate of growing individuals and showed the different effects on growth by the addition of the two splice variants of the one IGF-gene. We showed that MGF on its own cannot accelerate growth but it seems that it is a kind of enhancer for the impact of IGF-I on growth plate chondrocytes. If we assume that tissue repair reactivates the pathways, which are used during development, we may hypothesize, that MGF is a growth and repair factor, which initializes these processes not only in muscle, but plays also a pivotal role in growing paediatric tissues.

PO2-229 Growth Plate

Short term effects on height gain in idiopathic short stature: retrospective study with aromatase inhibitors

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Introduction: In most European countries the growth hormone treatment of patients with idiopathic short stature is not compensated by the insurance companies. In order to help these patients by increasing their predicted final height we retrospectively calculated data of single off-label treatments with aromatase inhibitors, which reduce the transformation of testosterone to estradiol leading to bone age retardation.
Patients: We included 9 boys (median age 14.7 years) with a prospective adult height below 168 cm. Treatment with 1.25-2.5 mg Letrozol was started in patients with a median bone age of 14 years. Results were calculated before and after 1 year of treatment.
Results: The treatment was well tolerated; adverse effects have not been observed. The boys' estradiol and testosterone levels were measured at the start of the treatment; median levels were 16 pg/ml and 83.7 ng/dl, respectively. At the end of 1 year, the levels were 32 pg/ml and 599 ng/dl, respectively. The median prospective adult height according to Bayley and Pinneau at the start of the trial was 163.1cm (145.6-167.8) and increased significantly (p=0.008) to 165.1 (157-173.2) (n=5) after 1 year, whereas the bone age remained unchanged 14 years in median. The median gain of height was 3.8 cm/year. The respective median H-SDS values increased from -2.95 to -2.49.
Conclusions: The treatment with aromatase inhibitors was well tolerated and seems to be effective to increase the prospective adult height even after a short term treatment in male patients with idiopathic short stature. The gain of prospected adult height is in a low-similar range compared to that reported after growth hormone treatment. However, our data can not give evidence regarding the final height of our patients.

PO2-230 Growth Plate

Do centimetres matter: inaccuracy of self-reported and estimated height measurements in parents?

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***Introduction*:** A discrepancy between reported and measured parental height is often observed.However, we often have to rely on the height of one absent parent as reported by the attending parent.The aims of this study were: a) to assess whether there is a significant difference between the reported and measured parental height; b) to focus on the reported and, thereafter, measured height of

the partner.
Methods and subject studied: A questionnaire was presented to parents whose children were referred to our clinics.All measurements were performed by a fellow specially trained in auxology using a calibrated wall mounted stadiometer.1542 individual parents were enrolled.The parents were subdivided into three groups:normal height (3-97th Centile), short (<3%) and tall (>97%) stature.
Results: Women of normal stature reported their height accurately, whereas tall females underestimated ($p<0.01$) and short females overestimated significantly ($p<0.001$) their measured height.Tall males underestimated their height, but normal and the short males overestimated their height ($p<0.0001$).Where both couples were of either normal, short or tall stature, the estimated heights of their partner were quite accurate.Females of normal stature underestimated the short partner and overestimated the tall partner significantly ($p<0.001$).Normal statured males overestimated both their short ($p<0.001$) as well as tall ($p< 0.01$) partners.Tall females estimated the heights of their short partners correctly, but underestimated those of normal statured males ($p<0.01$).Tall males overestimated the heights of their female partners of normal ($p<0.0001$) and short ($p<0.001$) stature.Short females estimated normal statured males adequately, and overestimated the heights of their tall partners ($p<0.001$).The short males significantly underestimated ($p<0.0001$) the normal but overestimated tall partners ($p<0.001$). ***Conclusion:*** Only measured heights should be used to perform accurate evaluations of height particularly when diagnostic tests or treatment interventions are contemplated.For clinical trials only quality measured parental heights are acceptable as the errors incurred in estimates may enhance/conceal true treatment effects.

PO2-231 Obesity, Fat II

Impact of serum adiponectin concentration on birth size and early postnatal growth

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Background In term neonates, the adiponectin concentration is higher than it is in adults. Our aim was to determine the effect of adiponectin on early neonatal growth in a cohort study. Methods and Results Fifty-two neonates at term, including 6 small for gestational age (SGA), 43 appropriate (AGA), and 3 large (LGA), were studied. Serum adiponectin concentrations, body sizes, and skinfold thickness were measured at birth and at 1 month of age. At birth, cord blood adiponectin concentration correlated positively with birth weight (r=0.484, P=0.0003), birth length (r=0.524, P<0.0001), and sum of the four skinfold thickness measurements (r=0.378, P=0.0057). In a stepwise regression, birth length was the only determinant of cord blood adiponectin concentration. However, at one month of age, serum adiponectin concentration correlated with no anthropometric parameter at all. Furthermore, there was no significant relationship between the individual changes in anthropometric parameters during one month and the change in serum adiponectin concentrations. In SGA neonates, cord blood adiponectin concentrations were significantly lower (16.3 ± 1.7 μg/ml) than those in AGA (23.8±1.2 μg/ml) and LGA (27.9 ± 2.6 μg/ml) (P = 0.0354) neonates. However, serum adiponectin concentration caught up in 1 month without catch-up growth in body size. Conclusion Serum adiponectin concentrations in cord blood have a strong relation to birth length rather than to body fatness, and this relation is not demonstrated in 1-month-old infants. In the early neonatal period, the hormonal regulation of growth and the physiological effect of adiponectin may change drastically.

PO2-232 Obesity, Fat II

Associations of obesity with metabolic risk factors in pre-pubertal Bulgarian children

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Background: Obesity and the regional distribution of adipose tissue (abdominal adiposity) are related to adverse health outcomes in adults. The association of obesity measures, i.e. body mass index (BMI), waist circumference (WC) with metabolic risk factors in pre-pubertal children is not extensively studied. Aim: To evaluate the relationship between anthropometric measurements and metabolic risk factors in pre-pubertal Bulgarian children. Design and research methods: A cross-sectional study of 168 pre-pubertal urban children (78 males; mean age 8.1±1.3 years) was conducted in 2007/2008. Body weight, height and waist circumference were measured; BMI was calculated. Blood pressure (BP) and pulse rate were determined in a seated position. Fasting serum levels of blood glucose, insulin, lipids (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol) and hepatic enzymes (ALT, AST) were measured. Insulin resistance was defined as HOMA-IR index. For the purposes of the analysis, children were divided into 3 groups according to their BMI and WC: 1st group (BMI≤85th P and WC≤75th P, n=53); 2nd group (BMI between the 85th and 95th P and WC≤90th P, n=46); 3rd group (BMI≥95th P and WC≥90th P, n=69). Descriptive statistics, one-way ANOVA and partial correlation analyses were applied, using SPSS for Windows version 15.0. Results: BMI and WC correlated well in both males (r=0.963, p<0.0001) and females (r=0.955, p<0.0001). Obese children (gr. 3) had significantly higher BP, triglycerides, ALT, HOMA index, insulin and blood glucose levels ($p<0.01$) as well as lower HDL-cholesterol levels ($p<0.05$), compared to non-obese subjects (gr.1). In males, after adjusting for age, the abdominal adiposity, measured as WC, was positively associated with systolic and diastolic BP (r=0.862 and 0.713, respectively, p<0.0001), triglyceride level (r=0.598, p<0.0001), insulin (r=0.609, p<0.0001) and HOMA index (r=0.566, p<0.0001), while HDL-cholesterol levels correlated negatively with WC (r=-0.317, p=0.046). The results in females were similar. Compared to WC, BMI correlated less significantly with the metabolic risk factors in both sexes.
Conclusion: The present study suggests that waist circumference correlates well with most of the adverse metabolic and cardiovascular parameters in pre-pubertal children, thus implying at further possibilities for identifying children at risk.

PO2-233 Obesity, Fat II

High birth weight protects obese adolescents from developing insulin resistance and metabolic syndrome

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Introduction**:** The incidence of obesity has increased exponentially in the last two decades worldwide; the current prevalence in the Spanish pediatric population being around 14%. Low birth weight (LBW) and the development of obesity in childhood have been associated to a higher risk for insulin resistance and cardiovascular disease of early onset. The effects of combined high birthweight (HBW) and childhood obesity have been explored to a lesser extent. Here, we hypothesized that obese adolescents with a history of HBW would be more insulin sensitive than those born with a LBW and presenting the same degree of obesity.
Methods: We studied 41 obese adolescents [age (m ± SD), 12.5 ± 2.0 yr], pubertal Tanner stage: 3.2 ± 0.3, body mass index (BMI): +3.4 ± 1.2 SD). The subjects were divided into three groups according to BW, with a similar gender distribution, and similar pubertal stage and BMI: HBW (≥+2 SD for gestational age; n=13), normal BW (NBW; between < +2 SD and >- 2 SD; n=14;), and LBW (<-2 SD; n=14). The following variables were assessed: weight, height, BMI, waist-to-hip ratio, blood pressure, fasting blood glucose, insulin, insulin sensitivity (HOMA), cholesterol, triglycerides, liver, renal and thyroid function; an oral glucose tolerance test was performed in all.
Results: Patients with HBW had lower insulin levels and were less insulin resistant than those with a LBW showing the same degree of obesity (HOMA: 3.3 ± 1.5 vs 5.7 ± 2.0, respectively, p=0.01). None of the adolescents with HBW showed components of the metabolic syndrome (based on the International Diabetes Federation criteria), or glucose intolerance; In contrast, in LBW adolescents, the prevalence of metabolic syndrome and glocose intolerence was 29% and 14% respectively.
Conclusion: Obese adolescents with a history of HBW are less insulin resistant than those born with LBW depicting the same degree of obesity, and thus are more prone to develop the so-called metabolically healthy obesity. This situation fits with the theory of expansibility of subcutaneous adipose tissue, according to which a higher prenatal weight gain favours a higher recruitment of

subcutaneous adipocytes that become more sensitive to the antilypolitic effects of insulin. Long-term follow up of these patients will allow to delineate the natural history and study the molecular and endocrine-metabolic mechanisms underpinning the expansibility of adipose tissue.

PO2-234 Obesity, Fat II

Psychological profile of obese adolescents involved in a care program

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Background: Psychological profile is an important aspect to take into account for the care of obese adolescents.
Objective: Description of the psychological profile of obese adolescents starting a program of therapeutic education in order to improve care effectiveness.
Population: 106 adolescents (46 boys and 60 girls; age (median [interquartile range]): 13.5 [12.6-14.7] years; IMC: 28.2 [25.8-31.4] kg/m²; zscore: 3.1 [2.7-3.9]) followed in our department of pediatrics were enrolled in the study. Adolescents with psychiatric pathology (according to the DSM IV-TR criteria) were excluded.
Method: Self-administered questionnaires assessing the following dimensions:
– Body satisfaction
Body awareness [BPQ: Body Prominence Questionnaire (Fisher 1970)]
Body worry [QPC: Questionnaire de Préoccupation Corporelle (Canestrari et al. 1980)]
Body perception [FRS: Figure Rating Scale (Stunkard et al. 1983)]
Self-esteem [SES: Self Esteem Scale (Rosenberg 1965)]
– Anxiety [R-CMAS: Revised Children Manifest Anxiety Scale (Reynolds et Richmond 1999)]
– Depression [CES-D: Center for Epidemiologic Studies – Depression scale (Radloff 1977)]
Results: Body awareness scores were rather low but higher in girls than in boys (2[1-4] vs 1[0-2] *P*=0.0032). The FRS indicated that 57% of girls and 89% of boys consider that they have an overweight or obese figure. Moreover, 60% of girls and 15% of boys would like to have a figure inferior to the norm or even thinner. Most frequent expressed body worries were "belly" (88% of girls and 83% of boys), "thigh" (82% of girls and 63% of boys) and "fatness" (88% of girls and 72% of boys). The SES showed that self-esteem was normal without gender difference (19.5[13.5-24] in girls vs 21[18-24] in boys *P*=0.1055). The RCMA-S showed that girls had a higher level of overall anxiety compared to boys (13[8-16.5] vs 8.5[5-13] *P*=0.0063). In addition, girls had a depression level greater than boys (30% vs 6.5% with moderate to severe depression symptoms [CES-D score ≥ 24] *P*=0.003).
Conclusion: This study showed that systematic assessment of various psychological dimensions is helpful in the care of obese adolescents. This protocol using standardized instruments is useful to modify our everyday practices in obesity diagnostic and clinical care.

PO2-235 Obesity, Fat II

Nampt (formerly visfatin) associations with insulin metabolism are secondary to strong associations with obesity in children

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Rationale: Nampt, formerly named visfatin, is an important enzyme in cellular NAD metabolism. Besides expression in adipose tissue, Nampt circulates in human serum and was hypothesized to be involved in the regulation of insulin secretion. There are only sparse data on Nampt association with obesity or insulin metabolism in humans applying valid assay systems. In this study we compared Nampt levels in normal lean with obese children and investigated the relationship with metabolic and cardiovascular comorbidities.
Results: In a cohort of n=138 healthy lean children, Nampt levels were not different between boys and girls matched for pubertal stage and there was also no dependency on pubertal development. Nampt levels were, however, significantly elevated in obese children compared to lean children (3.85±0.28, n=72 vs. 2.95±0.30, n=69, P=0.029) and correlated with BMI SDS (r=0.35, P<0.0001) and other parameters of obesity. We also identified significant associations with parameters of glucose (120 min BG r=0.23, P=0.006) and insulin metabolism (Matsuda ISI r=-0.23, P=0.007) in univariate analyses that were, however, mainly attributed to underlying associations with BMI. Similarly, Nampt associations with endothelial function (RHI r=-0.25, P=0.003) or 24h mean systolic blood pressure (r=0.23, P=0.022) were abolished after correcting for BMI.
In addition to parameters of body fat mass, Nampt levels correlated with the number of circulating endothelial progenitor cells (r_{corr}=-0.26, P_{corr}=0.016) and WBC (r_{corr}=0.48, P_{corr}<0.0001) that was robust after correction for BMI. To assess, which subpopulations of express Nampt, we isolated peripheral mononuclear blood cells and quantified Nampt expression. Monocytes and granulocytes had the highest Nampt expression (accounting for >90% of PBMC expression), while the contribution of lymphocytes and EPCs was marginal. Since Nampt was suggested to be related to insulin secretion, we determined Nampt during an oral glucose tolerance test in obese children (n=28) and found a significant decline of Nampt in response to glucose load. Time after glucose challenge (b=-0.25, P=0.003) and insulin levels (b=-0.23 P=0.042) were significant predictors of Nampt in multiple regression analyses, but not blood glucose.
Conclusions: Nampt levels were strongly related with obesity and peripheral leukocyte count in children. The association with insulin resistance in obese subjects was secondary to the underlying association with obesity.

PO2-236 Obesity, Fat II

Vaspin is associated with female sex, puberty, obesity and insulin sensitivity in children

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Background: Visceral adipose tissue-derived serine protease inhibitor (vaspin) has been suggested as a novel adipocytokine that is related to parameters of obesity and to exert insulin sensitizing effects in adults. So far, it is not clear whether the associations with metabolic parameters are secondary to an underlying association with obesity. Studies in children may help to dissect these potentially primary vs. secondary associations.
Methods: We quantified vaspin serum levels (ng/mL) in 66 lean (12.8±2.9 yr, BMI SDS -0.28±0.09) and 68 obese (12.2±2.7 yr, BMI SDS 2.2±0.06) children and assessed the association of circulating vaspin levels with normal physical development, obesity and metabolic and cardiovascular parameters.
Results: In normal lean children, vaspin levels significantly increased with pubertal development and age in girls (P1: 0.57±0.14, P2-4: 1.32±0.30, P5: 1.79±0.46, *P*<0.0001) but not in boys. Girls had significantly higher vaspin levels compared to boys (1.27±0.20 vs. 0.38±0.04, *P*<0.0001) as soon as they entered puberty.
In obese children, vaspin levels were significantly decreased (0.55±0.06 vs. 0.91±0.13, *P*<0.0001). The correlation with BMI SDS (r= -0.18, *P*=0.042) remained significant after adjustment for sex, age and pubertal stage. Multiple regression analyses confirmed an independent association of vaspin levels with sex, age and BMI SDS in children.
Relating to the sequelae of obesity, we identified a negative association with Matsuda insulin sensitivity index (ISI: r=-0.20, *P*=0.023 adj. for sex, age, BMI SDS) in the entire cohort, but this was not evident in boys, when stratified for gender. In multiple regression analyses, vaspin contributed independently to Matsuda ISI (*b=-0.19, P*=0.01) in addition to BMI SDS (*b=-0.59, P*<0.0001)

and pubertal stage (*b=-0.15, P*=0.03).
Vaspin also correlated negatively with mean systolic blood pressure (BP) in unadjusted analyses (r=-0.21, *P*=0.042) and the association was even stronger in adolescents (r=-0.43, *P*=0.035). In multiple regression analyses, vaspin marginally failed significance (*b=-0.15, P*=0.082), while BMI SDS, age, sex and height SDS were independent predictors of mean systolic BP.
Conclusion: The sexual dimorphism of vaspin develops during puberty. Vaspin is decreased in obese children and is independently associated with insulin sensitivity, particularly in girls.

PO2-237 Obesity, Fat II

Endocrine and metabolic sequelae in children treated for craniopharyngioma

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Introduction: Due to location of craniopharyngioma in the hypothalamic-pituitary region, various endocrine and metabolic disturbances may occur and worsen in the course of time, regardless the type of initial treatment with surgery and/or radiotherapy. The aim of this observational study was to evaluate co-morbidities in craniopharyngioma patients followed by a single institution.
Methods: The studied group comprised 15 children (median age at the diagnosis 10.1, range 1.4-16.7 years), with the mean follow-up period of 3.6 years. All patients had surgical resection of the tumour as the initial treatment: gross total resection in 7 and subtotal or partial removal in 8 cases. Surgery was followed by radiotherapy in 10 cases for tumour residue or progression. Sexual development and auxologic parameters (hSDS, height velocity delta hSDS, BMI) were evaluated at diagnosis and during follow-up. Levels of TSH, fT4, cortisol, LH, FSH, PRL, IGF-1 and GH were determined by chemiluminescent immunometric assays. Dysfunction of ADH was diagnosed on the grounds of clinical symptoms, water-electrolyte balance, urine specific gravity, and serum osmolality. Metabolic control was monitored by levels of glucose, insulin (fasting and/or OGTT), lipids and transaminases. Insulin resistance was expressed by HOMA index. **Results:** At the diagnosis median hSDS was -1.6 (range -4.5; 0.25), with 5 children being short-statured. After 12 months the median delta hSDS was 0.13 (range -1.0; 0.7), with only 4 children decelerating growth, subsequently qualified for GH therapy. Median delta hSDS for the whole follow-up period was 1.2 (range -1.1; 2.6). Diabetes insipidus was diagnosed in 8 patients (within 0-1.8 yrs of the follow-up; in 1 case as a sign of tumour progression). Hypocorticolism was found in 8 patients and hypothyroidism in 12 subjects (within 0-3.75 yrs for both endocrinopathies). During the follow-up 4 patients started sex hormone replacement therapy. At the diagnosis 5 children were overweight, however during follow-up only 4 children sustained normal BMI. Hyperinsulinaemia was found in 7 patients, with elevated HOMA in 4 cases, all receiving GH. Hypertransamineasemia was found in 3 and dyslipidaemia in 12 subjects. **Conclusions:** The results show that most children after craniopharyngioma treatment deteriorate their endocrine and metabolic status in various time intervals. Hence, interdisciplinary monitoring and treatment are necessary for optimal management of such patients.

PO2-238 Obesity, Fat II

Is cortisol a biological link between childhood obesity and depressive symptomatology? Positive associations between evening salivary cortisol and depressive scores in obese children and adolescents

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Background: Obesity is highly comorbid with depressive symptomatology in adults, while dysregulation of metabolic pathways may underlie both these associations. The Hypothalamic-Pituitary-Adrenal Axis (HPAA) is involved in the pathophysiology of depression and depressive disorders and cortisol abnormalities are potential links in mediating these relations. In children, several epidemiologic studies have reported high rates of a variety of emotional and behavioral problems comorbid with obesity, but the pathophysiologic pathways that mediate these relations are not fully understood.**Patients and Methods:** Fifty children and adolescents (20 males and 30 females, aged 8-15years), recruited from the pediatric obesity clinic were studied. BMI z-scores were calculated based on the Greek growth charts. Depressive symptomatology was assessed using the CDI (The Children's Depression Inventory) questionnaire. Serum morning cortisol concentrations were measured. Furthermore, salivary cortisol concentrations were measured serially, 5 times a day, to examine circadian variation. Serum and salivary cortisol was measured using an electrochemiluminescence immunoassay.
Results: In the entire population, a positive correlation was found between CDI total scores and evening salivary cortisol values (21.00hrs): (N=50, r=0.453, p=0.001). Positive correlations were also found between CDI total scores and salivary cortisol at 18.00hrs (N=50, r=0.312, p=0.03), 15.00hrs (N=50, r=0.439, p=0.002) and 12.00hrs (N=50, r=0.364, p=0.01). In contrast, no correlations were detected between CDI total scores and morning salivary or serum cortisol values.
Conclusions: Afternoon and evening salivary cortisol values correlate positively with depressive symptomatology in obese children. Longitudinal studies are needed to confirm a role for cortisol in mediating childhood obesity and depression.

PO2-239 Obesity, Fat II

Inconsistency of metabolic syndrome diagnosis and correlation of morning serum cortisol concentrations with stability of metabolic abnormalities in obese children and adolescents

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Background: The diagnosis of Metabolic Syndrome (MetS) in childhood and adolescence, despite the lack of a clear consensus-based definition, is controversial during changing periods of life. The aim of this study was to examine the stability of full and partial MetS in children and adolescents followed longitudinally at an obesity clinic and to correlate the presence of stability with an index of chronic stress such as serum cortisol concentrations.
Patients and Methods: Eighty-two children and adolescents, aged 6-18 y, with a full clinical and metabolic evaluation for obesity, were re-assessed one year later at the Pediatric Obesity clinic. IDF-adapted criteria were used for the definition of full and partial MetS. Groups were defined based on partial MetS consistency (at least two positive parameters in both assessments). Fasting morning insulin and cortisol concentrations were measured in both assessments.
Results: On admission, 11 % of the children had the full MetS and another 30% had partial MetS. In the longitudinal analysis, 16 children out of 82 had the full MetS either at the first (9) or the second (7) evaluation, but none in both assessments. Serum insulin was higher in the female group with consistent metabolic abnormalities (CoM) than those with the variable (VaM) and the non-metabolic complications (NoM) groups. A significant elevation of morning serum cortisol (p=0.04) was noted only within the CoM group in both genders.
Conclusions: MetS is an unstable diagnosis in childhood and adolescence. Cortisol seems to play a role in partial MetS maintenance through this period of inconsistent obesity-related changes.

PO2-240 Obesity, Fat II

Enhanced oxidative stress and platelet activation between obese adolescent and prepubertal girls with full or partial metabolic syndrome

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Background: In adults, obesity is a main factor implicated in increased oxidative stress(OS) and platelet activation(PA), both predisposing for cardiovascular disease leading to increased morbidity and mortality. Furthermore, the metabolic syndrome (MetS), which is an important cardiovascular risk factor in adults, is also highly prevalent among obese adolescents. However, scarce data on the presence of OS and platelet accumulation among obese adolescents and children with full or partial MetS exist. Objective: To evaluate OS and PA in obese adolescent and prebubertal girls with full or partial MetS. Methods: 96 obese adolescent and prebubertal girls and 23 normal-weight adolescent, (11,89±1,17 mean±sd years) (A-NW) and 21 normal-weight prebubertal girls-controls, (8,79±1,59) (PR-NW) were studied. Obese girls were subdivided in four groups, the first one comprising 31 obese prebubertal girls (8,81±1,58) and partial MetS (group PR-PMetS), the second group included 37 obese adolescents (11,55±1,56) and partial MetS (AD-PMetS), the third group comprising 10 obese prebubertal girls (9.02±1,24) and full MetS (PR-MetS), the fourth one included 18 obese adolescents (11,98±1,82) and full MetS (AD-MetS). The OS was evaluated by measuring plasma levels of 15-F2t-Isoprostane (15-F2t-IsoP), the most reliable biochemical index of the OS while PA by measuring plasma levels of TXB2, the biologically inactive metabolite of TXA2, using a reliable enzyme – immunoassay method. Results: Plasma 15-F2t-IsoP levels in the PR-PMetS, AD-PMetS, PR-MetS and AD-MetS groups were 20,19±5,03pg/ml, 23,77±5,48pg/ml, 32,50±4,19pg/ml and 34,28±9,56pg/ml respectively and were significantly higher than those in groups PR-NW and A-NW(13,89±2,68pg/ml and 13,58±2,74pg/ml). Plasma TXB2 levels in the PR-PMetS, AD-PMetS, PR-MetS and AD-MetS groups were 40,31±10,70pg/ml,47,68±10,72pg/ml, 68,83±12,58pg/ml and 71,26±14,33pg/ml correspondingly and were significantly higher compared to PR-NW and A-NW groups(26,78±5,49pg/ml and 27,05±5,34pg/ml). There was a positive correlation between 15-F2t-IsoP, TXB2 levels and BMI ($p<0,05$) and between 15-F2t-IsoP and TXB2 levels ($p<0,01$) in the four groups. Conclusions: OS and PA increase in obese girls, with increasing levels of obesity, being also higher among those with full MetS than partial MetS during adolescence and childhood. The coexistence of increased OS, rised PA and MetS constitute a further unfavorable prognostic set up for cardiovascular risk.

PO2-241 Obesity, Fat II

Magnetic resonance of abdomen correlates to auxological measurements and leptin/adiponectin levels in pre-school children

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Background
In adults, abdominal adipose tissue correlates to decreased insulin sensitivity better than Body mass Index (BMI, weight/height²) does.
Some studies in children show a stronger relation between cardiovascular and diabetes risk factors and visceral fat, compared to whole-body fat.
Correlations between magnetic resonance imaging (MRI) findings, dual energy X-ray absorptiometry (DXA) and auxological measurements have been studied previously, however not in pre-school children.
Aim
To compare measurement of abdominal adipose tissue assessed by MRI with measurements from DXA and simple auxological variables. To assess the correlation between VAT/SAT and leptin/adiponectin levels in healthy pre-school children.
Methods and population
Thirty-four 5-year old children (21 boys/13 girls) were recruited. A rapid MRI examination was performed using sixteen 10 mm thick T1-weighted slices at the L4-L5 level. SAT and VAT volumes were measured using manual image segmentation.
Body composition by DXA was measured with GE Lunar Prodigy DXA.
Leptin was analysed with RIA and adiponectin with ELISA.
Results
BMI (mean 15.3 kg/m², range 13.3-20.6), SAT (0.60 L, 0.22-1.72), and VAT (0.15 L, 0.08-0.37) was obtained for all children. The correlation between SAT and VAT was ($r=0.49$, $p<0.01$).
SAT correlated to body composition measured by DXA (total fat in gram ($r=0.96$) and truncal fat in gram ($r=0.95$)), BMI ($r=86$), waist circumference ($r=0.90$), waist height ratio ($r=0.84$), all $p<0.0001$.
The correlation between VAT and body composition measured by DXA was; total fat in gram ($r=0.57$), truncal fat in gram ($r=0.53$), and for BMI ($r=0.55$), waist circumference ($r=0.55$), and waist height ratio ($r=0.56$), all $p<0.001$.
Leptin (2.8 ug/L, 1.0-14.2) and adiponectin (8.3 ug/L, 1.4-17.5) was analyzed from all but two children. Leptin but not adiponectin correlated to SAT ($r2=0.75$) and to VAT ($r2=0.55$), both $p<0.0001$.
Conclusions
Subcutaneous adipose tissue can be estimated from auxological measurements, whereas for VAT neither auxological nor DXA measurements are enough accurate. Therefore, it is of importance to measure with MRI if the VAT is to be studied in pre-school children. However, neither leptin nor adiponectine correlated to VAT. Based on these data, whether MRI can be considered the best measure to estimate risk of health consequences in pre-school children is not answered.

PO2-242 Obesity, Fat II

Dietary calcium intake and ghrelin secretion dynamics in postpubertal adolescent females

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Recent research has linked calcium intake to the propensity to gain weight. Since appetite and hunger susceptibility play an important role in weight control, we explored whether they could be linked to dietary calcium. Ghrelin is an orexogenic hormone involved in a wide array of endocrine functions, including food intake and energy balance. Ghrelin levels rise before meals and are suppressed after carbohydrate consumption. In addition to meal-associated changes, Ghrelin concentrations spontaneously peak at night, a pattern that is poorly explained. The nocturnal increase of Ghrelin may have potential implications in appetite regulation and eating behavior. To further explore the association between dietary calcium intake and weight, we focused on postpu-

bertal adolescent girls because they experience a dramatic increase in fat mass during puberty.
In this study, we tested the hypothesis that dietary calcium intake would be a predictor of Ghrelin concentration measured two ways: 1. during overnight sampling averaging hourly values between 23:00 and 03:00 hours and 2. Sixty minutes after an oral glucose tolerance test following an overnight fast.
METHODS. We recruited 72 healthy adolescent females, ages 14 to 21y for an IRB-approved study on weight control. Blood was sampled overnight and after a supervised overnight fast in a subset (n =22) at which point 75 grams of oral glucose was administered. To calculate daily calcium intake (AvgCa) a 3-day food diary was provided and completed prospectively, supported by a one-on-one interview with a dietician and detailed analysis with the Nutrition Data System for Research software. Ghrelin was measured by a validated commercial assay. Body fat percentage was obtained by dual X-Ray absorptiometry (DEXA). Data was analyzed with SAS software.
RESULTS. Mean age was 18 (SD 2) years, BMI was 24 (SD 4) kg/m2, body fat percentage was 31 (SD 9) % and AvgCa 980 (SD 473) mg/day. AvCa was not related to BMI but was linked to percent body fat (p=0.049, R2=0.05) and the average nighttime Ghrelin (p = 0.001, R2 = 0.15). AvCa was also a predictor of Ghrelin concentration 60 minute after glucose challenge (p = 0.02, R2 = 0.24).
CONCLUSION. In this cross-sectional study, Calcium intake was related to two components of Ghrelin secretion dynamics: the overnight peak and the dynamic response to a glucose challenge. This might be one mechanism by which calcium intake would contribute to weight regulation in late adolescent females.

PO2-243 Obesity, Fat II

Effect of obesity on pulmonary function tests in overweight and obese children

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BACKGROUND: Although the influence of obesity on pulmonary functions tests (PFTs) in adults has long been recognized, the nature of relationship and mechanisms in children are not yet clear. OBJECTIVE: The purpose of this report was to examine the effect of overall obesity and fat distribution on pulmonary function tests in children. METHOD: A cross-sectional study of 25 overweight and obese male children, aged 7 to 15 years, with no history of asthma or other atopic diseases was conducted and compared with 25 normal weight, healthy male children. Height, weight, waist/hip ratio (WHR),body mass index (BMI) and PFTs were measured. PFTs were assessed by measuring FEV_1, FVC, PEFR, MVV and $FEF_{25-75\%}$ with the help of a computerized spirometer (MEDSPIROR). ANALYSIS: All these pulmonary functions were found to have statistically significant negative correlation with waist hip ratio and BMI in overweight and obese subjects (table 1) while in contrast, weight hip ratio and BMI had no statistically significant correlation with lung function tests in normal weights.

COMPARISON OF ANTHROPOMETRIC AND SPIROMETRIC PARAMETERS OF NORMAL WEIGHT AND OVERWEIGHT CHILDREN

Parameters	Control group	Study group	t Value	p value	Significance
Age	11.2±2.90	11.04±2.73	0.350	>0.05	NS
WHR	0.80±0.04	1.15±0.09	-19.19	<0.01	HS
BMI	19.64±2.11	28.7±2.09	-16.15	<0.01	HS
FVC	2.70±o.80	2.08±0.51	5.04	<0.01	HS
FEV1	2.29±0.72	1.80±0.48	4.637	<0.01	HS
PEFR	5.02±0.98	4.34±0.73	3.206	<0.01	HS
FEF25-75%	3.06±0.82	2.54±0.68	3.653	<0.01	HS
MVV	97.92±5.23	86.12±8.38	7.240	<0.01	HS

CONCLUSION: BMI and WHR are the simple measures of obesity and fat distribution and most strongly associated with lung dysfunction in obese children.

PO2-244 Obesity, Fat II

Successful use of restricted carbohydrate diet in treatment of obesity in Hispanic children in Brooklyn

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Introduction:
Incidence of obesity in Hispanic children in the USA is at least 22 % and constitutes significant socioeconomic and medical problem. Restriction of carbohydrates in diet was previously shown to be effective in treatment of obesity in adults.
Objective:
To evaluate the efficacy of carbohydrate-restricted diet in treatment of obesity in Hispanic children in Brooklyn, NY.
Materials and methods:
We conducted a retrospective review of 93 randomly selected charts of obese (body mass index (BMI) above 95%) Hispanic children (ages 4 to 17 years) who underwent treatment with carbohydrate-restricted diet. Our specifically designed carbohydrate-restricted diet was offered to patients as a written guide and its utilization was re-enforced during follow up visits every three months for a total duration of minimum three months and maximum two years. Patients with frank diabetes mellitus were excluded from the analysis. Nineteen of the selected patients were taking metformin because of comorbid conditions. Exercise above usual daily activities was not emphasized. The BMI values before beginning of treatment were compared with those after treatment. The differences between actual BMI and ideal BMI (50 percentile for age) before treatment were calculated for each patient and compared with those after treatment.
Results: The difference between actual and ideal BMI was expressed in median (25, 75). It reduced from 10.3 (8.7, 13.0) before treatment to 9.7 (7.6, 11.8). This difference was found to be significant by Wilcoxon Signed Ranked Test, P<0.001 and was not affected by gender. The group of children receiving metformin was analyzed separately. The treatment caused significant reduction of difference in BMI in this group (10.5 (9.23, 18.20) and 9.90 (6.90, 17.65)), P = 0.016. However, this reduction in BMI was not different from the one in the group of children who received dietary treatment only.
Conclusions:
The carbohydrate-restricted diet utilized in our treatment protocol is highly efficient in treatment of obesity in Hispanic children of Brooklyn, NY. The reduction of BMI after dietary treatment is significant and is not affected by administration of metformin.

PO2-245 Obesity, Fat II

Role of environmental pollutants in adipose tissue inflammation in obese patients

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Our diet is contaminated by a large number of Persistent Organic Pollutants (POPs). These lipophilic compounds are only partially metabolized and localized in the fat tissues of the body where they persist for a large number of years. Interestingly, epidemiological studies observed a positive relationship between serum concentrations of several POPs and obesity, type 2 diabetes, insulin resistance and metabolic syndrome. In human, it is difficult, for ethical reasons, to characterize the the toxic effects of dietary contamination by POPs by clinical assays. Therefore, we took advantage of a clinical protocol leading to a drastic weight loss of obese subjects which constitutes a unique model to trigger the release of POPs in blood and to assess the functional and toxic effects of such a redistribution.
First, we studied the functional effects of POPs release by assessing the expres-

sion of genes known to be specific targets for POPs by Q-RT-PCR on adipose tissue (AT) in obese patients *vs* age-matched lean patients and before and 6 months after the start of the weight loss. These *in vivo* studies were completed by *in vitro* studies in which we determined the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and PCB126, a PCB dioxin-like, on human preadipocytes and *in vitro* differentiated hMADS adipocytes.
In preadipocytes and in adipocytes, TCDD and PCB126 induced a dose-dependent increased expression of the dioxin receptor AhR-responsive CYP1B1 and pro-inflammatory cytokines associated with obesity such as IL8, MCP1 and COX-2. Other dioxin-inducible genes were also increased by TCDD and PCB126 such as CYP19A1 and NQO1. Interestingly, their overexpression was already been associated with the obesity. In parallel, in obese patients' AT, the expression of xenobiotics metabolizing enzymes gene family and the other genes that are targets of AhR were significantly increased : CYP1B1, NQO1, CYP19A1, PAI2. Furthermore, after 6 months following surgery, expression of COX-2, NQO1 and PAI2 was significantly decreased.
These data suggest a relative activation of the receptor in obese subjects AT by POPs that could be partially reversed after weight loss. Therefore, POPs could contribute to the increased incidence of obesity and its complications.
We intend to correlate the expression of these genes with the amount of pollutants in AT and in blood cells is completed to reinforce importance of POPs in the incidence of obesity and its complications.

PO2-246 Obesity, Fat II

Timing of adiposity rebound is associated with subcutaneous adipose tissue in young adult age – the GOOD study

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Early adiposity rebound, the nadir of body mass index (BMI) between 3 and 7 years of age, has been linked to obesity in both children and adults. Visceral (intraperitoneal, ip, and retroperitoneal, rp) adipose tissue is more strongly related to the metabolic syndrome than subcutaneous (sc) adipose tissue. Therefore, we wanted to study if age and BMI at adiposity rebound was associated with subcutaneous or visceral adipose tissue in young adult age in a well-characterized Swedish cohort.
Detailed growth charts were retrieved for the men participating in the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study (n=612). Body composition was analysed using Dual X-Ray Absorptiometry and adipose tissue areas using abdominal computed tomography at 18-20 years of age. Adiposity rebound was defined as the lowest BMI between 3 and 7 years of age.
In the GOOD cohort, age at adiposity rebound was 5.7±1.2 years of age, and BMI at adiposity rebound was 15.2±1.1 kg/m^2. When subjects were divided into tertiles according to age at adiposity rebound, the lower adiposity rebound tertile had a percentage body fat of 19.7%±7.9, the middle adiposity rebound tertile16.2%±6.4 and the latest adiposity rebound tertile 14.6%±5.9. Linear regression analysis demonstrated that when both age and BMI at adiposity rebound were included in the model, they both independently predicted young adult percentage body fat (standardized BETA=-0.26, p>0.001 for age; standardized BETA=0.20, p<0.001 for BMI). Similar results were seen for young adult sc adipose tissue (standardized BETA=-0.25, p>0.001 for age; standardized BETA=0.17, p<0.01 for BMI), but for young adult ip adipose tissue, only age at adiposity rebound independently predicted ip adipose tissue (standardized BETA=-0.15, p=0.04), and not BMI at adiposity rebound (data not shown). However, when young adult ip adipose tissue was adjusted for total abdominal adipose tissue (visceral + sc adipose tissue), age at adiposity rebound no longer predicted ip adipose tissue.
Thus, we demonstrate that age and BMI at adiposity rebound were independently associated with young adult percentage body fat and young adult sc adipose tissue, but that after adjusting for high total abdominal fat, age or BMI at adiposity rebound did not predict young adult ip adipose tissue. In conclusion, early adiposity rebound and high BMI at adiposity rebound were associated with a predominantly subcutaneous fat phenotype in young adult males.

PO2-247 Obesity, Fat II

Thyroid function in obese children

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Recent studies have shown influence of obesity upon the function of the thyroid gland. Some of obese children have subclinical hypothyroidism.
The aim of our study was to evaluate the thyroid function in a group of obese children.
Sixty nine obese children at the age of 12.3 2.6 years average (range 6-14 years) were referred for the evaluation of obesity. OGTT was performed. Peak insulinemia and HOMA index were correlated with the TSH and free T4 (fT4) levels. Standard ultrasound examination of the thyroid gland was performed. Thyroid antibodies were tested in children with altered thyroid structure.
The duration of obesity was 3.5 1.2 years (range 2.1-7.3 years) before evaluation. Average BMI was 29.2 1.8 kg/m2. Peak insulinemia was 129 33.2 mmols/dl, and HOMA index was 4.79 2.86. High TSH levels (4mIU/l) were found in 16 children (23.2%). Body mass index and insulinemia correlated with the levels of TSH significantly (p 0.01 and p 0.05 respectively). No significant correlation was found with the levels of fT4. The ultrasound findings were suggestive of inflammation in 29 children (42.0%). However, thyroid antibodies were elevated in 4 of them (13.4%, or 5.8% of the total cohort). The positive thyroid antibodies were found in children with highest BMI and higher TSH levels. Three children developed typical Hashimoto thyroiditis (4.7%). Their BMI was among the highest within the cohort. Only children with Hashimoto thyroiditis were treated with thyroxin.
In conclusion, obese children tend to have higher TSH levels that correlate with BMI. Findings on ultrasound confirm inflammation that might be due to the obesity. In obese children analysis of the thyroid gland function and structure should be a routine approach. Longitudinal studies are needed to explore the association of the duration of obesity with the thyroid function, and the influence of weight loss on the thyroid hormone levels.

PO2-248 Obesity, Fat II

Correlation of body mass index and insulinemia with adiponectin and leptin levels in obese children

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The epidemiological magnitude of obesity and its complications in children has initiated many studies of the metabolic state of these children.
The aim of our study was to explore the leptin and adiponectin levels in obese children, to correlate them with the body mass index and insulinemia, and to compare these findings with those in a group of age matched non obese children.
Sixty obese children (30 boys and 30 girls) and a control group of 40 children (20 boys and 20 girls) were included in the study.
Insulinemia was measured during standard OGTT test. BMI index was calculated according to the standard formula. Leptin and adiponectin were measured with ELISA method.
Age of the children was 12.6 ±2.6 years average (8-18 years) and 12.0 ±1.9 in the control group. BMI was 31.08 ±7.6 kg/m2 for girls and 29.87 ±4.4kg/m2 for boys. Body mass index for the control population was 16.0±2.7 kg/m2 and 17.5 ±2.3 respectively. Leptin level in the examined population was 36.1 ±13.7ng/ml versus 5.7 ±1.5 in non obese children (p<0.05). There was a significant correlation between the BMI and leptin (p<0.01 for girls and p<0.05 for boys) Leptin levels were higher in females than in males (p<0.05); that also holds for non obese group. Although we found correlation between the BMI and peak insulinemia, the correlation of the leptin with the insulinemia was significant only in male obese children (p<0.01). Adiponectin levels were

10.5±4.8 mg/ml average in obese children and 15.2±6.9 mg/ml in controls (p<0.05). There was a significant negative correlation of BMI with the adiponectin levels (p<0.05).
In conclusion, obesity in children is associated with high leptin levels and low adiponectin level. Follow up studies are needed in order to assess the changes of these hormone levels during the change of BMI and whether the risk for cardiovascular complications remains stable.

PO2-249 Obesity, Fat II

Abdominal height is associated with glucose tolerance in children

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Background: Obesity and patterns of lipid partitioning are important predictors of insulin resistance (IR) and cardiovascular disease risk in adults. Visceral adiposity is related to higher risk of type 2 diabetes in adults. Waist circumference and waist-to-hip ratio are the anthropometric measurements most commonly used to indicate visceral adiposity. Abdominal height, which measures abdominal thickness at waist level in the supine position, has been proposed to better correlate with visceral adiposity and diabetes risk (due to the theory that gravity moves the subcutaneous fat to the sides while visceral fat projects the abdomen vertically.) A few adult studies have examined abdominal height, but to our knowledge, no pediatric studies have correlated abdominal height with insulin sensitivity or glucose tolerance.
Methods: 39 obese adolescents (ages 8-17, Tanner stages 2-5) underwent oral glucose tolerance test (OGTT), frequently sampled intravenous glucose tolerance test (FSIGT), anthropometric measurements, and dual energy X-ray absorptiometry (DXA) scan. Continuous variables were analyzed by Spearman's rho correlation.
Results:

Correlations: anthropometric measures, insulin sensitivity and glucose metabolism

	SI	Fasting glucose	2-hour glucose
Abdominal Height	0.077 (-0.376)	0.68 (-0.74)	0.025 (0.39)
Waist Circumference	0.358 (-0.188)	0.879 (0.26)	0.146 (0.247)
Waist-to-Hip Ratio	0.718 (-0.076)	0.959 (-0.009)	0.975 (0.006)

All correlations: p(R); SI=Insulin sensitivity index (derived from FSIGT); fasting glucose and 2-hour glucose - pre- and post-glucose load (OGTT)

Conclusions: Abdominal height showed a stronger relationship with glucose tolerance (2-hour glucose as derived from OGTT) than did waist circumference or waist:hip ratio, and thus may be a better predictor of who is more at risk for impaired glucose tolerance (IGT) and type 2 diabetes. Abdominal height trended towards correlating with insulin sensitivity, far more than either waist circumference or waist-to-hip ratio. Abdominal height provides an additional tool to measure the risk of IGT and IR in obese adolescents.

PO2-250 Obesity, Fat II

Expression of genes *PPARG, CNR1, ADIPOQ* and *PBEF1* in subcutaneous and visceral adipose tissue and serum level of adiponectin in children

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Introduction. Obesity in both adults and children is increasing exponentially throughout the world. The role of adipose tissue is not simply to store excess energy but to secrete and to respond to a variety of metabolites, hormones, and cytokines, which play important role in the development of obesity-related pathologies. Adiponectin, an insulin sensitizer is decreased in obesity. Visfatin (PBEF1) - an adipokine with insulinmimetic, immunomodulatory and enzymatic function. PPARγ is a nuclear receptor associated with adipogenesis and insulin sensitivity and CNR1 is an endocannabinoid receptor associated with food intake and adipogenesis.
Objective. We investigated expression of genes *ADIPOQ, PBEF1, PPARG* and *CNR1*, in subcutaneous (SAT) and visceral adipose tissue (VAT) and serum levels of Adiponectin in obese and lean children.
Subjects and Methods. We examined 45 children with wide range BMI undergoing routine surgery procedures. Expression of genes was examined in paired samples of VAT and SATobtained from 38 lean (BMI < 75‰) and 7 obese and overweight (BMI >75‰) children. Expression of *ADIPOQ, PBEF1, PPARG* and *CNR1* was established at the mRNA level by RT-PCR. Serum levels of Adiponectin were quantified using ELISA.
Results. No significant difference in *ADIPOQ, PPARG and CNR1* mRNA was seen between SAT and VAT depots neither in lean nor in overweight patients. However, *PBEF1* mRNA in VAT was significantly higher than in SAT (p<0,001). We did not find any relation between expression of genes and gender, but there was a significant increase of expression of *ADIPOQ* (SAT and VAT), *PPARG (VAT) and CNR1*(VAT) with increase in age and pubertal stage (p<0,05). We found strong correlation between *PPARG* expression and *ADIPOQ* (r = 0,8) and *CNR1* (r = 0,7), but at the same time relationships between expression of *PPARG and PBEF1* were weaker. Serum levels of Adiponectin decreased from Tanner 1 to Tanner 3 in both lean and obese children (p < 0,05). Despite no significant difference between lean and obese children, serum Adiponectin resulted in significant negative correlation with BMI (r = -0,35) and WC (r = -0,33).
Conclusion. Based on our research we came to conclusion that activity of visceral adipose tissue increase with age, that may explain appearance of obesity-related co-morbidities in older age group. Reduction of serum adiponectin with increasing BMI and waist circumference may play a significant role in the development of complications of obesity in children.

PO2-251 Obesity, Fat II

Circulating adiponectin is related to osteocalcin in healthy children: perspective for an adiponectin-osteocalcin loop in humans

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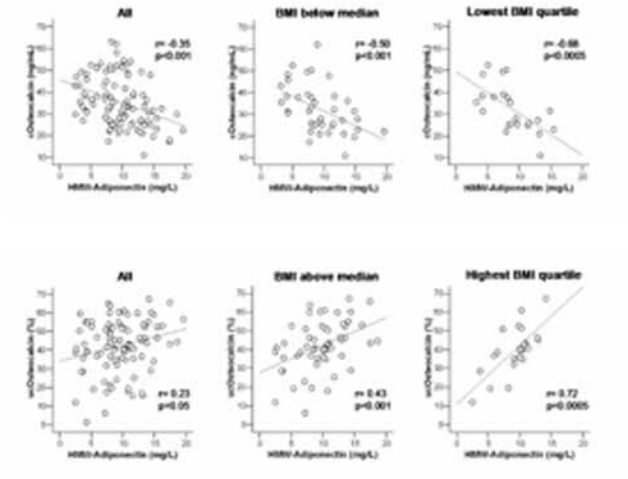
Background and Aim: Osteocalcin (OC), a bone-derived protein, was recently shown to increase adiponectin secretion. Adiponectin modulates bone mass, but it is unknown if it can also regulate OC metabolism, thereby defining an adiponectin-osteocalcin loop in humans.
Material and Methods: The clinical associations between serum total and high-molecular-weight (HMW) adiponectin and both serum carboxylated OC (cOC, the active form in the bone) and percent undercarboxylated OC (ucOC, a proxy for OC carboxylase activity) were investigated in a population-based sample of healthy prepubertal children (n=103; 49 boys and 54 girls).
Results: HMW adiponectin was independently related to cOC (β= –1.0, p<0.0001, R2=11%) with a remarkable association in lean children (β= –1.9, p<0.0001, R2=38%). HMW adiponectin was also independently related to percent ucOC and this association was most evident in overweight children (β=2.8, p<0.005, R2=34%).
Conclusion: In a weight-dependent manner, HMW adiponectin is in healthy children related to both cOC and to percent ucOC. These results point to a pos-

sible role of HMW adiponectin in the regulation of OC metabolism in humans.
Supported by grant no. 07/0404 (to A.L.-B.) from Carlos III National Institute of Health (Fund for Health Research FIS, Spain).
Figures: Correlation graphs of high-molecular-weight (HMW)-adiponectin with carboxylated osteocalcin (cOsteocalcin) and with percent undercarboxylated osteocalcin (ucOsteocalcin) in healthy children and in subgroups thereof according to cut-offs of BMI (below or above the median, and in the lowest or highest quartile).



PO2-252 Obesity, Fat II

Do ethnic specific differences in obesity complications exist in children?

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AIM: To characterize ethnic-specific prevalence of obesity complications and hormonal changes in children with BMI > 95 %.
SETTINGS: Urban Pediatric Endocrinology Clinic.
METHODS: 213 obese children were divided according to their ethnicity: African American (AA) group (n= 44, age 11.6±3.3 years), Arabic (A) group (n= 12, age 15.23±2.4 years), Caucasian (C) group (n=28, age 14.32±1.6 years), Hispanic (H) group (n=123, age 12.7±3.5 years) and South-Asian (SA) group (n=6, age 13.83±6.5 years). Anthropometric, biochemical and hormonal variables were compared among the groups.
RESULTS: Hispanic subjects were found to have highest prevalence of elevated cholesterol (40%), highest prevalence of low HDL (57%) and high TG (60%). Also prevalence of elevated ALT was maximal in Hispanics (30.3%). Statistically significant difference was found in average level of LDL in Hispanic group (93.9 mg/dl ±30) vs. Arabic patients (75.7 mg/dl ±11.5), and also average HDL is much lower in Hispanics (43.2 mg/dl ± 9.7) vs. African-Americans (49.1 mg/dl ±10.1, p=0.002).
African-American subjects have maximal prevalence of high LDL among all groups (35.1%). Arabic group had top elevated TSH prevalence (18.8%). Interestingly, all groups had very high prevalence of vitamin D deficiency, with total prevalence of 83.3%.
Statistically significant difference in average cholesterol level was found among Hispanics (160.17± 35.7mg/dl) vs. Arabic group (141.82±19.4 mg/dl). Average ALT in Hispanics (31.65±30.8 U/l) is significantly higher vs. African-Americans (21.6± 16.6 U/l, p= 0.025).
CONCLUSION: This study highlights discrete subgroups of children, who are more likely to present with fatty liver, metabolic syndrome, impaired glucose tolerance and require early screening and close observation.

PO2-253 Obesity, Fat II

Allelic variants of *INSIG2* gene are associated with glucose concentrations and HOMA-IR but not with BMI in Sardinian obese children and adolescents

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Background: Some genetic loci have been unequivocally associated to the obesity phenotype (i.e. the *FTO and the MC4R* genes), while the involvement of other genes is still controversial. INSIG2 protein regulates the transcription of genes promoting fatty acid synthesis and adipogenesis, making *INSIG2* a good biological candidate gene for obesity. Allelic variants of a polymorphism (rs7566605) located approximately 10 kb upstream *INSIG2* gene have been found in association with BMI and with other clinical features related to obesity in some populations but not in others. **Objective:** We tested the association of rs7566605 polymorphism with various clinical features related to obesity in a cohort of Sardinian obese children and adolescents. **Patients and Methods**: We genotyped rs7566605 in 711 Sardinian obese children and adolescents (330 males and 381 females, mean age 12.7 years ± 3.7 SD) and in 565 healthy controls. Genotypes did not deviate significantly from the expected Hardy-Weinberg frequencies. A chi squared test was performed to look for significant differences in allelic frequencies between patients and controls. Patients have been characterized for the following clinical parameters: BMI-SDS, waist circumference, systolic and diastolic blood pressure, insulinemia, glycaemia, HOMA-IR, cholesterol (total, HDL LDL), triglycerides, AST, ALT, TSH, FT3 and FT4. **Results:** Allele frequencies were not different between patients and controls (having 94% of power to exclude an OR=1.3 and 100% to exclude an OR=1.5, at the nominal level of significance P=0.05). Mean glucose concentrations was significantly higher in "CC" homozygous than in "GG" homozygous and "CG" heterozygous. Mean HOMA-IR was significantly higher in "CC" homozygous than in "GG" homozygous. **Conclusions:** Although *INSIG2* polymorphisms do not consistently associate with BMI, the observation of an association with glucose concentration and HOMA would support a role for this gene in the metabolic complications of obesity. Viewed in this light, glucose regulation might represent the link between INSIG2 and the obesity phenotype, thus explaining the inconsistency of association between INSIG2 polymorphisms and BMI by itself. Different prevalences of IFG and/or IGT in different populations might partially explain the contrasting results reported so far.

PO2-254 Obesity, Fat II

Experiences with laparoscopic adjustable gastric banding (LAGB) and sleeve gastrectomy (SG) in the treatment of patients with childhood craniopharyngioma and morbid obesity

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Craniopharyngiomas are embryogenic malformations which lead to eating disorders and morbid obesity due to hypothalamic involvement. From 1996 to 2007 we have recruited 401 patients with childhood craniopharyngioma in our trials (**HIT-Endo, KRANIOPHARYNGEOM 2000, KRANIOPHARYNGEOM 2007**). 40% of all patients with childhood craniopharyngioma developed severe obesity during long-term follow-up. The experiences with bariatric surgeries such as laparoscopic adjustable gastric banding (LAGB) and sleeve gastrectomy (SG) in obese craniopharyngioma patients are limited.
We are reporting on four patients with childhood craniopharyngioma diagnosed at age 2, 11, 12, and 21 years. BMI-SDS at diagnosis was +0.9, +4.5, +4.7 and -0.1SD. During follow-up, all patients developed morbid obesity (BMI-SDS: +13.9, +10.3, +11.4, +7.3) so that 11, 6, 9 and 3 years after diagnosis LAGB

were performed. After a follow-up of 4.5, 1.5, 3.0 and 2.5 years BMI decreased or stabilized continuously in all patients (BMI-SDS at latest visit: +9.9, +9.7, +9.5, +5.9 SD). The eating behavior changed in all patients profoundly. The addiction to food and especially sweets significantly improved based on self-assessment immediately after LAGB. In two patients a dislocation of the LAGB occurred and resulted in weight gain. A 20 years old female with craniopharyngioma and morbid obesity received a sleeve gastrectomy (SG) after dislocation of LAGB and experienced short-term weight loss (BMI: 6.57 SD at SG; BMI: 5.00 SD two months after SG). SG was well tolerated.
We conclude that LAGB could be effective in weight reduction of obese craniopharyngioma patients with hypothalamic syndrome. Close follow-up is necessary in order to analyze long-term effects and complications of LAGB in patients with childhood craniopharyngioma and morbid obesity. The question whether immediate LAGB effects on satiety and eating behavior are mediated by the bariatric procedure or by a functional vagotomy due to LAGB has to be further analyzed.
Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO2-255 Obesity, Fat II

Childhood pituitary adenoma – international registry of children and adolescents from Germany, Austria and Switzerland (HIT-Endo)

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Cases of childhood pituitary adenoma are systematically documented and registered by the German Paediatric Cancer Registry in accordance with international guidelines. In parallel to the surveillance study **KRANIOPHARYNGEOM 2000** and the randomized prospective trial **KRANIOPHARYNGEOM 2007** between 1997 and 2007 twenty-nine German, Austrian and Swiss patients with childhood pituitary adenoma were recruited in the **HIT-Endo** registry. Recruited patients with childhood pituitary adenoma were analyzed for the secreting type of adenoma and the chosen therapy based on the patients' records and the registration information.
Twenty-nine patients (16m/13f) were registered with a median age of 13 years (range: 1-18 years) at the time of diagnosis. Based on the records, visual impairment was a clinical manifestation at diagnosis in 16 patients (55%). In 13 patients treated surgically, a complete resection could be achieved in 7 patients, incomplete resection in 6 patients, no surgery in 10 patients (in 6 patients no information was available). One patient received local external irradiation, 22 patients were not irradiated (no information in 6 patients). Fifteen patients were diagnosed with a secreting adenoma, 8 patients with a non-secreting type of adenoma (no information was available in 6 patients). The patterns of hormonal secretion were: 2 patients GH, 3 prolactin, 1 prolactin + ACTH + GH; 2 prolactin + GH, 5 ACTH, 1 TSH, 1 ACTH + GH.
The completeness of data acquisition has to be improved, in order to draw valid conclusions on epidemiology, treatment and prognosis in patients with childhood pituitary adenoma. In regard to the rareness of the disease, international cooperation in registration of patients and data evaluation is warranted.
Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO2-256 Obesity, Fat II

Meningiomas in childhood and adolescence – prognosis in relation to histological grade and parasellar localization

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Meningioma (MG) are rare tumors during childhood. We analyzed the impact of treatment modalities and tumor localization on prognosis. 42 patients with childhood MG were included in a cross-sectional study (**HIT-Endo**). Pts with neurofibromatosis or MG as a second malignancy were excluded.
Median age at diagnosis of MG was 8.4 years (0.1-17.6 yrs). The histological diagnosis was confirmed by reference assessment in all pts (18 WHO I^o; 16 II^o; 3 III^o; 2 VI^o). The localizations were hemispheric in 25 pts, 6 optical tract, 5 parasellar and 2 cerebellar. Complete resection (CR) was achieved in 24 pts (19 I^o/II^o, 4 III^o/IV^b). Tumor progressions / relapses occurred in 11 of 19 pts after IR, in 10 of 24 pts after CR. Irradiation (XRT) was performed in 16 pts (38% after CR; 63% after incomplete resection [IR]), chemotherapy (XCH) in 6 pts (1 I^o/II^o after IR, 5 III^o/IV^b after CR). MG was non-responsive to XCH in 3 pts, in whom treatment response could be evaluated. 5-yrs-overall (OS) and event-free-survival rates (EFS) were lower ($p<0.001/p<0.05$) in III^o/IV^b (n=5; OS:0.27±0.23, EFS:0.40±0.22) in comparison with I^o/II^o (n=34; OS:0.97±0.3, EFS:0.48±0.11). 5-yrs-EFS in pts with WHO I^o/II^o was related to the degree of resection (CR: n=19, EFS:0.69±0.12; IR: n=15, EFS:0.34±0.15; $p<0.05$). XRT had an impact on EFS in pts with I^o/II^o after IR (XRT after IR: n=8, 3-yrs EFS:0.83±0.15; no-XRT after IR: n=7, 3-yrs EFS:0.33±0.26). The prognoses (OS/EFS) in 5 parasellar MG (1 I^o, 4 II^o) were comparable with I^o/II^o-MG of non-parasellar localization (1 complete remission, 3 stable disease; 1 progressive disease after surgery+XRT in 4 patients and multiple surgery in 1 patient). For MG WHO I^o/II^o a radical surgical strategy and XRT were feasible. MG WHO III^o/IV^b had the poorest prognosis regardless of treatment factors such as the degree of resection, XRT or XCH. Parasellar localization had no significant impact on prognosis. Novel and effective strategies are warranted in childhood MG III^o/IV^b.
Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO2-257 Obesity, Fat II

Rathke cleft cysts – results of a multicenter cross-sectional study on diagnostics, therapy and prognosis in 14 children and adolescents

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In **HIT-Endo** 14 patients (8m/6f) with a Rathke cleft cyst (RCC) were recruited. RCC were compared to 117 patients (60m/57f) with childhood craniopharyngioma (CRA) recruited in **KRANIOPHARYNGEOM 2000** and prospectively assessed for clinical manifestations, treatment and quality of survival (QoS). Histological diagnoses were assessed by a reference panel in all cases.
RCC was diagnosed at an age of 10.2 yrs (3-15), CRA: 10.0 yrs (1-18). The localization of RCC was in 57% intrasellar, 14% extrasellar and 29% combined intra-+extrasellar; CRA: 3% intrasellar, 23% extrasellar, 76% combined intra-+extrasellar ($p=<0.01$). No differences between RCC and CRA were found

in terms of duration of history, endocrine deficits, hydrocephalus, body mass index (BMI) and height at diagnosis and height at last evaluation. RCC patients presented with smaller masses than CRA patients (p=0.001) and without hypothalamic involvement (69% in CRA; p<0.001). Complete surgical resections were achieved in 46% RCC and 59% CRA. Local external irradiation was performed in 14% of RCC (1 pt after 4 relapses, 3 yrs after diagnosis; 1 pt after 2 relapses, 1.2 yrs after diagnosis) and 27% of CRA. We observed a 3-yrs-overall-survival of 1.00 and 0.97 in RCC and CRA, respectively. 3-yrs-event-free-survival rates were higher in RCC (0.82±0.12) when compared to CRA (0.44±0.06) (p=0.03). The follow-up in CRA (3.1 yrs [0.1-7.1]) was influenced by a higher degree of obesity (BMI-SDS at last evaluation: 2.9 [-1.8-14.6]) when compared with RCC (follow-up: 3.3 yrs [0.1-14.7]; BMI-SDS: -0.1 [-1.4-5.9]) (p=0.001). Differences in terms of QoS as measured by Fertikeitenskala-Muenster-Heidelberg (FMH) questionnaire at the time of last evaluation did not reach significance.
We conclude that radical resection is the therapy of first choice in RCC. Irradiation was effective in recurrent RCC. Due to the lack of hypothalamic involvement, obesity had no significant impact on QoS in RCC in contrast to CRA.
Supported by Deutsche Kinderkrebsstiftung, Bonn, Germany

PO2-258 Obesity, Fat II

Adiponectin, resistin, leptin levels in paediatric patients with chronic renal insufficiency: relationships with clinical, auxological and endocrine profiles

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Chronic renal insufficiency (CRI) compromises growth, puberty, glycometabolic, nutritional, endocrine profiles. Adipose tissue has a role, with interference on adipocytokines secretion, also mediated by cytokines as TNFα and IL6. Leptin (**L**) influences growth and puberty, resistin (**R**) the glycometabolic assess and insulin resistance, adiponectin (**A**) is an antiinflammatory and antiatherogenic factor. Few studies evaluated adipokines in paediatric CRI.
31 patients (19M;12F), age 12.1±4.5, with CRI on conservative management were divided in 3 groups: mild 16, moderate 14, severe 1. Clinical and biochemical data (e.g. creatinine, albumin, Hb, cholesterol, HDL, CRP, ferritin), auxological parameters (BMI, BA, Tanner stage, gonadal echographic diameters), fasting and post-prandial glucose, insulin, HOMA-R, HOMA B%, FSH, LH, E2, T, ACTH, cortisol, TSH, fT3, fT4, PRL, IGF1 were correlated with **L, A, R**. We compared all data with a control group of 30 healthy children matched for gender and age.
BMI was 20,5±5,1 (M≡F); 58% had BMI10°-85°centile. Insulin and HOMA-IR were significantly higher than in controls (13,2±4,9vs9,1±3,9; 3±1,3vs1,8±0,8).

		M	F	Controls
IGF1	346.1±211.5	282.7±196.3	409.6±215	
L	24.7±31	15.6±21.7	39.2±38.4	3,9±4,5
R	9±1.5	8.9±1.7	9.26±0.92	9,3±4,2
A	18.9±8.6	17.2±7.7	21.5±9.5	8,6±1,7

L was higher in F (p<0,001) and in CRI vs controls (p<0,001). **L** had a direct correlation with: creatinine (p<0,001), BMI (p0,000), BA (p0,042), pubertal stage (p0,047), IGF1 (p0,002).
R had correlation: direct with CRP (p0,04); inverse with Hb (p0,047), fT4 (p0,028).
A was significantly higher than in controls (p<0,001), and in patients with BMI<85°centile (20.3±8,7 vs 15,2±9,6), with an inverse correlation with BMI (p0,025), BA (p0,029), Hb (p0,041), ferritin (p0,023), proteins (p0,000), albumin (p0,006), urinary creatinine (p0,049).
Increased **L** depends on reduced glomerular filtration rate, chronic inflammation, hyperinsulinism: direct correlation with creatinine, insulin, HOMA-IR was relieved.
Normal **R** expresses adequate nutrition, metabolic assess, controlled inflammatory state: **R** could be an useful marker in follow-up.**A** was higher in patients with BMI<85°centile with a direct correlation with creatinine clearance (p<0,005). **A** could be a protective factor against cardiovascular diseases in this group. These relieves stress the importance of integrate follow-up, to maintain adequate nutritional state, to prevent overweight, reducing cardiovascular risk.

PO2-259 Obesity, Fat II

Comparison of the nerve conduction in obese children with and without diabetes as measured by the NC-Stat® system

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Background:
Diabetic peripheral neuropathy (DPN) is a frequent complication of DM2 in adults. It is characterized by a progressive loss of nerve fibers. Nerve conduction studies (NCS) are the most objective measure of DPN. Previous studies indicate that subclinical DPN is common among children with DM1.
Objective:
To evaluate the presence of subclinical DPN in obese children with DM2
To establish simple and accurate method of screening for DPN.
To identify patients at risk for DPN among obese patients.
Hypothesis:
Subclinical DPN is present in children at early staged of DM2. NC-stat System can identify nerve conduction abnormalities in children and therefore may serve as an effective screen for subclinical DPN in children with DM2
Design: Thirty subjects with normal neurological examination were studied with a NCS test on median, peroneal and bilateral sural nerves. Compound Motor Action Potential (CMAP), Sensory Nerve Action Potential (SNAP), Distal Sensory Latency (DSL), Conduction Velocity (CV) and F-wave parameters for nerves were measured. Children were divided into 3 groups, matched by age and height: Group 1 (DM 2) (n14, 16.9 years ±2.5 BMI 33.6±5), Group 2 (Obese control) (n13, 15.7 ±1.72, BMI 34.7±8.2), Group 3 (Lean control) (n 14, age15.9±2.1, BMI 21.2).
Results:
In Group 1 F wave mean of the median and peroneal nerves, SNAP of the median and sural nerves was decreased, when compared to Group 3 ($p < 0.05$). Girls from Group 1 had increased DSL and decreased CV and SNAP amplitude in the sural nerves compared to Group 3 girls ($p < 0.05$). Group 1 did not show difference compared to Group 2 in NCS.
Group 2 revealed lower DSL of the median nerve, as well as decreased CMAP area and amplitude of peroneal nerve ($p < 0.05$) when compared to Group 3.
Conclusions:
Obese children with DM2 are at risk of developing subclinical DPN. Nerve damages appear in children with DM2 much earlier than clinical symptoms. NC-Stat system is non-invasive, sensitive tool for measurement of motor and sensory nerve conductions and detection DPN in children and adolescents. According to the NCS analysis of motor and sensory nerves obesity along causes significant changes in conductivity regardless of presence or absent of diabetes. Studies from adults indicated that DPN may occur before diagnosis of diabetes had been made, therefore screening of obese children is important intervention that should be implemented when evaluating child for causes and complications of obesity.

PO2-260 Obesity, Fat II

Cardiometabolic risk factors, body mass index and uric acid levels in a cohort of obese Brazilian children

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INTRODUCTION: Obesity has adverse health repercussion in children and adults. Given adiposity follows from childhood into adulthood, and as confirmed by recent studies showing that cardiometabolic risk (CMR) develops during childhood, early recognition of these CMR factors is imperative.
OBJECTIVE: To evaluate the relationship between BMI Z-score and CMR factors as well as anthropometric and body composition parameters in a cohort of obese Brazilian children.
METHODS: Seventy-five subjects (42F, 33M) aged 12.2 ± 2.6 years (6.8-17.5) were evaluated concerning to BMI Z-score, waist circumference (WC), blood pressure (BP), fat mass (FM) and fat-free mass (FFM) percentages assessed by bioimpedance analysis, fasting insulin and glucose levels, 120-minute insulin and glucose response to an OGTT, alanine aminotransferase (ALT), serum uric acid (UA), leptin, triglyceride, total cholesterol, HDL and LDL cholesterol levels. Pearson correlation was used to find relations between continuous variables. ANOVA and Tukey's post-hoc analysis were used to determine differences between BMI tertiles.
RESULTS: BMI Z-score was positively related to WC (r=0.535, p<0.001), FM (r=0.516, p<0.001), UA levels (r=0.627, p=0.02), fasting insulin levels (r=0.260, p=0.045), 120-minute glucose response to an OGTT (r=0.323, p=0.04). It was negatively related to FFM (r=-0.434, p=0.001), and neared the threshold for statistical relation to diastolic BP (r=0.214, p=0.065). The tertile distribution of BMI Z-score showed striking significant differences between tertiles 1 and 3 in relation to WC (96.25 ± 9.04 *vs.* 112.4 ± 16.05 cm, p<0.001), FM (35.2 ± 4.4% *vs.* 40.2 ± 4.6%, p=0.002), FFM percentage (64.2 ± 5.1% *vs.* 59.8 ± 4.6%, p=0.011) and UA levels (4.03 ± 0.78 *vs.* 5.76 ± 1.01 mg/dl, p=0.008), and also between tertiles 2 and 3 for insulin (14.1 ± 9.3 *vs.* 25.3 ± 22.4 mIU/ml, p=0.047) and tertiles 1 and 2 for UA levels (4.03 ± 0.78 *vs.* 5.57 ± 0.92 mg/dl, p=0.017). There were marginally variations between tertiles 1 and 3 for ALT (16.7 ± 7.8 *vs.* 23.95 ± 11.7 IU/ml, p=0.056) and 2 and 3 for 120-minute insulin response to an OGTT (68.6 ± 35.8 *vs.* 120.3 ± 63.0 mIU/ml, p=0.055).
CONCLUSION: The prevalence of CMR factors increases with increasing serum UA. We found clear correlations in this cohort of children between BMI, WC, body composition parameters, fasting insulin and glucose response to an OGTT and also a strong significant association between BMI and UA levels.
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PO2-261 Obesity, Fat II

Effects of three year behavioral childhood obesity treatment on BMI SDS change: age at onset of obesity treatment is the most important predictive factor

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The standard treatment of childhood obesity involves behavioral interventions focused on eating habits and physical activity. Long-term studies are lacking despite that it is unlikely that a short-term treatment is the optimal way to treat a chronic disease.
The aim of this study was to evaluate factors of importance correlated to the effect of long-term behavioral obesity treatment. The primary aim was to study age at onset of treatment and its association with the treatment efficacy measured by BMI SDS. Secondary aims were to study if socioeconomic factors, parental obesity or age at obesity onset were correlated to change in BMI SDS during treatment.
Material and Methods
This is a longitudinal study in patients 6-17 y at the National Childhood Obesity Center, Stockholm (n=555).Patients with other treatments and with syndromal obesity were excluded. Subjects were divided into three groups, based on age at start of treatment, 6-9 (pre pubertal, n=125), 10-13 (pubertal n=263) and 14-16 (late/post pubertal n=167) An ANCOVA was used, with regard to the change in BMI SDS at follow up year 1, 2 and 3 compared to the first visit, including BMI SDS at the first visit as a covariate, and age at start of treatment, age of onset of obesity, gender, parental weight status, socio-economic status as fixed factors in the model.
Results
The mean BMI SDS decline was greater for the youngest age-group compared to the other age groups (p=0.001). In the age group 14-16, the mean BMI SDS decreased during the first year (p<0.05) but not thereafter. Only a weak correlation was found between change in BMI SDS from start to 1y and from start to 3y, r = 0.51 (p<0.001). Among children with no response to treatment during y1 (n=46), 40% had a clinically significant reduced BMI sds (-0.5) at the end of year 3. After 3y 19% of the children 6-9 y, 24% of 10-13 and 14% of 14-16 y old were no longer defined as obese. There were no gender differences. Analyses using LOCF replacement of missing data did not alter these results despite a high dropout rate, only 30% in the oldest age group remained in treatment for 3 years. Factors, such as SES and parental BMI status did not affect the effect of treatment.
Conclusion
Our results indicate that age at start is a key factor for successful behavioral treatment. The predictive value of the results of one year treatment for the long-term outcome was poor indicating that long-term studies are warranted.

PO2-262 Obesity, Fat II

A case of early-onset obesity, hyperfagia and ACTH deficiency due to a novel homozygous mutation in the POMC gene

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Proopiomelanocortin (POMC) is the precursor to five biologically active proteins, including ACTH produced in the pituitary and α-MSH produced in the hypothalamus. POMC has a prominent role in the leptin-melanocortin system: the neurons that expressed POMC are targets of adipocyte hormone leptin signalling, and the α-MSH is the product of POMC cleavage that activates melanocortin-4 receptor (MC4R). Prohormone convertase 1/3 (PC1/3) and PC2 are necessary for the proteolityc cleavage of POMC to each of the active peptides. Activation of MC4R in the hypothalamus by α-MSH relays a satiety signal and causes a decrease in food intake. Mutations in gene with critical roles in the leptin-melanocortin system cause early-onset obesity. We describe a male infant born from healthy Egyptian unrelated parents. Although the birth weight was normal, he was hyperphagic from the first weeks of life and subsequently his weight increased dramatically. He was observed at 8 month of age, showing fair skin, brown hair and an extreme obesity. During the first months of life hypoglycaemia and susceptibility to effects of infection were reported. He presented the cardinal features of congenital POMC deficiency: isolated ACTH deficiency, hyperphagia and severe early onset-obesity. Combined pituitary hormone deficiencies were excluded by pituitary hormone tests and a normal anatomy of the hypothalamic-pituitary region was confirmed by MRI. Direct sequencing of the coding sequence of the POMC gene revealed a homozygous single substitution C6902T in genomic DNA determining a Gln68X substitution. The generation of a premature stop codon predicts an aberrant protein composed of the first 67 amino acids and prevents the production of any normal POMC-derived peptides. The fair skin can depend on the role of POMC-derived peptides in the determination of phaeomelanin to eumelanin ratio in melanocytes. As regards dark hair, we follow the Farooqi and Coll. hypothesis that ascribes it to the genetic background: the retention of dark hair indicates that synthesis of eumelanin in humans is not dependent on the presence of melanocortin peptides. Thus, it can be assumed that, in ethnic groups predominantly characterized by dark hair, other genetic variants act to maintain eumelanin synthesis in absence of POMC-derived ligand. We can conclude

that, in presence of hyperphagia, severe early-onset obesity and ACTH deficiency in a young child, a genetic diagnosis should be considered.

PO2-263 Obesity, Fat II

Effect of weight loss on total and high molecular weight (HMW) adiponectin levels in obese children

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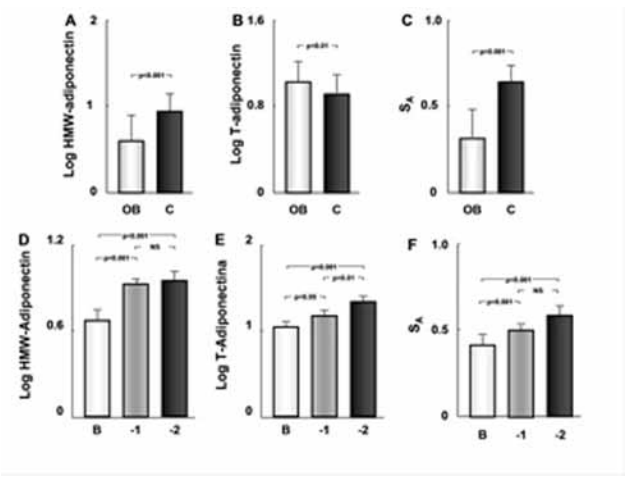
Adiponectin plays a major role in carbohydrate metabolism regulation. It forms high molecular weight (HMW) oligomers after intra-adipocyte post-translational processing. Their quantification and the calculation of the HMW- to total (T-) adiponectin ratio (S_A) could be useful in addressing the risk of early development of carbohydrate metabolism impairment in obese children.
Thus, our aim was to investigate serum HMW-adiponectin levels and S_A in obese children at diagnosis and after weight reduction, analyzing their relationship with insulin sensitivity.
Seventy obese children (**OB**, Tanner I, 48 boys/22 girls) were followed for 2 years and studied at baseline (**B) and after moderate (-1** SD, n=51) and severe BMI reduction (**-2** SD, n=21), and compared with 16 control children (**C**). After overnight fasting, serum T- and HMW-adiponectin levels were determined by RIA and EIA, respectively (Linco®, USA) and HOMA index calculated as (glucose [mmol/l] x insulin [µUI/ml] / 22.5).
Patients showed higher T-adiponectin and lower HMW-adiponectin levels and S_A than controls (Fig.). Weight reduction increased T-adiponectin levels progressively whereas HMW-adiponectin increased at a weight loss of -1SDS, but did not change further after -2SDS of weight loss. (**Fig.**). In the whole study population BMI (SDS) was negatively correlated with HMW-adiponectin levels ($r = -0.31$; $p<0.001$) and S_A ($r = -0.47$; $p<0.001$), but not with T-adiponectin. Also, HOMA index negatively correlated with HMW-adiponectin ($r = -0.33$; $p<0.001$) and S_A ($r = -0.40$; $p<0.001$), as did T-adiponectin and glycemia ($r = -0.22$; $p<0.05$). In the obese cohort no correlations between BMI and HMW-, T-adiponectin or S_A were found, but there was a negative correlation between HOMA index and T-adiponectin levels ($r = -0.35$; $p<0.01$).



HMW-adiponectin levels and S_A index could be useful in the evaluation of obese children as they demonstrate a closer relationship with carbohydrate metabolism status and body fat content in pediatric population than do T-adiponectin levels.

PO2-264 Obesity, Fat II

Serum proteomic profiles of lean and obese children: effect of weight loss

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In childhood obesity, subtle changes often precede overt impairment of carbohydrate or lipid metabolism. The identification of new proteomic biomarkers could be helpful to predict the risk to develop them or to identify early stages of metabolic complications in obese children.
To investigate the influence of obesity and weight loss, the serum proteomic profile of 21 lean controls (7 males (M)/14 females (F), BMI -0.48 ± 0.81 SDS) and 22 obese prepubertal, Caucasian children (13 M/ 9 F, BMI 3.43 ± 1.08 SDS) were compared. Also, a second population of 20 obese prepubertal, Caucasian children (16 M /4 F) was studied at diagnosis (BMI 4.77 ± 1.30 SDS) and after extensive (> 2SDS) BMI reduction. After albumin and IgG depletion, serum samples were subjected to two-dimensional gel electrophoresis. Protein 'spots' displaying significant ($P<0.05$) intensity changes between comparison groups were analyzed by mass spectrometry (MS) and tandem-MS. Protein identities were established using the online softwares Mascot (*[http://www.matrixscience.com]*) and MS-Seeker (*http://www.msseeker.com*).
Significant intensity differences were found in 30 protein 'spots' between lean and obese children. Among them, 23 were down-regulated in obese children including several binding proteins (transferrin, transthyretrin, ceruloplasmin, vitamin D binding protein), 5 apolipoprotein (Apo) A1, and 2 clusterin (Apo J) isoforms. Also, 3 haptoglobin (HP) isoforms with molecular weight (MW) > 60kDa were down-regulated, whereas 4 HP isoforms with lower MW (15-50 kDa) were among the 7 up-regulated 'spots' found in obese children.
Weight loss resulted in significantly increased expression of 6 protein 'spots', including 2 high molecular weight isoforms of HP and 2 isoforms of ApoA1 (MW ~29kDa, pI ~5.4 and MW ~29kDa, pI ~6, respectively). However, no differences in total ApoA1 or HDL cholesterol levels were found after weight reduction by using standard immuno and enzymatic assays.
Changes in several proteins, including some related to lipid metabolism, accompany the onset of childhood obesity, and weight loss can reverse them, at least partially. In addition, proteomic analysis (in particular, detection of isoforms of known proteins) seems to be more sensitive than routinely used assays to detect these early alterations in serum proteins. Quantification of these changes could result in novel biomarkers for the risk of development of obesity related metabolic complications.

PO2-265 Obesity, Fat II

Metformin does not improve markers of cardiometabolic disease (CMD) in children with simple obesity: a 6-month randomized controlled trial

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Background: Obesity is a growing epidemic in children and predisposes them to premature CMD. Our earlier studies showed a significant increase in markers of inflammation and thrombosis in children with simple obesity (i.e. with no other components of the Metabolic Syndrome (MS)), as compared to age-matched lean controls.
Objective: To determine if lifestyle modification with diet and structured exercise, with or without Metformin (500-2000mg/d), improves inflammatory and pro-thrombotic markers, as well as intrahepatic fat contents in children with simple obesity.
Subjects and Methods: 66 children with simple obesity between the ages of 7-18 yrs. were recruited (30 boys, 36 girls; 29 prepubertal, 37 pubertal; mean age ± SE: 12.2 ± 0.3 yrs). Dietary and exercise (both aerobic and strength training) counseling were provided, and subjects given a free YMCA member-

ship. Subjects were followed every 3 months with biweekly phone calls. Body composition was measured by DEXA, intrahepatic fat by fast MRI. Parametric student's t test was used for comparisons of protocol outcomes.
Results: 42 subjects completed 6 months and are included in the analysis. Subjects on Lifestyle/Metformin lost 4.3 ± 1.0 kg vs. 2.0 ± 1.1 kg on lifestyle alone (p=0.14 between group). BMI improved in both groups, with non-statistically significant improvements in waist circumference and intrahepatic fat. The pubertal group on lifestyle alone showed greater improvements in CRP and fibrinogen compared to those on Lifestyle/Metformin (p<0.05). While insulin resistance (HOMA-IR) did not change in either group, adiponectin was favorably modified in both groups. Modest changes were seen in other pro-thrombotic and pro-inflammatory markers (see Table).

Comparison of Results Between Lifestyle/Metformin and the Lifestyle Group (Mean ± SE)

	Lifestyle/Metformin		Lifestyle	
	Baseline	6-months	Baseline	6-months
Weight (kg)	75.4 ± 5.3	71.1 ± 5.3*	83.4 ± 3.8	81.3 ± 3.7
BMI (kg/m2)	30.9 ± 1.3	28.7 ± 1.5*	33.1 ± 0.9	31.9 ± 1**
Waist Circumference (cm)	97.7 ± 2.8	91.1 ± 3.1**	103.5 ± 1.8	101.7 ± 2.4
% Fat Mass (DEXA)	40.6 ± 1.2	36.6 ± 1.6*	40.6 ± 1.1	38.2 ± 1.3**
Intrahepatic Fat (%)	8.6 ± 1.1	8.2 ± 0.4	10.1 ± 1.1	8.7 ± 1
CRP (mg/dL)	2.1 ± 0.4	2.5 ± 0.6	4.7 ± 0.9	3.4 ± 1
Fibrinogen (mg/dL)	386 ± 14.4	384 ± 15.1	411 ± 28.1	364 ± 21.8
IL-6 (pg/mL)	2.6 ± 0.5	3.5 ± 1.1	3.5 ± 0.7	2.1 ± 0.3
PAI-1 (ng/mL)	96.3 ± 8.8	85.5 ± 6.6	91.6 ± 8.6	66 ± 6.1**
TNF-α (pg/mL)	16 ± 5.2	18.9 ± 5.4	12.6 ± 5.5	4.3 ± 1.6
Adiponectin (µg/mL)	12.4 ± 1.7	14.2 ± 1.3**	7.7 ± 1.2	10.4 ± 1.1**
HOMA-IR	4.8 ± 0.5	5.7 ± 0.8	5.5 ± 0.9	6.8 ± 0.8
ALT (IU/L)	26.4 ± 2.8	21.7 ± 1	29.7 ± 3.9	19.3 ± 1.3**

No significanct difference found between pubertal and prepubertal groups
*p<0.001, **p<0.05

Conclusion: Lifestyle modification with diet and structured exercise decreased adiposity in subjects regardless of the addition of Metformin. Although some inflammatory and thrombotic factors showed favorable changes, these interventions produced only modest improvements in these markers, with no difference in those taking Metformin.
Funding: Thrasher Research Fund, WJ Wadsworth Research Gift, Young Men Christian Association (YMCA)

PO2-266 Obesity, Fat II

Aerobic fitness in adolescents with simple obesity: effects of lifestyle modification, with and without metformin

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Objective: To determine if lifestyle modification, consisting of dietary changes and structured exercise with or without the addition of Metformin, improves aerobic fitness and body composition in pubertal children.
Subjects and Methods: 37 children with simple obesity (i.e. no other feature for the Metabolic Syndrome) in late puberty, between the ages of 11-18 were recruited (16 boys, 21 girls, mean age: 14 ± 0.3 yrs). Subjects were counseled regarding proper diet and exercise to promote weight loss and enhance physical fitness. They were provided a free supervised YMCA membership and encouraged to attend 3 times per week for at least 30 minutes per session, with structured exercise including both aerobic and strength training. Patients were randomized to lifestyle modification with or without Metformin (500-2000mg/d divided BID) for 6 months. Cycle ergometer maximal oxygen consumption at exhaustion (VO_2max) was measured in all subjects at baseline and at 6 months. Body composition was measured by DEXA. Parametric student's t test was used for comparisons of protocol outcomes.
Results: 21 of 37 subjects completed 6 months of the study, 16 completed the exercise testing and are included in the analysis. 16 children did not complete the study due to family and social issues. Data in all 16 subjects that completed VO_2max determinations showed that weight decreased by 4% (p=0.01), BMI by 5.5% (p=0.002), absolute % body fat decreased by 3.6% (p=0.002), and VO_2max improved 10.3% (p=0.061). There was no difference in benefit in those that had lifestyle modifications and also took Metformin.
Conclusions: Lifestyle modification with dietary counseling and structured exercise clinically significantly increased aerobic fitness, as measured by VO_2max, after 6 months with modest weight loss and favorable changes in body composition. However, Metformin did not have any additional effect above lifestyle modification for improving VO_2max or body composition.
Funding: Thrasher Research Fund, Young Men Christian Association (YMCA)

PO2-267 Obesity, Fat II

Autonomic dysfunction in the overweight adolescent

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It is known that there is an association of obesity with hypertension and diabetes in adults. This association becomes stronger as the degree of obesity worsens. However, the etiological link between obesity and hypertension is not well defined. One possible explanation is an imbalance of parasympathetic and sympathetic tones in the body with either a relative increase in sympathetic activity, a decrease in parasympathetic activity, or both. This autonomic dysfunction(AD) can be determined non-invasively using heart rate variability. Low frequency domain or LF is a measure of sympathetic nervous activity while high frequency domain or HF is a measure of parasympathetic nervous activity. Both of these are determined by power spectral analysis of RR intervals. The low frequency to high frequency ratio or LF:HF is measure of overall sympathovagal balance and will be one of the main outcome variables of this study.
There are studies that have shown overweight children and adolescents have an imbalance in parasympathetic and sympathetic tones, however no published studies have analyzed correlations of AD with biochemical or hormonal abnormalities. While the proximate cause of the autonomic imbalances is not known, our study further characterizes AD in obese adolescents.
In this study, we evaluated 25 overweight adolescents with BMI>85%. These patients underwent evaluation with fasting glucose, insulin, lipid panel, and heart rate variability testing. Heart rate variability data was gathered at rest, with deep breathing and valsalva maneuvers, and with standing. All 25 individuals were found to have some quantifiable abnormality in one or more of testing stages, however not all had elevated blood pressure. This suggests that other factors, perhaps degree of adiposity and duration of obesity also play a role in development of early cardiovascular changes. Our data did not show any statistically significant correlation between LF, HF, or LF/HF ratio with HOMA or with the presence or absence of metabolic syndrome; however this may be attributed to the power of study at the time of this analysis. Our data did find a statistically significant negative correlation with BMI and LF/RF ratio. Based on these results, we suggest that measurement of AD may become a clinically relevant study, offering independent information, relevant to the early detection of individuals at higher risk of developing obesity related cardiovascular disease.

PO2-268 Obesity, Fat II

Does leptin levels affect cardiometabolic factors independently of adiposity in obese children?

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BACKGROUND: Increased body mass index (BMI) and waist circumference (WC) are related to cardiometabolic risk (CMR) factors. Studies assessing whether leptin independently affects CMR factors are controversial. Some studies assert that leptin is not an independent factor of adiposity affecting CMR, while others affirm that it aids in predicting a deterioration of the CMR features independently of obesity. It is well known that leptin is synthesized in

white adipose tissue and correlates with its level. Therefore, because BMI is not a precise measure of fat mass (FM), it is possible that even after adjusting the associations between leptin and CMR factors for BMI, residual confounding by adiposity could persist.
OBJECTIVE: To evaluate the relationship between leptin levels, leptin levels adjusted for FM and anthropometric body composition and CMR profile in a cohort of obese Brazilian children.
METHODS: Seventy-five subjects (42 girls and 33 boys) aged 12.2 ± 2.6 years (6.8-17.5) were submitted to anthropometric (BMI Z-score, WC), body composition (FM and fat-free mass [FFM] percentage assessed by bioelectrical impedance analyzer) and CMR profile (fasting insulin and glucose levels, 120-minute insulin and glucose response to an oral glucose tolerance test [OGTT], HOMA-IR, blood pressure [BP] and lipid profile). Pearson correlation was then used to determine associations between continuous variables.
RESULTS: Leptin levels were positively related to HOMA-IR (r=0.409, p=0.005), insulin levels (r=0.439, p=0.002), WC measurement at the midpoint between lowest rib and iliac crest (r=0.358, p=0.017), WC at the iliac crest (r=0.323, p=0.03), 120-minute glucose response (r=0.689, p<0.001) and 120-minute insulin response (r=0.599, p<0.001) to an OGTT. Leptin levels were negatively related to FFM percentage (r=-0.324, p=0.036). A trend was documented between leptin levels and FM percentage (r=0.294, p=0.058), but leptin levels showed neither a relationship to systolic and BP percentile, fasting plasma glucose nor to triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol levels or BMI Z-score. Furthermore, all correlations were lost when the analysis was performed with leptin levels adjusted for FM.
CONCLUSION: In summary, though some studies assert that leptin is an independent factor adversely affecting CMR factors, our study suggests that leptin adjusted for FM does not have a regulatory role for CMR in obese Brazilian children.

PO2-269 Obesity, Fat II

Prevalence of the metabolic syndrome (IDF Criteria, 2007) in obese Spanish children and adolescents

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The metabolic syndrome (MS) is a constellation of risk factors for the development of atherosclerotic cardiovascular disease and type 2 diabetes mellitus. Excessive abdominal fat is the main independent risk factor for insulin resistance and the MS. Therefore, The International Diabetes Federation (IDF) recently recommended including waist perimeter measurements as the main diagnostic criterion in the definition of MS.
AIMS: To ascertain the prevalence of MS in obese Spanish children and adolescents and establish its correlation with the degree of obesity.
PATIENTS AND METHODS: Three hundred and forty-six obese Caucasian patients (180 males) aged from 6 to 20 years (mean age: 11.7±2.9) were studied. BMI distribution was: BMI between +2 and +3 SD: 36%, BMI between +3 and + 4 SD: 32% and BMI > 4 SD: 32%. IDF 2007 criteria were used for MS classification: abdominal obesity (waist perimeter >P90 for age and sex) and two of the following criteria: triglycerides >150mg/dl, fasting glucose >100 mg/dl, HDL-C <40 mg/dl, systolic BP >130 mm Hg or diastolic BP > 85mm Hg. For patients over the age of 16, waist perimeter was modified: women > 85 cm and men > 95 cm and HDL-C in women < 50 mg/dl.
RESULTS: The overall prevalence of the MS was 10.7%, with no inter-sex differences. The prevalence of systolic BP was 19.9%, diastolic BP: 7.3%, hypertriglyceridemia: 10.9%, HDL-C < 40 mg%: 24.7% and glucose > 100 mg/dl: 2.9%. The prevalence of the MS by age group was: children < 10 years: 3.5%, from 10 to 16: 11.1% and > 16: 21.7% (p= 0.01) and BMI by groups was: between +2 and +3 SD: 5.5%, between +3 and +4 SD: 8.2% and > 4 SD: 19% (p= 0.003).
CONCLUSIONS: 10.7% of obese Spanish children and adolescents in our study met MS criteria (IDF 2007). The degree of obesity and age are major factors in the rise of MS prevalence.

PO2-270 Obesity, Fat II

Adipocytokines (adiponectin, IL-6, RBP4), uric acid and HOMA index as predictors of the metabolic syndrome in obese children and adolescents

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Introduction: Detection of the metabolic syndrome (MS) in obese children and adolescents is essential to prevent the development of cardiovascular disease and type 2 diabetes later in life.
Aims: To ascertain whether the adipocytokines: total adiponectin (total Adp), high-molecular-weight hexamer (HMW Adp), interleukin 6 (IL-6), retinol-binding protein (RBP4), serum uric acid and HOMA index predict the MS in obese children and adolescents.
Patients and Methods: Prospective study of 346 Caucasian obese patients (180 males) aged between 6 and 20 years (mean age: 11.7±2.9) with BMI 3.6±1.3. The area under the ROC curve (AUC ROC) was calculated to determine the capacity of each of the adipocytokines analyzed, and uric acid and HOMA index to predict the presence of the MS in obese children and adolescents. IDF criteria (2007) were used for the pediatric population to establish the presence of the MS. Plasma total Adp and HMW Adp levels were determined by ELISA, IL-6 by the highly sensitive quantitative sandwich enzyme immunoassay technique and RBP4 for nephelometry.
Results: The number of determinations used, AUC ROC value and its confidence interval are shown in the table.

Uric acid and cytokine predictive capacity of the metabolic syndrome

	n	AUC ROC	Confidence Interval
HOMA	283	0.674	0.573-0.775
Uric acid (mg/dl)	236	0.682	0.579-0.786
RBP4	245	0.579	0.454-0.706
Total Adp	76	0.703	0.528-0.877
HMW Adp	76	0.757	0.614-0.900
IL-6	83	0.744	0.566-0.922
Uric acid + RBP4	236	0.701	0.575-0.828
Uric acid + total Adp	72	0.780	0.643-0.917
Uric acid + HMW Adp	72	0.797	0.666-0.928
Uric acid + IL-6	81	0.794	0.653-0.936
Uric acid + IL-6 + HOMA	81	0.864	0.733-0.996

Conclusions: The predictive capacity of the presence of the metabolic syndrome in obese children and adolescents increases significantly when serum uric acid, IL-6 and HOMA index values are available.

PO2-271 Obesity, Fat II

Increased hepcidin levels in childhood obesity: the missed link between adiposity and disrupted iron metabolism

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Obesity and iron deficiency are two of the most common nutritional disorders worldwide. Several studies have consistently found higher rates of iron deficiency in obese than in normal weight children. Although, in the past, eating unbalanced meals have been typically proposed to explain the association between iron deficiency and obesity, recently some studies have supported the idea that, among obese subjects, iron deficiency could represent one of the co-morbidities associated to the typical low-inflammation state of this disease. Hepcidin, a recently discovered peptide produced mostly in the liver but also in the adipose tissue, represents the main inhibitor of intestinal iron absorption. Hepcidin is increased in generalized inflammatory disorders and its expression is increased in adipose tissue of obese patients. It has been also demonstrated that leptin is able to raise hepcidin expression using the STAT3 signalling

pathway.
We have investigated iron status and absorption in 64 obese children (28 girls; mean age: 11.3 +/- 2.5 years; mean BMI-SD: 2.7 +/- 0.5) and in 50 sex and age matched lean controls. In the same subjects we have evaluated plasma hepcidin and leptin concentrations. Iron status has been assessed by serum iron, transferrin and ferritin concentrations. Iron absorption has been studied administrating to the children ferrous sulphate (1 mg/kg) and calculating the increase of iron concentration in the plasma 2 hours after the loading (i.e.; delta iron). Leptin was evaluated by ELISA and the concentration of hepcidin was determined by using a combination of weak cation exchange chromatography and surface enhanced laser-desorption/ionization time-of-flight mass spectrometry. We found lower iron and transferrin saturation (both $p<0.05$) in obese children compared to lean controls. Obese children showed higher serum hepcidin compared to controls ($p=0.02$). Furthermore, we observed statistically significant inverse correlations between serum hepcidin and iron (r2: -0.14, p= 0.05), hepcidin and transferrin saturation (r2: -0.22, p= 0.005), hepcidin and delta iron (r2: -0.37, p= 0.003) and a direct correlation between hepcidin and BMI-SD (r2: 0.33, p: 0.0015). Finally, we observed a direct correlation between leptin levels and hepcidin (r2: 0.27, p= 0.006). We suggest that, in obese patients, increased hepcidin production, likely leptin mediated, can represent the missed link between adiposity and disrupted iron metabolism.

PO2-272 Obesity, Fat II

Overweight adolescents: a group at risk for metabolic syndrome (Tehran adolescent obesity study)

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Background: Metabolic syndrome not only is a serious problem for adults, but is also afflicting an increasing number of children and adolescents. This syndrome is a risk factor for type 2 diabetes mellitus and cardiovascular diseases. The aim of this study was to estimate the prevalence of metabolic syndrome in a sample of Iranian adolescents.
Methods: A total of 554 overweight adolescents (aged 11 – 17 years) participated in a
community-based cross sectional survey. Anthropometric examinations including height, weight, body mass index, and blood pressure were assessed. A fasting blood sample was taken for measurement of glucose and lipid profile. Metabolic syndrome was determined by the definition released by the National Cholesterol Education Program Adult Treatment Panel III, which was modified for age.
Results: The overall prevalence of metabolic syndrome was 26.6%. There was no gender difference in the distribution of metabolic syndrome. When stratified by body mass index, 22.5% were overweight (BMI≥95th percentile) besides having the criteria for metabolic syndrome, while the remaining 4.1% of the adolescents were at risk for overweight (BMI between 85th and 95th percentile) together with metabolic syndrome. Hypertriglyceridemia was the most common and high-density lipoprotein was the least common constituent of metabolic syndrome.
Conclusion: This study suggests a high prevalence of metabolic syndrome among overweight Iranian adolescents. This poses a serious threat to the current and future health of Iranian youth.

PO2-273 Obesity, Fat II

Two years follow up results of a multidisciplinary primary care intervention for childhood obesity

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Objective: to present the 2-years follow-up results of a multidisciplinary primary care intervention for childhood obesity. The evaluation focused on diet, physical activity habits and BMI Z-score evolution.
Patients and method: 83 children (of whom 41 overweight and 42 obese) aged 2 to 17, had a coordinated multidisciplinary approach initiated by their primary care physician, helped by other health professionals (dietician, psychologist, physiotherapist) under coordination by a regional network for prevention and treatment of childhood obesity (RePPOP in Franche-Comté). The therapeutic intervention, scheduled over a 2 years period, is based on patient education in order to modify food intake and life habits. A food behavior score from 0 to 10 was calculated, based on dietary habits including snacking or breakfast skipping habits, meal frequency, sizes of portions, and sugar sweetened beverage intake (0: diet habits were out of range of the recommendations for health nutrition and 10: diet habits were adapted). Fruit and vegetable intake was recorded and physical activity amount evaluated.
Results: mean follow up time was 20 months (1-33) and 54 children (65,1%) were followed during 2 years. 29 children (34.9 %) stopped the follow-up before 24 months for these reasons: 9 were lost to follow-up immediately after the first visit and 11 later because lack of motivation, 3 stopped because of success of the educational care and 3 because of failure, 3 had bad relationships with health professionals. At the beginning of the study, the mean BMI Z-score was 3.38 (1.14-6.25), mean food behaviors score was 5.3, mean fruit and vegetable consumption was 1.54 per day and mean physical activity was 3.5 hours per week. After two years: the average BMI Z-score decreased from 3, 38 to 2,93 (p=0,0001), physical activity improved (3.5 to 4.83 h per week, p=0.015), food behavior scores rose significantly (5.29 to 7.88, p=0,00001), as did fruit and vegetable intake (1.54 to 2.54 per day; p=0.0001).
Conclusion: a close multidisciplinary approach of childhood obesity involving the primary care physician under supervision and coordination of a specialized network can ensure, not only BMI improvement in substantial proportion of patients, but also a significant change in life habits. These results and the 65% compliance rate achieved in this program warrant confirmation on a larger scale.

PO2-274 Obesity, Fat II

Sex dimorphism in fetal programming of metabolic syndrome in rats

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Introduction
Epidemiological evidence suggests that early-life events play an important role in determining the risk for common cardiovascular and metabolic disorders in adulthood. In particular, intrauterine growth restriction (IUGR) newborns are associated with a substantially greater incidence of adult hypertension, insulin resistance/type 2 diabetes mellitus and cardiovascular disease. The mechanisms that link IUGR and adult disease remain unknown but are of clear importance. We have shown that there were sex differences in terms of sensitivity to be metabolic syndrome in later life in IUGR model rat. The aim of this study was to investigate whether female and male offspring rats show metabolic disorders in adulthood.
Material and Methods
Pregnant rats received control(C) or restricted food(R) from day 7 of pregnancy to term gestation. After weaning, offspring were given control(C) or high fat diet(H).They were assigned to 4 groups : i CC, ii CH, iii RC and iv RH(the first letter indicating dams' and the second representing pups' diet, respectively). Blood pressure and blood glucose were measured at the age of 11 weeks.
Results
Birth weights of both male and female offspring of restricted dams were about 20% less than those of control dams. However, at 11 weeks of age only in male offspring of restricted dams, body weight reached the same levels as control counterparts. Blood pressures were higher in restricted groups than control groups especially in male rats. Blood glucose levels were significantly increased in RH group compared with other groups in male rats. There were no significant changes in corticosterone levels between restricted and control groups in male rats, whereas corticosterone levels were greatly higher in high fat-fed groups(CH,RH) than their corresponding control groups(CC,RC) in female rats.
Conclusions
Present data showed that only male IUGR offspring revealed increased weight gain, blood glucose levels and high blood pressure in adult. Our result suggests that male IUGR offspring are easy to develop metabolic disturbances and females have much tolerance to metabolic syndrome in later life.

PO2-275 Obesity, Fat II

Association between the hepatosteatosis and impaired glucose tolerance and insulin resistance in obese adolescents

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We aimed to show the hepatosteatosis and the grade of it in obese adolescents and also show the relation between hepatosteatosis and impaired glucose tolerance and insulin resistance.
A two hours oral glucose tolerance test and abdominal ultrasonography were performed for 98 obese adolescents (56 females, 42 males) whose body mass indexes were above 95. percentile, between the ages of 11 and 18 years. All participants were evaluated for glucose intolerance and insulin resistance (HOMA-IR).
The mean age was 13,15±3,8 years. Hepatosteatosis was detected in 36 (36,7%) of the obese adolescents.
Impaired glucose tolerance during fasting was found in 19 (19,3%) and insulin resistance was found in 14 (14,3%) of them. Hypertriglyceridemia was found in 12 (12,2%) and hypercholesterolemia was found in 9 (9,1%) of them. Thirtytwo (32,3%) of the participants had obese parents.
Hepatosteatosis was found in considerable ratios (36,7%) in our obese adolescents. The hyperinsulinemia and impaired glucose tolerance prevalence was higher in cases who had hepatosteatosis. There was a direct relationship between the grade of hepatosteatosis and body mass index.

PO2-276 Obesity, Fat II

Impact of a high-fiber nutrition plan (HFNP) in the management of obese minority, low-income children

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Childhood obesity (CO) is a world wide epidemic, and is the result of a confluence of factors: increased consumption of cheap, energy dense fast foods, and decreased physical activity are common and have been documented throughout the world.
While the consequences of CO are formidable and costly, resources for nutrition education and dietary counseling are limited.
Objective To study the impact of increased daily fiber (HFNP) on body mass index (BMI) and metabolic parameters in a low-income, at risk, outpatient pediatric population.
Intervention Participants and their parents were instructed to incorporate (age in years plus 5) grams of fiber per day into their daily diets, using the USDA publication, Fiber Content of Foods (Nut-GEN-033-2005). Each participant was provided with a logbook and instructed to record the grams of fiber consumed daily, as well as daily exercise using a pedometer.
Design, Setting, and Participants Subjects were recruited from the Pediatric Diagnostic Center in Ventura, California. Twenty-two Hispanic patients, 14 girls and 8 boys, participated. Data collected at baseline and at 3 months included age, sex, BMI, fasting glucose, serum insulin, lipid profile and HgbA1c. Compliance was assessed by review of patient logs.
Results Subject BMIs ranged from 25.0 kg/m^2 to 48.6 kg/m^2 with a mean z-score at initial visit of 4.6 $\pm$ 2.81. Compliance was nearly 60%. Changes in BMI were 0.18 kg/m^2, 0.25 kg/m^2, and -0.45 kg/m^2 for ages 5-10 years, 11-15 years, and 16-18 years respectively. For children ages 12-17 years, 58% lost weight during the intervention period. Pre-intervention BMI was 32.5 $\pm$ 6.69 kg/m^2 vs. 32.08 $\pm$ 6.57 kg/m^2 ($p < 0.09$) by paired t-analysis.
Serum insulin levels decreased after three months (29.16 $\pm$ 15.82 mU/ml vs. 27.33 $\pm$ 4.04 mU/ml) as did mean triglycerides (198 mg/dL to 159 mg/dL). LDL cholesterol (106.9 mg/dL to 92.8 mg/dL) was reduced by 13.16%.
Summary and Conclusion HFNP simplifies the selection of foods that are low in fat and simple carbohydrates. **P**reliminary findings suggest that HFNP is associated with good compliance and promotes healthful changes in the pediatric population. Future prospective studies are warranted to evaluate potential long-term endocrine and cardiovascular benefit.

PO2-277 Obesity, Fat II

BMI changes and metabolic factors in children with Prader Willi syndrome (PWS)

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PWS is characterised by growth failure, hypotonia, hypogonadotrophic hypogonadism and bi-phasic body composition with early feeding problems and failure to thrive and late hyperphagia and obesity. Previous reports demonstrate increase risk of type 2 diabetes in PWS in the apparent absence of insulin resistance. We studied our PWS cohort for confirmation of these findings and assessed evidence of early metabolic complications.
We studied children (27, male 16, age range 1.4-16.3 y) with PWS (confirmed by genetic testing) using records on our clinical database at the Mater for age (CA), sex, height, weight, BMI, BMI SDS (CDC 2000) and fasting insulin, glucose, cholesterol and triglyceride. We compared BMI SDS, fasting glucose and insulin in two groups age <8y (n = 16) and >8 y (n =11). Statistical analysis for linear regression was performed using SPSS.
Overall there was a positive association between age and BMI SDS (r= +0.26, P= 0.00). This was not associated with the onset of puberty, GH therapy or sex hormone replacement. Mean BMI SDS was – 0.44 (± 0.71) in <8 y and +2.64 (± 1.52) in >8 y (P=0.009). Despite the significant differences in BMI SDS the mean fasting serum insulin level in <8 yr of 5.23 mIU/L (±2.75) was not different (P=0.63) from the >8 yr group 7.23 mIU/L (±5.20). Mean fasting blood glucose level for <8 yr, 4.61 (±0.41) was not clinically significantly different compared to >8 yr was 4.49 (±0.39) (P=0.06). There was no correlation between insulin and BMI SDS in <8y group (r= 0.26, P= 0.55) nor in the >8y group (r= 0.0.23 P= 0.39). Cholesterol {<8 yr, 4.39 mmol/L (±0.81), >8 yr, 4.28 (±0.63), P= 0.63} and triglyceride {<8 yr, 1.10 mmol/L (±0.73), >8 yr, 1.09 (±0.62), P= 0.98} for the two groups were also not different. No patient had an elevated HbA1c.
We conclude that older children with PWS despite of BMI increase the expected metabolic defects associated with obesity of elevation of fasting serum glucose, insulin, cholesterol and triglyceride were not seen. In other populations at high risk of Type 2 diabetes such as indigenous children in Australia there is a very close relationship between these factors observed by 10 yr. We hypothesise that these differences may be due to dysautonomia seen in PWS.

PO2-278 Obesity, Fat II

Abstract Withdrawn

PO2-279 Obesity, Fat II

Serum leptin and ghrelin levels in infants with protein energy malnutrition

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Protein-energy malnutrition (PEM) is a clinical problem caused by inadequate intake of one or more nutritional elements, and remains as one of the most important health problems in developing countries. Leptin is a protein hormone secreted by adipocytes, regulating body fat and food intake. Ghrelin, another appetite-regulating peptide appetitate hormone, plays an important role in controlling the feeding and nutritional states by signaling hypothalamic centers.

This study was performed to investigate the relationship between body composition and serum leptin, ghrelin concentrations in protein energy malnutrition. The study group consisted of 15 children diagnosed with PEM and 15 healthy controls. Ghrelin and leptin levels were independent of age and sex ($P > 0.05$). Although serum leptin levels of infants with PEM were significantly lower, the mean serum ghrelin levels were significantly higher than controls.
Serum ghrelin is elevated in PEM as an adapting consequence of the malnutrition rather than a primary event. The decrease of energy intake and adipose tissue in infants children with PEM may result in decrease of leptin secretion. Decrease in serum leptin levels may initiate food intake by increasing appetite and it may be an important signal to reflect the metabolism of children with PEM.

PO2-280 Obesity, Fat II

Oxidative stress, obesity, and features of the metabolic syndrome in children: is there a link?

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Introduction: ~ 1/3 of obese adolescents are affected by the metabolic syndrome, a cluster of risk factors for cardiovascular (CV) diseases caused by insulin resistance. Obesity and insulin resistance track from childhood into adulthood and are associated with an increased risk for type 2 diabetes and CV diseases. Oxidative stress is implicated in the pathogenesis of diabetes mellitus, hypertension, and atherosclerosis. Studies in adults have shown a link between obesity, insulin resistance and oxidative stress. In contrast, little is known on the presence of this association in children
Methods: Children with BMI > 95th percentile attending the outpatient clinic of the Section of Endocrinology and Diabetes at St. Christopher's Hospital for Children were prospectively enrolled in this IRB-approved study. Exclusion criteria included: history of hypertension, diabetes, or medications known to affect glucose metabolism. In all enrolled subjects, the following parameters were recorded: weight, height, BMI, and waist circumference. Fasting blood glucose, insulin, lipid panel, and urine microalbumin and 8-isoprostane (a marker of oxidative stress) were collected. For some of the enrolled subjects, an OGTT was performed and the 24-hour ambulatory blood pressure was monitored at home.
Results: 25 children (14 males), 12.3 ± 2.5 yrs. old (mean ± SD) were included in the study. 44% were Hispanic, 40% African-American, and 16% Caucasian. Their BMI-SDS was 2.4 ± 0.4. When we analyzed the urine 8-isoprostane levels according to the subjects' BMI SDS, those with more severe obesity (BMI-SDS >2.5) had a higher 8-isoprostane compared to subjects with mild/moderate obesity (BMI-SDS < 2.5) ($p< 0.004$). When we grouped the study sample according to the HOMA quartile (lower vs. highest), there was no difference in the urine 8-isoprostane between the two groups. Lastly, we found no correlation (by Pearson correlation) between 8-isoprostane and the following parameters: BMI-SDS, fasting glucose and 2-hour glucose (by OGTT), peak insulin, HOMA, WBISI, triglycerides, HDL- and LDL-cholesterol, urine microalbumin, mean 24-hour systolic or diastolic blood pressure.
Conclusions: Our findings suggest the occurrence of increased oxidative stress in children with more severe obesity. The lack of correlation between oxidative stress and several markers of the metabolic syndrome must be re-evaluated using additional markers of oxidative stress in a larger population sample.

PO2-281 Pancreas II

Global outcomes for congenital hyperinsulinism

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Congenital hyperinsulinism (HI) is a rare genetic disorder with a spectrum of symptom and treatment severities. It is mainly characterized by a dangerously low blood glucose level with inappropriately high levels of insulin. Although the endocrine or metabolic dysfunction is understood in some cases, most cases of HI are formally diagnosed only through fasting challenges and/or appropriately timed blood tests. In the most severe cases, HI presents in the first few days of life and may need partial or nearly complete pancreatectomy to be treated. Many cases of HI go undiagnosed or are misdiagnosed for several months or years.
Because the condition is so rare, the causal links often unknown, and the effects of chronic HI-induced hypoglycemia on a developing brain not understood, there are large gaps in our knowledge of neurological and physical outcomes. Most medical studies are restricted to single countries, single HI subtype, and small sample sizes. We report on a much larger set of 139 patient outcomes from an informal international community of families from 20 countries. Although our sampling methods are not statistically rigorous (we are not a research organization), our collection represents the largest and broadest sample of HI patients in existence. We wish to open a dialogue and explore future steps we may take as a patient/support organization that will be of greater help to the HI research community.

PO2-282 Pancreas II

Donor treatment with cobalt-protoporphyrin prolonged islet allograft survival by HO-1 expression

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Background
Islet transplantation is a promising treatment for diabetes. However, besides the rejection, the implanted islet cells also will lose glucose-stimulated insulin release by the proinflammatory cytokines and free oxygen radicals released in transplanted sites. HO-1 has been described as an inducible protein capable of cytoprotection via radical scavenging and apoptosis prevention. This study examined whether only treating donor with cobalt-protoporphyrin induce HO-1 and prolong the allogenic islet graft survivial.
Methods
Islets were isolated from BN rats receiving a two intraperitoneal dose of CoPP (2.5mg/kg) at 3 days and 1 day prior to islet isolation. Cytoimmuno-fluorescence and western blot were used to detect the HO-1 expression on the islets. Glucose-stimulated insulin release was measured in an ELISA kit. Lewis rats were rendered diabetic by intravenous administration of freshly prepared alloxan (60mg/kg).HO-1 expressed islets were implanted into renal subcapsular of Lewis rats.
Statistical analysis
All values were expressed with mean ±SE. Difference between groups were evaluated by an analysis of variance, *P* value less than 0.01, was considered significant.
Results
HO-1 expression was found on the Islets isolated from CoPP-treated rats by cytoimmunofluorescence. Additionally, HO-1 protein was also manifested by western blot. The glucose-stimulated insulin release was used to assess the function of cultured rat islets. A significantly insulin release response was detected in HO-1 gorups which treated with CoPP. The glucose stimulation index was 2.98±0.10. However, the glucose stimulation index of the control groups, was significantly lower (1.55±0.01, $P<0.05$). When the HO-1 expressed islets (1500 IEQ) were transplanted into the diabetic rats, the recipients achieved ecuglycemia within 2 days, but returned to a diabetic state by 12.20±5.67 days post transplant. However, in the control groups, the recipients achieved ecuglycemia within 3 days, but returned to a diabetic state by 5.60±1.14 days post transplant. Compared with the two groups, islet allograft survival of the HO-1 group was significantly prolonged. The histology analysis revealed the presence of more islands of insulin-positive cells and considerably fewer lymphocytes or inflammation infiltration than the controls.
Conclusions
We conclude that only donor treatment with cobalt-protoporphyrin enhances the islet function and prolonged islet allograft survival by HO-1 expression.

PO2-283 Pancreas II

Silencing epigenetic modifications are responsible for decreased Pdx-1 expression in liver compared to pancreatic islets

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Background: *Pdx1* is a pancreatic and duodenal homeobox transcription factor that is obligate for beta cell function and development, and is preferentially expressed in pancreatic beta cells. Absence of *Pdx1* leads to pancreatic agenesis in both humans and animal models, and haploinsufficeincy of *Pdx1* in humans and animal models leads to b-cell dysfunction and type 2 diabetes. Epigenetic modifications play a key role in regulation of *Pdx*1 transcription in islets. In previous studies we found that robust histone acetylation and methylation (H3Ace, H3K4me3) and absence of DNA methylation at the proximal promoter of *Pdx1* is requisite for normal expression in islets. In contrast, the silencing histone modification, H3K9 methylation and DNA methylation, result in a marked decrease in *Pdx1* expression. The aim of this study was to map and compare the histone code of the *Pdx1* proximal promoter in tissues with variable levels of *Pdx1* gene expression, namely pancreatic islets and liver.
Hypothesis: 1) Silencing histone modifications, including decreased histone H3 acetylation, decreased histone H3K4 methylation and increased histone H3K9 methylation, are present at the proximal promoter of *Pdx1* in liver samples compared to islets. 2) DNA methylation within the CpG island at the *Pdx1* proximal promoter is increased in liver as compared to islets.
Methods: Liver and islets samples from male Sprague-Dawley rats were harvested at 6-8 weeks of age. Gene expression was measured by quantitative PCR analysis. Histone modifications were evaluated by chromatin immunoprecipitation assay and quantitative PCR analysis. DNA methylation was measured by pyrosequencing analysis.
Results: Pdx1 gene expression was decreased significantly in liver compared to islets (5000 fold decrease, p=0.005). Liver H3 acetylation is decreased approximately 90% and H3K4 trimethylation was 30% decreased at the proximal promoter of *Pdx1* compared to islet samples. H3K9 dimethylation was increased approximately 4 fold and binding of the transcription factor USF-1 was decreased 65% in liver compared to islet samples. Interestingly, DNA methylation at the proximal promoter of *Pdx1* in liver did not differ from islets suggesting that DNA methylation does not play a role in determining tissue specific expression of Pdx1.
Summary: Histone modifications play a role in determining tissue specific expression of Pdx1.

PO2-284 Pancreas II

Congenital hyperinsulinism associated with signs of cerebellar dysfunction not related to hypoglycaemic brain injury

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Background: Congenital Hyperinsulinism (CHI) is a major cause of hypoglycaemic brain injury in the newborn and infancy period. The hypoglycaemic brain injury presents with seizures, developmental delay, cognitive defects and cerebral palsy. Magnetic resonance imaging (MRI) of the brain in patients with CHI typically shows involvement of the parieto-occipital regions with sparing of the cerebellum. Patients with CHI usually show no signs of cerebellar dysfunction. The commonest genetic cause of CHI is due to loss of function mutations in *ABCC8 and KCNJ11* (which encode the SUR1 and KIR6.2 components of the pancreatic beta-cell K_{ATP} channel).
Aims: to describe a unique cohort of patients with CHI and cerebellar dysfunction not associated with hypoglycaemic cerebellar brain damage.
Research design and methods: all 4 patients were diagnosed with CHI at a mean age of 4.5 months. Their mean birth weight was 3.68 Kg with no family history of diabetes mellitus. The CHI was fully responsive to diazoxide (10-15 mg/kg/day). These patients developed clinical signs of cerebellar dysfunction (broad based gait, intention tremor and slurred speech) at a mean age of 34.5 months (18-54 month). Other known causes of ataxia were excluded in these patients. The MRI scan in each patient was completely normal with no evidence of cerebellar hypoglycaemic brain injury. Each patient was negative for mutations in *ABCC8 and KCNJ11*. A candidate gene (NeuroD1) which is highly expressed in the pancreas and cerebellum was also sequenced but was negative.
Conclusions: This unique clinical phenotype of diazoxide responsive CHI and cerebellar dysfunction suggests a novel syndrome linking unregulated insulin secretion from pancreatic beta-cells to defects in cerebellar function. The genetic aetiology probably involves defects in gene/s specifically expressed in the pancreatic beta-cell and components of the cerebellum. Understanding the genetic aetiology of this syndrome will give novel insights into the developmental biology and function of the pancreas and cerebellum.

PO2-285 Pancreas II

Hyperinsulinism-hyperammonaemia (HI/HA) syndrome: novel mutations in the *GLUD1* gene and genotype-phenotype correlations

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Background: Activating mutations in the *GLUD1* gene (which encodes for the intra- mitochondrial enzyme glutamate dehydrogenase GDH) cause the hyperinsulinism-hyperammonaemia (HI/HA) syndrome. Patients present with hyperammonaemia and leucine sensitive hypoglycaemia. GDH is regulated by another intra-mitochondrial enzyme Sirtuin 4 (SIRT4). *SIRT4* knock out mice demonstrate activation of GDH with increased amino acid stimulated insulin secretion.
Objectives:
1) To study the genotype-phenotype correlations in patients with *GLUD1* mutations
2) To report the phenotype and functional analysis of a novel mutation (P436L) in the *GLUD1* gene associated with absence of hyperammonaemia
Patients and Methods: Twenty patients with hyperinsulinism from 16 families had mutational analysis of the *GLUD1* gene in view of hyperammonaemia (n=19) or leucine sensitivity (n=1). Patients negative for a *GLUD1* mutation had sequence analysis of the *SIRT4* gene. Functional analysis of the novel P436L *GLUD1* mutation was performed.
Results: Heterozygous missense mutations were detected in 15 patients with HI/HA, two of which are novel (N410D, D451V). In addition, a patient with a normal serum ammonia concentration (21µmol/l) was heterozygous for a novel missense mutation P436L. Functional analysis of this mutation confirms that it is associated with a loss of GTP inhibition. Seizure disorder was common (43%) in our cohort of patients with a *GLUD1* mutation. No mutations in the *SIRT4* gene were identified.
Conclusion: Patients with hyperinsulinism due to mutations in the *GLUD1* gene may have normal serum ammonia concentrations. Hence *GLUD1* mutational analysis may be indicated in patients with leucine sensitivity; even in the absence of hyperammonaemia. A high frequency of epilepsy (43%) was observed in our patients with *GLUD1* mutations.

PO2-286 Pancreas II

Prevalence of HNF4A gene mutations in patients with diazoxide responsive hyperinsulinism

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Background

We previously reported that *HNF4A* gene mutations can cause diazoxide responsive congenital hyperinsulinism (1, 2) with onset of diabetes (MODY) at a later age. In this study we investigated the prevalence of *HNF4A* mutations in a large cohort of patients with diazoxide responsive hyperinsulinism (HI).

Methods

We selected 208 probands with diazoxide responsive HI and sequenced the *KCNJ11/ABCC8* genes and/or *GLUD1* (in patients with hyperammonaemia or leucine sensitivity). The 10 exons and P2 promoter of the *HNF4A* gene were sequenced in those without a mutation in *KCNJ11, ABCC8 or GLUD1*.

Results

Mutations were identified in the *KCNJ11 or ABCC8* gene in 35 probands (16.8%) and 13 had a *GLUD1* mutation (6.2%). Thirteen different *HNF4A* mutations were detected in 14 unrelated probands (6.7%). Eight of these mutations are novel; C106S, M116I, L263P, Q362X, S371R, R80W, L332_P333insLL and H378del.

Six of the 14 probands with *HNF4A* mutations (42%) were known to have a family history consistent with MODY. Two mutations occurred de novo and 5 patients inherited the mutation from an unaffected parent (median age 36 years, grandparental samples awaited).

The 14 *HNF4A* mutation carriers were diagnosed at a median age of 1 day (IQR 1-5days) and were macrosomic (median 96th centile, IQR 81-99). Eight had persistent HI requiring diazoxide treatment for >1 year.

Conclusions

In this large series, *HNF4A* mutations account for 6.7% of cases of diazoxide responsive HI. The identification of an *HNF4A* mutation is important because of the likelihood of later diabetes which is likely to respond to low dose sulphonylureas. We recommend that *HNF4A* sequencing is considered in all patients with diazoxide responsive HI presenting in the neonatal period irrespective of a known family history of diabetes.

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PO2-287 Pancreas II

Familial post prandial hyperinsulinaemic hypoglycaemia

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Background: Postprandial hyperinsulinaemic hypoglycaemia (PPHH) is characterised by hypoglycaemia with an exaggerated insulin response in the postprandial phase. In the childhood period it is commonly observed with the 'dumping syndrome' following gastro-esophageal surgery and in association with protein sensitivity in congenital hyperinsulinism.

Objective: To describe a family with PPHH, not associated with any known causes of the condition. Patients and Methods: The index patient presented with recurrent episodes of hypoglycaemia at 10 years of age. There was a strong family history of hypoglycaemia (2 other siblings, mother, maternal aunt and grandmother). The index patient was investigated with a 24 hour blood glucose profile, a controlled fast and a protein load. Serum Glucagon Like Peptide-1 (GLP -1) levels was measured in the mother of index case in the postprandial phase. A prolonged oral glucose tolerance test was then conducted on the index patient and the four other affected members of the family. An intravenous glucose tolerance test was also conducted in two of the affected members.

Results: The index case had normal fasting tolerance and no hypoglycaemia in response to a protein load. Total active serum GLP-1 levels in the mother of index case were within the normal adult range (22-33pg/ml) throughout the postprandial phase. A prolonged oral glucose tolerance test provoked severe symptomatic hypoglycaemia between 3-4 hours, with elevated serum insulin levels in the index case, 2 siblings, mother and maternal aunt (grandmother not tested). Intriguingly hypoglycaemia was not provoked by the intravenous glucose tolerance test.

Conclusions: The history of PPHH in this extended family suggests a novel dominant genetic cause. The PPHH in this family does not seem to be related to changes in postprandial GLP-1 levels. Further genetic studies are required to understand the aetiology of the PPHH in this family. From the clinical point of view a prolonged oral glucose tolerance test should be conducted in children with episodes of recurrent hypoglycaemia that are not provoked by a controlled fast.

PO2-288 Pancreas II

Two siblings with pancreatic agenesis/hypoplasia, cardiac malformation, gallbladder agenesis and congenital diaphragmatic hernia: a new syndrome or variations on a theme?

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Case reports:

A symmetrical IUGR male (birth weight 1.74 kg) born to non-consanguineous parents at 37 weeks was found to have hyperglycemia (19 mmol/L) in the first day of life and insulin therapy was commenced. Further examination revealed a non-dysmorphic child with an inguinal hernia, atrial-septal defect and pulmonary valvular stenosis (PVS). C-peptide was not detectable and the pancreas was not visible on ultrasound. This child had significant difficulty with weight gain despite treatment with insulin and pancreatic enzymes. At 6 months of age he weighed only 4.7 kg with a acute on chronic malnutrition. The patient was admitted to hospital for deterioration thought secondary to cardiac failure and poor nutrition. On admission, a chest x-ray revealed a delayed presentation of left congenital diaphragmatic hernia (CDH). Surgical repair of the AV canal and CDH allowed abdominal exploration, which revealed pancreatic and gallbladder agenesis. Intestinal malrotation was corrected at this time. The patient is now 2 years old and thriving on insulin pump therapy. He is developmentally normal. PDX1 gene sequencing and karyotype are normal.

The fetus in the second pregnancy in this family was found to have a complex cardiac abnormality. The pregnancy was terminated at 20 weeks. The autopsy revealed a well formed male fetus with Tetrology of Fallot, a CDH (with stomach, spleen, and portion of liver found in the left chest), pancreatic hypoplasia, absent gallbladder, malrotation of the intestine and accessory spleen. A skeletal survey and karyotype was normal. A small residual pancreas was identified and staining for beta cells was positive. Focal somatostatin and glucagon positive cells were also identified in relation to the islets.

Conclusion:

We report two cases of pancreatic agenesis/hypoplasia associated with CDH, gastrointestinal and cardiac abnormalities. We compare the findings of our cases to those previously described in the literature. Although there are similarities amongst reported cases, genetic heterogeneity is clearly present. This is the first case of CDH associated with neonatal diabetes and we propose that it represents a new syndrome rather than a variation on those previously reported. These rare cases will help to further delineate the genes involved pancreatic development.

PO2-289 Pancreas II

Diabetes mellitus and congenital hyperinsulinism. An epidemiological study from two different data sources

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Diabetes has been frequently reported caused by subtotal resp. near total pancreatectomy after a variable time interval with euglycemia. Epidemiological analysis of risk factors and natural course is hampered by unknown prevalence. Capture-recapture analysis enables to adjust for the extent of incompleteness using different sources. Two independent registries, (1) the Diabetes Patienten-Verlaufsdokumentationssystem (DPV) initiative and the (2) German registry for congenital hyperinsulinism (CHI) were screened for diabetes manifestation after pancreatectomy. Initiated in 1990, the DPV program is an ongoing, prospective long-term longitudinal follow-up study to benchmark the quality of care provided to pediatric diabetes patients. 269 diabetes centres participated with a total of 50,645 patients (52% male) in the nationwide data collection. The German CHI registry compiles 212 patients with a mean age of 12.9 y. (5 months to 35 years). In 81 pancreatectomy was performed, including 64 patients with at least subtotal resection. In this registry in 12 out of 64 patients diabetes has been documented so far. A lower gestational age of 34.4 vs. 38.4 weeks (p=0.059) was found in pancreatectomized children with diabetes vs. non-diabetic. In the DPV registry 25 children (12 male) from 20 centres were revealed as diabetic after pancreatectomy; recapturing 12 identical cases in both registries. Compared to children with T1DM significant differences (non parametric Kruskal-Wallis-test) had been found: age at diagnosis 7.3 (0 to 17.7) vs. 9.8 years, follow-up time 7.6 (0.92 to 17.4) vs. 5.5 y., BMI-SDS 0.84 (-1.05 to 3.5) vs. 0.49 SDS, height-SDS -0.62 (-2.2 to 1.1) vs. -0.02 SDS, mean daily insulin dose 0.7 (0.1 to 1.2) vs. 0.79 U/kg body weight, HbA1c 7.6 (5.4 to 12) vs. 7.9 %. **Conclusions**: (1) Pancreatectomized patients with CHI have an increased risk for diabetes mellitus during prepubertal age. (2) In addition in these children lower gestational age predestines to diabetes manifestation. (3) Compared to T1DM pancreatectomized children are more often obese and had a reduced height. (4) Insulin dose and HbA1c are indistinguishable from the findings in type 1 diabetes.

PO2-290 Pancreas II

Insulin gene (INS) deletion causes neonatal and adult-onset diabetes

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Background: Mutations of the insulin gene (INS) have recently been found in patients with permanent neonatal diabetes (PND) and adult-onset diabetes. We now report on a large family with deletion of exons 1 and 2 of the INS gene and the phenotype of complete (DEL/DEL) or heteroyzgous loss (INS/DEL) of the insulin gene.

Methods: We studied a large family with frequent adult-onset diabetes and permanent neonatal diabetes in two children. DNA was analysed for mutations of the INS-gene and the cases with neonatal diabetes had no PCR-product of exon 2. Individuals with heterozygous INS-deletion were then identified by breakpoint PCR spanning the 446 bp fragment. To asses the clinical impact of heterozygous INS-deletion, medical history, genetic and metabolic (HbA1c) analyses were performed in 44 adults of the family.

Results: Exact sequencing of the neighbouring INS gene region indicated a small 446 bp deletion including exon 1 and exon 2 of the INS gene (chr11:g.2138434_213908del446) and results in complete lack of signal peptide, B-chain and half of the c-peptide of the preproinsulin protein. Complete absence of exons one and two of the INS-gene was found in the two children with PND who presented with reduced birth weight and hyperglycemia within the first 24hrs of life. One child with PND had [^{18}F]DOPA PET-CT-scan imaging normal size of his pancreas and no decrease in [^{18}F]DOPA uptake. Thus islet content was not found to be decreased. The PND cases had no detectable c-peptide or insulin while one heterozygous mother and one hetereozygous sister had normal oral glucose tolerance tests. In extended family members however, diabetes, as characterized by treatment (hypoglycemic agents or insulin) or an HbA1c > 6.2 % was more frequent ($p<0.05$) in members with INS/Del genotype (5 of 14) than with normal INS/INS (2 of 30) genotype. Clinical characteristics of INS-Del-subjects with diabetes / INS-Del-subjects without diabetes / normal subjects were older age (50.3+-2.2 / 33.1+-3.5 / 33.6+-2.3 yrs), increased HbA1c (9.1+-1.4 / 5.5+-0.3 / 5.0+-0.2%) but normal BMI-SDS (-0.15 / +1.0 / +1.5).

Conclusion: Complete loss of the INS gene in humans results in neonatal diabetes, reduced birth weight and complete absence of c-peptide and insulin function. Furthermore, also heterozygous INS-deletion is a strong risk factor for developing diabetes at older age despite normal BMI.

PO2-291 Pancreas II

Neonatal diabetes: a subtype of monogenic diabetes

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Neonatal diabetes (NDM) is a heterogeneous group of disorders that traditionally have been defined as diabetes diagnosed during the first months of life with two different forms clinically recognized, transient (TNDM) and permanent (PNDM). Advances have been made in understanding the clinical and genetic characteristics of these patients that in many cases have a monogenic etiology resulting from mutations that primarily reduce β cell function. Abnormalities of the imprinted region 6q24, which encompasses the *ZAC and HYMA1* genes, mutations of K_{ATP} channel subunits (Kir6.2 subunit-*KCNJ11* gene; SUR1 subunit-*ABCC8* gene) and mutations at insulin gene (*INS*) are the most common etiologies.

Objective: Our study aimed was to investigate the genetic etiologies in a group of NDM patients from different hospitals of Spain. We also tried to find a correlation between clinical phenotype and genetic findings.

Research design and methods: We screened the imprinted region 6q24 by methylation and microsatellite analysis, and *KCNJ11, ABCC8* and *INS* genes by direct sequencing in 41 patients diagnosed with diabetes before 6 months of age. Clinical data in relation to diabetes (glycaemia, ketoacidosis, age at diagnosis and duration of insulin treatment) and birth anthropometry (weight, length and ponderal index) were obtained from clinicians. SPSS.15 for Windows was used for statistical analysis (Kruskall Wallis or Mann-Whitney U test).

Results: We identified heterozygous mutation in *KCNJ11* gene in 14 patients (5 TNDM) and in *ABCC8* in 6 patients (3 TNDM). We found a reccesive mutation in *INS* in 4 children (1 TNDM). Finally, 8 patients had an abnormality of chromosome 6q24 associated with TNDM. The most common cause of TNDM and PNDM were abnormalities in 6q24 and mutation in *KCNJ11,* respectively. Patients with abnormalities in 6q24 were diagnosed earlier than K_{ATP} mutation (3 vs. 42 days of life; $p = 0.003$) and had less weight and ponderal index at birth (1864 vs. 2590 g; $p = 0.001$ and 1.9 vs. 2.4; $p = 0.011$). Clinical characteristics of patients with *INS* reccesive mutations were similar than in 6q24 abnormalities.

Conclusions: We are able to establish a monogenetic cause in 76% of patients

diagnosed with diabetes before 6 months of age. The most common etiology is K_{ATP} channel mutations and the second, abnormalities in 6q24. Some aspects of clinical phenotype are correlated with genetic etiology. Genetic finding is valuable for guiding prognostic about duration of diabetes.

PO2-292 Pancreas II

An exploratory investigation into the cognitive profile of children with congenital hyperinsulinism

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Background: Congenital Hyperinsulinism (CHI) is a rare disorder of dysregulated insulin release associated with severe, recurrent hypoglycaemia. Earlier psychometric studies reported significant learning difficulties in 30-50% of children with CHI, and cognitive deficits in a majority (Rother et al., 2001; Jack et al., 2003). However a more recent study reported good neurodevelopmental outcomes, although 29% children had some form of learning problem (Mazer Aronovitch et al 2007). The aim of this study was to perform a detailed analysis of the cognitive profile in a contemporary cohort of children with CHI from a single UK centre (Northern Congenital Hyperinsulinism Service- NORCHI).
Methods: All patients over 6 years of age (N= 17) registered with NORCHI were invited to attend a neuropsychological assessment which included the Wechsler Intelligence Scale for Children – 4th edition (WISC-IV), the Test of Everyday Attention – Child version (TEA-Ch) and subtests from the Wechsler Individual Achievement Test – 2nd edition (WIAT-II).
Results: Eleven children (2 girls, 9 boys) at a mean age of 9 years (range 6 – 15) whose educational achievement was "normal" were brought for assessment. Four children (36%) (1 girl, 3 boys) had mean Full Scale Intelligence Quotient (FSIQ) in the Learning Disability range: 68.3(range 63 – 73) [Normal range=85 – 115] and a mean Word Reading Index score in the borderline range: 79.5 (69 – 91) [Normal range= 85 – 115]. Seven children (64%) (1 girl, 6 boys) had satisfactory mean FSIQ: 102.7 (87 to 127) and satisfactory mean Word Reading Indices: 93.6 (69-128). However, on assessment of a measure of sustained attention from the TEA-Ch, ten children (90%) scored below the average scale: 4.3 (3 -7) [Normal range 8–12]. All four children with Learning Disability FSIQ scores fell below the 5th centile on the TEA-Ch scale: 2.3 (1–4).
Conclusions: In our preliminary study of apparently educationally normal children with CHI, 90% had cognitive difficulties in the selective and sustained attentional domains and one third had Learning Disability. These findings have significant clinical implications. Further detailed evaluation of the genetic, physiological and therapeutic factors which may affect cognitive development in children with CHI is required and we plan to address this through a collaborative, prospective study of the longitudinal neurodevelopment of children with congenital hyperinsulinism in the UK.

PO2-293 Pancreas II

Insulinomas in childhood: twenty year experience at the Children's Hospital of Philadelphia

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Background: Insulinomas are a rare cause of hyperinsulinemic hypoglycemia in children. Diagnosis of an insulinoma in children is more challenging than in adults due to its rarity in comparison to other causes of hypoglycemia, its vagueness of symptoms, and the difficulty of confirming the lesion by imaging. Our institution's extensive experience with hyperinsulinemic hypoglycemia provides an opportunity to better define some aspects of pediatric insulinomas.
Objective: We review our institution's experience to describe the range of presentation and clinical course, to identify distinctive features, and to devise an efficient evaluation method for suspected insulinoma.
Methods: We conducted a retrospective chart review of all cases of insulinoma at The Children's Hospital of Philadelphia between 1987 and 2008. Demographics, clinical features, biochemical studies, imaging studies and pathology results were collected.
Results: Seven patients (3F:4M) were identified, between 4 and 16 years of age. All had been referred for evaluation of hypoglycemia. All had objective neuroglycopenic symptoms: syncope, seizures, confusion, or other mental status change. Whipple criteria were met in all. Median length of time between onset of symptoms and diagnosis was 6 months. Diagnostic fasts confirmed hyperinsulinism in all. Ability to fast ranged from 3-25 hrs. Insulin levels at hypoglycemia ranged from 7.3 to 292uIU/ml. Six of the 7 were confirmed to have an insulinoma by diagnostic study prior to surgery. Insulinomas were found in all regions of the pancreas. All patients were screened for MEN1 and were negative. Surgical resection was curative in all but one, whose persistent hypoglycemia required diazoxide.
Conclusion: Insulinomas are a surgically curable cause of hypoglycemia even in early childhood, and must be considered in the evaluation of hypoglycemia causing objective neuroglycopenic effects. No cases were malignant, and no cases were due to MEN1. No simple imaging method revealed all lesions, but endoscopic ultrasound may be the best current method. Biochemical studies were characteristic of hyperinsulinism, but in contrast to some forms of childhood hyperinsulinism, insulin levels were invariably measurably elevated at the time of hypoglycemia.

PO2-294 Pancreas II

The histological architecture of alpha-cell distributions differs in focal and diffuse forms of congenital hyperinsulinism of infancy (CHI)

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Congenital Hyperinsulinism of Infancy (CHI) is a rare disorder associated with dysregulated insulin release and recurrent hypoglycaemia. More than half of patients have mutations in *ABCC8 or KCNJ11* genes encoding subunits of ATP-sensitive potassium (K_{ATP}) channels. Histologically, CHI pathology is subdivided into focal or diffuse disease; the former characterized by clusters of islet cell proliferation with normal nuclei and the latter showing disorganization of islet structure, islet cell hyperplasia and enlarged nuclei.
Aims and methodology: This prospective ethically approved study, purposed to examine the distributions of alpha and beta-cells in pancreatic tissue from 7 patients with focal or diffuse CHI requiring therapeutic surgical pancreatic resection (median age at surgery 2.5 (2, 30) months). Using standard immunofluorescence (IF) detection procedures, pancreatic alpha and beta-cells were identified using antibodies raised against glucagon or insulin. To quantify differences in antibody distributions across the islets, multiple IF images were photographed and analyzed using Image J software.
Results: All patients had K_{ATP} channel mutations (5 *ABCC8, 2 KCNJ11*). 3 patients had focal disease confirmed on ^{18}Fluoro (F)-DOPA PET-CT scanning, of whom 1 had a mosaic *de novo ABCC8* mutation. IF image analysis on resected pancreatic tissue showed insulin-positive beta-cells distributed throughout islets in all patients. However, marked differences in distributions of glucagon-positive alpha-cells were noted between focal and diffuse pancreatic tissue. In focal cases, alpha-cells were principally detected at the islet periphery whereas in diffuse cases, these cells were distributed throughout the islet. Both distribution patterns were demonstrated in the mosaic case.
Conclusions: Glucagon producing alpha-cells appeared to have a peripheral islet distribution in tissue from focal CHI patients when compared to diffuse CHI patients, where a more homogenous distribution of alpha-cells was noted. This may reflect differences in the ontogeny of the pancreas in focal CHI, which may affect glucose counter-regulatory mechanisms.

PO2-295 Pancreas II

The spectrum of disease in diazoxide responsive hyperinsulinaemic hypoglycaemia

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Hyperinsulinaemic hypoglycaemia (HH) has traditionally been divided into milder cases that maintain euglycaemia on Diazoxide therapy (Diazoxide Responsive Hyperinsulinaemic Hypoglycaemia [DRHH]) and more severe unresponsive cases. Loss-of-function mutations in the *ABCC8 and KCNJ11* genes, encoding subunits of ATP sensitive (K_{ATP}) potassium channels, are the commonest genetic cause for HH. Genetic analysis and pancreatic imaging are not routinely performed in DRHH; however it is recognized that channel mutations and HH due to focal lesions can be associated with mild disease. It has therefore been our policy to genetically test all HH patients and perform pancreatic imaging in those with a genotype consistent with possible focal disease.
Aim: To assess the spectrum of HH disease in patients with DRHH presenting to the Northern Congenital Hyperinsulinism Service (NorCHI) over a 2.5 year period (April 2006-November 2008).
Results: 26 patients with DRHH were identified. 12 patients (46%) had a genetic abnormality (11 heterozygous mutations: 7 in *ABCC8, 3 in KCNJ11*, 1 in *GLUD1* and 1 unbalanced translocation between chromosomes 9 and 13). 7 patients with either no mutation (n=4) or a paternal heterozygous K_{ATP} mutations (n=3) underwent ^{18}Fluoro [F]-DOPA Positron Emission Tomography (PET) CT which identified 3 with resectable lesions (2 tracer positive, 1 tracer negative). All 3 patients underwent surgical lesionectomy and remain euglycaemic off treatment. Histology confirmed the presence of non-malignant insulinomas in 2 of these patients. 8 patients (31%) had late resolution of disease and discontinued treatment within a year of therapy, of whom 4 are mutation positive.
Conclusions: 46% of our DRHH patients had a genetic abnormality associated with their disease. 31% of patients had late resolution of their disease, including mutation positive patients. 12% had a lesion amenable to surgical cure. Mutation screening and appropriate use of ^{18}[F]-DOPA PET-CT imaging in DRHH is helpful and can influence the approach to management.

PO2-296 Pancreas II

Congenital hyperinsulinism of infancy: a dominant K_{ATP} channel mutation in an Italian family

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Congenital Hyperinsulinism of Infancy (CHI) (1:50,000 births) is the most important cause of recurrent and profound hypoglycaemia in early infancy. Prompt recognition, diagnosis and therapy are necessary to prevent or minimize permanent neurological complications due to prolonged and repetitive hypoglycaemia.
Two histologically and genetically distinct groups are recognized among patients with HI due to K_{ATP} channels defect: a diffuse type (Di-HI) and a focal form (FoHI). The diffuse form appears to be associated with autosomal recessive and dominant mutations in the ABCC8 and KCNJ11genes. The Fo-HI has been demonstrated to arise in individuals who have a germline mutation in the paternal allele of ABCC8 or KCNJ11 in addition to a somatic loss of the maternally derived chromosome region 11p15 in pancreatic beta cells.
Here we describe the clinical and molecular findings in an Italian child and his family who presented hypoglycemia in the neonatal period.
The propositus is an Italian boy born from non consanguineous parents with family history of occasionally mild symptomatic hypoglycaemia episodes (mother). He was born at 34^{+6} weeks of gestation, and his birth weight was 3690 g (>90°ple). He presented from birth hypoglycaemia. A glucose infusion rate of 16 mg/kg/min (0.88 mmol/kg/min) and enteral feeding were necessary to maintain euglycaemia. Investigations performed during an episode of non chetotic hypoglycemia (glycemia 1.16 mmol/L) showed inappropriately elevated serum insulin concentration (30 mUI/mL). Inborn errors of metabolism and metabolic disorders related to amino acid, organic acids, or fatty acid oxidation were no detected. The exams performed suggest diagnosis of hyperinsulinemic hypoglycemia and diazoxide therapy was started at 21th day of life.
Genetic mutation analysis identified a single I1512T mutation in the ABCC8 gene, which had been previously reported to be associated with dominant hyperinsulinism. The proband's mother carries the same mutation despite a milder phenotype.
After two years of follow-up the patient always maintains a good glycometabolic control with pharmacological therapy (diazoxide 8.5 mg/kg/day).
Children with these dominant K_{ATP} hyperinsulinism mutations seemed to have a milder hypoglycaemia phenotype than that seen in children with hyperinsulinism resulting from recessive K_{ATP} mutations. Most cases appeared to be responsive to treatment with diazoxide, suggesting a greater degree of residual channel function.

PO2-297 Pancreas II

Prevalence of mutations in the HNF4A gene among Japanese patients with congenital hyperinsulinism

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[Background]
Congenital hyperinsulinism (CHI) is the leading cause of persistent hypoglycemia in infancy. Hypoglycemia extending beyond several months after delivery usually has a genetic etiology. The pancreatic KATP channel genes, ABCC8 and KCNJ11, are the most common causative genes followed by the GLUD1 gene. However, overall, mutations in known genes explain less than half of the patients with CHI. Recently, mutations in the HNF4A gene were reported in selected families with CHI characterized by prenatal overgrowth and a family history of diabetes mellitus (MODY1).
[Aim]
To assess the prevalence of mutations in the HNF4A gene among Japanese CHI patients.
[Subjects and Methods]
Twenty six Japanese patients without mutations in the ABCC8, KCNJ11, and GLUD1 genes were analyzed. Briefly, all exons, exon-intron boundaries, and the core promoter region of the HNF4A gene were amplified from genomic DNA and directly sequenced. The clinical course of the identified patient was reviewed from the medical record.
[Results]
During the last five years, we have performed mutational analysis of 45 patients with CHI. Among them, mutations were identified in 19; 1 in KCNJ11, 13 in ABCC8, and 4 in GLUD1. Out of the remaining 26 patients, one splice site mutation, IVS4+1G>A, was identified in the HNF4A gene. The patient was actually the first child of a dizygotic twin born to healthy Japanese parents. She was born after an uneventful, 35weeks of pregnancy with the birth weight of 2888g. Fasting blood glucose of <20 mg/dL was identified on day 1, and continuous intravenous glucose infusion was initiated. The patient remained hypoglycemic for ~40 days following delivery with 4-5 mg/kg/min of glucose infusion to keep her blood glucose at 40-60 mg/dL. Then the hypoglycemia resolved gradually and she was discharged on day 60 without any medication. There was no family history of diabetes. However her father with the same mutation was found to have low insulinogenic index (0.22) on 75g oral glucose tolerance test.
[Conclusion]

The prevalence of mutation in HNF4A was one in 45 in our series. Contrary to previous reports, the clinical manifestation of our patient was much milder in terms of the severity of hypoglycemia, prenatal overgrowth, and family history of diabetes. Considering the fact that most of these patients with milder phenotype do not undergo genetic analysis, the frequency of mutations in HNF4A might be higher than previously postulated.

PO2-298 Thyroid II

Prognostic factors of Grave's disease antithyroid drug treatment outcomes for children and adolescents: retrospective study

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Objective: to define determinants of Grave's disease (GD) antithyroid drug treatment (ADT) outcomes for children and adolescents.
Study methods. We analysed initial data (sex; pubertal status; BMI, kg/m^2; BSA, m^2; levels of fT4, TPOAbs, TgAbs and TBII; ophtalmopathy presence; thyroid volume and "goiter index" (GI=TV/BSA, ml/m^2) by US) as well as autoantibodies dynamics during 6 months ADT of 67 children (51F/16M) of ages from 1,5 to 17,6 years (Me – 14,2). We aimed to assess influence of the data on GD outcomes. We compared two groups: 1) children in remission during at least one year after therapy cessation (n = 31); 2) children with relapse (n=36). The group 2 contained 17 children with relapse after ADT cessation and 19 children with uncontrolled relapsing thyrotoxicosis on the background of one or more years of therapy. The results were processed using non-parametric criteria and reported as Mediana and 25-75% quantiles.
Results: 48 of 67 children have ADT discontinued after 17,4 months [11,1; 31,8]. 31 children of them were being in prolonged remission with mediana 25 months. 17 of 48 have developed relapse within one year after ADT discontinuation. Other 19 children of "relapse" group had uncontrolled relapsing course during 15,5 months of ADT that was a reason to direct them to radical treatment. A percentage of prolonged remission cases among 23 children prepubertal at the moment of GD manifestation was 65,2 % that was higher than this one among 44 pubertal patients – 36,4 % (p=0,012). Patients achieved prolonged remission had TV and GI lower than children of "relapse" group (16,9 ml vs 27,6 ml, p=0,0003 and 13,5 ml/m^2 vs 18,2 ml/m^2, p= 0,005). 94,4% of patients without TBII decrease at least for 50 % of baseline have developed relapse. In the pubertal group relapse developed more frequently at enlarged goiter size (GI>18 ml/m^2; TV>27-30 ml), male sex and high TPOAbs level (>1000 IU/l)
Conclusion: 1) Drug treatment at GD manifestation is more effective for prepubertal children and for children with small goiters (by US).
2) Because of high risk of relapse at ADT initial choice of radical treatment is more reasonable for adolescents with large goiter and high initial TPOAbs level as well as for males. 3) In the case of ADT is chosen it's reasonable to be guided by TBII level regardless initial clinical factors. Negative dynamics of TBII in 6 months of treatment determines high risk of relapse that requires changes of further therapeutic tactics.

PO2-299 Thyroid II

MCT8 mutation in a family leading to severe cognitive impairment in affected males and mild cognitive deficits in affected females

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Background: Monocarboxylate transporter 8 (MCT8) has been identified as an active and specific thyroid hormone transporter. The MCT8 gene is located on the X chromosome and mutations in this gene have been shown to cause X-linked severe psychomotor retardation and consistently elevated serum T_3 levels. Since MCT8 facilitates neuronal T_3 uptake, its inactivation results in an impaired supply of T_3 to neurons.
Case report: Generalized axial hypotonia developed in a term born boy shortly after birth. Hypotonia persisted and at the age of four months thyroid function was tested, which revealed elevated total T_3 (4.3 nmol/l; N 1.4-2.5) but low free T_4 (8.7 pmol/l; N 11-25) levels. Newborn thyroid screening (TSH) was normal. The boy's mother and grandmother showed mild cognitive deficits resulting in a need for special education. Moreover, mother's brother suffers from severe cognitive impairment and spasticity. The uncle also has high serum T_3 (3.5 nmol/l) and low FT_4 (8.1 pmol/l) levels, and the mother has normal T_3 (2.2 nmol/l) but low FT_4 (10.8 pmol/l) levels.
Laboratory evaluation: The MCT8 exons were amplified by PCR and directly sequenced. The patient was identified with a hemizygous mutation (c.C733T, p.R245X) resulting in a premature stop codon. This mutation has been reported before and shown to completely inactivate MCT8 (Jansen et al, JCEM 2007;29:2378-81). The patient's mother was heterozygous for the same mutation.
Conclusion: We describe a boy with severe psychomotor retardation, high T_3 and low FT4 levels because of a nonsense mutation in the MCT8 gene. Since the patient's mother is heterozygous for this mutation, our findings suggest that heterozygous female carriers of MCT8 mutations may have a significant cognitive impairment.

PO2-300 Thyroid II

Severe neurologic damage in a patient with normal growth and high serum T3: a new mutation in *MCT8*

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Introduction: Monocarboxylate transporter 8 (*MCT8*) is a specific thyroid hormone (TH) transporter that facilitates the cellular entry of TH. The *MCT8* gene is located at Xq13.2. We describe a new mutation of *MCT8.*
Case report: We evaluated a male infant (9 months old) who was referred to us with neurologic delay, generalized hypotonia, strabismus and brain malformation. Pregnancy and delivery were uneventful, and his birth weight (3096 g) and body length (51 cm) were normal. His parents are healthy and nonconsanguineous. His two brothers are healthy. His growth was normal (74 cm, +0.46 standard deviation score [SDS]), and his weight was 9 kg (-0.38 SDS). No goiter was found. The laboratory evaluation showed high serum T3 (285.5 ng/dL; normal: 70-200 ng/dL), reduced total T4 (3.4 µg/dL; normal: 6.4-13.3 µg/dL) and free T4 (0.5 ng/dL; normal: 0.6-1.54 ng/dL) and mildly increased TSH (6.8 µU/mL; normal: 0.5-4.2 µU/mL). A computed tomography brain scan showed a rigid ventricular system and a bilateral posterior hypodense extra-axial collection.
Treatment with levothyroxine further increased the T3 serum levels with no changes in the other thyroid function tests. As the clinical and hormonal features suggested a mutation of *MCT8*, a molecular study was performed. It showed a new mutation, c.630insG, in exon 1 of *MCT8*. His mother was heterozygous for the mutation and his brothers did not have it.
Conclusions: In our patient the severe neurologic damage in spite of normal growth and absence of myxedema suggests that *MCT8* has a very important role in the transport of TH in brain. In bone, muscle and adipose tissues, the high serum T3 compensated for the inactive *MCT8*.

PO2-301 Thyroid II

Medical care of young adult patients with congenital hypothyroidism

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Neonatal thyroxine replacement therapy effectively reduces morbidity in patients with congenital hypothyroidism (CH). However, careful long-term follow-up of the appropriateness of treatment is also required to reduce the risks of impaired neurocognitive functioning and poor long-term outcome. Few studies have examined continued care after the transition from pediatric practice. In this study, we aimed to evaluate prospectively the medical follow-up of a population-based cohort of young adult patients with CH.

Patients and methods: All patients listed in the French national registry who were born during the first 10 years of the screening program were invited by letter to participate in the study. Responses were obtained from 1169 (63% of the cohort, n = 864 female patients) of the 1839 selected patients. These patients completed the questionnaire at a median age of 23 years.

Results: In the responding patient population, 1047 patients (90%) said they had their TSH levels checked within the past two years, for 791 patients (68%) of whom the results were available. 171 patients for whom we had no access to the results obtained or who had not had their TSH levels checked over this time period agreed to do so in the following weeks. Patients were assigned to four groups on the basis of TSH values.

TSH values mIU/l	≤0.1	0.11-5.0	5.1-14.9	≥15
median TSH values (25-75th p.)	0.04 (0.02-0.06)	1.4 (0.6-2.6)	8.2 (6.1-10.4)	38 (21-95)
number of patients	70	686	119	87

Although 71% of the patients had appropriate TSH levels in the normal range, 29% had impaired TSH values, which were abnormally undetectable (7.3%) slightly high (12.3%) or very high (9%). Most participants were followed by general practitioners (63%) or endocrinologists (32%). It is reasonable to assume that the follow-up of non-participants was less adequate than that of participants. Relationships between the appropriateness of treatment and clinical characteristics will be quantified.

Conclusion: These findings suggest that approaches to the care of about one third of patients with CH require revision to improve medical care during the transition period, particularly as appropriate thyroxine treatment is essential in women to prevent possible fertility loss and pregnancy complications and adverse effects on the outcome of offspring associated with maternal thyroid dysfunction.

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PO2-302 Thyroid II

Prediction of congenital hypothyroidism (CH) based on initial screening TSH in the Ontario newborn screening program (ONSP)

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Background: Since the introduction of thyroid-stimulating hormone (TSH)-based CH screening programs in the 1970's, optimal TSH-screening cutoffs, and the predictive value of various screening TSH levels have been the subject of debate. The principal aim of this study is to examine these values within the ONSP.

Methods: The initial screening and follow-up data of 366,658 full term infants born in Ontario, Canada April 1, 2006 to November 30, 2008 were used for the analysis. The screening cut-off used was ≥17 mIU/L. Confirmed CH cases were based on local endocrinologists' report.

Results: There were a total of 327 positive screening tests (~1/1000 live term births). Follow-up data is available on 259 subjects of which 149 were true positives (~1/2000 live term births). Subjects were further subdivided based on TSH with positive predictive values as indicated in the table below:

TSH Screening Range (mIU/L)	Number if Subjects	PPV (%)
17-19.9	93	27
20-29.9	56	38
30-39.9	15	73
>40	95	97

Increasing the TSH-screening cutoff from ≥17.0 to ≥20.0 mIU/L decreases the sensitivity of the test from ~100% to 83%. Ninety seven percent of neonates with an initial screening TSH of ≥ 40 mIU/L and 73% of those with ≥ 30 mIU/L were later confirmed to have CH.

Conclusions: This supports the notion that a lower initial TSH screening cutoff of at most ≥17 mIU/L is necessary to detect an acceptable percentage of patients with CH. Twenty seven percent in the 17-19.9 mIU/L range were true positives and this group needs to be examined in more detail with extended follow-up data to determine if they have transient or permanent CH. Samples collected after 28 hours of life are more likely to represent true CH cases in those with modestly elevated TSH values of 17-30 mIU/L. The very high frequency of true positives in term newborns with initial TSH values ≥ 30mIU/L suggests that this group should be referred directly to a pediatric endocrinologist in an effort to expedite further assessment and treatment. The abstract presentation will be updated based on data available on currently pending cases.

PO2-303 Thyroid II

Remission rate and remission predictors of Graves disease in children and adolescents

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Purpose: Medical therapy is chosen as the initial treatment in children to avoid complications of surgery or radioiodine. However, the optimal treatment for childhood Graves disease is controversial because most patients require relatively long periods of medical therapy and relapse is common after discontinuing medication. Therefore, this study aimed to search clinical or biochemical characteristics which could be used as remission predictors in Graves' disease.

Methods: We retrospectively studied children who were diagnosed as Graves disease and treated with anti-thyroid agents. Enrolled patients have been observed at least 3 years. Patients were divided to remission and no remission group, and we compared these two to determine which variables were associated with remission.

Results: Sixty-four patients were enrolled, 37 (57.8%) achieved remission and 27 (42.2%) could not achieve remission until last visit. Normalization of TBII after treatment in remission group was faster than non-remission group (remission group; 15.5±12.1 vs. non-remission group 41.69±35.70 months). TRH stimulation test was done in 28. Only 2 (8.33%) of 26 patients who showed normal or hyper response in TRH stimulation test relapsed. Multiple logistic regression analysis identified rapid achievement of TBII normalization by 18 months after treatment as a significant predictor of remission. Six percent of patients achieved a remission within 3 year, and 55% within 6 years.

Conclusion: Rapid achievement of TBII normalization can be a predictor of remission in childhood Graves' disease. TBII stimulation test can be a predictor whether remission will be maintained after remission is achieved.

PO2-304 Thyroid II

Recently increasing congenital hypothyroidism with delayed rise in serum TSH missed on newborn screening and necessity of 2nd newborn screening

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Mental retardation is a major sequela of delayed treatment of congenital hypothyroidism(CH). And irreversible brain damage can be caused by CH. But, newborn screening does not offer hundred percent safety. We reviewed 594 CH children who were treated in Soonchunhyang University Hospital, from January 1997 when started newborn screening test by government in Korea, to March 2009. Among them, 31 patients(5.2%) were diagnosed after 1 month because of persistent jaundice and constipation despite of normal newborn screening result. 3 patients were diagnosed CH performing thyroid function test because other siblings were treated by CH. From 1997 to 2006, CH patients with delayed TSH rise were 12 cases. But in 2007 and 2008, there were 7 and 10 CH patients with delayed TSH rise, respectively, with increasing incidence. Among 31 patients, 9(29.0%) were preterm baby and 3(9.6%) had maternal antibody. In terms of type of CH in 31 patients, 12 were dyshormonogenesis and 19 were hypoplasia. All CH due to ectopic thyroid and aplasia were diagnosed on newborn screening. Initial average TSH level is 33.62±43.29 μIU/ml in patients with delayed TSH rise and 124.34±455.1 μIU/ml in patients diagnosed on newborn screening. There is no statistically difference between 2 patients groups(P=0.280). Initial average free T4 level is 1.05±0.33 ng/dL in patients with delayed TSH rise and 0.79±0.81 ng/dL in patients diagnosed on newborn screening. There is no statistically difference between 2 patients groups(P=0.105).
Regardless of the result of newborn screening, infant with any symptoms suspected CH, should be carefully examined for possible CH. Furthermore, with increasing incidence of CH with delayed TSH rise, maybe due to premature hypothalamus-pituitary-thyroid axis, thyroid disrupting chemicals or any environmental factor, performing newborn screening test 2 times, at 3-5 days after birth and at 2-3 weeks of infant age, is most safe for all newborn baby. So we should consider the cost effectiveness and quality of life in diagnosis delay in CH patients. And also we should study the cause of increasing incidence of CH with delayed TSH rise.

PO2-305 Thyroid II

Normal thyroid ultrasonography, but no uptake on ^{99m}technetium scan in congenital hypothyroidism, what is the cause?

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Given normal thyroid ultrasonography(USG), but no presentation on ^{99m}technetium scan in congenital hypothyroidism(CH), the well known causes of this discrepancy are typically abnormal iodine transport defect, receptor defect and presence of maternal thyrotrophin blocking antibodies(TRBAb). We aimed to investigate the causes behind the lack of uptake on thyroid scan. We reviewed 605 CH children who were treated in Soonchunhyang University Hospital, from January 1997 to April 2009. Among them, 52 patients(8.6%) showed mismatching USG and scan. 4(7.7%) patients of 52 were central CH and 4(7.7%) patients had betadine umbilical dressing for some time. 10(19.2%) patients had maternal antibody. 3 were positive for TRBAb, thyroglobulin antibody and microsomal antibody while 3 were positive for thyroglobulin and microsomal antibody. And another 3 patients presented with only positive thyroglobulin antibody and 1 patient with only positive TRBAb. 34 patients(65.4%) had unknown causes, maybe due to iodine transport defect or TSH receptor gene mutation. From 1997 to 2004, CH patients with mismatching imaging finding were 9 cases, but from 2005 to 2009, April, there were 25 cases with increasing incidence maybe due to competition or blocking of NIS(Sodium-Iodine Symporter) by thyroid disrupting chemicals or any environmental factor adding to iodine transport defect. 9(17.3%) CH patients had normal newborn screening result. Initial average TSH level is 41.96±139.27 μIU/ml in patients with mismatching imaging finding and 127.69±464.91 μIU/ml in other CH patients. There is no statistically difference between 2 patients groups(P=0.219).
There are some cases with mismatching imaging finding, so if possible, we should perform both thyroid USG and scan at the time of diagnosis and check microsomal Ab and thyroglobulin Ab of the baby and mother with TRBAb, the well known cause of lacking uptake on ^{99m}Technetium scan. Furthermore with increasing incidence of these mismatching cases, we should study the cause of lacking uptake on scan in CH.

PO2-306 Thyroid II

Raised TSH serum levels in AGA and SGA children born prematurely: a follow-up study

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The natural history of the non autoimmune hyperthyrotropinemia, found in some children born prematurely is not known. We evaluated therefore 74 children (26 AGA and 48 SGA) born prematurely (33 ± 2.2) for the first time at the age of 7.6 ± 2.3 years (phase 1) and subsequently at the age of 11.3 ± 2.3 years (phase 2). TSH, fT3, fT4, TPO-Ab and TG-Ab were evaluated and a thyroid ultrasound was performed. Two patients in the SGA group were excluded from the follow-up study, due to the diagnosis of Hashimoto's thyroiditis at an intermediate visit.
In the AGA group, mean TSH serum level (normal range: 0.4-3.6 mU/l) was similar in both phases (3.1 ± 1.2 vs 3.0 ± 0.9 mU/l; NS) and it was above the upper normal limit in 4 (15.4%) subjects at phase 1 and in 6 (23.7%) subjects at phase 2 (NS).
In SGA group (46 subjects), mean TSH serum level was similar in both phases (2.85 ± 1.25 vs 2.54 ± 0.99 mU/l; NS) and it was above the upper normal limit in 11 (23.9 %) subjects at phase 1 and 5 (10.8 %) subjects at phase 2 (NS).
In AGA group, during the follow-up 18 (69.2 %) patients remained euthyroid, 2 (7.7 %) showed a persistently raised TSH, 2 (7.7 %) normalized and in 4 (15.4 %) TSH increased above the upper normal limit. Free T4 and fT3 were always in the normal range and TPO- and TG-Abs were absent.
In SGA group, during the follow-up 32 (69.6 %) patients remained euthyroid, 2 (4.3 %) showed a persistently raised TSH, 9 (19.6 %) normalized and in 3 (6.5 %) TSH increased above the upper normal limit. Free T4 and fT3 were always in the normal range.
In AGA group, mean thyroid volume increased during the follow-up study (p=0.025), but remained significantly lower than that of matched controls (phase 1: -0.67 ± 0.49 SDS; p=0.000; phase 2: –0.25 ± 0.54 SDS; p=0.005).
In SGA group, mean thyroid volume increased, but not significantly, during the follow-up study and it was never different from matched controls (phase 1: -0.31 ± 1.36 SDS; phase 2: –0.03 ± 1.49 SDS). Thyroid ultrasound showed a normal thyroid structure in all children.
In conclusion AGA and SGA children born prematurely frequently show alterations of the pituitary-thyroid axis. The small thyroid volume together with its normal structure would support the concept of a partial refractoriness of the gland to TSH action which however seems to improve during the follow-up.

PO2-307 Thyroid II

Impairment in the performance on dynamic psycho-pedagogical evaluation of children with congenital hypothyroidism

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Mental retardation and cognitive impairment due to congenital hypothyroidism (CH) can be avoided if hormonal replacement with levothyroxine is introduced in the first weeks of life. Children with CH with normal Intelligence Quotient (IQ) may present attention deficit disorders, behavior problems, speech dif-

ficulty, memory and visual spatial impairments.
The objective of this study was to evaluate cognitive functions in CH patients followed in the Pediatric Endocrinology Unit at Unicamp using psychometric tests and dynamic psycho-pedagogical evaluation.
Methods: Forty-three patients and 43 paired controls aged 5 to 15 years were evaluated. Cognitive evaluation was performed using 3 tests: Visual Attention, Rey Complex Figure and Raven's Progressive. Dynamic psycho-pedagogical evaluation using mathematical challenges based on Vigotsky's Theory was used. Wilcoxon non parametric statistical test was used. This study was approved by the local ethics committe.
Results: Patients with CH presented a similar intelectual performance, motor visual coordination and execution time when compared to children in the control group. However, they had a significantly worse performance on visual memory test (p=0.030) and concentrated attention time (p=0.003). The dynamic evaluation showed a significant impairment in information reception (p= 0.019), integration of the information (p=0.034), initial representation (p= 0.01), performance memory (p=0.001), exploratory action (p= 0.046) and utilization of high-order scheme (p= 0.003). Consequently, the evaluation of production and final execution of tasks were impaired in the group of patients (p= 0.009 and p=0.039 respectively).
Conclusion: Although patients with Congenital Hypothyroidism have a normal IQ, they present cognitive functions and task execution impairments which may result in bad performance at school.

PO2-308 Thyroid II

Paediatric thyroid nodules and multinodular goiter: incidence, management and outcome: a French collaborative multicentric study

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Clinical diagnosis and further management of thyroid nodule (TN) and/or multinodular goiter (MNG) may be sometimes a crucial challenge in pediatric population.
Aim of the study: evaluation of incidence, type of nodule and management practices within 6 French pediatric endocrinology clinics.
Design of the study: retrospective study including patients suffering from TH or MNG between 2000 and 2009.
Results: 60 patients (46F, 16M) were included, mean age : 12.9 yrs (1.9 - 17.9), 6/60 had PMH of neoplasia. They came to consultation for: goiter: 24 (40%), nodule:18 (30 %) and adenopathy : 6(10%). As expected, US improved clinical examination: MNG: 18, solitary nodule: 27, multiple nodules: 15, adenopathy: 24 vs 17. Classical characteristics of malignancy were found. On surprise, only 9 patients had fine-needle aspiration cytology (FNAC): 36% were carcinoma, 27 % were adenoma and 27 % were not contributive. In 4 cases, local anesthesia using lidocaine was proposed. No complication of FNAC was noted. 46 % of these children had scintigraphy: 9 patients presented cold nodules and 6 hot nodules. Follow-up was proposed for 32 patients (53%) (thyroiditis: n=14;nodules < 1 cm n=10; MNG: n=3, adenoma on FNAC: n=2), 9/32 received LT4. 28 patients (47%) underwent thyroidectomy (13/28 had initial total thyroidectomy, 5/28 had a second look because of carcinoma). 13 of our 60 patients (22%) had thyroid cancer: 5 had positive FNAC; 1 had no contributive FNAC; 7 were diagnosed on the piece of thyroidectomy. All of these cancers were carcinomas. There were 8 girls and 5 boys. The risk of malignancy in girls is 17% and in boys 31%. Off interest, 4 of the 6 patients having PHM of neoplasia present thyroid carcinoma with nodes metastasis at time of diagnosis but only one was diagnosed during systematic follow-up.
Conclusion: Thyroid nodules are not so rare in French children. As shown in the present study, they occur during prepubertal period and the risk of malignancy is high (near 20% for our series). We must improve diagnosis before surgery using more often FNAC that is save. We also should take care of patients with past medical history for another malignancy and propose systematic US thyroid during long term follow-up. Thyroid nodules in children need more aggressive investigations than during adulthood because of the high risk of malignancy.

PO2-309 Thyroid II

Heavy metal poisoning causing organification defect of the thyroid

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Case Report: a 16 yo boy presented to the endocrinology clinic with rapid onset of goiter. T4 of 0.7, (0.9-1.4), T3 total of 177 (97-186), and a TSH of 45.95 (<4.3). His pediatrician began thyroid hormone (TH) replacement. His initial exam revealed tachycardia (120/min), tremor and low weight. His thyroid measured 11 cm diameter, 7 cm diagonal each lobe. Lab tests 1 wk after starting treatment T4 was 0.75 and his TSH is 68.5, antibodies negative. His TH dose was increased. US showed homogenously enlarged thyroid. Perchlorate washout showed The 6 hour uptake was 34 % (normal is 3 - 25%). diffuse, homogeneous uptake within an enlarged thyroid gland. The concentration of I-123 within the thyroid gland decreased by 80% over 30 minutes, which was consistent with congenital organificatioin defect.
Tachycardia could not be explained.
He was noted to have hct of 55mg% confirmed which also could not be explained, his erythropoetin level was 10 (not suppressed)
Three months after his initial evaluatioin he reported loss of visual acuity, uncorrectable. An MRI was ordered but could not be completed because of "anxiety" which mom noted was accompanied by abdominal pain. A plain film revealed 2 large cattle magnets in his stomach which were corroded. The components of the magnet were Cobalt, Aluminum, Nickel, Copper,and Iron.
The magnets were removed surgically after endoscopic removal failed. Chelation therapy was completed post-operatively. His goiter has resolved, hct has decreased and tremor resolved. His vision has improved.
Cobalt is known to cause thyroid dysfunction by interrupting iodine incorporation. It can also cause decreased O2 dissociation from Hgb thereby causing tachycardia and stimulating Hgb production. Aluminum can cause neuropathy. This is an unusual presentation for heavy metal poisoning and should be kept in the differential for thyroid dysfunction.

PO2-310 Thyroid II

Rare cases of "fulminating" auto-immune thyroiditis in infants

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Autoimmune thyroiditis is uncommon in infants and evolution toward myxoedema is rare.
2 cases of infants are reported which occurred during the first year of life, one with familial history.
Case 1. S… an 9 month old infant presented in paediatric surgery for a umbilical hernia…, and a typical myxoedema confirmed by TSH 680 mU/l free T4 < 2 mcmol/l and positive thyroid antibodies. Neonatal screening was negative and was controlled. Thyroid ultrasound and Tc scintigraphy did not show any thyroid gland. Nevertheless thyroglobulin was at a significant level. Thyroxine therapy treatment was administered. Unfortunately, the child died 2 months later due to myocarditis. Autopsy showed remnants of thyroid tissue with lymphocytic infiltration suggesting thyroiditis autoimmune process.
Her brother B. was hospitalized at 10 years of age with myxoedema and typical auto-immune thyroiditis. Family investigation only showed antistomach antibodies in the father, and there was no evidence of IPEX syndrome.
Case 2. L... 6 months old infant was hospitalized for myxoedema (TSH 407 mU/l free T4 = 0). Neonatal screening was normal and was controlled. Thyroid antibodies were positive. Iodine scintigraphy revealed an heterogeneous but reduced thyroid gland and the patient is currently under treatment.
This destructive auto immune process leading to myxoedema within the space of a few months has been very rarely reported in the literature.

PO2-311 Thyroid II

Tissue-specific knockout of TSHr in adipocytes increases white adipocyte size and decreases TSH induced lipolysis

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Background
The primary function of TSH is to activate TSH receptors (TSHr) in the thyroid gland and thereby stimulate thyroid hormone synthesis and secretion. TSHr are also expressed in other organs, such as human heart and bone but their physiological importance is still unclear. We have previously shown that TSHr, expressed in human adipocytes, are of potential importance for lipolysis and extrauterine adaptation during the neonatal period
Methods
To further study the role of TSHr in adipocytes we selectively removed the TSHr gene in mice brown and white adipocytes by using the Cre-loxP recombination system. TSHr knockout (KO) newborn mice were phenotypically characterized. Epididymal isolated adipocytes from eight-week-old male mice were studied in terms of cell size and metabolism. Cell diameters (n = 1000) were measured in each of eight eight-week-old knockout and eight eight-week-old wild-type mice.
Principal Findings
Mice lacking TSHr in adipocytes were apparently normal at birth and at eight weeks of age. No differences in thyroid gland histology were observed and thyroid hormone levels were normal. Southern blot analysis of DNA from isolated adipocytes demonstrated that the somatic inactivation of the TSHr gene was effective (70% - 80%), although not complete. TSHr transcript was less abundant *(<75%)* in adipocytes from TSHr *knockout* as compared to *wild-type littermates* Sensitivity for TSH induced lipolysis was ten times lower in adipocytes from targeted animals compared to wild-type indicating that adipocytes had shifted from the physiological range of TSH. Catecholamine-induced lipolysis and insulin-induced inhibition of lipolysis were unaltered. Adipocyte size was increased in the targeted animals compared with wild type animals. (mean ± SEM 53.77 ± 0.18 µm and 49.10± 0.227 µm, respectively, P<0.001). Basal lipolysis was increased in targeted animals as a result of the increased adipocyte size.
Conclusion
Our results indicate that adipocyte TSHr under normal conditions affects white adipocyte growth and development.This confirms that TSHr in extrathyroidal tissues might be of physiological importance

PO2-312 Thyroid II

22 years of untreated congenital hypothyroidism

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Since the introduction of the newborn screen program in the US in 1970's, undiagnosed and untreated adult-age patients with congenital hypothyroidism have not been described.
We present a case of a 22 years old Mexican male with previously undiagnosed congenital hypothyroidism.
The patient was diagnosed with Down syndrome at 3 years of age. He was brought to the ED because of respiratory distress. His past medical history was significant for developmental delay and life-long constipation. Physical examination findings were not consistent with trisomy 21. This non-ambulatory boy was very petite, measuring 77 cm in length (Z-score= -13.18) and weighed 14.6 kg (Z-score= -23.96). He had coarse facial features, macroglossia, depressed nasal bridge, dry skin and hair. His cry was hoarse. Dentition was delayed with most teeth being deciduous. Thyroid gland was not palpable, but marked myxedema and small umbilical hernia were present. He had Tanner stage 1 genitalia development. The relaxation phase of deep tendon reflexes was prolonged. His chest radiography showed massive cardiac silhouette due to pericardial effusion, confirmed by echocardiogram.
Clinical course:
Laboratory studies revealed TSH of 101 uIU/mL (reference range 0.35-5) and free T4 of 0.24 ng/dL (reference range 0.71-1.85), consistent with primary hypothyroidism. A thyroid gland was not visible by ultrasound. Technetium thyroid scan had no uptake. Hand radiography was notable for markedly delayed bone age close to 3 months.
From the spectrum of medical problems related to congenital hypothyroidism, this patient had profound developmental and growth retardation, bilateral hip dysplasia, anemia, hearing loss on BEAR testing and pericardial effusion. A brain MRI demonstrated cerebral volume loss and mild cerebellar hypoplasia without enlargement of the sella turcica.
During the 9-month follow-up on levothyroxine the patient has grown 9 cm. He exhibited improvement in cognitive and motor skills as demonstrated by increased vocabulary and ability to ambulate with assistance. This case confirms crucial role of thyroid hormone for normal cerebral, skeletal and gonadal development.

PO2-313 Thyroid II

Chronic lymphocytic thyroiditis (CLT): clinical characteristics in children and adolescents from an urban Spanish population

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Background: Chronic Lymphocytic Thyroiditis (CLT) is the most frequent cause of goiter and acquired hypothyroidism in children and adolescents in non-deficient iodine areas. We present the clinical features of a series of patients diagnosed of CLT in an urban Spanish population.
Material and methods: Retrospectively, we studied 123 patients who had been diagnosed of autoimmune thyroid disease at our Hospital. Amongst these, 106 (86%) corresponded to girls (ages: 1 to 16 years old), and 17 (14%) to boys (ages: 5 to 16 years old). First medical appointment reason included: obesity, growth retardation, family history of autoimmune disease and co-existence of an associated autoimmune pathology. We recorded patients' clinical characteristics, laboratory and imaging results, and incidence of replacement therapy.
Results: Incidence of TLC was higher in girls between 7 and 15 years of age, with a clear pick at 9-10 years, while in boys higher incidence was detected between ages 10 to 14 years. At time of diagnosis, 14% of the patients were obese, independently to age or sex. Furthermore, unconnected to obesity, non-familial hypercholesterolemia was detected in 24% of the cases. Eighty-nine patients (75%) showed goiter. Among these, 79 (88%) corresponded to girls, 52 (65%) of them in puberty ages; in boys, goiter did not show any correlation to puberty. Eighteen patients (14%) suffered of associated autoimmune pathology such as celiac disease, vitiligo, type 1 Diabetes Mellitus and alopecia. All patients showed a positive result for anti-thyroid antibodies: 81 children (65%) were positive for both anti-TG and anti-TPO antibodies, 29 patients (23%) for anti-TG antibodies alone and 13 patients (10%) for anti-TPO antibodies alone. Ultrasound imaging was performed in 73 patients. Amongst these, 44 (60%) showed a pathological thyroid gland, with a chess-like pattern and nodular configuration. Thirty-nine patients (32%) were treated with L-T4.
Conclusions: In our series, we have observed a higher positivity for anti-TG antibodies compared to anti-TPO antibodies; thus, presence of anti-TG antibodies alone may reveal a diagnosis of TLC. We have not identified any TLC case in boys younger than 5 years of age. Goiter does not correlate to puberty in boys as it does in girls. Testing for anti-thyroid antibodies seems mandatory in patients with another autoimmune disease to rule out TLC diagnosis.

PO2-314 Thyroid II

Heterophile antibody: pitfalls in interpretation of thyroid functions

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Serum thyrotropin measurement is a critical requirement in diagnosis and management of patients with variety of thyroid dysfunctions –hyperthyroidism, hypothyroidism, and resistance to thyroid hormone. Heterophile antibodies are antibodies against specific animal immunoglobulins or against immunoglobulins of various animal species, depending on the cross-reactivities between

species immunoglobulins. It has been recognized, for the past several years, that the presence of heterophile antibodies may interfere with currently used, two-site immunometric assays for TSH measurement. The most common heterophile antibodies are human anti-mouse monoclonal antibodies (HAMA), which can react with mouse monoclonal antibodies that are used in many immunometric assays.
We describe a case of 12 yr old girl who presented with goiter, tachycardia, elevated free T4, and T3, and inappropriately normal TSH. Moreover, two additional TSH assays done at two Reference labs - Quest Diagnostics and Esoterix also reported similar TSH values. Further investigations were carried out to look for a TSH producing pituitary adenoma and resistance to thyroid hormone, which were negative. Subsequently, a sample sent to Dr Roy E Weiss' lab at the University of Chicago, reported the TSH to be suppressed. Furthermore, dilution assays done at Esoterix and in our local lab confirmed the presence of HAMA. Patient was then started on Methimazole, which led to euthyroid status within 4 weeks.

Table 1

Dilution	Observed TSH	Expected TSH	Suggestive of HAMA	Free T4
Patient #1	1.48uU/ml			2.64ng/dl
with 5% Mouse Serum	0.06uU/ml	1.41uU/ml	Yes	1
with 50% Mouse serum	0.05uU/ml	0.74uU/ml	Yes	

Performed at Esoterix Lab, 4301 Lost Hills Road, Calabasas Hills, California 91301, with help from Dr Kelly Y. Chun, Ph.D

Two additional patients under 18 years were falsely given the diagnosis of subclinical hypothyroidism due to mild elevation of TSH and were later recognized to be due to HAMA interference, as well, based on dilution studies.
The prevalence of HAMA interference may be rising because of increasing use of Monoclonal murine antibodies for immunotherapy. We recommend that when faced with discrepant thyroid function results, awareness of possible heterophile antibody interference and need for additional testing to prove non-linearity with sample dilution, would be much efficient way to avoid expensive testing and delayed or unnecessary treatment.

PO2-315 Thyroid II

Prevalence of goiter among children of age 11-16 years in Ahwaz, Iran

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Objective: This cross-sectional study was conducted in Ahwaz, Iran to find out the prevalence of goiter among guidance school children in the age group of 11-16 years.
Method: Multistage sampling methodology was followed for selecting the study population.
Results: A total of 1950 children were clinically assessed for thyroid gland enlargement. Data were collected on age, sex, weight, height, iodized salt consumption, family history
of goiter, and thyroid size by palpitation. 146 children showed various grades of goiter, giving a prevalence of overall goiter of 7.48%. Of 146 persons with goiter, 31.5% (46 cases) were male and 68.5% (100 cases) were female. Prevalence of goiter among male and female students was 5.11% and 9.52%, respectively. Prevalence of grade 1, 2, and 3 goiter was 56.8%, 37.7%, and 5.5%, respectively. We found a considerable difference between height of students with grade 1 goiter and students with grade 2 and 3 goiter ($p<0.001$). A significant difference was found between weight of students with grade 1 goiter and students with grade 2 goiter ($p<0.002$).
Conclusion: These results suggest that it is unlikely that Ahwaz city is an endemic area to iodine deficiency, and it is probably because of the geographic location of this area (low height of the sea level) or nutritional habits of its population (consumption of fish and iodized salt). The observed differences between height and weight of children reflect the effect of thyroid hormones on growth of children and further support the need for thyroid examination and correction of probable hormonal disturbances.

PO2-316 Thyroid II

Prevalence of thyroid dysfunction in newly diagnosed type 1 diabetic Iranian children

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Objective: Thyroid disease may affect diabetes management. The aim of this study was to investigate the prevalence of thyroid dysfunction among children and adolescents with newly diagnosed type I diabetes in Iran.
Materials and methods: A case-control study was done between March 2005 to August 2008 in the pediatric ward of a tertiary Hospital, Ahwaz, Iran. 75 consecutive newly diagnosed type 1 subjects were selected and were compared with 105 healthy control children. Physical examination for signs of thyroid dysfunction and estimation of thyroid size by using palpation were performed and TSH and T3 and T4 were measured to evaluate prevalence of thyroid dysfunction.
Results: Prevalence of thyroid dysfunction in diabetics was 14.6%; of them 9.3 % were subclinical hypothyroidism, 4% hypothyroidism and 1.3% subclinical hyperthyroidism which were higher than normal controls. Goiter prevalence by palpation was the same in both groups.
Conclusions: Because of higher prevalence of thyroid dysfunction in newly diagnosed type 1 diabetes than in controls, we confirm the recommendation that thyroid testing be done at diagnosis and routinely in children and adolescents with type I diabetes.

PO2-317 Thyroid II

Phenotypes, genotypes and outcome of neonatal hyperthyrotropinemias

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Neonatal hyperthyrotropinemia is the congenital elevation of TSH (CETSH) with thyroid hormone levels in the normal range. In contrast to congenital hypothyroidism (CH), the therapeutic approach towards CETSH has not been clearly established. In regions with iodine deficiency, etiology of CETSH is suggested environmental, but evidence accumulates of a genetic component of the disease.
Aim. Prospectively determine the incidence of CETSH in the population, its transient or permanent course, and its possible molecular origin.
Patients & **Methods.** All babies positive for CH screening (filter paper TSH ≥ 10 mU/L) born in Navarra region (Spain) between the years 2000 and 2006, with serum confirmatory TSH ≥ 10 mU/L and normal FT4 levels were followed by FTFs during the first semester of life. When TSH was ≥ 3 DS or FT4 was ≤ -1 DS for age (according to Spanish normative values) treatment with levothyroxin was initiated. At 3-5 years of age, reevaluation was performed after 1 month L-T4 withdrawal with TRH test (7 mg/kg, iv), thyroid ultrasounds and scintigraphy, and molecular study of thyroid hormonogenesis.
Results. Over a population sample of 42,321 newborns, 23 cases of ECTSH were identified, representing a prevalence of 1:1,804 neonates. At reevaluation of children, TRH test detected overstimulation of the pituitary-thyroid axis (peak TSH ≥ 35 mU/L) and thus permanent CETSH in 80% of cases (17/21). Average duration of T4-treatment in transitory CETSH was 37±20 months. Children with CETSH are carriers of heterozygous mutations in DUOX2 (E641K, A728T, H678R, R701Q, P982A, L1243R), DUOXA2 (R132W) and TSHR (R519H) genes which are not described as polymorphisms in genetic databases.
Conclusions. CETSH is a very common neonatal disorder whose outcome is mostly permanent. Considering CETSH as part of the wide spectrum of CH, real prevalence of CH is 1: 1,175, triplicating the generally accepted prevalence for this disorder. The molecular screening of CETSH suggests moderate dyshormonogenetic forms, and the morphological study identifies mild dysgenetic alterations of thyroid development.

PO2-318 Thyroid II

TSH deficiency and macroorchidism: clinical description of a novel phenotype of central hypothyroidism

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Congenital hypothyroidism (CH) and pseudo-precocious puberty was a frequently reported association before the CH screening era. Typically, prepubertal boys with primary hypothyroidism had large testicles, together with physiologically low levels of gonadotrophins and testosterone. Thyroid hormone inhibits proliferation and stimulates differentiation of Sertoli cells. Thus, longstanding hypothyroidism in these boys caused over-proliferation of Sertoli cells, major contributors to testicular volume. We present 2 boys diagnosed with central CH who pre-pubertally developed abnormal testicular enlargement. Hypothyroidism was treated from the beginning of their lives, and cannot be invoked for the pathogenesis of their macroorchidism.
Patient 1 was undetected by a TSH-based CH screening program, but presented clinical hypothyroidism at 14 days. Patient 2 was identified in a T4-based CH screening. In serum, both had low-normal TSH (1.4 and 2.6 mU/L) and low FT4 (7.2 and 10 pmol/L). TRH tests confirmed central hypothyroidism with distinct type 2 response (Table). Prolactin responses were not diminished. L-T4 was started at 8-10 mg/kg/day but, remarkably, very low doses (1.4- 2 mg/kg/day) were needed to maintain euthyroidism, and strongly suppressed TSH (< 0.005 mU/L). Pituitary MRI was normal in both cases. Patient 1 had a mildly hypoplastic thyroid at scintigraphy.

TRH test

	Age	TSH 0 '	TSH 20 '	TSH 30 '	TSH 60 '	TSH 180 '
Patient 1	3 yr	1.07	3.28	-	2.69	-
Patient 1	6 yr	1.45	5.56	-	4.69	-
Patient 2	1 1/2 mo	3.8	9.2	10.6	5.4	4.1

Patient 1 showed progressive bilateral testicular growth (4 yr 3-4 ml; 8 yr 5 ml; 10 yr 6 ml; 11 yr 8 ml) always with prepubertal FSH (0.66-1.1 UI/L), LH (0.10-0.18 UI/L), and testosterone levels (< 0.10 nmol/l), and response to GnRH. Patient 2 had abnormally large (6 ml) testes at 11 yr, with hormonal profiles completely prepubertal. Patients spontaneously entered puberty at 12.5 years, with testes of 12 and 10 ml, respectively. Mid-pubertal volumes are currently 35 and 30 ml. Testicular ultrasounds proved normal in patient 1.
We hypothesize that this phenotype is caused by the secretion of an abnormal TSH molecule, with low bioactivity towards the TSH receptor but aberrant stimulation of the testicular FSH receptor. Since macroorchidism was absent in males reported with isolated TSH deficiency due to mutations in the TSHβ gene, we propose this phenotype might represent a genetically distinct entity.

PO2-319 Thyroid II

Large-scale molecular screen of DUOX2 and DUOXA2 genes in thyroid dyshormonogenesis

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H_2O_2 generation is a crucial step in thyroid hormonogenesis. The proteins responsible for this activity are DUOX2 and its maturation factor DUOXA2. After the initial description of 4 nonsense mono- and biallelic DUOX2 mutations in patients with transient or permanent congenital hypothyroidism (CH), a few compound heterozygous missense mutations have been reported linked to a permanent mild hypothyroidism. However, the molecular clues underlying transiency or permanency of this type of hypothyroidism, including the severity of each mutation and the number of alleles affected, remain to be established.
Methods: We screened DUOX2 and DUOXA2 genes in a large international cohort of 97 patients with permanent or transient CH (including hyperthyrotropinemia) of proven dyshormonogenetic origin.
Results: We identified 18 novel heterozygous mutations in DUOX2 and 2 novel mutations in DUOXA2 in a total of 37 patients. Two DUOX2 mutations are *nonsense*, generating prematurely truncated proteins (K530X, Q1301X), 14 change one amino acid (P311L, R354W, R422S, Q570E, E641K, H678R, R701Q, R726W, A728T, M883I, P982A, R1110Q, A1127G and L1343F) and 2 (IVS13-1C, IVS27+2A) alter a splice-site. Topographically. 6 mutations are located in the peroxidase-like domain, 6 in the 1st intracellular, EF-hand-containing loop, and 6 in the oxidase (NOX) domain of DUOX2. SIFT, Polyphen and Blosum62 computer programs predict damaging or possibly damaging effects of most of these mutations (n=12) on DUOX2 function. Interestingly, mutations H678R, R701Q and P982A appear genetically linked and, in 50% of cases, are associated with another mutated DUOX2 in this cohort In DUOXA2, two novel mutations were identified (W132L, G294E) in patients with permanent congenital hyperthyrotropinemia and euthyroid goiter.
Conclusions: DUOX2 mutations are frequently present (30%) of CH patients with a gland *in situ,* while DUOXA2 defects are more rare (2%) in this cohort. Only DUOX2 mutations are found in either transient or permanent CH cases. Most mutations are predicted to be pathogenic. *In vitro* determination of functional impact of individual (and linked) mutational changes will allow adequate genotype-phenotype correlations in thyroid H_2O_2 generation defects.

PO2-320 Thyroid II

Elevated free thyroxine levels detected by a neonatal screening system

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In Sapporo city of Japan, neonatal screening for congenital hypothyroidism has employed the measurement not only TSH but also free T4 in filter-paper blood spot. This system has enabled us to detect hyperthyroxinemic disease. Between January 2000 and December 2006, filter-paper blood samples was collected from newborns at 4-6 days of age and neonates who showed elevated free T4(>4.0gn/dl,4SD above the mean) was studied. 11 infants were identified with persistent hyperthyroxinemia. 8 had neonatal Grave's disease. 7 of 8 mothers were treated with antithyroid drug in pregnancy, and only one mother was detected after neonatal screening. One infant with slightly elevated free T4 and normal TSH was diagnosed as familial dysalubuminemic hyperthyroxinemia (FDH). 2 neonates showed elevated free T4 without suppressed TSH and were considered as resistance of thyroid hormone (RTH). One had a heterozygous point mutation (T277I) and the other had a heterozygous single base insertion(C) in codon 446-447 of the TRβ gene, respectively.
In conclusion, free T4 screening system enables for early awareness of RTH and FDH. Regarding neonatal Grave's disease, the benefit of elevated free T4 screening is small, because most mothers with Grave's disease were almost treated. As there have been few studies of long-term psychomotor development of neonatal Graves' disease after recovery, it is important to follow up these patients continuously.

PO2-321 Thyroid II

Diagnostic accuracy of four-modality (Quattro mode) assessment in determining the cause of congenital hypothyroidism in the newborn

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Introduction Determining the unequivocal cause of congenital hypothyroidism (CH) in newborns referred with TSH elevation is helpful in establishing the lifelong diagnosis, assisting with genetic counselling, and ascertaining thyroxine requirement.

Aims To compare the diagnostic success and propensity to mislead using one, two, three or all of the following four modalities ('Quattro mode'): pretreatment venous thyroid function tests (TFTs), quantitative thyroglobulin (Tg), thyroid ultrasound scan (USS) and ^{99m}Pertechnetate radio-isotope scan (RIS).

Methods Data were re-assessed retrospectively in 46 newborns (29 girls) born from January 2004 to May 2008 who had attended our centre for diagnostic imaging. Radiologists were blinded as to the initial interpretation of scans.

Results Three cases of transient elevated TSH were found (one with no uptake on RIS) and one patient has probable CH, requiring diagnostic challenge at a later date for definitive proof. Final diagnosis in the 42 children with definite CH was thyroid dysgenesis in 32 (76 %) and dyshormonogenesis in 10 (24 %), including twins with thyroglobulin gene defect causing obstructive goitre. Causes of dysgenesis were: thyroid ectopia (14); hypoplastic gland *in situ* (4, with TSH-R defect in 1); true athyreosis (defined as undetectable fT4 and Tg with no uptake on RIS) (2); apparent athyreosis with measurable Tg (5); and unclassified dysgenesis due to incomplete Quattro analysis (7: 6 with no Tg value and 1 where TSH was within the normal range - 1.89 mU/l - on day of scan).

A definitive diagnosis was made in 100 % of cases when full Quattro mode assessment was performed. The diagnostic rate using other combinations of tests was: TFTs alone 0 %; TFTs + Tg 2.9 %; TFTs + USS 40 %; TFTs + RIS 75 %; TFTs, USS + RIS 86 %; TFTs, Tg + USS 43 %; TFTs, Tg + RIS 75 %.

Incorrect causes suggested by these combinations were: TFTs alone 17 %; TFTs + Tg 3.8 %; TFTs + USS 37 %; TFTs + RIS 11 %; TFTs, USS + RIS 0 %; TFTs, Tg + USS 0 %; TFTs, Tg + RIS 11 %.

ConclusionQuattro mode assessment optimises diagnostic accuracy and may suggest targets for mutational analysis in biosynthetic defects. Measuring Tg in infants with suspected CH indicates the presence of thyroid tissue, regardless of uptake on RIS and/or image interpretation on USS.

PO2-322 Thyroid II

Molecular biomarkers of malignancy (BRAF, RET/PTC-1, RET/PTC-3, AKAP9/BRAF and PAX8/PPARgamma) in childhood thyroid nodular disease: analysis of 119 aspirates from biopsy

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Oryginality: The genetic abnormalities in childhood vs adult thyroid cancer are different and therefore it is a challenge to investigate preoperatively biomarkers of malignancy in these age groups.

Methods: Preoperative material from fine-needle biopsy (aspirates) of childhood thyroid nodules was studied (n=119). Both, DNA (for BRAF gene mutation) and RNA (for fusion oncogenes - RET/PTC-1, RET/PTC-3, AKAP9/BRAF, PAX8/PPARgamma - expression analysis) were isolated. From the total DNA, the exon 15 of BRAF gene was amplified to investigate the T1799A (V600E) mutation based on PCR-sequencing technique. Total RNA underwent reverse-transcription and polymerase-chain-reactin (RT-PCR) to obtain cDNA and to analyse the expression of markers of thyroid cancer (candidate genes) (Qiagen OneStep RT-PCR).

Analysis: In all samples the analysis of control genes: GAPDH (for the qualitative and quantitative evaluation of RNA) and thyroglobulin (control marker of thyroid cells) were performed. Aspirates used for studies were verified histologically in the vast majority of patients and represented all histological types of thyroid disease including 11 PTC, 1 FTC and 2 MEN2A.

Results: T1799A (V600E) BRAF mutation was negative in all 119 samples. Among fusion oncogenes the positive result for RET/PTC-1 was seen in 3 samples of 119 (only in PTC), in 4 samples/119 was positive for RET/PTC-3 (PTC, follicular adenoma and in 2 aspirates verified postoperatively as colloid goiters). PAX8/PPARgamma was positive in only 1 patient with congenital hypothyroidism and coexisting thyroid nodules in both lobes (histologically: dyshormonogenetic goiter). AKAP9/BRAF was negative in all analysed aspirates.

Conclusions: 1. BRAF gene mutations were not responsible for thyroid cancer in the studied children. 2. RET/PTC-1 rearrangements proved the sporadic nature of this type of thyroid cancer and had 100% specificity compared to 25% of RET/PTC-3 positive result. 3. The assessment of biomarkers in aspirates from thyroid nodules may improve preoperatively the classical cytological evaluation however the risk of false-positive result should also be considered.

PO2-323 Thyroid II

Six new mutations of the thyroglobulin gene discovered in Taiwanese presenting with thyroidal dyshormonogenesis

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Background: Thyroglobulin (Tg) defect is a rare cause of congenital hypothyroidism. While a few mutations of the human Tg gene have been identified, we have suspected a Tg defect in 38% of Taiwan Chinese presenting with moderate or severe thyroidal dyshormonogenesis.

Study objective: To report the discovery of new Tg gene mutations and associated clinical manifestations of Tg defect.

Patients and results: In 7 patients from 6 families, we detected 6 new Tg gene mutations, including c.1348 del T, p.R451X, IVS3 +2T>G, c.1712del T, p.Q1784X, and c.6047 del A. The c.1348 del T and p.R451X mutations were the most common, respectively detected in 33% and 25% of alleles studied. Haplotype analysis revealed that the c.1348delT mutation is due to a founder effect, while p.R451X is probably due to independently recurrent *de novo* mutations. mRNA transcript of the IVS3 +2T>G mutant, detected in blood by RT-nested PCR, showed skipping of exon 3 and a frameshift, with a terminal signal after 17 altered amino acid residues. **Conclusions:** Tg defect plays is important cause of severe thyroidal dyshormonogenesis in Taiwan Chinese. Its genetic characteristics are markedly different from those described in other populations presenting with mutations of the Tg gene.

PO2-324 Thyroid II

Transient acidosis in infancy with a novel variant in MCT8 (Monocarboxylate transporter 8) gene

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MCT8 (Monocarboxylate transporter 8) has important role of triiodothyronine(T3) uptake into central neurons. Impairment of MCT8 function induces several neurological symptoms (psychomotor retardation, hypotonia, epilepsy), and patients show characteristic pattern of serum thyroid hormone level (elevated T3, lowT4, and normal TSH). The symptoms except for neurological findings have not been noticed in this disorder, although many organs express MCT8. Here, we examined the Japanese boys having a novel SLC16A2 mutation (L304P) responsible for MCT8 dysfunction, about their acid excreting function at renal tubules, adding longitudinal blood lactate levels. As a result, they had shown distal renal tubular acidosis (dRTA) ascribable to acid excreting dysfunction in infancy. Moreover, transient hyperlactacidemia

was also seen. These findings were dominant in infancy, and became latent gradually. These are interesting findings clinically suggesting hypofunction of Monocarboxylate and proton transport in his other organs, especially kidney.

PO2-325 Thyroid II

Differentiated thyroid cancer in childhood: review of the Hospital for Sick Children, Toronto experience from 1984 to 2006

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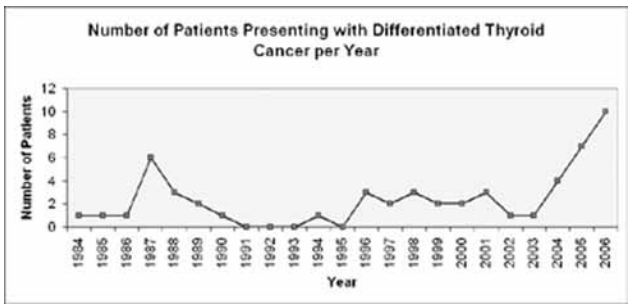
Background: Differentiated thyroid cancer (DTC) is uncommon in childhood. The reported age standardised incidence rate was 5.3/million/year in Canada from 1997-2001, accounting for 3.4% of childhood solid tumors. We aimed to describe our experience in the management of pediatric DTC from 1984-2006, and to explore if the incidence at our institution is changing.
Methods: Cases were identified by searching pathology databases for cases of DTC following surgical intervention between 01/1984-12/2006. Retrospective review of health records for demographics, presenting features, pathology, treatment and outcomes to age 18y was conducted.
Results: 54 subjects were identified: 36 F, 18 M (p=0.01); aged 13.3±3.0 years (no sex difference) and followed for 3.5±2.5 years. 6 had history of therapeutic radiation exposure for previous malignancies. 6 were not born in Canada, including 3 born in Eastern European regions with high thyroid cancer endemnicity. Year of presentation is shown. 4/5 patients with maximum tumor diameter <1cm presented in 2005-06.
Presentation was as an asymptomatic thyroid nodule (N=42) and cervical lymphadenopathy (N=12). 4/54 patients had a family history of thyroid cancer. All patients underwent total thyroidectomy for surgical tumour excision (including 14 patients who underwent a second procedure for completion thyroidectomy): 11 had postoperative hypocalcemia (transient in 4, persistent in 7) and 1 had transient hoarseness. 52/54 received radioactive I-131 (RAI) therapy (range 1-4 doses, cumulative RAI dose 13372 mCi).
Pathology showed: 38 pure papillary; 3 papillary-diffuse sclerosing variant; 7 papillary-follicular; 6 pure follicular. 25/54 had pathological evidence of tumor metastases to lymph nodes. 8/54 patients demonstrated distant RAI uptake after RAI therapy (including 5 with lung metastases). 7/54 more patients developed distant metastases during follow-up.
Discussion: These data support suggestions that the incidence of DTC is increasing and this does not appear to be related to identification of smaller tumors. Consistent with previous studies, many patients had metastases at presentation, but mortality was low.



PO2-326 Thyroid II

A multisystem disorder associated with defective selenoprotein synthesis and a thyroid signature

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The "superfamily" of ~25 human selenoproteins includes antioxidant and oxidoreductase enzymes together with other proteins of unknown function. We describe a child with a multisystem disorder involving deficiencies of several selenoproteins, identified on the basis of abnormal thyroid function.
A 3.6 year old male was referred with elevated free thyroxine {FT4-44.4pmol/L (N 12-22)}, low free triiodothyronine {FT3-1.9pmol/L (N5.2-10.2)} and normal TSH {2.9mU/L (N<6)} concentrations, indicating reduced T4 to T3 conversion. Deiodinases (DIOs) are selenoenzymes, and low circulating selenium 0.06µmol/L (N 0.5-1.3), undetectable glutathione peroxidase (GPx) & reduced selenoprotein P concentrations, suggested multiple selenoprotein defects. Incorporation of selenocysteine (Sec) during protein synthesis requires binding of a multiprotein complex that includes the **SElenoCysteine Insertion Sequence Binding P**rotein 2 (*SECISBP2*) to a stem loop structure in the 3'-untranslated region of their mRNAs. *SECISBP2* gene sequencing identified a heterozygous missense mutation (Cys691Arg) in the proband and his mother, involving a highly conserved cysteine residue within the C-terminal RNA-binding domain of this protein; an additional defect, involving aberrant *SECISBP2* splicing, was identified in the proband's other allele and his father. Additional features in the index case included short stature (height -2.4SDS), mild global developmental delay, and proximal muscle weakness. Selenoprotein N (SEPN) is essential for normal muscle function, and SEPN expression was markedly reduced in cultured fibroblasts from the proband. Progressive failure to thrive in infancy led to a diagnosis of eosinophilic colitis at 24 months. Body composition was abnormal, with markedly increased fat mass (+2SDS), but associated with a propensity to non-ketotic fasting hypoglycaemia requiring supplementary enteral nutrition. Auditory assessment suggested bilateral high frequency hearing loss, which is also a feature in DIO2 null mice. Normalisation of FT3 levels following commencement of liothyronine treatment, was associated with improvement in linear growth, speech and neurodevelopment.
This unusual genetic disorder highlights the diverse roles of selenoproteins in biological processes and may also be a useful paradigm to model consequences of human selenium deficiency.Àβµ

PO2-327 Thyroid II

Pediatric differentiated thyroid cancer (DTC): presentation and follow up

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DTC is the most frequent endocrine malignancy in childhood.
Presentation and long term outcome vary in different series.
To report the clinical findings, treatment and follow-up of pediatric DTC, we analyzed the clinical charts and anatomopathological reports of 43 patients with DTC (9 males) (6,2-19,5 years)(36 pubertal) followed up for 0,16 -13,1 years at the Endocrinology Unit between 6/88 and 12/08.
Results: 32 patients consulted with a thyroid mass, 8 had also palpable cervical lymph nodes (CLN) and 11 multinodular goiters. Tumor size by palpation was <1cm: 2, 1-4cm: 30 and >4cm: 10. All underwent total thyroidectomy; 3(7%) presented recurrent laryngeal nerve paralysis and 8(18,6%) permanent hypoparathyroidism.19 had CLN involvement, 4 distant LN metastases (MTS) and 5 lung MTS. Out of the 37FNAB performed 37,8% were diagnostic and 37,8% suspicious. Anatomopathological studies revealed 38 papillary and 5 follicular carcinomas, 25 multicentric and 21 with extrathyroidal extension. All underwent postoperative radioiodine remnant ablation and l-thyroxin suppressive therapy. 6 recurred: 3 regional LN, 1 mediastinal LN, 1 in lung and 1 indeterminate location. At 5 years of follow up (n: 20) 70% had no evidence of disease. In children DTC mainly presented as a palpable single thyroid mass

(32/43) had already LN involvement (45%) and was predominantly papillar (38/43) multicentric (59,5%) with pulmonary MTS (14,3%). FNAB was a useful tool for diagnosis and therapeutic approach allowed eradication of the disease in 70% of patients followed up for 5 years.

PO2-328 Turner, Noonan I

Spontaneous regular menstruation is expected, if LH is <5 mIU/ml, and FSH is <10 mIU/ml continuously from 10 to 12 years of age in Turner syndrome

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Background:
Primary hypogonadism of Turner syndrome (TS) is variable. Prediction of the presence of spontaneous regular menstruation in their prepubertal or early pubertal age is useful in clinics, although studies aiming this kind of prediction have scarcely been done. The aim of the present study is to investigate whether karyotype, LH, FSH, E_2 concentrations, and bone mineral density (BMD) data can predict spontaneous regular menstruation from 10 to 12 years of age in TS.
Subjects:
50 TS patients (without Y fragment) who were able to examine retrospectively about spontaneous menarche and menstruation cycle were the subjects. Their current ages were from 12 to 30 years of age. They were divided into 3 groups, Group A (n=37) with no spontaneous menarche, Group B (n=6) with secondary amenorrhea or irregular menstrual cycle, and Group C (n=7) with spontaneous menarche and regular menstrual cycle for more than 18 months. Median of the chronological age at menarche was 14 years and 3 months of age in Group B, 12 years and 6 months of age in Group C. Median of the duration of spontaneous menstruation was 1 year and 3 months (only once in 2 cases) in Group B, and 4 years and 1 month in Group C.
Method:
Karyotype was analyzed in a blood lymphocyte culture in all cases. LH, FSH, LH/FSH, E_2 and BMD were analyzed respectively at 10 and 12 years of age (before starting hormone replacement therapy or menarche). BMD was checked by quantitative computed tomography (QCT mg/cm^3) and dual energy X-ray absorptiometry, DXA (g/cm^2)/BMI(kg/m^2) and DXA(g/cm^2)/BSA(m^2).
Results:
In Group C, 5 with mosaicism without structural abnormality of the second X (45,X/46XX, 45X/47,XXX), and 2 with mosaicism with structural abnormality of the second X were observed. LH was <5 mIU/ml, and FSH was <10 mIU/ml continuously in Group C, except for a case that developed secondary amenorrhea 2 years and 1 month after menarche. These data were significantly different from LH and FSH in Group A or B. The data of LH/FSH, E_2 and BMD in Group C overlapped those in Group A or B. There were no differences in LH, FSH, LH/FSH, E_2 and BMD between Group A and B.
Conclusion:
Patients with karyotype of 45,X/46,XX or 45,X/47,XXX were the most likely to have spontaneous regular menstruation as were previously reported. Spontaneous regular menstruation is expected, if LH is <5 mIU/ml, and FSH is <10 mIU/ml continuously from 10 to 12 years of age in TS, which is expected but has not been published.

PO2-329 Turner, Noonan I

Increased abdominal adiposity and insulin resistance in patients with Turner syndrome after growth hormone treatment

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Background: Patients with Turner syndrome (TS) are at increased risk for impaired glucose tolerance and diabetes mellitus. It is not clear, whether growth hormone (GH) treatment commonly used for short stature in TS affects this risk. The objectives were to study glucose tolerance and body composition in formerly GH treated adolescent TS patients.
Method and Patients: In a cross-sectional, single center analysis, formerly GH treated TS patients (n= 56; age 17.29 ± 2.41 years) were tested for glucose tolerance. Local body composition was determined at the non-dominant forearm using peripheral quantitative computed tomography (pQCT). Patients had been treated with GH for a mean of 6.05 ± 3.01 years and were off treatment since 1.72 ± 1.18 years.
Results: Mean BMI-SD in all patients was high (1.24 ± 1.70, p<0.001). An elevated waist-hip ratio (>0.8) was detected in 74% of patients. Oral glucose tolerance testing revealed an increased HOMA (>2) in 43% and a reduced Insulin Sensitivity Index (ISI, Matsuda < 5) in 53% of patients, both suggesting insulin resistance. As expected, congruent data for HOMA and ISI were found in 73% of patients. Only the HOMA correlated with the HbA1c level (r=0.28, p=0.038). None of our patients had an impaired glucose tolerance. Data on body composition showed no correlation with parameters of insulin resistance. There was a significant inverse correlation of final height and waist-hip-ratio (r= -0.33, p=0.014) and a trend towards a lower local fat cross-sectional area (CSA) with higher final height (not significant). Muscle CSA (0.045 ± 1.12 SD) was adequate for patients' height in comparison to a healthy reference population (p=0.52). Cortical bone mineral density (BMD) z-score (-2.15 ± 3.14, p<0.001) and trabecular BMD-z-score (-0.40 ± 1.02, p=0.005) were significantly reduced. Genotype (45,X0 vs. mosaics), duration or time since cessation of GH treatment had no influence on body composition or metabolic parameters.
Conclusion: More than one year after cessation of GH treatment half of TS patients were insulin resistant. Local body composition did not correlate with markers of insulin resistance. Cortical BMD was severely reduced. All TS patients should be followed regularly for metabolic disturbances and bone development after cessation of GH treatment.

PO2-330 Turner, Noonan I

Sensorineural hearing loss in Turner syndrome: ten years of follow-up

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Sensorineural hearing loss (SNHL) is a characterist feature of subjects with Turner syndrome (TS). The onset is in childhood, adolescence and young adult age, remaining stable or progressing slowly with time.
The aim of this study was to evaluate the hearing impairment in a group of young subjects with Turner Syndrome (TS) with sensorineural hearing loss (SNHL) during a ten year follow-up.
Subjects and Methods: 17 pts with SNHL; mean chronological age 17.99±6.18 (range 7-30 yrs); karyotype distribution was: 45,X0 in 52.%, X-structural anomalies in 35%, X-mosaicism in 6%, Y-mosaicism in 6%. Patients were submitted to different therapy regimens: GH and EE or no therapy. Otological examination and audiometric measurement were performed for a period of ten years.
Results: SNHL in TS consists of two patterns: a typical dip involving mid-frequency (MF-HL; 0.5-3 kHz) and a high-frequency loss (HF-HL). 12 patients (70%), 5 with typical dip MF-HL and 7 with HF-HL, remained stable in time, instead 5 patients (30%), all with typical dip (HF-HL), had a progressive decline of hearing. Degree of hearing loss was mild-moderate 20-60 decibel hearing level (dBHL) in 67%, severe (45-60 dBHL) in 29%, profound (>85 dBHL) in 4%.
Conclusions: In our series we observed an early hearing impairment in patients with a typical mid- frequency dip, probably of a genetic origin, instead the HF-HL, probably age related, remain stable with time, probably due to the young age of our patients.
We recommend a regular audiological follow-up, to identify HL at an early stage; in particular the MF-HL is an important predictor for a future hearing decline with an impact on social life.

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PO2-331 Turner, Noonan I

Adult height in patients with Ullrich-Turner-syndrome – with (N=35) and without (N=13) growth hormone treatment

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BACKGROUND: Turner's syndrome (TS) is one of the common genetic disorders characterized by an absent or abnormal second X-chromosome. Short stature is the main clinical feature and in some extent due to the haploinsufficiency of the short stature homeobox-containing gene on the X-chromosome (SHOX). Untreated women are approximately 20 cm shorter than healthy women. In TS girls body length and mass deficits already exist at birth in relation to gestational age.
MATERIAL AND METHODS: In a retrospective study we assessed the effect of growth hormone (GH) on final height gain in girls with TS (n=35) and we evaluated 13 untreated patients with TS who attained adult height. Data of length and weight at birth, gestational age, parents height, karyotype, auxological data and clinical symptoms, especially SHOX-features were obtained from 70 patients.
RESULTS: Age at the beginning of GH treatment was 10.8 ± 2.9 yr (range 5.7-16.4yr) with a GH daily dosage of 6 - 7.5mg/m²/week and a mean duration of therapy of 5.4 ± 2.2 yr (range 2- 9.6 yr). Mean adult height of the treated TS group was 150.6 ± 4.8 cm (H-SDS -3.2) vs. 148.5 ± 4.8 cm (H-SDS -3.6) in the untreated group. There is no significant difference in adult height between the two groups, but there was a significance in target height (TH). TH of TS patients in the GH treated group was 162.5 ± 5.3 cm (H-SDS -0.86) and TH of TS girls in the untreated group was 166.1± 5.6cm (H-SDS -0.18) (p=0.0498). The height gain over the TH was 8.1cm in the treatment group vs. 2.4 cm in the untreated group (p=0.0047).
Birth weight of 63 newborns (gestational age 40 ± 1 week) was 2941 ± 436 g and birth length was 48.8 ± 2.3 cm. In 40% of TS newborns we found a weight or length below 10th centile (SGA, small for gestational age). All of the 7 preterm infants (gestational age 35.6 ± 0.8 weeks) were SGA with a middle birth weight of 1814 ± 226 g and birth length of 43.6 ± 2.4 cm.
CONCLUSION: Growth hormone therapy in TS led to an increase of adult height of 5.7 cm with respect to TH. Adult height correlates with parental height but not with karyotype or number of SHOX features. SGA appears to be one of the symptoms of TS.

PO2-332 Turner, Noonan I

Case report: Noonan syndrome in a patient with two novel heterozygous substitutions of neighboring nucleotides in the PTPN11 gene

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Background: Noonan syndrome (NS) is an autosomal dominant (AD) disorder with an incidence of 1/1,000-2,500 live births, characterized by facial dysmorphism, congenital heart defects, developmental delay, and short stature. The phenotype is variable and may also include skeletal defects, cryptorchidism, and bleeding diathesis. Neurological defects including Arnold-Chiari malformation have also been reported. Four genes are implicated in NS, with the majority of patients (~50%) harboring dominant gain of function mutations in the PTPN11 gene. Many affected individuals have *de novo* mutations; however, an affected parent is recognized in 30-75% of families. We report on genetic/clinical features of 2 members of a family with NS. Case report: Our patient is a 4-year-1-month old male evaluated in endocrinology clinic for growth failure/short stature. His clinical findings, consistent with NS, include typical facial features, pulmonary stenosis, asymmetric septal hypertrophy, lower sternal indentation, wide spaced nipples, 5th finger clinodactyly, one café-au-lait spot, and multiple moles. He has a history of mild speech delay. His response to growth hormone therapy was typical of NS patients, with a good initial response followed by a subsequent decline in growth velocity. The family history is positive for NS in the child's mother, as well as questionable symptoms in the maternal grandmother. Patient's mother: Has features of NS. The head CT scan obtained for worsening headaches showed aqueductal stenosis, obstructive hydrocephalus, abnormal appearance to a vascular structure in the left perisellar region, and soft tissue crowding of the foramen magnum. VP shunt placement improved symptoms. This case adds to previous few reports of NS with neurological defects. Genetic testing: PTPN11 gene sequencing in the child revealed heterozygosity for two novel substitutions of neighboring nucleotides, c.1402A>G and c.1403C>A. These mutations could potentially give rise to either two missense mutations if in *trans (T468A and* T468K) or one missense mutation if in *cis* (T468E). Parental studies were performed to determine the phase of these mutations: The patient's father tested negative, while the symptomatic mother was positive for both mutations, indicating they were inherited on the same maternal allele. We report the details of the clinical and novel genetic findings in this case.

PO2-333 Turner, Noonan I

The influence of clinical and auxological parameters on thyroid size and structure in patients with Turner syndrome

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The phenotypic heterogeneity in Turner syndrome (TS) regards also thyroid function. The frequency of thyroid abnormalities ranges from 4% to 40% in different series. Thyroid enlargment usually is not present at clinical evaluation, so suggesting that predominant form of autoimmune thyroid disease (ATD) in TS is represented by atrophic thyroiditis.
The aim of the study was to determine thyroid volume and structure by ultrasound (US) in patients with TS and to evaluate the association with clinical and auxological parameters.
Seventy-three patients with TS (aged 25.01 ± 10.43 yrs; karyotype 45,X in 38) were included in the study. ATD was present in 32 TS (45,X in 24 pts). At the moment of the US all pts were euthyroid (12 with therapy with L-tiroxine). Ninety-three healthy female subjects height and age matched were used as control group. None of the controls had an acute illness or a history of thyroid disorders. None of them were receiving any drugs affecting thyroid functions or size.
Thyroid evaluation comprised clinical evaluation, measurement of basal serum TSH, FT3, FT4 and anti-TSH-R, anti-TPO and anti-Tg antibodies and execution of thyroid US.
No patient presented goiter. Thyroid volume in TS pts was higher than in controls (7.11± 2.39 vs 6.13±2.25; p=0.007) in particular thyroid size was higher in TS with ATD than in TS without (7.77± 2.35 vs 6.59±2.4, p=0.03) and controls (7.77± 2.35 vs 6.13±2.25, p=0.006). Thyroid volume was significantly influenced by height (p=0.001) and data stratified for height and adjusted for age showed that thyroid size in TS with thyroiditis decreased increasing age (table1).

Age	TS with ATD	TS without ATD	Controls
1-11	6.94±1.21	4.04±1.6	4.48±1.09
11-20	8.26±2.84	6.67±2.1	5.41±1.0
20-27	7.76±2.66	7.21±1.7	8.38±2.0
27-45	7.7±2.2	7.25±2.38	8.72±2.03

No significative difference was present in pts in therapy with L-tiroxine respect to girls without (p=0.21).
Dyshomogeneous thyroid structure was present in all TS with ATD, but also in

34 pts without autoimmune thyroiditis. Dyshomogeneity was associated with significantly higher BMI (p=0.002), volume (p=0.005) and age (p<0.001) and pterygium colli (p<0.0001).
Our data suggest that in the evaluation of thyroid volume in TS pts height and not only age must be taken into account. Structural dyshomogeneity can be related not only to ATD, but also to pterygium colli and BMI.

PO2-334 Turner, Noonan I

Five patients with suggestive Noonan-related syndromes and atypical molecular findings

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Noonan Syndrome and related disorders (LEOPARD, Costello, and CFC syndromes) are entities characterized by the presence of facial dysmorphism, congenital heart defects and short stature. Mutations in genes coding for different components of the RAS/MAPK signalling cascade (PTPN11, SOS1, RAF1, HRAS, KRAS, MEKs) have been described, but still new genetic causes for mutation-negative patientes remain to be identified. Herein we describe 5 cases with atypical findings in molecular diagnosis. Some indirect approaches have been performed to explore the effects of these variants.
Patient 1 had suggestive facial dysmorphism, severe short stature and bilateral cryptorchidism. DNA testing identified an inframe 3pbinsertion in exon7 of PTPN11 gene (p.Q255_Q256insQ). The variant segregates in two other affected members of the family. Informatic protein model showed mutation falls into a conserved domain.
Patient 2 presented with facial dysmorphism and mild short stature. PTPN11 assays were performed, revealing a 3pbdeletion in intron12 (c.16_18delATG). *In silico* modelling predicted an alternative splicing for this variant.
None of these PTPN intronic variants have been encountered in 400 normal chromosomes.
Patient 3 displayed craniofacial anomalies with severe short stature, pulmonic stenosis and hypertrophic myocardiopathy, moderate developmental delay, and severe linfatic dysplasia. PTPN11 gene analysis revealed the presence of a de novo single codon double nucleotide substitution in exon 3 of PTPN11 (p.T73L).
Patient 4 had typical facial phenotype, pulmonic stenosis, hypertrophic myocardiopathy and severe feeding difficulties. Molecular diagnosis showed a heterozygous 6pbdeletion in intron1 5'UTR region in HRAS gene (c.84_89delGGGCCT). Mutations in 5'UTR might affect regulation of gene expression.
Patient 5 had typical facial dysmorphism, severe short stature and retarded development, with no congenital heart defect associated. She developed neonatal thrombocytopenia and juvenile myelomonocytic leukaemia. Molecular analysis revealed a double mutation in exons 3 (Y63C) and 8 (M311V) of PTPN11 gene. Another 4 affected relatives shared both the same mutations, which did not segregate separately.
Mutation-based differencial diagnosis may play an essencial role in patients with borderline clinical manifestations. Atypical molecular findings should be reported in order to expand the genotypic spectrum of these disorders.

PO2-335 Turner, Noonan I

Glucose homeostasis in Turner syndrome

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Introduction
Women with TS have been reported to have 11.5% relative risk of type 1 diabetes mellitus (DM) and 4.3% relative risk of type 2 DM (1). However, it is now evident that the traditional categorisation of DM is not completely appropriate as the defect of glucose homeostasis may be an insulin secretary defect rather than impaired glucose tolerance (IGT) (2). So far little is known about the natural history of DM in TS in the paediatric population. The aim of our study was to evaluate alterations of glucose metabolism in TS since childhood.
Subjects and Methods
10 subjects aged 8 - 25 years were studied. We evaluated dynamic testing of glucose homeostasis such as oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT), full metabolic blood screen with HOMA index1,HOMA index2, QUICKI and auxological parameters.
Results
Primary outcome
IVGTT: 30% of the group, aged 16-20 years, despite normal glucose tolerance, HOMA index1 and not obese, exhibited a reduced FPIR (Mean FPIR =52.6 <3° centile compared to the reference population)(3).
OGTT: One patient exhibited IGT (BMI SDS = 0.63);one patient showed a peak glucose value of 199 mg/dl after 30' with normal value at 120'. Six patients showed elevated levels of insulin with normal glucose tolerance, and two patients showed normal glucose tolerance and normal insulin levels. Two patients in treatment with GH and BMI SDS > 2, exhibited HOMA index1 of 2,99 and 2,59(cut-off= 2,5)(4). Correlation between HOMA index 1 and BMI SDS was significant (r=0,81).
Secondary outcome
Metabolic profile: HbA1c, IGF1, lipids were normal in all patients except one who exhibited an increase of total and LDL cholesterol.
Conclusion
The group studied showed different defects of glucose homeostasis. High HOMA index 1 was correlated with high BMI. Insulin insufficiency was more prevalent than IGT and observed in subjects with normal glucose tolerance, not obese and with normal insulin sensitivity.
Our findings confirm that the defect of glucose homeostasis in TS may consist in impaired insulin secretion and that b-cell dysfunction or insufficiency may be a primary feature of the Turner metabolic syndrome since childhood.
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PO2-336 Turner, Noonan I

Combined therapy of growth hormone and low dose stanazololum increases adult height of Chinese girls with Turner syndrome

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Purpose: to evaluate the effect of Combined Therapy of Growth Hormone(GH) and Low Dose Stanazololum on the adult height of girls with Turner syndrome.
Methods: From 1994 to 2007, twenty-three girls with Turner syndrome who received the combined therapy of GH and low dose stanazololum for at least 1 year and had reached the near adult height were enrolled in the study. The dosage of GH was 1U/kg/week, and 20-40μg /kg/d stanazololum was given at the same time.
Results: Twenty-three girls started the combined therapy of GH and low dose stanazololum at the age of 12.17 ±3.25(7-15.9) years. The duration of therapy was 3.02 ± 1.45(1-6.75) years. The bone age at the beginning of the treatment was 10.27 ± 1.8(4.2-12.3) years, at the end of the treatment, which increased to 12.89±0.59(12-14) years. The near adult height of the twenty-three girls was 150.82 ± 1.45(146-156.8)cm, which was significantly higher than the (140.0 ± 7.9) cm of the adult height of Chinese girls with Turner syndrome upon spontaneous growth. No major adverse events were detected during the therapy.
Conclusion: The combined therapy of GH and low dose stanazololum is safe and effective for the promotion of growth in children with Turner syndrome.

PO2-337 Turner, Noonan I

Linear growth according to the genotype of patients with Noonan syndrome

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Introduction: Noonan syndrome (NS) is a heterogeneous syndrome which is frequently associated with short stature. In addition to the PTPN11 and KRAS mutations, other 2 mutated genes SOS1 and RAF1 belonging to the conserved Ras-MAPK signaling pathway have been identified two years ago. The causative influences of these gene mutations on the linear growth are not currently well known. The aim of this study was to investigate the linear growth of NS according to their molecular characterization.

Methods and Results: The cohort of this study included 34 subjects (17F, 17M), age range:3 days - 30 years, 13 (38%) carrying a PTPN11 mutation (11 probands and 2 affected mothers), 9 (26%) cases with a SOS1 mutation (2 sporadic and 2 familial with 5 affected members in their pedigree), 1 of these patients carried also a RAF1 mutation, and the remaining 12 (35%) subjects negative for gene mutations. The diagnosis of NS was proposed according to the clinical criteria defined by van der Burgt.

Short stature with the height < 3rd percentile was detected in 8 (61%) out of 13 cases carrying the PTPN11 mutations. The mean H-SDS was lower than target height (TH) (-1,9±0,88 *vs* 0,43±1,26; p=0,0012). A growth hormone deficiency (GHD) was found in 2 probands and in one mother PTPN11 mutation-positive. The height of all 9 the cases carrying a SOS1 mutation, one of these carrying also a RAF1 mutation, was > 3rd percentile. The mean H-SDS was close to the TH (-0,63±0,49 *vs* -0,53±0,37; p=ns). The affected mother of 1 proband had manifested a transient GHD secondary to the pubertal delay. Short stature with the height < 3rd percentile was detected in 6 (50%) out of the 12 cases mutation-negative, 2 of them showed a GHD. The H-SDS was lower than TH (-2,18±1,4 *vs* -0,33±1,2; p=0,0021). The H-SDS of patients with SOS1 mutations was significantly higher than that of subjects with PTPN11 mutations or mutation-negative (p=0,0005 and p=0,0051, respectively). No significant differences were found between H-SDS of PTPN11 and mutation-negative patients.

Conclusions. In this cohort of NS patients a high frequency of short stature with or without GHD has been detected in PTPN11 positive and in mutation-negative subjects. In contrast, normal stature has been detected in all SOS1 cases and in the child carrying a double heterozygosis for SOS1 and RAF1 mutation. Therefore, these last 2 mutations do not seem to be associated with short stature in NS.

PO2-338 Turner, Noonan I

Videocapillaroscopy in Turner syndrome considering dyslipidemia and HOMA IR

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INTRODUCTION: Turner syndrome patients (TS) have an increased prevalence of impaired glucose homeostasis and dyslipidemia. Endothelium abnormalities are present in groups with insulin resistance and dyslipidemia.

OBJECTIVES: The value of measuring projected area of transverse segment of hand nailfold capillary loops using videocapillaroscopy was studied in TS and healthy controls, considering dyslipidemia and HOMA IR.

METHOD: Videocapilaroscopy in rest conditions and post-occlusive reactivity test was performed in 50 TS and 50 controls to study: transverse segment area (TSA), the maximum post- ischemia area (MAIt) and the maximum increment percent (MAIp).We analyzed serum lipids and glucose, the use of medications. The significance level accepted was $p < 0.05$.

RESULTS: The mean age of patients and controls was 13.4±3.8 and 13.2±3.8 respectively (p=0.85). Thirteen patients in each group had dyslipidemia. The HOMA IR was higher (p=0.0005) in controls than in patients (1.9±0.89 and 1.3±0.69). There were no differences in TSA, MAIt and MAIp between controls and TS patients. There were no differences in TSA, MAIt and MAIp between patients with insulin resistance with HOMA IR and controls. There were no differences in TSA, MAIt and MAIp between TS patients with high cholesterol and controls. MAIt was significantly increased in TS with high triglycerides compared to control group(p0,04).

CONCLUSION: There are alterations in MAIt in the Turner Syndrome with high triglyceride compared with healthy people but there are no differences in capillaroscopy between TS and controls considering HOMA IR values.

PO2-339 Turner, Noonan I

Left ventricular dimensions in Turner syndrome under GH treatment

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Background:

Patients with Ullrich-Turner syndrome (UTS) are frequently affected *by* congenital and by acquired cardiovascular diseases. This study was performed to assess the effect of growth hormone(GH) treatment on left ventricular dimensions.

Patients and methods:

Echocardiographic evaluation was performed in 47 girls with UTS (8 patients with karyotype 45,XO and 39 patients with mosaicism) aged 5.6-22.6 years, treated with a mean GH dose of 0.33 mg/kg/week. Interventricular septal (IVS) and posterior wall (PW) thickness, left ventricular internal diameter (LVID) was measured using 2-dimensional echocardiography. Fractional shortening index (FS) and left ventricular mass (LVM) was calculated. LVM was indexed with body surface area (BSA).

Results:

echocardiographic data (SD scores)						
uring treatment years	End-diastolic IVS	End-diastolic LIVD	End-systolic LIVD	End-diastolic PW	FS (%)	LVM/BSA (g/m2)
Before of GH- treatment n=36	0.57 (1.1)	-0.66 (1.2)	-0.22 (1.1)	-0.17 (0.8)	35.7 (5.5)	59.6 (15.3)
1 year of GH- treatment n=22	0.64 (1.4)	-0.29 (1.2)	-0.15 (0.9)	0.59 (1.1)	37.0 (5.9)	66.0 (13.59)
2 year of GH- treatment n=18	0.05 (0.7)	-0.28 (1.6)	-0.27 (1.5)	0.05 (0.9)	37.9 (5.5)	60.5 (15.7)
3 year of GH- treatment n=9	0.12 (1.2)	-0.56 (1.3)	-0.29 (1.3)	-0.44 (0.9)	37.2 (4.6)	59.6 (18.9)
4 year of GH- treatment n=4	0.78 (1.3)	-1.07 (0.3)	-0.68 (0.9)	0.26 (1.0)	35.0 (7.5)	63.7 (12.7)
5 year of GH- treatment n=4	0.24 (1.6)	0.77 (0.8)	-0.93 (0.4)	-0.14 (0.7)	38.3 (2.1)	59.8 (15.0)
6 year of GH- treatment n=4	-0.24 (1.4)	-0,19 (0.2)	0.39 (0.7)	0.02 (0.7)	33.1 (3.5)	62.5 (15.5)
After GH-treatment (mean duration 2.8 year) n=8	-0.1 (1.5)	-0.24 (1.3)	0.39 (0.6)	-0.1 (0.8)	32.6 (4.1)	65.8 (8.6)

The left ventricular (LV) dimensions of almost every girl were within the normal range. Before, during and after GH treatment the mean SD scores were as follows*:* end-diastolic IVS 0.35 ± 1.5; end-diastolic LVID -0.45±1.2; end-systolic LVID -0.21±1.1; end-diastolic PW 0.02±0.8; FS (%) 36.1±5.2 and LVM/BSA (g/m²) 61.6±14.47.

Conclusion: GH-treatment up to 6 years does not result in LV hypertrophy in girls with Ullrich-Turner syndrome.

PO2-340 Turner, Noonan I

Cardiovascular assessment of girls with Turner syndrome. Is echocardiography sufficient for early surveillance?

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Background
Risk factors for aortic dissection in Turner syndrome (TS) include hypertension, bicuspid aortic valve (BAV) and aortic arch abnormalities. Pregnancy is a time of especially high risk. As pregnancy using donor ovum IVF is now a realistic option for young women with TS, adequate evaluation of the cardiovascular system prior to conception is essential. Recent literature suggests that echocardiography (Echo) is insufficient to detect all aortic root (AoR) abnormalities in TS. Consensus guidelines recommend MRI for surveillance.
Aims
To audit our cardiac evaluation in TS in a large tertiary paediatric and young adult endocrine service, where early MRI is becoming part of routine surveillance at age >10 years or earlier where clinically indicated.
Methods
Subjects with TS were identified through our clinic databases. Relevant information was extracted from casenotes, cardiology and radiology databases. Hypertension was defined using height-related standards. AoR dilatation was defined as being >95th percentile for body surface area (BSA).
Results
121 subjects were identified with TS (53 XO, 37 mosaic, 15 Y-positive). Mean (SD) age at most recent appointment was 19.8 (10.2) years and mean height SDS -2.2 (1.1). Results of local cardiac evaluation were available in 103 (85%), 15% were unavailable. Hypertension was present in 28, with 14 on treatment, 3 had long QT syndrome. Mean age at Echo was 19.1 (10.9) years. A total of 32 abnormalities were found on Echo: coarctation (CoA) (13), BAV (13), other valvular abnormalities (6). AoR diameter was documented in 76, mean AoR diameter was 2.5 (0.5) cm, and 4 had AoR dilatation for BSA, of whom 2 had associated risk factors – 1 was hypertensive, 1 had a BAV on Echo. Thus far 11 patients have had cardiac MRI of whom 45% have abnormalities that were not detected on Echo including previously undiagnosed CoA and BAV. MRI surveillance in this cohort is ongoing and further data will be presented.
Conclusions
In this paediatric and young adult cohort we detected AoR dilatation in 3.9% of those screened by Echo, of whom half had risk factors. Given that we have detected new and previously unrecognised abnormalities in 45% of those screened by MRI so far, we believe that Echo is not sufficient for diagnosis and follow up of cardiovascular anomalies in TS. In particular, to decrease risk of young women commencing a pregnancy without prior surveillance, we recommend routine MRI evaluation in all TS girls from puberty onwards.

PO2-341 Turner, Noonan I

Cholesterol and apolipoprotein levels in a contemporary cohort of children with Turner syndrome

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Background: The prevalence of ischaemic heart disease in women with Turner Syndrome (TS) is 7 times higher than the normal population. The reasons for this are likely to be multifactorial; hypertension, obesity, insulin resistance and diabetes occur with increased frequency. Blood cholesterol is raised in the adult TS population, however little is known about lipid profiles in children and adolescents with TS.
Design: Retrospective case note review
Objectives: To describe the levels of cholesterol, apolipoprotein A1 and apolipoprotein B, in children and adolescents with TS and to compare these to normative data.
Patients: 68 lipid profiles taken from 34 girls with TS, age 11.64 ± 4.05 years at initial sampling.
Measurements: Total cholesterol, Apolipoprotein A1, Apolipoprotein B, Apolipoprotein B/A1 ratio.
Results: Blood cholesterol SDS was (mean ± 1SD) 0.35 ± 1.16 in subjects aged less than 12 years. In subjects aged more than 12 years blood cholesterol SDS was significantly higher (1.39 ± 0.63, p=0.02), than in younger subjects, persisting after correction for BMI. Apolipoprotein B/A1 ratio was not raised in either age group. Blood cholesterol exceeded the American Academy of Pediatrics recommended upper limit of 5.1mmol/L in 9.5% of girls age less than 12 and 33% of older girls. Subjects more than 12 years treated with oestrogen had a higher cholesterol SDS compared to those not treated (1.5 ±1.3 vs. 0.55 ±1.6, p= 0.05).
Conclusion: Prepubertal girls with TS have a trend towards higher cholesterol which increases with age however apolipoprotein B/A1 ratio is unchanged. There is a paradoxical increase in cholesterol in those on oestrogen which may in part reflect sampling bias, but may also demonstrate higher cholesterol in those girls with a more severe phenotype. This data suggest that whilst it is true that girls with TS have a different lipid profile to the normal population, it may contribute less to the overall cardiovascular risk than previously thought.

PO2-342 Turner, Noonan I

Final height in Turner syndrome is improved by oxandrolone but not late pubertal induction (14 vs 12 years): results of a UK randomised, double-blind, placebo-controlled study

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Growth hormone (GH) therapy increases height velocity & improves final height (FH) in girls with Turner syndrome (TS). However, the benefit of Oxandrolone (Ox) & the timing of pubertal induction remain controversial. The UK Turner Study examines the impact on FH of Ox & Ethinylestradiol (E2) therapy at 12y vs 14y. **Methods:** Girls aged 7-13y receiving a standard dose of GH [10mg/m^2/week in daily injections] were randomised to receive Ox [0.05mg/kg/day, maximum daily dose 2.5mg] or placebo from 9y (or from enrolment if aged >9y). Girls requiring oestrogen were further randomised to begin oral E2 [Year 1: 2µg daily; Year 2: 4µg daily; Year 3: 4 months each of 6/8/10µg daily] or placebo at 12y, with the placebo group beginning E2 at 14y. **Results:** From 1999-2003, 106 TS girls were recruited at 36 UK hospitals of whom 15 have withdrawn & 62 have reached FH. No serious adverse events (AEs) have been reported; in particular, no voice deepening or clitoromegaly. The effect of Ox is shown in Table 1 & timing of E2 in Table 2; data as mean (SD).

Characteristics of participants randomised to Ox or placebo (n=91)		
	Ox (n=42)	Placebo (n=49)
Enrolment age (y)	10.4 (1.5)	10.2 (1.6)
Enrolment height (cm)	127 (9)	125.2 (8)
Enrolment Ht SDS	-2.1 (0.8)	-2.2 (0.8)
Age at GH start (y)	7 (2.5)	7 (3.1)
Latest age (y)	15.6 (1.6)	16.1 (1.6)
Latest/maximum height (cm)	152.7 (7.2)	148.7 (6.9)
Latest/maximum Ht SDS	-1.4 (0.8)	-2.2 (1.1)
Δ Ht SDS	0.7 (0.8)	0 (0.7)

Characteristics of participants randomised to E2 at 12y or 14y (n=55)		
	E2 at 12y (n=28)	E2 at 14y (n=27)
Enrolment age (y)	9.6 (1)	9.6 (1.2)
Enrolment height (cm)	122.4 (6.7)	123.8 (6.9)
Enrolment Ht SDS	-2.2 (0.8)	-2 (0.9)
Age at GH start (y)	6.7 (2.1)	5.6 (2.5)
GH prior to E2 (y)	5.3 (2.1)	8.4 (2.5)
Latest age (y)	15.4 (1.2)	16 (1.7)
Latest/maximum height (cm)	149.1 (7.3)	151.3 (8.5)
Latest/maximum Ht SDS	-2 (1.1)	-1.7 (1.1)
Δ Ht SDS	0.2 (0.7)	0.3 (0.9)

Based on t tests, the effect of Ox on maximum height was +3.9 SE 1.5 cm ($p < 0.01$) & on FH +5.9 SE 1.7 cm ($p = 0.001$). Once all participants reach FH, it is estimated the Ox effect will be nearer 4cm. No effect could be shown between inducing puberty at 14 vs 12y. **Conclusions:** Ox has been shown to have a positive effect on FH with no serious AEs over a 10-year period. We have been unable to show any FH benefit from delaying pubertal induction to 14y.

PO2-343 Turner, Noonan I

"The Gap" – are young people and their families satisfied with endocrine healthcare?

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Background
The challenge of engaging young people with endocrine conditions is key in the process of transition. However current healthcare organization often neglects to address the needs of young people. There is now an increased focus on patient satisfaction with healthcare as a way of adapting services to meet user's requirements.
Objectives
To assess satisfaction with endocrine care from the perspective of the young person and also their parents attending paediatric endocrine clinics.
Methods
Young people (YP) and their families were asked to complete a "Mind the Gap" questionnaire. The questionnaire addresses aspects of care (27 for parents and 21 for YP) relating to staff, environment and process in the clinic. Participants using a Likert scale of 1-7 (strongly agree to strongly disagree) rate what is important for best endocrine healthcare and what they feel about their current endocrine healthcare. The discrepancy between 'best' and 'current' represents "The Gap" in current endocrine care.
Results
40 YP (21 male) and their parents took part, aged between 11 and 19 years. The YP had a wide range of endocrine conditions, hypothalamic pituitary disease (27%), adrenal conditions (20%) and Turner syndrome (18%). For best endocrine healthcare, YP agreed that staff were more important than environment which in turn was more important than process ($p<0.007$), whereas parents agreed that staff and process were equally important and both more important than environment ($p<0.0001$). There were significant differences for both YP and parents between best and current endocrine healthcare for staff, process and environment, i.e. a "Gap" in endocrine healthcare. For the parents, the largest "Gap" was in the provision of both information about other people and organizations who could support them and help in planning for the future. The YP rated opportunities to meet others and age appropriate physical environment as least important, knowledgeable staff, provision of honest explanations, help with preparation for adult services and informing other professionals involved in their care as most important.
Conclusions
There is a "Gap" in current endocrine healthcare which may affect YP engaging with services. There is clearly a need to improve provision of information and help with preparation for the future and for adult services. Although environment needs improving this is considered least important by both YP and parents compared with staff and process.

PO2-344 Turner, Noonan I

PTPN11, SOS1, KRAS and RAF: genotype-phenotype correlations in Noonan syndrome

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The autosomal-dominant Noonan Syndrome (NS) is characterized by multiple abnormalities like congenital heart defects, short stature and distinct facial anomalies. NS is genetically heterogeneous with four genes currently known to be involved in its pathogenesis (PTPN11, SOS1, KRAS and RAF1). This phenotype is overlapping with others syndromes as Leopard Syndrome (LS), Costello Syndrome (CS), Cardio-facio-cutaneous Syndrome all of them related with RAS-MAPK pathway. The new genetic information has allowed investigators to establish genotype-phenotype correlations.
Objectives:1) Describe the clinical features of NS. 2) Genetic Studies of PTPN11, SOS1, KRAS and RAF genes. 3) Analyze genotype-phenotype correlations.
Methods: We studied 68 patients with clinical suspicion of NS who come from the North of Spain (Galicia, Asturias, País Vasco). 1) Molecular Study: in all of cases was performed direct bidirectional sequencing of the coding region of the PTPN11, SOS1, RAF and KRAS genes. 2) Study of clinical characteristics and biochemical and hormonal data. 3) Genotype-phenotype correlations and statistic analysis.
Results: 1) Genetic study: We found disease-causing mutations in 24 (35%) patients, which were located in the PTPN11 (14), SOS1 (8), RAF (1) and KRAS (1). 2) Genotype-phenotype correlations: 31 patients were re-evaluated with the clinical criteria defined by van der Burgt and we found NS at 21, 2 of them LS and 1 CS and 6 patients not full fill criteria. PTPN11 mutation was found in 7/31 patients, two of them not described previously and SOS1 (5/31 patients). There are a good correlation between pulmonic stenosis and broad thorax with SOS1 mutation. Similarly, cardiac defects and typical face dysmorphology correlate with PTPN11. Also, mutations prevalence depends on the place of birth.
Discussion: In conclusion, great progress in the detection and analysis of NS-causative genes has been achieved in recent years, but NS is still a challenging disorder and overlapping with other syndromes. We found less percentage of genetic mutations than reported. As well, the prevalence depends on the area people come from. Besides, there is good correlation between genotype-phenotype with SOS1 and PTPN11.

PO2-345 Type 1 Diabetes II

A novel GATA4 gene mutation in a patient carrying atrial septal defect and pancreas agenesis

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Congenital heart defects are among the most common developmental anomalies affecting around 1% of newborns. Three genes, *NX2.5, TBX5 and GATA4*, are considered as the most frequently involved in these pathogenesis. Permanent Neonatal Diabetes is a rare condition affecting 1 in 300,000 to 400,000 live births and only in 30-40% of cases it is possible to identify the underlying genetic defect. More rare is the condition of neonatal diabetes due to pancreas agenesis. Only two genes are known to determine this phenotype: *PDX-1 and IPF1a*. Coexistence of pancreas agenesis and congenital heart defect has never been reported so far. We tried to genetically characterize this phenotype. A patient with neonatal diabetes due to pancreas agenesis and atrial septal defect was genetically investigated. Three genes, *NX2.5, TBX5 and GATA4*, plus a panel of pancreas development genes, including *GCK, Kir6.2, IPF1a, PDX-1, HNF-1A, NgN3, SOX17, SOX7, SOX9* and *INS*, were screened for genetic characterization of atrial septal defect and pancreas agenesis, respectively. A novel mutation in *GATA4* (c1512C>T) was detected, and functional characterization showed a reduction of transactivation ability due to a combined defect in nuclear translocation and reduced binding affinity to *GATA4* recognition site. Screening of genes causing permanent neonatal diabetes was negative. We describe the first case of pancreas agenesis with associated atrial septal defect, carrying a novel mutation in the *GATA4* gene.

Clinical characteristics and main laboratory data of the patient		
APGAR SCORE	at 1' / at 5'	8/9
BIRTH WEIGTH	1620 g	⟨3rd centile
BIRTH LENGTH	45 cm	⟨3rd centile
BIRTH HEAD CIRCUMFERENCE	30 cm	⟨3rd centile
BIRTH GLYCAEMIA	500 mg/dl	
HbA1c	10.2%	n.v 4-6%
SERUM INSULIN	⟨2 U/mL	n.v 4-6 U/ml
SERUM LIPASE	⟨10 U/L	n.v 10-85 U/L
IAA	undectable	n.v ⟨10%
IA-2A	undectable	n.v 0.0-3.0 AU/mL
GADA	undectable	n.v. 0.0-3.0 AU/mL

Our effort to identify the genetic defect causing pancreas agenesis was unsuccessful but further studies could show a relationship between *GATA4* gene and permanent neonatal diabetes phenotype.

PO2-346 Type 1 Diabetes II

Folic acid, vitamin B_{12} and homocysteine levels fasting and after methionine load in patients with type 1 diabetes mellitus

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High plasma homocysteine (Hcy) concentrations are associated with cardiovascular disease, atherothrombosis and cardiovascular mortality in the general population. Studies of plasma Hcy concentration in people with type 1 diabetes mellitus (T1DM) yielded conflicting results. The aims of this study were to assess plasma concentrations of folic acid, vitamin B12 and total plasma homocysteine (tHcy) during fasting and after methionine load in young patients with T1DM.

Methods: We enrolled 41 young patients with T1DM without any sign of microvascular complications and 123 healthy controls in a 1:3 cross-sectional case-control study. Fasting and post-methionine load (PML) tHcy, folic acid and vitamin B12 levels were measured in both groups. Data regarding chronological age, metabolic control (assessed by mean values of Hemoglobin A1c in the last 12 months), disease duration, were also recorded.

Results: fasting and PML tHcy were significantly lower in patients than in controls: 7.3 ± 2.7 μmol/l vs 8.3 ± 2.5 μmol/l, p=0.01; and 16.7 ± 5.8 μmol/l vs 17.3 ± 4.3 μmol/l, p=0.01, respectively. No correlation was found between tHcy levels (fasting and PML) and chronological age, disease duration, metabolic control and insulin requirement. Patients had significantly higher vitamin B12 levels than controls (767 ± 318 vs 628 ± 236 pg/ml, p=0.003, while folic acid turned out to be lower in patients than in controls (5.3 ± 1.9 vs 7.5 ± 2.6 nmol/l, p<0.0001).

Conclusions: Adolescents and young adults with T1DM without microvascular complications show lower tHcy both fasting and PML. Moreover, lower folate concentrations in these patients might require more investigations.

PO2-347 Type 1 Diabetes II

Cardiovascular screening in type 1 diabetes mellitus – evaluation of carotid artery

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Introduction: Macrovascular complications of diabetes resulting from atherosclerosis are the leading cause of morbidity with a prevalence of 4-21% in childhood diabetics. Early changes of atherosclerosis are arterial vessel stiffening and increased carotid artery intimo-medial thickness (cIMT) followed by cardiac dysfunction. This study was aimed to screen our type 1 diabetics.

Material and methods: Thirty Type 1 diabetic patients [15females and 15 males] in the age group of 10-18 years attending Pediatric Endocrine Clinic were screened. Their lipid profile and glycated hemoglobin [HbA1C] were measured. The arterial distensibility before and after sublingual nitroglycerine, cIMT and left ventricular parameters-ejection fraction, internal diameters, inflow velocities; were assessed using echocardiography. The correlations of cardio-vascular functions with cardiac risk factors like blood pressure, dyslipidemia and HbA1C were evaluated.

Results: The mean age of the patients was 14.30±3.09 years with mean diabetes duration 5.35±2.1 years. Their systolic and diastolic blood pressures were normal-111.46±12.52mmHg and 70.48±9.16mmHg. The mean HbA1C was 8.01%. The mean lipid profile-cholesterol, triglyceride, HDL and LDL were all normal. The cardiovascular parameters revealed a mean arterial distensibilty of 0.097±0.064 mm which didn't vary with either of the risk factors. Two patients had longer disease duration of >8years and HbA1C>10% and had a low distensibility of 0.02mm. The mean cIMT measured was 0.698±0.233 mm. The above two patients had cIMT>1mm. cIMT was significantly higher in patients with elevated blood pressures [SBP-p=0.039, DBP-p=0.010] and in subjects with poor glycemic control [p=0.019]. The cIMT was significantly influenced [p<0.05] by serum cholesterol and LDL. The cardiac functions showed a poorer ejection fraction with higher HbA1C [p<0.05]. No change was observed in other cardiac parameter in relation with blood pressure or lipid profile.

Conclusion: The magnitude of cIMT [versus arterial distensibilty] as a sensitive marker for cardiac dysfunction in type 1 diabetes was established. The cardiac functions were impaired in our subjects with inferior glycemic control. The role of dyslipidemia and hypertension in predicting poorer cardiovascular status was also recognised.

PO2-348 Type 1 Diabetes II

Initiation of a continuous subcutaneous insulin infusion rapidly improves vascular function independent of changes in HbA1c

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Whilst chronic sustained hyperglycaemia in type 1 diabetes (T1DM), as mea-

sured by HbA1c, predicts the risk of future vascular disease, the role of glucose variability as an independent risk factor is still unclear. We aimed to investigate whether continuous subcutaneous insulin infusion (CSII), as an intervention to reduce glucose variability, improves vascular function independent of changes in HbA1c.
In an ongoing study, fifteen children with T1DM (5 males, aged 12.6 ± 3.2 years) who had been referred for commencement on a CSII were reviewed immediately prior and 3 weeks after initiation on CSII. Vascular function (flow mediated dilatation [FMD] and glyceryl trinitrate mediated dilatation [GTN]), glucose variability using a Minimed continuous glucose monitoring system [Medtronic], clinical and biochemical variables were assessed at each visit. Methods used to measure glucose variability were mean of daily differences (MODD), mean amplitude of glycaemic excursions (MAGE) and continuous overlapping net glycaemic action (CONGA n4).
Vascular function, HbA1c, and blood pressure improved after initiation of CSII. Glucose variability did not significantly decrease between the two assessments (Table 1). However, at both baseline and 3 weeks, GTN related strongly to glucose variability (MAGE r =-0.64, p<0.01; MODD r =-0.71, p<0.01; CONGA n4 r =-0.51, p = 0.04), but not HbA1c (r =-0.19, p = 0.5). In addition, the improvement in vascular function was associated with the reduction in MODD (p = 0.05) but did not relate to the improvement in HbA1c.

Table 1

	Pre-CSII	3 weeks post CSII	p
FMD (%)	5.0 (4.1)	7.1 (2.5)	0.08
GTN (%)	23.2 (7.0)	26.7 (6.1)	0.02
HbA1c (%)	8.7 (1.7)	8.1 (1.1)	0.01
Systolic blood pressure (mmHg)	110.4 (11.4)	103.8 (13.1)	<0.01
Diastolic blood pressure (mmHg)	61.4 (6.5)	57.7 (6.3)	<0.01
MODD (mmol/L)	4.0 (1.1)	3.6 (1.9)	0.3
MAGE (mmol/L)	8.8 (3.0)	8.5 (3.9)	0.5
CONGA n4 (mmol/L)	4.6 (1.1)	4.2 (1.5)	0.2

Mean (SD)

Initiation of CSII rapidly improves vascular risk factors, including blood pressure and vascular function. The improvement in vascular function is independent of changes in HbA1c and relates to glucose variability.

PO2-349 Type 1 Diabetes II

Extreme self-monitoring, fear of hypoglycemia and obsessive-compulsive disorder in type 1 diabetes mellitus: when less is better

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Background: Pre-existing psychological factors can strongly influence coping with type 1 diabetes mellitus and interfere with self-monitoring. Psychiatric disorders seem to be positively associated with poor metabolic control. We present a case of extreme compulsive blood testing due to obsessive fear of hypoglycemia in an adolescent with type 1 diabetes mellitus. **Case report:** Type 1 diabetes mellitus (anti GAD-antibodies 2624 U/l, norm < 9.5) was diagnosed in a boy aged 14.3 years [170 cm (+ 0.93 SDS), weight 50.5 kg (+ 0.05 SDS)]. Laboratory work-up showed no evidence for other autoimmune disease. Family and past medical history were unremarkable. Growth and developmental milestones were normal. Insulin-analog based basal-bolus regime was initiated, associated to standard diabetic education. Routine psychological evaluation performed at the onset of diabetes revealed intermittent anxiety and obsessive-compulsive traits. Accordingly, a close psychiatric follow-up was initiated for the patient and his family. An adequate metabolic control (HbA1c drop from >14 to 8%) was achieved within 3 months, attributed to residual β-cell function. In the following 6 months, HbA1c rose unexpectedly despite seemingly adequate adaptations of insulin doses. Obsessive fear of hypoglycemia leading to a severe compulsive behavior developed progressively with as many as 68 glycemia measurements per day (mean over 1 week). The patient reported that he could not bear leaving home with glycemia < 15 mmol/l, ending up with school eviction and severe intra-familial conflict. Despite intensive psychiatric outpatient support, HbA1c rose rapidly to >14% with glycemia-testing reaching peaks of 120 tests/day. The situation could only be discontinued through psychiatric hospitalization with intensive behavioral training. As a result, adequate metabolic balance was restored (HbA1c value: 7.1 %) with acceptable 10-15 daily glycemia measurements. **Discussion:** The association of overt psychiatric disorders to type 1 diabetes mellitus is very rare in the pediatric age group. It can lead to a pathological behavior with uncontrolled diabetes. Such exceptional situations require long-term admissions with specialized psychiatric care. Slow acceptation of a "less is better" principle in glycemia testing and amelioration of metabolic control are difficult to achieve.

PO2-350 Type 1 Diabetes II

Social deprivation and low parental education levels are associated with lack of success of insulin intensification; a longitudinal observational cohort

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Objectives: To evaluate the interaction between social deprivation indices and intensification of insulin therapy (≥three injections per day or pump therapy) on glycaemic control in children and adolescents with type 1 diabetes.
Methods: We retrospectively collected data on deprivation (scored using the National Index of Multiple Deprivation 2004), insulin therapy and HbA1c levels from three paediatric clinics (n=282) between 2005 and 2007. Linear mixed modelling was used for longitudinal analysis of (1) the whole cohort and (2) a subgroup that was intensified from conventional regimens during follow-up.
Results: Overall mean HbA1c levels were 8.96% (SD 1.39). Intensification therapy increased during the study from 49 to 72% (χ^2=32.98, P=<0.001), but there was no corresponding improvement in HbA1c levels. By the end of follow-up, the frequency of intensification therapy was least in children from the most deprived families (most deprived quintile [n=26, 42.6%] v least deprived quintile [n=42, 66.7%], χ^2=11.74, P=0.019). In linear mixed modelling, factors independently associated with poor glycaemic control were; age (P=0.006), greater social deprivation indices, particularly lower parental educational levels (P=0.003), but not intensification therapy except for lack of insulin pump use (P=0.004).
In the sub-group of patients intensified during follow-up; HbA1c levels actually deteriorated (mean difference +0.24% [95%CI -0.17 to 0.51], P=0.067), and in those who did not (n=39) compared with those who did (n=36) demonstrate improved HbA1c levels post- intensification; social deprivation was greater (P=0.032).
Conclusions:
In this longitudinal observational study, children with type1 diabetes who came from families that were most deprived and had the lowest parental educational levels had the lowest uptake of and demonstrated least improvement in glycaemic control with insulin intensification.

PO2-351 Type 1 Diabetes II

5mm needles reliably inject into subcutaneous fat, are remarkably well tolerated and result in minimal leakage at doses up to 60 units

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Intruduction: Inadvertent intramuscular (IM) injections of insulin in children and adolescents with type 1 diabetes mellitus (DM) are common and cause variability in glycaemic control. While shorter needles reduce IM injections, concerns about dermal injections and insulin leakage have been raised. There also remains debate as to the most reliable technique to administer insulin. The aim of this study was to assess the injection technique most reliably delivering insulin into subcutaneous (SC) fat using NovoFine® 5mm 32G needles.
Methods: Subjects had 8 injections of air (200ml); 4 into the lateral thigh and 4 into the abdomen using either angled or vertical insertion and using either a pinched skinfold or no skinfold. Immediately after each injection an assessment of injection depth was made by ultrasonography by a radiologist. At a separate visit 20, 40 or 60 units of test medium was administered using the

same techniques into the thigh and abdomen. Leakage was assessed by blotting the injection site and weighing the filter paper.
Results: 102 children (age 6-19 years, 52% male, BMI 14.4-38.3) were recruited. IM injections occurred at the lowest rate using pinched angled abdominal injections (2%) and at the highest rate using unpiniched vertical thigh injections (12%). IM injections were associated with reduced SC fat thickness ($p<0.0001$), BMI ($p<0.0001$) and male gender ($p<0.001$). There was only one dermal injection. No clear preference for injection technique was shown, although the abdominal site was preferred over the thigh ($p=0.01$). There were no differences in pain perception using an analog pain scale between the different injection techniques. An overall pain score was compared to a previous study (n=56) using 6 and 8 mm needles. The 5mm needles were much less painful (1.51±0.5cm versus 2.51±1.2cm and 2.85±1.6cm against both 6 and 8 mm needles respectively, $p<0.001$). Leakage studies using 20U (n=102), 40U (n=25) and 60U (n=45) of test medium showed minimal loss equating to less than 0.5U of insulin.
Conclusion: Even with 5 mm needles, IM injections occur in thin subjects. The injection technique used minimized this risk with an angled insertion and pinched skinfold being the best. All 8 injection techniques reliably injected into subcutaneous fat in the majority of children. With less pain and the lack of leakage we recommend 5mm needle length in all children using insulin needles for insulin administration.

PO2-352 Type 1 Diabetes II

Continuous glucose monitoring in children and adolescents with type-1 diabetes mellitus: recent analysis from the DPV diabetes documentation and quality management system including 49305 patients from Germany and Austria

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Introduction
The use of real-time continuous glucose monitoring (CGM) in pediatric patients with type-1 diabetes is increasing in Germany and Austria. In this study the frequency, duration and influence on glycemic control of CGM, dependent on age, diabetes duration and type of insulin therapy are analyzed.
Method
In a large prospective multicenter database (DPV-Wiss) 935,498 data from 49,305 children, adolescents and young adults with type-1 diabetes from 131 diabetes centers in Germany and Austria are documented. In a multivariate analysis (F-test) the possible influence of the following parameters on glycemic control were evaluated: CGM, age, sex, duration of diabetes, insulin regimen, insulin dose and treatment modality. For statistical analysis using nonparametric statistics the SAS 9.1 software was used.
Results
2121 (4,3%) out of 49305 patients used the sensor for 112349 sensor-days. 1352 patients (64%) of the sensor wearing group were equal or less than 18 years old. The mean age was 11,98 years, 662 (49%) of them were girls, 690 (51%) boys, 748 (55%) were 12-18 years old, 440 (33%) 6-12 years and 157 (12%) < 6 years. The mean diabetes duration was 4,58 years, 811 (60%) were on MDI-, 473 (35%) on CSII-therapy, 68 (5%) had 3 or less injections daily. 906 (67%) of the patients used the sensor less than 30 days. A significant influence on HbA1c was detected for age ($p<.0001$) and insulin dose ($p<.0001$). HbA1c was significant lower in patients < 12 years of age and with lower insulin dose. CGM use, sex, duration of diabetes, insulin regimen and treatment modality were not significantly associated with glycemic control.
Conclusions
Although CGM devices are more frequently used in the pediatric age group, only 1352 (2,7%) of all pediatric patients with type-1 diabetes in Germany and Austria use CGM. CGM was used for less than 30 days, long-term use is still rare in children. No significant influence of CGM on glycemic control could be found. To evaluate whether more and longer use of CGM can improve glycemic control, further studies are needed.

PO2-353 Type 1 Diabetes II

Regular exercise strongly associated with an "excellent" self-rated health among young Egyptians with type 1 diabetes; a pilot study

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Introduction: Patients diagnosed with type 1 diabetes mellitus (T1DM) face increased stress when dealing with their condition, which can have a negative effect on their quality of life. Health-related quality of life among Egyptian children with T1DM has not been addressed before. Self-rated health (SRH) is a global, self-assessment of an individual's current health status that reflects internalized calculations encompassing lived experience and knowledge of disease causes and consequences. It is based on the question, "In general, how would you rate your health?" and subjects rate their health as excellent, very good, good, fair or poor. **Objective:** To assess the SRH among Egyptian children and adolescents with T1DM seen at our clinic and determine factors associated with each health category. **Subjects and methods:** Subjects with type 1 diabetes aged ≥9 years and with >1 year diabetes duration were included. Participants were asked to rate their health in comparison with others their age. Additional lifestyle related questions were asked. Diabetes duration, regimen type and metabolic control data were obtained from the patients' records. **Results:** Our population consisted of 40 subjects, of which 21 (52.5%) were males & 19 (47.5%) were females. The average age in years was 13.52 ± 4.3 with an average diabetes duration of 6.53 ± 5.31 years. The average HbA1c value was 7.91 ± 1.99. The majority of our subjects classified their health as "good" representing 32.5% (n=13), while the second largest group were those who ranked their health as "excellent" representing 30% (n=12). Those who answered "very good" represented 15% (n=6), while those who answered "poor" and "very poor" followed, each representing 10% (n=4 in each group, respectively). Only one subject (2.5%) replied "I do not know". Analysis showed that age, sex, diabetes duration, HbA1c and regimen were not significantly different among SRH categories ($p>0.05$). Whereas, independent of other factors, regular exercise was strongly associated with an "excellent" sense of well-being, with 66.7% (n=8/12) of subjects in the "excellent" category reporting regular exercise, and this was highly statistically significant ($p< 0.001$). **Conclusions:** This is the first study to assess quality of life among young Egyptians with T1DM. Based on our results, we strongly recommend regular exercise to improve health-related quality of life among T1DM patients. We plan to further recruit more subjects for additional analyses.

PO2-354 Type 1 Diabetes II

Lipide profile, antioxidant factors and endothelial function in children and adolescent with type 1 diabetes mellitus

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Type 1 diabetes mellitus (T1DM) is associated with the development of micro- and macro-vascular complication generally related to the duration of disease and glycemic control. Chronic hyperglycemia increases production of free radical intermediates which in turn augments oxidative stress. The oxidative stress can play a key role in the development of diabetic complications affecting also vascular function.
In this study we evaluated the lipid profile, the parameters related to oxidative stress and the endothelial function to assess the relationships between these parameters and metabolic control in children and adolescents with T1DM. A total of 58 T1DM patient (11,5±3,49 yr) and 36 healthy children (9,60±3,21 yr) were studied.
In all children serum concentration of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), Lipoprotein(a) (Lp(a)), homocysteine, fibrinogen, MDA, Vitamin E (VitE), beta-carotene, lycopene, retinol, Vitamin C reduced

(Vit C-Red) and oxidized (VitC-Oxid) and hemoglobin glycosilated ($_{HbA1c}$) were measured. Vascular function was assessed by measurement of endothelium-dependent vasodilatation of brachial artery (FMD) using a high-sensibility of ultrasound system. There were no significant difference in the levels of TC, LDL-C and HDL-C between diabetic patients and the controls. Serum concentrations of TG in T1DM subjects were significantly lower than in control ones. Total antioxidant status (VitE, beta-carotene, lycopene, retinol, Vit C-Red) don't showed significant difference between groups. Only levels of VitC-Oxid were significant higher in patiens with T1DM. Moreover, prepuberal T1DM subjects showed higher MDA serum concentration than controls (p<0,01). FMD (7.99±1.06%) was impaired in 46% of T1DM subjects. There was negative correlation between FMD and serum levels of LDL-C. In our T1DM patients the glycemic control (mean value of HbA_{1c}= 8,33%) was associated with normal lipid profile and normal antioxidant levels. Despite of these data diabetic patients show increased oxidative parameters (MDA, VitC-Oxid). Even if FMD was not apparently related to antioxidant status, the relationship with lipid values emphasizes the role of a global metabolic control to optimize the vascular health of these patients.

PO2-355 Type 1 Diabetes II

Progressive loss of regulatory T cell function in the first year from diagnosis in children with type 1 diabetes mellitus

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In subjects with new onset Type 1 Diabetes Mellitus (DM1), there commonly occurs a remission a few months after onset, characterized clinically by a transient lower requirement for exogenous insulin and an improvement in metabolic control. The immunologic mechanism that underlies this remission period has not been elucidated. The status of Regulatory T cells (Treg) activity in DM1 remains controversial and has been limited mainly to cross-sectional analysis of adults. We have performed a longitudinal study of children with GAD and/or IA-2 antibody positive DM1 (n=21, 15 males, 6 females, average age = 12.8 +/- 2.8 years) from diagnosis for 12 months. To characterize the remission in these subjects, we measured glycosylated hemoglobin (HbA1c) levels at diagnosis, 3, 6, 9 and 12 months, and tracked exogenous insulin requirements at these time points. HbA1c levels were lowest in the group at 6 months, and insulin requirements lowest between 3 and 9 months. Treg assays were designed to test the suppressive function of equivalent numbers of Foxp3+CD25+CD4+ T cells from diabetic and healthy controls in single cell assays of CD4+ target cell proliferation. Stimulations were performed across a number of anti-CD3/anti-CD28 mAb concentrations to determine if strength of signal may reveal differences in Treg function. We found no difference in the frequency of Foxp3+CD25^{high}CD4+ T cells in the peripheral blood of DM1 versus healthy controls nor in the susceptibility of DM1 CD25-CD4+ T cell targets to suppression by Tregs from healthy controls. A small but significant number of patients presented with defects in Treg function at diagnosis. More strikingly however, of those DM1 individuals that did not present with Treg defects at diagnosis, over 40% did develop Treg defects by 6 months, which were sustained through 12 months. These results suggest that the classic "honeymoon" remission period encompasses changes in immune function that paradoxically result in a decline in regulatory function in the peripheral blood.

PO2-356 Type 1 Diabetes II

The persistence of mood, anxiety and posttraumatic stress disorders among mothers of diabetic children and its relation to their children's glycemic control: results of a pilot prospective follow-up study

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A high incidence of psychological impairment and posttraumatic stress disorder (PTSD) has been reported among parents of diabetic children that may impair their children's glycemic control. The aim of this study was to investigate the presence of depression, anxiety and PTSD among mothers of newly diagnosed and long-standing diabetic children and prospectively study the evolution of the psychological burden and its possible impact on their children's glycemic control. The sample consisted of 123 children (54 boys), 51 (61.5%) newly diagnosed, 72 (58.5%) with long-standing diabetes, all followed at the Diabetes Center of the First Department of Pediatrics of Athens University with a mean age of 10.9±4.2 years. Their mothers' mean age was 37.9±6.6 years. The questionnaires used were the 17- item PTDS scale, the Beck Depression Inventory (BDI), the Spielberger's modified Greek state-trait anxiety inventory (STAI) and a Major Life Events Questionnaire. Mothers of long-standing diabetic children filled the questionnaires once, mothers of newly diagnosed ones filled them 4 weeks after diagnosis and 6 and 12 months thereafter. State, trait, depression and PTSD were significantly and positively inter-correlated with correlation coefficients ranging from 0.37 to 0.67 (p<0.001), indicating that greater levels of PTSD are accompanied with greater levels of depression and anxiety. A significant negative correlation was found between PTSD and BDI scales with the age of the mother (r=-0.22, p=0.023 and r=-0.20, p=0.038, respectively), suggesting that younger mothers had more severe PTSD and depression. A significant negative correlation was found between PTSD and duration of disease (r=-0.23, p=0.046), indicating that mothers of newly diagnosed children had more severe PTSD. On follow-up of newly diagnosed diabetic children, HbAIc reduction from baseline to 6 months was significantly correlated with state (r=0.69, p=0.028) and PTSD (r=0.75, p=0.012) at 6 months, indicating that better glycemic control is associated with reduction of psychological burden. Specifically, depression level decreased from baseline to 6 months for mothers whose children had HbAIC <7.8 (BDI_6-BDI_0 = -3.4±5.6) and increased for mothers whose children had HbAIC >7.8 (BDI_6-BDI_0=6.7±3.7, p=0.005 and p=0.017). In conclusion, depression, anxiety and PTSD are frequent among mothers of diabetic children that may persist over time aggravating their children's glycemic control.

PO2-357 Type 1 Diabetes II

Study on the prevalence of celiac disease in Greek children with type 1 diabetes mellitus (DM1)

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Background: DM1 is associated with other autoimmune diseases, among which is celiac disease (CD). The most reliable serological marker for the screening of CD is the presence of tissue transglutaminase antibodies (tTG).
Aims-Methods: The aim of this study was: i.to assess the prevalence of CD in Greek children and adolescents with DM1, ii.to evaluate its possible association with diabetes duration and gender, iii.to study the effect of CD on growth and glycaemic control. The study group included 144 DM1 children (male/female:77/67), aged (mean±SD)12.3±4.6yrs, with a diabetes duration of 4.6±3.9yrs. During each hospital visit growth and puberty was assessed. For the diagnosis of CD, tTG IgA autoantibodies were measured.
Results: 9/144 DM1children (6.25%) were found to have tTG IgA auto-

antibodies. These were predominantly girls [boys(5.6%) vs girls(11.8%), p=0.049], with shorter height (htsds:-1.01vs+0.21, p=0.006), shorter diabetes duration (2.3vs4.6 years, p=0.042) and similar HbA1c levels with the rest of the DM1children (HbA1c:7.75 vs 8.15%, p=NS). Multiple logistic regression analysis showed that the presence of tTG IgA autoantibodies was only associated with female gender (OR:1.50,95%CI:0.89,22.31). Only 5/9 (55.5%) tTG IgA positive children developed mild gastrointestinal symptoms, anaemia and growth retardation. All children with high titres of tTG IgA underwent celiac biopsy (4/9) and all had histological findings typical of CD.
Conclusion: CD seems to be more common in female children with DM1 and may present early in the course of the disease. It usually presents with mild symptoms or is asymptomatic. Thus the importance of its early diagnosis by regular autoantibody screening, is underlined.

PO2-358 Type 1 Diabetes II

‘*Sweet* program’: – effective transition of young people with diabetes to adult care in Queensland, Australia

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Young people with diabetes are often lost to diabetes specialist follow-up during the transition from paediatric to adult diabetes care. These patients often re-present as adults with diabetes-related complications due to poor diabetes control[1].
Aim
The aim of the *Sweet* Diabetes Transition project was to develop an appropriate model of care, supported by *Best Practice Guidelines* (BPG), for the effective transition of young people with diabetes to specialist adult care.
Methods
· An audit via questionnaire of 15 regional diabetes centers in Queensland to assess availability of transition and multidisciplinary services.
· An audit at 3 diabetes centres (1 metropolitan and 2 regional) to establish the rate of young people ‘dropping out’ of specialist care.
· A clinical survey to determine where 15-17 yr olds with diabetes, are accessing diabetes care.
Results
Only 1 of the 15 regional diabetes centers had a formal transition process in place but was lacking the appropriate supporting resources. Of the 3 centers audited to establish ‘drop-out’ rates, the metropolitan hospital had the lowest rate at 30%, with the 2 regional centres reporting rates up to 50%. Data collection for the state-wide clinical survey has been completed with the collection of data on 1359 (70%) young people with diabetes aged < 18 yrs. Preliminary analysis has found that clinical data was collected on 60% of young people ≥ 15 yrs of age compared to 48% (p=<0.0001) in a previous survey in 2002. The increase in the capture rate for this age group may be attributed to the launch of the *Sweet* project during the study period. The following resources have also been developed:
· *Best Practice Guidelines* on transition for health professionals (including GP’s) caring for young people with diabetes
· A Diabetes Transition Progress Sheet to document the young person’s progress through the transition process
· A website called *Sweet* at www.sweet.org.au, incorporating fact sheets, learning tools and other resources for young people and their families.
Summary
In the absence of *Best Practice Guidelines* and associated resources, a significant percentage of young people with diabetes are being lost to appropriate medical follow-up. The key to effective transition may be having *Best Practice Guidelines,* resources and an effective roll-out of the model to health professionals, young people and their families.
[1] *Can J Diabetes Care* (1996);20(3):13-20

PO2-359 Type 1 Diabetes II

A 10 year review of clinical outcomes and complication screening practices in young people with diabetes

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In the past decade there have been many changes in diabetes care aimed at improving the outcomes for young people with diabetes including the development of complication screening guidelines and lower $HbA1_c$ goals. Queensland has a widely distributed population, with 40% living in rural and remote areas. Distances are great and provision of diabetes care in these circumstances is difficult. We therefore developed and implemented a state-wide complication screening guideline and a survey to measure health outcomes since the implementation of these guidelines in 2000.
Aims
The aims of this study were to: audit clinical management practices; review diabetes related health outcomes; to assess changes in complication screening practices for young people with diabetes over a 10 year period.
Methods
Health professionals caring for young people with diabetes were asked to participate in three state-wide clinical audits. These audits collected data on every young person with diabetes <18 years seen during three, four-month periods in 1999, 2002 and 2008. The audit collected information on age, date of diagnosis, where diabetes care was being accessed, $HbA1_c$, associated medical conditions, severe hypoglycaemic events, diabetes related admissions and the presence of diabetes complications.
Results
There was a significant increase in the capture rate of subjects (53% to 70% p= <0.0001) between the 1999 and 2008 audits. The majority of subjects in all 3 audits had Type 1 diabetes (> 97%). There was no improvement in mean $HbA1_c$ results between the three audits (8.7%; 8.9%; 8.9%). Over 96% of subjects in the three audits were under paediatric supervision. There was minimal change in the rate of severe hypoglycaemia between the audits (8.5%; 8.4%; 7.8%). The rate of complication screening between the 1999 and 2002 audits rose from 37% to 58% (p=<0.0001) but decreased to 33% (p=<0.0001) in the 2008 audit.
Summary
These results from Queensland highlight that we should not assume that therapeutic advances for the treatment of diabetes will, of themselves, result in improved outcomes. In order to achieve the maximum benefit from therapeutic advances or altered clinical strategies, the changes must be adopted, resourced, implemented and include ongoing education and support. There may be additional benefit in the development of a state-wide diabetes register which includes an annual complication screening recall system.

PO2-360 Type 1 Diabetes II

Transition from a pediatric diabetes centre to diabetologists for adults: longidutinal metabolic control data from 135 patients with type 1 diabetes

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More than 95% of patients in the greater region of our city are treated in the diabetes centre of the University Children’s Hospital. The transition takes place at age 18-20 years. 178 patients were originally selected for this study, but longitudinal clinical and laboratory data could be collected retrospectively from 135 patients. After transition patients were followed by a specialist in a private office or in the outpatient diabetes center of the University Hospital at least two years before the study began. Data were available from 8 years before to 6 years after transition. At transition data from 82 women and 96 men were available. The mean values of glycosylated hemoglobin (HbA1c) in the year before

transition was 7.8% in men and 8.2 % in women (p<0.05). HbA1c values increased towards transition, correlated positively with diabetes duration, reached its maximum at transition and decreased gradually thereafter during 6 years after transition. The difference between men and women remained unchanged (p<0.05) until the age of 24 yrs, when they were no longer different. Data analysis in groups according to HbA1c-values showed a sex difference only in the group with HbA1c levels between 7.5-9.5% before transition. In this group there is no significant difference between HbA1c before and after transition. In conclusion these longitudinal data give insight in the quality of treatment and the effect of transition on patients with type 1 diabetes showing no sharp bend in the curve of HbA1c before and after transition and a gradual trend to better HbA1c values in young adult age only after several years.

PO2-361 Type 1 Diabetes II

Oral glucose tolerance testing in children with cystic fibrosis

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Background:
Cystic fibrosis related diabetes (CFRD) is the most common co-morbidity in persons with CF. Several studies have provided evidence for the clinical utility of OGTT screening in adolescents and adults with CF and guidelines recommend annual OGTT screening for all CF patients age 10 and older. This recommendation does not address younger patients with CF. Frank diabetes is rare prior to puberty in CF and usually has clear clinical signs. However, OGTT screening in children could be recommended if, as in adults, the glucose tolerance category provided clinically relevant prognostic information. This current retrospective chart review was performed to determine whether the baseline OGTT category in CF children age 6-10 years predicts subsequent clinical course.
Methods:
Data were retrospectively retrieved from the Minnesota CF Center Database on all patients who had an OGTT performed between Jan 1, 1998 and Dec 31, 2003, to identify individuals between the ages of 6-10 years. Children were classified as normal glucose tolerance (NGT) or abnormal glucose tolerance (AGT) based on OGTT. Height, weight, % predicted forced expiratory volume in 1 second (FEV1), % predicted forced vital capacity (FVC) and hemoglobin A1c (HbA1c) were collected at baseline and 5 years later. Children without 5 years follow-up data were excluded. All patients and their parents gave informed assent/consent for their records to be reviewed.
Results:
Of 43 children who had at least one abnormal OGTT between 1998-2008, 35 had sufficient data to be included. 35 children with NGT matched for age and gender were included as controls. At baseline, no children in either group had fasting hyperglycemia or impaired fasting glucose. There was no significant difference in height, weight, BMI or FEV1 at baseline between AGT and NGT. Diabetes subsequently developed in 14 children (40%) in the abnormal glucose tolerance group and in one child (3%) in the control group. (p< 0.001) The average age of onset was 13 +/- 1 years in boys and 11+/- 1 years in girls, considerably earlier than the average diabetes onset of 23 years in the UM CF population. At 5 year follow up there was no significant difference in height, weight, BMI or FEV1 between AGT and NGT.
Conclusions:
Children with abnormal glucose tolerance are clearly at high risk for progression to early onset frank diabetes compared to children with NGT. OGTT screening identifies these high-risk children.

PO2-362 Type 1 Diabetes II

Differential diagnosis of newly diagnosed type 1 diabetic ketoacidosis with stress hyperglycemia in critical children patients

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Objective: To inquiry into the differential diagnostic indexes of newly diagnosed type 1 diabetic ketoacidosis (DKA) with stress hyperglycemia(SHG) in critical children patients.
Methods: Thirty cases with DKA aged (6.5±3.6) years and 20 critical cases with SHG patients aged (5.8±3.1)years were prospectively studied. Glycosylated hemoglobin (HbA1c), fasting blood glucose(FBG), carbon dioxide combining power(CO2-CP), anion gap(AG), fasting C -peptide(FCP), fasting insulin(FINS),insulin resistence index(IRI), cortisol(COR) and if insulin dependence were compared between DKA and SHG group. Thirty healthy children aged (6.1±3.4)years served as normal controls(C group).There were no difference among groups in age and gender, p>0.05.
Results: (1)The concentrations of FBG, AG and COR were significantly higher, CO2-CP were significantly lower in both group DKA and SHG than in group C(p<0.001). (2)The concentration of HbA1c in DKA was significantly higher than both group SHG and C (p<0.001).There was no significantly difference between group SHG and C(p>0.05).And there was no overlap of HbA1c between DKA and SHG at 95% CI. HbA1c>7.3% in DKA,and HbA1c <6.5% in SHG.(3)The concentrations of FINS,FCP and IRI were significantly lower in group DKA than in both group SHG and C(p<0.001), and there were no overlap of three between DKA and SHG at 95% CI. FINS<2.6U/L, mg/L, IRI<2.7(mM.U/L) in group DKA;and FINS>9.3U/L, mg/L, and IRI>5.3(mM.U/L) in group SHG. (4)The blood glucose easily control in group SHG after intravenous 0.9%NaCL, reducing the intravenous glucose infusion and insulin-controversary regulating hormones, short-term small dosage insulin transfusion were needed when blood glucose>12mmol/L after above treatment in SHG, but the blood glucose control dependent on the insulin injection in group DKA.
Conclusions:(1) The FCP, FINS, IRI and HbA1c are simple and specific differencial indexs for differencial diagnosis between DKA and SHG. FCP, FINS, IRI,were decreased significantly and HbA1c were elevated significantly in DKA, but FCP, FINS, IRI were increased significantly, and HbA1c<6.5% in SHG .(2) Whether depending on insulin treatment or not is a reconfirming index for differencial diagnosis between DKA and SHG.

PO2-363 Type 1 Diabetes II

New onset diabetes mellitus in a young girl, type 2 with DKA and hyperosmolar hyperglycemic state or type 1 with DKA and insulin resistance?

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Introduction/Background
The boundaries between Type 1 and Type 2 can blur with overlapping clinical characteristics. We describe a case of a girl with features of both diabetic ketoacidosis (DKA) and hyperosmolar hypergylycemic state (HHS) in light of positive family history for both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) in first degree relatives.
Case presentation
EL, a10 year old Hispanic female, presented with 3 weeks of polyuria, polydipsia and 1 day of emesis. Physical exam revealed tachycardia, hypertension, elevated BMI of 26 kg/m2 and acanthosis nigricans on neck and axilla. Laboratory studies revealed pH of 6.99, serum glucose of 623 mg/dL and anion gap of 28. After 2 days of IV insulin therapy and hydration, the acidosis, anion gap and tachycardia persisted until hydration increased. First degree relatives exhibit comorbidities of T2DM (hyperlipidemia, hypertension, and obesity). Further results are ICA negative, GAD 65 AB >30 U/mL, and C-peptide 0.7 ng/mL.

Comparison of Characteristics			
	Type 1	Type 2	Patient
Pathophysiology	Autoimmune destruction of pancreatic β-cells	Insulin resistance (multifactorial-diet, genetics, sedentary lifestyle) Obesity predisposes to insulin resistance via adipokines	Insulin resistance and autoimmune destruction based on low c peptide and postive GAD AB
Inheritance/Genetics	HLA-linked DR3 and DR4, GAD-65, ICA-125. (polygenic)	Family History (40-80% incidence of children with parents with the disease) ethnicity (Hispanic Native American, African American) female gender (1.7 x); also polygenic	positive GAD AB; Hispanic, female, first degree relatives with Type 1 and Type 2 DM, HLA pending
Age of Onset	Bimodal: 4-6 years old and 10-14 years old	Adolescence (mean =13.5 years old)	10 years old
Insulin	Deficient	Resistant and decreased secretion	Low C-peptide level
Body Habitus	Thin	Obese with BMI>24-27, Acanthosis nigricans	Obese, BMI=26, Acanthosis nigricans

Discussion
This young, Hispanic female has features of HHS, DKA, T1DM and T2DM. Type 2 young, Hispanic females are more prone to DKA. EL improved with insulin administration, but her acidosis remained refractory, suggesting concurrent Hyperosmolar Hyperglycemic State. Conclusive evidence would be positive HLA DR3/DR4 genes.
Conclusion:
Genetic screening for pediatric patients with acanthosis nigricans, elevated BMI, and positive diabetes antibodies can assess predisposition to both T1DM and T2DM. This case deserves further investigation.

PO2-364 Type 1 Diabetes II

Insulin dependent non-immune diabetes mellitus may be caused by mutations in the *WFS1* gene

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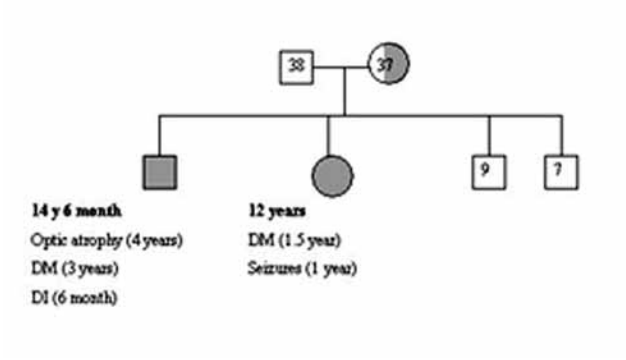
Introduction:
Wolfram Syndrome (WS), otherwise known as Diabetes Insipidus Diabetes Mellitus Optic Atrophy and Deafness (DIDMOAD) Syndrome, is an autosomal recessive condition associated with mutations in the *WFS1* gene. Wolframin protein is expressed in the brain and pancreas. Minimal criteria for WS include familial juvenile-onset insulin-dependent diabetes mellitus and progressive optic atrophy. WS patients frequently present with additional nondiagnositic phenotypes such as renal anomalies, psychiatric disorders, and a variety of other neurologic symptoms (ataxia, peripheral neuropathy and seizures).
We report two siblings with distinct clinical scenarios both harboring identical compound heterozygous *WFS1* mutations (E169K and V412fxX440).
Results:
Case 1: 10 year old boy presented to our service in severe DKA with coma. He had a history of optic atrophy diagnosed the previous year. At 13 years of age, he developed diabetes insipidus. Formal evaluation revealed normal sensorineural hearing, and CT of the brain was unremarkable.
Case 2: 10 year old girl, sister to the previous patient (case 1), presented with new onset of non-immune diabetes mellitus. Three month after later, she had two seizure episodes (not associated with hypoglycemia). A brain MRI was normal as was formal hearing evaluation.
Otherwise, their family history was not significant for any neurologic or psychiatric illness.



Case 1 met minimal criteria for WS and case 2 did not. However, mutation analysis in both siblings revealed compound heterozygous mutation with missence mutation: c.505G>A (E169K) and deletion c.1230_1233delCTCT (V412fxX440), which have already been identified in WS patients.
Discussion:
Our report of this illustrative sibling pair suggests that *WFS1* gene analysis should be considered in cases of non-immune insulin dependent diabetes in children, particularly if seemingly unrelated neurologic manifestations are present. Obtaining a through family history of WS-associated criteria is critical in all patients with non-immune DM. Finally, knowing the status of *WFS1* is critical for anticipating and possibly treating related clinical manifestations.

PO2-365 Type 1 Diabetes II

An independent association of maternal ambulatory blood pressure with albumin excretion in young offspring with type 1 diabetes

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Familial predisposition to hypertension has been associated with the development of diabetic nephropathy in adults, but there are limited data in adolescents with type 1 diabetes (T1D). Our aim was to assess whether parental ambulatory blood pressure (ABP) was associated with ABP and albumin excretion in young offspring with T1D.
24-hour ABP monitoring (ABPM) was performed in 509 T1D offspring, 311 fathers and 444 mothers from the Nephropathy Family Study, for a total of 295 matched fathers-offspring and 411 mothers-offspring. Mean systolic (SBP) and diastolic (DBP) BP during 24-hour, day (d) and night-time (d) were calculated. 3 early morning urinary albumin-creatinine ratios (ACR), HbA1c and anthropometric parameters were available for the offspring.
The offspring's mean age (±SD) was 15.8±2.3yr and T1D duration 6.4±3.9yr; mothers' age was 44.7±5.3yr and fathers' age: 46.9±5.9yr. All paternal ABPM parameters (age-adjusted), except for nSBP, were independently related to the offspring's ABP (24h SBP: β=0.17, 24h DBP: β=0.20, dSBP β=0.21, dDBP β=0.20; nDBP β=0.18, all p<0.01), after adjusting for the offspring's age, gender, BMI and HbA1c. Maternal 24h DBP (β=0.13 p=0.01) and nSBP (β=0.14 p=0.007) were related to the offspring's corresponding ABP parameter.
Significant independent associations were found between the offspring logACR (adjusted for age, gender, HbA1c) and maternal ABPM parameters (model 1), and they persisted even after adjusting for the offspring's ABP (model 2).

	model 1	model 2
24h SBP	β=0.12 p=0.01	β=0.09 p=0.06
24h DBP	β=0.12 p=0.01	β=0.10 p=0.04
dSBP	β=0.10 p=0.04	β=0.09 p=0.07
dDBP	β=0.12 p=0.01	β=0.11 p=0.03
nSBP	β=0.14 p=0.006	β=0.12 p=0.02
nDBP	β=0.11 p=0.03	β=0.10 p=0.04

Mothers of offspring with microalbuminuria (MA+, n 62) had higher ABPM parameters, except for nSBP, than mothers of MA– offspring (all p<0.05). In contrast, there was no significant association between paternal ABP and the offspring's ACR.
In this cohort of young people with childhood-onset T1D, parental ABP significantly influenced the offspring's ABP, therefore confirming its heritability. In addition, maternal ABP was closely related to ACR in the offspring, suggesting a dominant effect of maternal genes or an effect of intrauterine factors on MA risk.

PO2-366 Type 1 Diabetes II

Longitudinal changes in asymmetric (ADMA) and symmetric (SDMA) dimethylarginines in young people with type 1 diabetes

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Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is a risk factor for cardiovascular disease (CVD). The ADMA stereoisomer, symmetric dimethylarginine (SDMA), has also been associated with CVD, but it has mainly emerged as a potential new plasma marker of glomerular filtration rate (GFR). ADMA and SDMA measurements can be combined by using mass spectrometry technology, thus representing a valuable tool to assess renal function and CVD in populations at risk. Our aim was to assess the relationship of plasma ADMA and SDMA with glycaemic control, clinical variables, and the development of microalbuminuria in a cohort of young people with type 1 diabetes (T1D).
Plasma ADMA and SDMA levels were measured, by stable isotope dilution mass spectrometry, in 1105 samples collected longitudinally from 417 subjects with T1D from the Oxford Regional Prospective Study, (an inception cohort of children followed for a median of 10.3[7.1-12.3]yrs). Additional data concerning annual assessments of height, weight, HbA1c, urinary albumin/creatinine ratio were available.
Significant differences were found between males (n=239) and females in plasma levels of ADMA (0.478±0.08 vs 0.462±0.08 μM/L; p<0.001) and SDMA (0.420±0.06 vs 0.385±0.06 μM/L; p<0.001). Changes in ADMA and SDMA levels over time were negatively related to HbA1c (B±SE: -0.017±0.002/1% and -0.011±0.002/1%, p<0.001). ADMA, but not SDMA, was also independently related to diabetes duration (B±SE: -0.016±0.002/1yr, p<0.001). ADMA and SDMA levels were significantly lower in microalbuminuric (n=116) than in normoalbuminuric (n=301) subjects (0.455±0.08 vs 0.477±0.08 μM/L, p=0.01 and 0.385±0.06 vs 0.412±0.06 μM/L, p<0.001; respectively), probably reflecting hyperfiltration in subjects developing microalbuminuria. However, these differences persisted for SDMA (p=0.02), but not for ADMA (p=0.07) after adjusting for confounding factors (age, gender, duration, HbA1c).
In this longitudinal study, plasma ADMA and SDMA levels were higher in males, reflecting gender related differences in their production rate. HbA1c was an independent negative predictor of changes in ADMA and SDMA levels over time, suggesting that chronic hyperglycaemia might down-regulate mechanisms implicated in their synthesis/production or stimulate their metabolism/clearance.
SDMA, but not ADMA, was also related to microalbuminuria, probably reflecting changes in renal function associated with this complication.

PO2-367 Type 1 Diabetes II

Balancing risks and grocery lists in pediatric diabetes

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Diabetes (DM) management requires close attention to diet and insulin therapy. DM is particularly difficult for families with limited financial resources. Household food security (FS) exists when "all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life". FS was assessed in 2004 as part of the Canadian Community Health Survey 2.2 (CCHS), showing 9.2% of Canadian households were food insecure (FI), with a provincial rate of 14.6% in Nova Scotia (NS). This study was designed to: 1) examine the prevalence of FI in households with a DM child compared to NS and Canada, 2) determine if FI is associated with worse glycemic control (A1c), and 3) describe characteristics of, and strategies used by FI families.
Parents of consecutive patients attending the provincial tertiary care pediatric DM clinic, along with those followed for DM by pediatricians at one regional site were approached to participate in a telephone interview. Questions comprised the validated CCHS survey for FS status, with additional questions addressing demographics, and DM management strategies. Charts were reviewed for A1c.
The DM children (mean age 12.2±4.1 years) had a mean duration of DM of 5.5±3.6 years. The rate of FI in DM families was 20.6% (26/126, 95%CI = 13.6-27.7%), 6% higher than the NS average, and more than twice the national average. FI was associated with higher A1c (mean 9.75% in FI vs 8.90% in FS families (P=0.0056)). On average, FI families had 1.6(0-5) additional children, with a household income in the $30 000 - $40 000 range, and the majority from single income households (15/26). In 58% (15/26) of FI families, parents reported eating less so that the child with DM would have enough food, and 50% (13/26) were unable to purchase the types of food they knew were best due to the expense of DM supplies. Only a few families endorsed being unable to buy supplies (1/26), reusing needles (2/26) or glucose testing less often than recommended (2/26) to manage finances.
These preliminary data suggest FI is associated with worse DM control and that FI is higher in DM families in NS where health care, but not drug/DM supplies, are publicly funded. With further recruitment the strength of the association will be reassessed. This study has important implications for clinician awareness and advocacy for financial support for families with a child with DM.

PO2-368 Type 1 Diabetes II

Pulmonary function in children and adolescents with type 1 diabetes

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Pulmonary complications of type 1 diabetes mellitus (DM1) have been poorly characterised in children and adolescents. However, lung function abnormalities have a clear incidence in diabetic adult population.
AIMS: To assess lung function in children and adolescents with DM1 and identify risk factors associated with the development of this complication.
SUBJECTS AND METHODS: In a cross-sectional study, a hundred diabetic patients and 77 match controls were studied. Age of study population ranged 8 to 18 years (mean 12.8±2.6, 55.9% females) and none had evidence of lung, allergic, cardiac, neuromuscular, connective tissue or obesity disorders. All were non-smokers. Age, sex, pubertal stage and anthropometric data were analyzed. Pulmonary function was studied by spirometry [forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1) and FEV_1/FVC ratio], plethysmography [total lung capacity (TLC), residual volume (RV), RV/TLC ratio and airway resistance (R0.5)], diffusing capacity for carbon monoxide [total transfer of carbon monoxide (TLCO), alveolar ventilation (VA) and TLCO/VA ratio] and exhaled nitric oxide (NOe). Values were expressed as SD score corrected by height and pubertal stage, according with *Rosenthal et al (1993)*. In diabetic patients, duration of diabetes, metabolic control [mean HbA_{1c} (%) in the previous year], insulin dose, diabetic complications and biochemical data were analyzed. Statistics were performed with SPSS.
RESULTS: There were not differences in age, sex, pubertal state and BMI-SD between diabetic patients and controls. Mean duration of diabetes was 6.2±3.8 years and mean HbA1c levels 7.23±0.8 %. FEV_1/FVC ratio was found to be significantly higher in diabetics (p 0.005). Diabetic patients also had a trend towards lower FVC, FEV_1, TLC, R0.5 and TLCO values and higher RV, RV/TLC and NOe values than controls, but not reaching statistical significance. There

were no differences in pulmonary performance among diabetic patients based on duration of diabetes or level of metabolic control. We found a negative correlation between degrees of microalbuminuria and TLCO in diabetic patients. **CONCLUSIONS:** This study suggests that the lung is functionally involved in children with DM1 early in the course of the disease. Performance at these lung parameters must be followed up in time when reaching adulthood to establish long-term implications of what seem to be subtle initial alterations of pulmonary function in diabetic children.

PO2-369 Type 1 Diabetes II

Neurophysiologic findings in a group of Venezuelan adolescents with insuline-dependent diabetes mellitus

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Introduction: Neuropathy is a common complication of insulin-dependent diabetes mellitus (IDDM), but uncommon in children and adolescents with short duration of diabetes. It is assumed that symptomatic neuropathy, is preceded by a subclinical form which is important to be detected even at this age. Electrophysiological studies of nerve conduction velocities are probably one of the most sensitive tools to detect early abnormalities of nerve dysfunction. **Objectives:** Evaluate neurophysiologic findings using electromyography in adolescents with IDDM, its relationship with age, glycemic control, duration of diabetes, and the presence of neuropathy and other chronic complications. **Methods and Subjects:** Nervous conduction velocities compound motor action potentials, distal latencies and sensory nerve action potentials were assessed in 13 adolescents with IDDM attended at the Diabetes Unit. A Pearson correlation statistical analysis was performed with a significance level of 95 and 99%. **Results:** 13 adolescents, five (38.46%) females and 8 (61.46%) males, of age between 10.6 to 20.4 years with a mean of 15.46±3.2 were studied. The mean duration of diabetes was 6.18± 3.75 years. Five patients had good metabolic control according to the ISPAD guidelines, and 8 had a poor control. Twelve patients (92.3%) had normal nervous conduction velocities, 6 (50%) of which, had incipient nephropathy and none had retinopathy. One patient, the one with longest duration of diabetes, a poor glycemic control and incipient nephropathy, had electrophysiological changes compatible with incipient polineuropathy (7.7%). **Conclusion:** Our results are similar to worldwide reports in the sense that diabetic neuropathy is present in patients with longest duration of diabetes and is rarely evident during the first five years of the disease. The fact that only one adolescent had distal nerve dysfunction, is in accordance with previous reports, showing that few adolescents with IDDM have neuropathy. The majority of our patients had normal nervous conduction velocities in spite of poor glycemic control, therefore we can assume electrophysiological changes are not always accordant with metabolic control at this age, and, that neuropathy, is not a common complication during early stages of the disease. Clinical application of electromyography can be done after five years of the disease. However, more electrophysiological studies should be performed at this age, in order to know its prevalence.

PO2-370 Type 1 Diabetes II

Bone age corresponds to chronological age at onset of type 1 diabetes in youth

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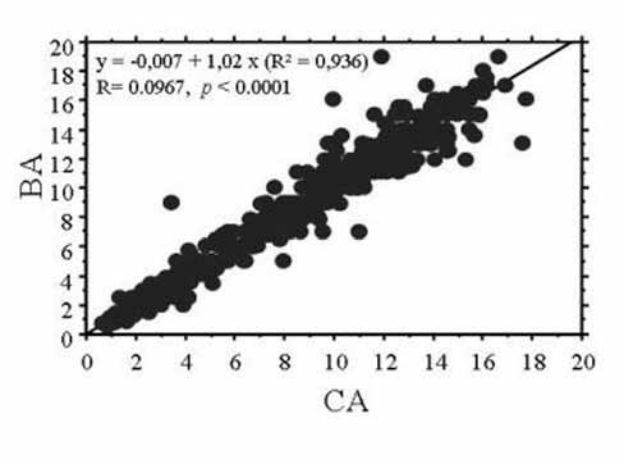
INTRODUCTION – There are only few data on skeletal maturation in diabetic children and these are controversial: bone age (BA) has been reported advanced or delayed. The aim of this study was to compare chronological ages (CA) and BA and to evaluate the impact of glycated hemoglobin (HbA1c) on BA at diagnosis of type 1 diabetes.
METHODS – This study included 496 diabetic patients [age 8.7 ± 4.0 years (mean ± SD)], 289 (58%) boys and 207 (42%) girls, 376 (76%) age < 12 years and 120 (24%) age ≥ 12 years. Standing height was measured and transformed into standard deviation score (SDS) according to the British 1990 growth reference. HbA1c levels and radiographies of left hands and wrists were obtained at diagnosis of type 1 diabetes. BA was determined according to Greulich and Pyle.
RESULTS – At diagnosis, height SDS (mean ± SD) was 0.35 ± 0.95 in girls and 0.37 ± 1.07 in boys (p: NS). In the whole population, CA was 8.7 ± 4.0 years and BA 8.8 ± 4.3 years. In girls CA was 8.12 ± 4.1 years and BA 8.4 ± 4.4 years and in boys CA was 9.0 ± 3.9 years and BA 9.1 ± 4.2 years. In <12 years of age CA was 7.0 ± 3.2 years and BA 7.2 ± 3.5 years and in ≥12 years of age CA was 13.8 ± 1.3 years and BA 14.0 ± 1.6 years. There was a strongly significant correlation between CA and BA in the 496 patients (r=0.967, p<0.001).



BA corresponded to CA: Δ (BA-CA) [median (25th, 75th percentiles)] was in the whole group 0.0 years (-1.0, +1.0). There was no correlation between Δ (BA-CA) and HbA1c (p: NS).
CONCLUSIONS – For each group the difference between CA and BA was in the range of the standard deviations for skeletal age. This study showed that bone maturation is normal for age and gender and independent of HbA1c at diagnosis of type 1 diabetes. These findings are compatible with the fact that the mean duration of typical symptoms (polyuria, polydipsia, weight loss, tiredness) of type 1 diabetes before diagnosis has been evaluated to 3 weeks (1,8) [median (25th, 75th percentiles)] in children. The short exposure to important insulin deficiency does not impair the mechanisms by which the GH-IGF-I axis allows normal growth.

PO2-371 Type 1 Diabetes II

Leptin before and after insulin therapy in children with type 1 diabetes

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Introduction: Several reports suggest that insulin may regulate serum leptin levels. In children with new-onset Type 1 Diabetes, leptin levels are low and increase with insulin therapy to normal levels.
Objectives: To compare leptin levels in diabetic children before and 3 months after insulin therapy in children with new onset type 1 diabetes. To evaluate the variables that can affect the increase of leptin levels with insulin therapy.
Methods: It was performed a prospective study, of 51 diabetic children attending at Paediatric Hospital in new-onset of type 1diabetes. At diagnosis were assessed sex, age, A1c, severity of presentation and leptin level. Three months after were evaluated leptin level, insulin doses, insulin regimen and A1c. Data was analysed using SPSS-15.
Results: Fifty one children were evaluated, 26 girls and 25 boys. Mean age of type 1 diabetes diagnosis was 8,1±3,2 years and previous duration of symptoms 17±13 days. At new-onset diabetes, 14 (27%) children presented ketoacidosis. They presented mean osmolality levels 302±8mOsm/L, mean glycaemia levels 506±157mg/dl and mean A1c 10,9±2,6%. Fifteen (28,4%) started conventional insulin therapy and 36 (70,6%) functional insulin therapy. Three months after diagnosis, the mean A1c were 7±1,1%.

Leptin levels before and after 3 months were 1,47±1,37 versus 5,1±4,3ng/ml (p<0,0001).
At three months, the mean rise of the leptin was 3,7±4,1ng/ml. The mean increase of leptin levels at three months presented a positive correlation with symptoms duration (r=0,46 and p=0,001) and initial A1c (r=0,41 e p=0,003). The rise of leptin levels was 4,3±4,6ng/dl in the functional insulin therapy group and 2,1±1,9 ng/dl in the conventional insulin therapy group (p=0,02).
Conclusions: This study showed lower leptin levels in newly diagnosed children with type 1 diabetes that increase with insulin therapy. This increase was associated with longer diabetes duration and higher initial A1c. Conventional insulin therapy is associated with lower rise of leptin levels.

PO2-372 Type 1 Diabetes II

Analysis of insulin requirements for adolescents with diabetes type 1 during a 3-day camp

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Background: Diabetes camping (DC) is often associated with increased physical activity. During DC, the goal is to balance the insulin dosage with the activity level to avoid risk of hypoglycemia.
Objective: To evaluate the effect of free physical activity on insulin requirements.
Study design: Cross-sectional study.
Setting: Diabetes Camp of the Chilean Diabetes Foundation, Summer 2009.
Subjects and Methods: Forty-four adolescents enrolled (Female 17/44). The average age was 15 years (Range, 13 to 18 years). From the clinical chart, the following data were analyzed: basal (BAI) and bolus insulin (BOI) requirements on day#0 (before camp), day#1, #2 and #3 of camp; age; gender; HbA1c; insulin schedule; BMI. The insulin requirement on day#0 was consider as 100%. To show the diffencences of insulin doses, the delta was calculated between day#0 and day#1, #2 and #3. Non parametric test was used for statistical analysis.
Results: Are presented as mean (standard deviation). The BAI decreased in -0.31% (±2.51%); -2.66% (±7.83%) and -6.82% (±15.59%) during Day#1 (p=0.59); #2 (p=0.025) and #3 (p<0.001) respectively. The BOI variations were +0.95% (±23.2%); -12.86% (±32.8%) and -17.4% (±34.4%) during Day#1 (p=0.66); #2 (p=0.01) and #3 (p<0.001) respectively. The highest BAI decrease was observed in people with higher HbA1c (Spearman' Rho= 0.396; p=0.02); no association was observed relation to gender (p=0.2), age (p=0.07), BMI (p=0.3) and years of duration of diabetes (p=0.2).
Conclusion: Insulin requirements, mainly bolus of insulin, decrease with free physical activity during the 3-day camp, and this has been was associated with previous glicemic control (HbA1c).

PO2-373 Type 1 Diabetes II

Identification of factors that influence parental attitudes toward clinical research for type 1 diabetes

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Objectives
To identify factors that influence parental willingness to enroll their children in Type 1 Diabetes clinical trials (T1DCTs).
Methods and Materials
Input from local diabetes experts was used to identify key domains that were expected to influence parent attitudes. Survey questions were then generated to probe these domains. The survey was refined through feedback from parent focus groups and a pilot test at a diabetes day camp. The final survey consisted of 48 questions including open-ended, yes/no, and Likert response formats. Surveys were then distributed at Diabetes Family Day (DFD) (n=21) and the Eskind Pediatric Diabetes Clinic 11/12/08-11/21/08 (n=67). All participants were parents of pediatric T1D patients.
Results
Response rate was 57% and respondents were predominantly Caucasian (95%) females (79%). 75% of respondents reported awareness of T1DCTs at Vanderbilt. Nearly 50% described themselves as willing to enroll. Willingness to enroll was positively influenced by whether participants had received easy-to-understand information about T1DCTs from a healthcare provider (r=0.55, p<0.01). Self-reported income and concern about diabetes complications positively correlated with willingness (r=0.25 and 0.33, p<0.05). Only 20% recalled being asked to enroll a child in a T1DCT by a healthcare provider. Less than 30% of parents reported comfort with T1DCT protocols using IV medications, vaccines, or placebo, procedures that are common in current trials. Parents reporting themselves as more willing to enroll in T1DCTs were more likely to accept these trials (r=0.26-0.49, p<0.01).
Conclusions
Parents report willingness to enroll their children in T1DCTs. However, only a minority accept methods in current trials. Information from healthcare providers can significantly influence parent willingness. Thus, efforts by healthcare providers to increase awareness of T1DCTs and their methods may accelerate testing of new therapies for T1D.

PO2-374 Type 1 Diabetes II

Successful transition from insulin to glyburide in 2 siblings with neonatal diabetes mellitus due to *KCNJ11* gene mutation encoding Kir6.2

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Background/Aim: The most common cause of permanent neonatal diabetes is an activating mutation in the ***KCJN11*** gene, encoding Kir6.2, a subunit of the K^+ATP channel of the ß cell. The activating mutation maintains a high intracellular ADP/ATP ratio, thereby preventing depolarization of the ß cell and consequently limiting insulin secretion.
We report the 2-year follow up of successful transition from insulin to oral sulfonylurea in 2 siblings with neonatal diabetes secondary to mutation in ***KCNJ11*** gene. Both siblings presented in the neonatal period. Father and paternal grandmother had infantile onset of diabetes. Detailed clinical and molecular finding was previously reported (Diabetes, 2004;53:2713-8).
Case1: A full term boy presented at 6 weeks of age with fever and serum glucose of 850 mg/dl. He was treated with different insulin regimens until the age of 5 5/12 years when he was transitioned to Glyburide. His mean HbA1c improved from a mean of 9.3 % on insulin therapy to 6.3 % on Glyburide 5.5 mg daily (0.23 mg/kg/day). He has experienced fewer hypoglycemic episodes and less erratic glycemic control on Glyburide. Currently, he is 7½ years old and has normal growth but borderline mental retardation of unknown cause.
Case 2: The younger sister was monitored from birth and diagnosed at 4 days of age with serum glucose of 230 mg/dl. She was started on Glargine and Lispro on 4th DOL until the age of 4 2/12 years, when she was transitioned to Glyburide. Mean HbA1c improved from 9.4 % on insulin to 7.8% on current dose of Glyburide 4.5 mg/daily (0.18 mg/kg/day). Currently, she is 6 4/12 year old and has normal growth and development.
Both siblings were found to have a mutation in ***KCNJ11*** gene resulting in amino acid substitution Y330C.
Conclusion: We provide long-term follow up of 2 additional cases of children with neonatal diabetes mellitus due to ***KCNJ11*** mutation who transitioned successfully from insulin oral sulfonylurea.

PO2-375 Type 1 Diabetes II

Vitamin D status in a new England pediatric population with type 1 diabetes

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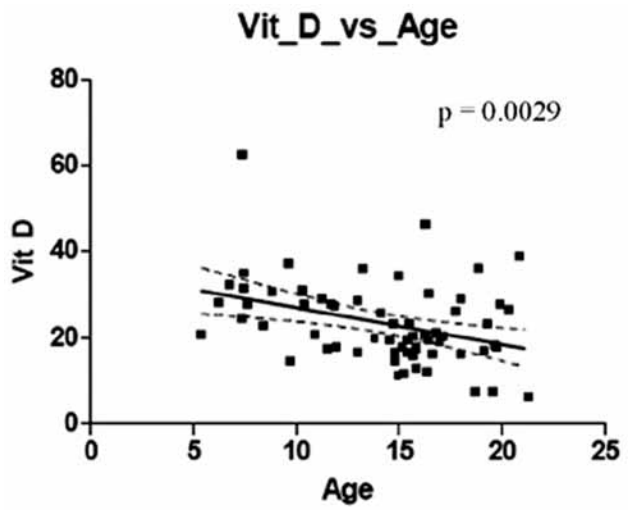
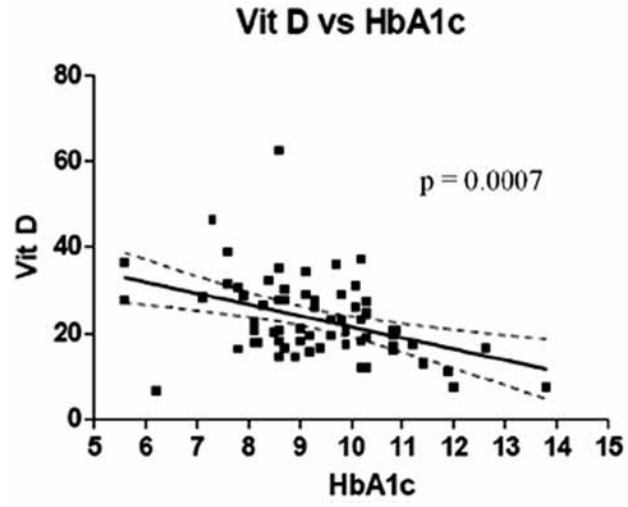
Background: Vitamin D deficiency and insufficiency in children and adolescents is a growing problem, particularly in children with chronic illness. Patients with Type 1 diabetes (T1DM) are at risk for future bone disease, but the factors accounting for this are unknown. Given this risk of metabolic

bone disease and the high prevalence of vitamin D deficiency in children with chronic illness, we have been measuring 25-hydroxy vitamin D (25-OH D) levels in our diabetic population. A recent study has shown a high incidence of Vitamin D deficiency in New England children with T1DM (J Pediatr, 2009;154(1):132-4).Therefore we retrospectively examined the 25-OH D levels in our clinic patients.

Methods: We reviewed 64 charts of T1DM patients aged 6 months to 22 years and examined their 25-OH D levels that were measured September '08 to March'09. We then correlated the 25-OH D level with HbA1c, age of patient and duration of diabetes.

Results: Overall, 27 patients (42.1%) had vitamin D deficiency (25-OH D ≤20 ng/mL), 24 patients (37.5%) had vitamin D insufficiency (25-OH D 21- 29 ng/mL) and only 13 patients (20.3%) were vitamin D sufficient (25-OH D ≥30 ng/mL). After linear regression analysis of our data, we found a negative correlation between 25-OH D level and age ($p = 0.0029$; $r^2 = 0.13$). In children age 5-11 years, 35% were sufficient, 50% deficient and 15% insufficient. By comparison, in children aged 12-21 years, 13.6% were sufficient, 31.8% were insufficient and 54.5% were deficient. In addition, 25-OH D level and HbA1c were correlated ($p = 0.0007$; $r^2 = 0.16$).





No correlations were found between vitamin D status and duration of diabetes, between HbA1c and duration of diabetes or between HbA1c and the age of the patient.

Conclusion: Almost 80% of our cohort had vitamin D insufficiency or deficiency, which is comparable with the results of the recent study from Boston in a similar population. These results are concerning, particularly in a population with a high baseline risk for future osteoporosis. Further studies are required but regular measurements of 25-OH D status appear to be warranted in this population.

PO2-376 Type 1 Diabetes II

Estimated average glucose in children and young people with type 1 diabetes mellitus derived from continuous glucose monitoring

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Estimated average glucose (EAG) as described by Nathan et al. is an alternative way of expressing HbA1c, which may improve patients, understand of their glycemic control. The dataset that was used to derive the EAG equation [EAG= 1.59*(HbA1c)-2.59] consisted of adults with type 1 and 2 diabetes along with controls. To determine whether the EAG equation is applicable for children and young people (CYP) with type 1 diabetes (T1DM) we compared continuous glucose monitoring (CGM) with HbA1c measurement in all CYP with T1DM and derived EAG from our dataset and compared it to that predicted by the Nathan equation. As part of a larger prospective cohort 85 children 44 (52%) male with a mean age 12.97 (range 7.84-18.43yrs), underwent CGM for 72hours and a measurement of HbA1c using a DCCT aligned system (Bayer 2000) was obtained. Mean glucose was derived from the CGM and compared with HbA1c using linear regression. Average HbA1c was 8.9% for this cohort with mean blood glucose averaging between 10.0 and 14.5 mmol/l. In contrast the population in the Nathan study had an average HbA1c of 6.8%. Mean insulin dose in our population was 1.0 Units/kg/day. The average number of glucose readings available for analysis was 945. Linear regression of CGM glucose and HbA1c yielded the following equation for Paediatric EAG (PEAG): PEAG= 0.53 (HbA1c) +6.1. There was no evidence of bias when PEAG versus Nathan EAG were compared (mean PEAG 11.72 mmol/l, Nathan EAG 11.70 mmol/l). The 95% limit of agreement for the two methods was 3.82mmol/l. There was no effect of gender or insulin therapy modality effect on PEAG. These data suggest that the Nathan EAG can be used in CYP with T1DM. Caution should still be exercised in the estimates derived for EAG as the dataset is skewed in both Nathan and PEAG in opposite directions due to the differences in average HbA1c. Larger cohort studies should help resolve this and allow for refinement of the relationship between PEAG and Nathan EAG. However, the level of agreement that we have demonstrated for CYP suggests that the Nathan EAG can be used in diabetes education and management for CYP with T1DM.

PO2-377 Type 1 Diabetes II

Continious subcutaneous insulin infusion (CSII) treatment in children and adolescents with type 1 diabetes in Siberia

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Introduction: Insulin pump therapy is more effective than multiple daily injections for achieving optimal metabolic control of diabetes but because its price it is not available to all children and adolescents with type 1 diabetes. In Russia more than 1000 children use the insulin pump therapy. In Moscow 15% of pediatric diabetes population have CSII. In Siberia less than 1% children with type 1 diabetes (200 pediatric patients) use insulin pump therapy (in Novosibirsk region - 30 patients; in Omsk -15; in Krasnoyarsk – 12, in Irkutsk - 8).

Aims. To investigate the differences in data of metabolic control, insulin doses and quality of life children and adolescents with type 1 diabetes before and after the start of using CSII.

Methods. We compared the data of HbA1c, insulin doses and quality of life in nine children and adolescents (5M/4F) with type 1 diabetes on CSII. Our patients were mean 12.1 years old, with average duration of diabetes 4.5 years. The insulin pumps "Accu-Check Spirit", "Medtronic Paradigm 712" and "Medtronic Paradigm 722 (PRT)" was used. HbA1c level before and in 3, 6 months after the start of CSII; the frequency of severe hypoglycemia and diabetic ketoacidosis, quality of life (by written questionnaires) were analysed.

Results. Mean HbA1c in our patients before CSII was 8.2 ± 1.9%; in 3 months after the start of CSII - 8.0 ± 2.1%; in 6 months - 7.7 ± 1.3% respectively. Daily insulin dose decreased in all patients from 15 to 20%. Diabetic ketosis

and severe hypoglycemia occurred in 2 patients. CSII improved the quality of life in all children due to reduction in the number of injections, flexibility of eating and better metabolic control.
Conclusion: By insulin pump therapy we significantly reduce daily insulin dose, improve metabolic control of diabetes and quality of life in our patients .

PO2-378 Type 1 Diabetes II

Are there any risks of hyperglycaemia during acute febrile illness at pediatric emergency department?

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Introduction: Hyperglycemia may predict the severity of the disease as well as the possibility of developing diabetes and impaired glucose metabolism.
Objectives:We examined the prevalence and risk factors for stress hyperglycemia in children with fever whom admitted to the emergency department. This study also aimed to assess the clinical conditions associated with stress hyperglycemia and to evaluate the prediction of diabetes mellitus.
Design:Patients who admitted to the emergency department of Ege University Children's Hospital with acute febrile illness(axillary temperature 37,3 C) within 6 months period enrolled and screened prospectively for hyperglycemia. All febrile children classified into 5 groups according to Emergency Severity Index v4. Severity was determined as severe (level 1 and 2) and nonsevere (level 3,4 and 5). Children were judged as hyperglycemic if capillary blood glucose level was higher than 140 mg/dL.If it was detected by the capillary sample then confirmed by venous blood sample.Confirmed hyperglycemic patients investigated for impaired glucose metabolism or the possibility of developing diabetes mellitus by pediatric endocrinology department.Regarding demographic characteristics, history, clinical findings, diagnosis and severity of the disease were ascertained.
Results:A total of 185 patients (113 male, 72 female) with a mean age of 4.46 4.08 years were enrolled. Body temperatures and blood glucose values respectively ranged from 37.5 C to 40.8 C (mean: 38.6 0.57 C) and from 56 mg/dL to 307mg/dL (mean:139.21 48.53 mg/dl).In 22 patients (11.8%) venous hyperglycemia dedected.None of those patients had either impaired glucose metabolism or diabetes mellitus.Autoantibodies were all negative. The prevalence of stress hyperglycemia was significantly increased among patients with temperatures (1) > than 38.6 C (36%) than temperatures < 38.6 C (30.6%) ($p = 0.025$), (2) with higher severity disease than lower severity group ($p = 0.036$) and (3) with patient who presented with seizure than the other group ($p = 0.016$).There was no correlation between stress hyperglycemia and CRP level.
Conclusions:Stress hyperglycemia does not appear to be associated with a particular diagnostic category but is significantly associated with severity of illness as measured by elevated temperature and severity of the disease.There is no data to support the development of diabetes in the follow up period.

PO2-379 Type 2 Diabetes, Insulin Resistance II

The first report on the prevalence of impaired fasting glucose and type 2 diabetes in a population-based sample of overweight/obese children in the Middle East

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Background: Type 2 diabetes mellitus (DM2) is increasing in young population because of high prevalence of obesity in this age group. Obese children with impaired fasting glucose (IFG) are at increased risk for developing diabetes. The aim of this study was to determine the prevalence of IFG and DM2 for the first time in a representative sample of Iranian children and adolescents with obesity.
Methods: A total number of 672 overweight and obese Iranian children, aged 6-19 years (mean, 12.89±3.10 years) selected from 7554 students, were included in this cross-sectional, population-based study. Fasting plasma glucose (FPG) and lipid profile were measured in all participants. Oral glucose tolerance test and insulin level were measured in those children with IFG (100≤ FPG<126mg/dl). Insulin resistance defined by HOMA-IR more than3.10.
Results: The prevalence of IFG and DM2 were 4.61% (31 persons; mean age, 13.23±3.58 years) and 0.14% (1 person; age, 18.00years), respectively. There was no significant difference in lipid profile between IFG group and other children. Impaired glucose tolerance and insulin resistance were detected in 3 and 6 participants with IFG, who consisted 0.4 %and 0.8% of total obese and overweight students, respectively.
Conclusion: Although the prevalence of DM2 is low in Iranian obese children and adolescents, IFG is not uncommon. Preventive measures notably lifestyle modification and regular screening of FPG should be considered to these children.

PO2-380 Type 2 Diabetes, Insulin Resistance II

Adult and infant islet morphology in a monkey model with diminished glucoregulation

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Although the pathogenesis is still unknown, offspring of women with polycystic ovary syndrome (PCOS) are more likely to develop the syndrome and its metabolic complications. Adult, prenatally androgenized (PA) female rhesus monkeys exhibit PCOS-like phenotypes, including insulin resistance and impaired insulin response to glucose on the continuum towards type 2 diabetes. In infancy, PA monkeys demonstrate both increased insulin sensitivity and hypersecretion, suggesting that fetal imprinting of pancreatic function underlies the evolution of pancreatic dysfunction in adult primates. Islet amyloid polypeptide (IAPP) and insulin are normal products of the pancreatic beta cell and are useful in quantifying islet morphometry. The deposition of IAPP as amyloid has been linked to beta cell death in the pathogenesis of type 2 diabetes. Our aim was to determine 1) if adult glucose dysregulation was associated with altered islet morphometry assessed by differences in IAPP and insulin staining, and 2) if infantile morphometry revealed evidence of islet changes. Pancreatic tissue from female infant (n=5) and adult (n=6) PA rhesus monkeys and from infant and adult age and BMI similar controls (C; n=4, n=7, respectively) were sectioned and stained using IAPP or insulin antibodies. Islet count, size, and area fraction were quantified using Image Pro software. In PA monkeys, islet count per µm2 and fractional area in insulin- and IAPP-stained adult and infant sections were similar to those of controls. Total islet area per islet count, reflecting average islet size, was increased (p=0.022) in IAPP-stained adult PA (10.80[8.17, 16.00] x 102 µm2) versus adult C (6.09[4.91, 706] x 102 µm2). No difference in average islet size was observed in the adult insulin- or infant insulin- or IAPP- stained sections. Assessment of islet size distribution (small: <500, intermediate: 501-1000, large: > 1000, and very large: > 8,000 µm2) showed adult PA insulin-stained sections with fewer intermediate islets (C: 451.7±60.2; PA: 244.2±65.0 x 106, p=0.039) and increased (p=0.043) numbers of very large islets (C: 10.6±8.2; PA: 38.1±8.8 x 106, p=0.043). There were no differences in islet size distribution in adult IAPP or infant IAPP or insulin-stained sections. These results suggest that PA female monkeys with PCOS-like phenotypes and perturbed insulin-glucose homeostasis leading to type 2 diabetes, are predisposed to develop larger islets with greater expression of IAPP after infancy.

PO2-381 Type 2 Diabetes, Insulin Resistance II

High normal fasting glucose level in youth: a marker for insulin resistance and beta cell dysregulation

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Background:High but normal fasting glucose (NFG) level in adults is a risk factor for future development of Type 2 diabetes (T2DM). Thus, before fasting glucose (FG) reaches the diagnostic range for impaired glucose tolerance (IGT), derangements in insulin sensitivity and secretion might be present.
Objective: We investigated whether NFG levels, i.e. (FG< 100mg/dl), could be used to identify children with cardiovascular and metabolic disease risk factors. Specifically we investigated whether FPG could identify children with insulin resistance (IR), reduced beta cell function and thus at risk for IGT.
Design: Setting: Yale Pediatric Obesity and Lipid Disorder clinic, a referral center for the greater New Haven (CT) area.
Patients: A multiethnic cohort of One thousand and ninety-three children and adolescents (boys=39%) with NFG underwent a standard oral glucose tolerance test (age 13±3 years, BMI Z 2.35±.5) with calculation of indices of insulin resistance (IR) and beta cell function. In addition, anthropometric indices were measured. Of them, 150 had IGT, while 6 had T2DM. Quartiles of NFG were calculated (I 61-86 mgdl-1; II 86.5-90 mgdl-1; III 90.5-95 mgdl-1; IV 95.5-99.9 mgdl-1).
Results: Interestingly, in children with normal glucose tolerance, we observed a significant increase across quartiles for IR ($p<0.0001$), 2-hour glucose ($p<0.0001$), and HbA1C ($p<0.0001$) and a significant decrease in the disposition index ($p<0.0001$) and whole body insulin sensitivity ($p<0.0001$). Moreover, as FPG increased, the odds ratio of presenting with IGT was 1.655 (95% CI 1.2-2.3).
Conclusions: These data suggest that in children and adolescents, independent of age, BMI, gender and ethnicity, insulin sensitivity and secretion decline progressively when moving from low to high NFG. The simple measure of FPG may assist clinicians in identifying children for targeted diabetes screening and subsequent lifestyle management.

PO2-382 Type 2 Diabetes, Insulin Resistance II

A novel heterozygous mutation in the glucokinase gene is responsible for an early-onset mild form of MODY 2 diabetes

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MODY diabetes usually presents as mild asymptomatic hyperglycemia in non-obese children, adolescents and young adults with a positive family history of diabetes indicating an autosomal dominant trait. The most frequent type is MODY 2, caused by heterozygous mutations of the glucokinase gene. Homozygous mutations lead to permanent neonatal diabetes whereas heterozygous activating mutations lead to PHHI. Glucokinase is mainly expressed to the pancreatic β-cell and the hepatocyte, where it catalyzes the first and most important stage in glucose metabolism, which is the production of 6-P-glucose. Glucokinase dysfunction causes insufficient insulin secretion and glucogen synthesis in the β-cell and the hepatocyte respectively.
We present a 5 year old boy (BMI 15.2 Kg/m^2, -0.24 SDS) with fasting hyperglycemia 100-120 mg% and slightly elevated HbA1c 6-6.5% since the age of 2. OGTT (0', 60', 120', 180') showed glucose intolerance: 115, 249, 192, 94 mg% with a low insulin response: 5, 23, 20, 4 IU/L and analogous c-peptide levels: 0.5, 3.1, 2.5, 0.8 ng/ml. HbA1c was 6.4%. Genetic analysis of the HLA region did not reveal any high risk aplotypes for type 1 diabetes, but there were positive anti-GAD antibodies.
The boy's father aged 42, his sister aged 44 and her daughter aged 17, have a mild form of diabetes. The father reports fasting hyperglycemia and elevated HbA1c from infancy and is currently under sulfonylurea treatment. He has negative anti-GAD antibodies. The paternal grandmother was diagnosed with gestational diabetes at 28 years, which proved persistent - currently treated with sulfonylureas at the age of 72.
Sequencing of the glucokinase gene revealed a novel heterozygous mutation in exon 8 c.908G>T, Arg303Leu. It has not been found as a polymorphism, whilst a different mutation at the same codon in an unrelated family with MODY 2 has been published. Analysis with Polyphen and Alamut predicts a damaging effect on the glucokinase protein (score 3.1). The mutation is coded as follows:
DNA Level (cDNA): **NM_000162.3:c.908G>T**
DNA Level (genomic):**Chr7(NCBI 36):g.44152698G>T**
Protein Level:**p.Arg303Leu**
The boy is now 7 years old and his fasting plasma glucose and HbA1c are maintained in satisfactory levels only with a normocaloric low glycaemic index diet. Detailed examination of the living carriers in this family will probably reveal the long term sequellae of this mutation.

PO2-383 Type 2 Diabetes, Insulin Resistance II

Congenital insulin receptor mutations: variability in clinical course and diagnostic utility of serum biomarkers

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Background: Donohue Syndrome (DS) and Type A insulin resistance (IR) are syndromes of congenital insulin receptor (INSR) dysfunction representing two ends of a spectrum of INSR disorders. DS is reported to have a poor prognosis and life expectancy of only 1-2 years. Type A IR presents peripubertally with PCOS-like symptoms. There is little consensus about optimal management. Recently, serum biomarkers were shown to be predictive of INSR mutations.
Objectives: 1. To present a case of DS with a favorable course despite no intervention. 2. To report a case of Type A IR with a novel INSR mutation. 3. To show the diagnostic utility of serum adiponectin, IGFBP-1, and SHBG in predicting INSR mutations at both ends of the clinical spectrum.
Design/Methods: Case series. Standard biochemical assays. Direct sequencing of INSR.
Results: Patient 1 presented at birth with IUGR, dysmorphism, hirsutism, breast buds, clitoromegaly, decreased subcutaneous fat, polycystic ovaries, hyperglycemia, fasting hypoglycemia, insulin of 4925 uU/mL, and developed severe acanthosis nigricans. INSR sequencing revealed a 1bp insertion in exon 2, causing an immediate stop codon (Y60X). Mother is a heterozygote. Patient 1 likely has a second cis-acting mutation. At 14 mos, her blood glucoses range from 100-200 mg/dl without intervention, and she has not required hospitalization for metabolic decompensation. Patient 2 presented at 14 years with primary amenorrhea, acne, and hirsutism. She had ovarian hyperandrogenism, severe IR, and diabetes, despite a BMI of 20 kg/m2. Maternal aunt and mother had diagnoses of PCOS; mother had symptoms of fasting hypoglycemia. INSR sequencing revealed heterozygosity for W1175R, a novel point mutation. Both patients had elevated adiponectin, IGFBP-1, and SHBG, highly unusual for common forms of IR.

Biomarkers Predictive of INSR Mutations

	Patient 1	Patient 2	Value Predictive of INSR Mutation
BW (kg)	1.6	2.16	
Age (Sex)	14 mos (F)	14 years (F)	
Insulin (uU/mL)	2855-2631	124-154	
Adiponectin (mcg/mL)	51	19-27	>7*
IGFBP-1 (ng/mL)	179-575	36	>30
SHBG (nmol/L)	561-988	64-242	>70

*based on Institute of Metabolic Science Assay

Conclusions: 1. There is much variation in clinical course and prognosis even among DS cases. 2. We report a novel INSR mutation. 3. Adiponectin, IGFBP-1 and SHBG levels are predictive of INSR mutations in both conditions, with the degree of elevation consistent with clinical severity.

PO2-384 Type 2 Diabetes, Insulin Resistance II

Waist-to-height ratio is useful as BMI to identify high metabolic risk but does not predict ghrelin and adiponectin regulation in paediatric obesity

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Introduction. Waist-to-height ratio (W/Hr) has been recently proposed to be a greater value for predicting abdominal obesity, metabolic syndrome (MS) and cardiovascular disorders in adults and children. Circulating ghrelin and adiponectin levels are decreased in obesity and MS and negatively correlate with BMI. The role of W/Hr in predicting ghrelin and adiponectin secretion in comparison to BMI has not been investigated yet.

Aim. In order to understand the usefulness of W/Hr in MS in childhood, we measured AG, UAG, obestatin, total adiponectin (Tad), high molecular weight adiponectin (HMW), glucose, insulin, lipid profile and liver enzymes at fasting in 155 prepubertal (PP; 75) and pubertal (P; 80) children. Auxological parameters, blood pressure, HOMA, QUICKI and acanthosis indexes were also evaluated. We divided patients according to paediatric IDF 2007 criteria for MS.

Results. In all the group, 74 children were obese (34 PP and 40 P) and 59 (79.7%) had MS and 8 of them had glucose intolerance (IFG or IGT). In the 81 normal weight children (41 PP and 40 P) nobody had MS or glucose intolerance. According to not normal distribution, all the parameters were log transformed.

Log W/Hr well correlated with anthropometric variables, components of MS, the number of IDF07 criteria other than large waist (>90th percentile), acanthosis, HOMA and QUICKI indexes (Pearson higher than 0.300) but not with total and LDL cholesterol, and AST. The associations were maintained when weighted for pubertal status an/or sex. Log BMI presented similar correlation to Log W/Hr also when weighted. Log W/Hr correlated with UAG (r: -0.271; $p<0.02$), Tad (r: -0.412; $p<0.02$) and HMW (r: -0.403; $p<0.02$) but not with AG and obestatin. When weighted for puberty and/or sex, Log W/Hr maintained its correlation only with Tad and HMW. Conversely, Log BMI strongly correlated with UAG (r: -0.582; $p<0.0001$), AG (r: -0.356; $p<0.001$), obestatin (r: -0.153; $p<0.02$), Tad (r: -0.469; $p<0.008$) and HMW (r: -0.458; $p<0.001$) and the correlations were maintained with the same strength after corrections.

In a model composed by ghrelin and adiponectin, the best predictors were HMW (β:-0.438) for Log W/Hr (r2: 0.192) and much more UAG (β:-0.572) and HMW (β:-0.479) for Log BMI (r2:0.453).

Conclusions. W/Hr is useful as BMI in identifying MS and insulin resistance but seems to be not able to predict the alterations in ghrelin and adiponectin secretion in obesity and MS in childhood.

PO2-385 Type 2 Diabetes, Insulin Resistance II

Screening overweight school-aged students at high risk for impaired glucose metabolism in the Buffalo public schools

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Background: Insulin resistance (IR) in obese youth is a growing public health problem. Acanthosis nigricans (AN) is an early sign of insulin resistance, but is often overlooked. It may be a quick and reliable screening tool to identify youth who are at high risk for impaired glucose metabolism. Methods: During the academic school years 2006-2007 and 2007-2008, trained nurse practitioners screened 623 overweight youth at high risk for type 2 diabetes mellitus (T2DM) part of the Diabetes Prevention grant funded by the NYSDOH. The screening took place in 12 urban elementary, middle and high schools in Buffalo,NY. Students were eligible for a random blood glucose (RBG) drawn by finger stick if they had enrolled in the school based health center, had a BMI >85th percentile for age/sex plus one other risk factor for T2DM outlined in the 2006 ADA screening criteria. Results: Ninety five percent of all students were of minority ethnic status. The mean BMI was 28.1 kg/m^2. Fifty-nine percent were female. Age ranged from 4 to 18 years (mean age 11.1 ± 3.2) and 46% being ≥ 12 years old. Acanthosis nigricans was identified in 153/623 (25%) of the students, 92% of whom were African American. Sixty percent of students who presented with AN were ≥ 12 years of age. While students with AN were significantly older than students without AN (12.1 vs. 10.8 years, respectively, $p<0.001$), it was detected as early as 6 years of age. AN was twice as common among secondary school aged children (41%) compared with elementary and middle aged school combined (21% $p<0.001$). The age-adjusted mean BMI was significantly higher among students with AN (32.0 kg/m^2) compared to those without AN (27.0 kg/m^2), $p< 0.001$. Eighteen percent of students with AN reported a family history of diabetes compared with 5.0% with no AN, $p < 0.001$. RBG was obtained on 463 (75%) of the students: 1.1% (n=7)had RBG ≥ 140 mg/dl including one student ≥200 mg/dl. and 5/7 (71%) had AN. Conclusion: In this urban public school population of overweight youth the prevalence of AN was comparable to other reports. Acanthosis nigricans was highest in African American children ≥12 years. AN can be an early sign of IR. Thus, primary care health professionals within and outside the school system should screen overweight children for AN and note its presence in the physical exam. Moreover, the family and the child should be made aware of the significance of AN as it relates to insulin resistance and counseled appropriately

PO2-386 Type 2 Diabetes, Insulin Resistance II

Prevalence of type 1 (T1DM), type 2 (T2DM), type 1.5 (T1.5DM) and MODY diabetes in a multi-center diabetes practice in Brooklyn

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Background:

The differential diagnosis of major forms of childhood diabetes includes type 1, type 2, type 1.5 and MODY. The increased prevalence of childhood obesity and acanthosis nigricans made the clinical presentation of these forms of diabetes overlap.

Aim:

To analyze the clinical prevalence of different types of diabetes among different races in our diabetes practice in Brooklyn.

Patients and methods:

285 children with diabetes were classified as T1DM by positive islet cell antibodies, BMI< 95th percentile; T1.5 DM by positive antibodies and BMI > 95th %, +/-AN; T2DM in case of negative islet cells antibodies, presence of AN, +/- obesity; and as MODY by negative islet cell antibodies and autosomal dominant inheritance at least in 3 generations.

Results:

285 subjects were included in this analysis. Results of the distribution of the different types of diabetes in the different ethnic groups(African-Americans of Caribbean (AAC) descent, African-Americans (AA), Caucasians, Hispanic, and Arabic) and the age of onset breakdown are in (Table 1) section, copy and paste the following tag, including brackets, where you would like your first table to appear.

Distribution, Age of Onset of Diabetes in Different Ethnic Groups				
	Number of patients	Type 1 diabetes	Type 2 diabetes	MODY
Group	Total N	N (%) Age (yrs)	N (%) Age (yrs)	N (%) Age (yrs)
Carribean Black (AAC)	73	37 (50%) 11.7 +/- 5	12 (16%) 14.8 +/- 1.9	20 (27%) 10.4 +/- 4.4
Caucasian	106	70 (66%) 7.8 +/- 3.6	10 (9.4%) 15 +/-1.8	4 (3.7%) 13.5 +/- 2.4
Hispanic	64	37 (58%) 6.7+/- 4.2	9 (14%) 13.6 +/- 3	1 (1.5%) 13
African American (AA)	29	16 (55%) 8.5+/- 3.7	6 (20%) 12.3 +/- 2.8	0 --- ---
Arabic	13	10 (77%) 6.2 +/- 2.7	2 (15.3%) 12.9 +/- 0.8	0 --- ---

Type 1 diabetes has prevalence around 50-55% across all races with similar age of onset.
Type 1.5 diabetes has prevalence around 20% among Caucasians, AA, and Hispanics, comparing to~5-10% in other races.
Type 2 diabetes has a lower incidence in Caucasianscompared to other races and later age of onset than either type 1 or type 1.5 diabetes.
MODY was surprisingly highly prevalent among AAC, and presented earlier compared to Caucasian and Hispanic patients.

Conclusion:

Different racial groups have remarkable differences in the distribution of diabetes variants.Our study reveal differences in diabetes prevalence, age of onset between distinct ethnic groups. Further studies are needed to confirm our findings.

PO2-387 Type 2 Diabetes, Insulin Resistance II

Insulin resistance parameters in first degree relatives of women with PCOS

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First degree relatives of women with PCOS have been shown to have increased risk for diabetes and glucose intolerance. Normal glucose tolerant relatives have evidence of insulin resistance.

Objective: Cross-sectional study looking at insulin resistance parameters in early adolescent girls who are first degree relatives of women with PCOS and compare them with healthy age-matched girls.

Design/Methods: Cross-sectional study involving early adolescent girls who were first degree relatives of PCOS patients. Healthy cohort was obtained from 6th to 8th grade children enrolled in a school based intervention study. Relevant clinical and biochemical parameters including FSIVGTT were obtained.

Results: A total of 15 first degree relatives and 21 age-matched girls were evaluated. All had anthropometric measurements and frequently sampled IVGTT testing. There was no difference in age or BMI Z score as shown in table 1.

Comparaison of differents clinical and biochemical parameters betwen first degree relatives of PCOS and control age- matched patients		
	Age-Matched Controls (N=21)	First degree relatives of PCOS patients(n=15)
Age(years)	12.1(0.4)	11.6(1.1)
BMI Z Score	0.83(0.8)	0.97(0.8)
Acute Insulin Response	100(71)	120.4(84)
Disposition Index	2.92(0.19)	2.73(0.32)
HOMA*	2.62(1.67)	3.69(1.88)

First degree relatives showed significantly higher HOMA, reflecting more insulin resistance and lower disposition index, suggesting increased risk of developing glucose intolerance and diabetes.

HOMA* :Homeostasis model assessement :Simple fasting method to measure insulin resistance.

Conclusion: Early adolescent girls who are first degree relatives of patients with PCOS seem to have a higher risk of developing diabetes or glucose intolerance as compared to healthy girls of the same age. This warrants more aggressive screening of early adolescent first degree relatives of patients with PCOS.

PO2-388 Type 2 Diabetes, Insulin Resistance II

Parental diabetes, pubertal stage, and extreme obesity are the main risk factors for prediabetes in children: a simple risk score to identify children at risk for prediabetes

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Objective: The current worldwide increase of prediabetes (impaired fasting glucose or impaired glucose tolerance) coincide the increase of obesity. However, it is unclear which overweight children should be screened for prediabetes.

Methods: We studied 437 overweight children to identify risk factors for prediabetes. A risk score for prediabetes was developed based on the strongest correlation to prediabetes. This score was examined in a second, independent cohort of 567 overweight children. History of type 2 diabetes mellitus in parents and grandparents, degree of overweight, age, pubertal stage, birth weight, hypertension, dyslipidemia, acanthosis nigricans, and abdominal obesity were considered as potential risk factors.

Results: The frequency of prediabetes was 6% in sample 1 and 17% in sample 2. The strongest association was observed for history of parental diabetes with an adjusted odds ratio (aOR) of 9.5 (95%CI 2.5-36.4) in sample 1 and 6.3 (95%CI 3.7-10.7) in sample 2 followed by pubertal stage with an aOR of 5.5 (95%CI 0.7-45.4) in sample 1 and 6.2 (95%CI 2.4-15.6) in sample 2 and by extreme obesity with an aOR of 5.0 (95%CI 1.7-15.3) in sample 1 and 3.3 (95%CI 2.0-5.4) in sample 2. A risk score of ≥2 based on history of parental diabetes (2 points), extreme obesity (1 point), and pubertal stage (1 point) yielded a sensitivity of 88% for prediabetes.

Conclusions: Screening for prediabetes seems meaningful in overweight subjects with either parental history of diabetes or a combination of extreme obesity and pubertal stage detected nearly 90% of the overweight children with prediabetes.

PO2-389 Type 2 Diabetes, Insulin Resistance II

How is type 2 diabetes impacting upon paediatric tertiary hospital diabetes services?

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Introduction

Numerous groups have reported a global increase in the prevalence of Type 2 diabetes mellitus (T2DM) in children and adolescents. These patients have specific clinical needs that are different from those with Type 1 diabetes mellitus (T1DM) – particularly in the management of associated weight problems, wider metabolic health and early diabetes-associated complications. It is therefore important that we correctly identify these individuals and tailor management programs to their specific needs.

Aims

To correctly identify all children and adolescents with T2DM within our large paediatric diabetes service, and assess whether these individuals are receiving appropriate disease-specific management within a service primarily developed for T1DM.

Methods

An audit of our paediatric diabetes service was undertaken (study period Jan 2001 – Dec 2006) and clinical data collected on all autoantibody-negative patients. Diabetes sub-classification was undertaken by 2 diabetologists using American Diabetes Association guidelines.

Results

Within our diabetes database of 1574 patients (aged 0-18 years), 935 (59.4%) had autoantibody results recorded. 170 (18.2%) were autoantibody negative with 14 (median [range] age 12.5 [9.3-16.8] years; 10 female) satisfying criteria for a diagnosis of autoantibody-negative T2DM. At diagnosis, mean (SD) age- and gender-adjusted Body Mass Index (BMI-Z) was +2.0 (0.5) and median (range) HbA1c was 6.3 (5-12.4)%. Median (range) duration of follow-up was 3.7 (1.4-8.1) years. Mean (SD) BMI-Z at follow-up was +1.7 (0.8) and median (range) HbA1c was 8.4 (5.6-13.6)%. Metformin had been prescribed to 13/14 with 5 also receiving subcutaneous insulin. Blood pressure had been recorded in all while 11/14 (79%) had undergone screening for dyslipidaemia and 9/14 (64%) had been screened for nephropathy and retinopathy. 3/14 (21%) had hypertension, 3/11 (27%) had a cholesterol >5.2mmol/l, 3/9 (33%) had microalbuminuria and 4/9 (44%) had abnormal retinal findings.

Discussion

These data indicate that at present T2DM represents a small case workload (approximately 1.5%) within our paediatric diabetes service, compared with T1DM. It is not yet clear whether, with the forecasted increase in T2DM, this will change over the next few years. An increase in HbA1c in these patients over time however, along with a high prevalence of early complications, means that we now need to develop strategies for optimising clinical management in these patients.

PO2-390 Type 2 Diabetes, Insulin Resistance II

Which factors are associated with early complications in children and adolescents with type 2 diabetes?

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Introduction

Compared with Type 1 diabetes, young people with Type 2 diabetes mellitus (T2DM) have an increased propensity for early complications. Identification of those most at risk of early complications will help focus valuable resources, as well as furthering our knowledge of factors that underpin these processes.

Aim

To investigate factors associated with an increased likelihood of having T2DM-related complications.

Methods

Data on all young people enrolled in our paediatric diabetes service with T2DM (n=33 defined by American Diabetes Association criteria) were analysed to identify factors associated with the presence of hypertension (defined using recent UK centiles), dyslipidaemia (cholesterol ≥5.2mmol/l ± triglycerides ≥1.7mmol/l), microalbuminuria and/or evidence of retinopathy. Parameters assessed were age at diagnosis, current age, gender, initial and current Body Mass Index (BMI), change in BMI, initial and current age- and gender-adjusted BMI (BMI-Z), change in BMI-Z, initial and current HbA1c, change in HbA1c, treatment modality and duration of follow-up. Statistical analysis was by independent samples t-tests and Chi-square. Data reported are mean [SD] unless otherwise stated.

Results

33 youth with T2DM (median [range] age at diagnosis 13.4 [9.2-17.4] years; 22 female) received follow-up for an average of 2.2 [2.1] years. Data were available for blood pressure in 30/33, lipids in 27/33, urinary albumin in 20/33 and retinal screening in 16/33. Hypertension was more likely in those with greater initial and follow-up BMI (36[5] vs 28[6]kg/m^2; p=0.001, and 37[5] vs 28[8] kg/m^2; p=0.007, respectively) and greater initial and follow-up BMI-Z (2.3[0.3] vs 1.7[0.7]; p=0.01, and 2.3[0.3] vs 1.4[0.9]; p<0.001, respectively). Those with dyslipidaemia were older (17.5[2.1] vs 15.9[1.6]; p=0.03) and more likely to be receiving combination insulin+metformin than metformin alone (p=0.03). There were no significant predictors of microalbuminuria. Retinopathy appeared more likely in those with higher current HbA1c values (11.2[2.5] vs 7.6[2.1]%; p=0.01) and those with greater changes in HbA1c over time (4.1[3] vs 0.2[1.9]%; p=0.009), while no association was seen with BMI or BMI-Z.

Discussion

These data highlight difficulties in identifying those with early T2DM complications. The association seen with hypertension and weight, while retinopathy appears more dependent on HbA1c, demonstrates that optimal T2DM management must not only focus on glycaemic control.

PO2-391 Type 2 Diabetes, Insulin Resistance II

Phenotypical variety of insulin resistance in a family with a novel mutation of insulin receptor gene

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A novel mutation of insulin receptor gene (INSR) was identified in a three generation family with phenotypical variety. Proband was a 12-year-old Japanese girl with type A insulin resistance. She showed diabetes mellitus with severe acanthosis nigricans and hyperinsulinemia without obesity. Using direct sequencing, a heterozygous nonsense mutation causing premature termination after amino acid 331 in the α subunit of INSR (R331X) was identified. Interestingly, her healthy father and grandmother with type 2 diabetes also had the same mutation. The proband also showed low level C-peptide/insulin molar ratio, indicating that this will be useful index for finding genetic insulin resistance patients. We experienced another Japanese patient with leprechaunism bears homozygous mutation at codon 331, indicating INSR(R331X) is responsible for insulin resistance. The same mutation was not detected in 92 non-diabetic adults and 180 type 2 diabetic adults. In conclusion, R331X is a novel mutation of type A insulin resistance and type 2 diabetes but the variance of clinical phenotype suggests that other factors may influence the onset period of diabetes.

PO2-392 Type 2 Diabetes, Insulin Resistance II

Intracellular magnesium of obese and type 2 diabetes mellitus children: the change of magnesium after treatment

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Background: Magnesium is a critical cofactor in numerous enzymatic reactions. The diabetic patients and obese subjects are reported to have intracellular magnesium ([Mg2+]i) deficiency. Platelets are often used in the study of cellular cation metabolism, because they are thought to share a number of features with vascular smooth muscle cells. In addition, human platelets are shown to have insulin receptors. We have postulated that insulin could modulate levels of [Mg2+]i in platelets and that levels of [Mg2+]i are lower in children with diabetes mellitus and obesity. We study the role of magnesium in the pathogenesis of insulin resistance in patients with type 2 diabetes (DM2) and obese subjects.
Subjects and Methods: Eleven subjects with simple obesity [male 9, female 2; age 17±8 years, medication period 1.0±0.6 years], 9 subjects with DM2 [male 6, female 3; age 16±2 years, medication period 1.0±0.7 years] and 20 healthy controls [male 10, female 10; age 17±7 years] were enrolled in the study. Using a fluorescent probe, mag-fura-2, we examined the basal and insulin-stimulated levels of [Mg2+]i in platelets. Plasma levels of leptin, ghrelin, adiponectin, and redistin were determined by ELISA. As the intervention strategy, modification of lifestyle (diet and therapeutic exercise) was instructed not only to the patients, but also to the entire family. In patients with DM2, metformin(n=5) and glibenclamide(n=1) were administered.
Results: Mean basal levels of [Mg2+]i were lower in subjects with obesity (162±65 μmol/L) and those with DM2 (140±31 μmol/L) than those in the control (375±42 μmol/L). The peak values of [Mg2+]i after insulin stimulation, were also lower in subjects with the obesity (417±140 μmol/L) and DM2 (327±69 μmol/L) than those in the control (802±72 μmol/L). The stimulated

levels of [Mg2+]i increased significantly after successful intervention in the patients with the obesity.

The effect of treatment				
	Obesity	Obesity	DM2	DM2
	Before	After	Before	After
BMI(Kg/m²)	33.2±4.7	31.5±3.8	28.9±7.3	29.9±7.6
HbA1c (%)	5.0±0.3	5.1±0.3	7.8±3.7	5.9±1.5
Basal [Mg²+]i (μmol/l)	162±65	205±80	140±31	181±40
Stimulated [Mg²+]i (μmol/l)	417±140	541±277†	327±69	356±75

†p<0.05 Before vs. After

Conclusions: It is further suggested that magnesium plays an important role in the pathogenesis of insulin resistance and that levels of [Mg2+]i could be a good index of successful intervention of obesity and DM2.

PO2-393 Type 2 Diabetes, Insulin Resistance II

Clinical and metabolic phenotype of obese children and adolescents with normal glucose tolerance, impaired glucose tolerance and type 2 diabetes mellitus

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The epidemic of childhood obesity in has been accompanied by an increase in the prevalence of type 2 diabetes mellitus (DM) among children and adolescents. We performed a retrospective chart review of pediatric patients who had clinical features of insulin resistance (IR) ± family history (FH) of type 2 DM that underwent a 2-hour oral glucose tolerance test (OGGT) at our institution between the years 2007-2008. Out of a total of 201 patients, 87 % had normal glucose tolerance (NGT), 11 % were found to have impaired glucose tolerance (IGT), and 2 % had silent type 2 DM. Female to male distribution was 73% vs. 27%. Race distribution for the whole group was approximately 61 % non-Hispanic white, 26 % African American, 8 % Hispanic and 3 % Asian. For the IGT group race distribution was 64% non-Hispanic white and 36 % African American. In the diabetic group there were 2 non-Hispanic whites, 1 Hispanic patient and 1 Asian patient. 34.5 % patients in the NGT group had a first degree relative with type 2 DM vs. 50 % patients in IGT group and 33.3 % in the DM group. Only 2 patients in the DM group and 3 patients with IGT had impaired fasting glucose (IFG). The prevalence of IGT was higher in adolescents than prepubertal children (9.45% vs. 1.49%). Patients with IGT had significantly higher 2-h post-OGGT insulin levels (105±82 vs 303±255, p<0001), max OGGT insulin levels (335±270.4 U/ml vs. 129 ± 91.69 U/ml, p<0001) and HOMA-IR (7.004 ± 6.521 vs. 3.308 ± 3.260, p<0001) but a lower QUICKI (0.126 ± 0.017 vs. 0.139 ± 0.014, p<0001) compared with subjects with NGT. The IGT group had a significantly higher (p=0.0001) percentage of HBA1c values >6 % (38.89%) compared with NGT group (10.14%). A slight increase (p=0.0476) in AST levels in IGT versus NGT group was noted. No differences were found in the male-to-female ratio, age, ethnicity, birth weight, FH of type 2 DM in first/distant degree relatives or maternal gestational diabetes, BMI, BMI-z score, hypertension, presence of acanthosis nigricans, Tanner stage, levels of HDL, triglyceride, and ALT in IGT vs. NGT group.**Conclusion:** IR is highly prevalent in obese children and adolescents. Severe hyperinsulinemia is associated with the onset of IGT. There are no cut point values for HOMA-IR and QUICKI which can predict IGT. FBG, insulin levels, HOMA-IR, and QUICKI are not effective screening tools for IGT. An OGTT is required in all subjects at high risk of developing type 2 DM.

PO2-394 Type 2 Diabetes, Insulin Resistance II

Effects of high fat/high energy and high protein diets from neonate to adulthood on insulin and ghrelin expression in rats

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The aims were to investigate long-term effects of high fat/high energy and high protein diets on insulin secretion, ghrelin expression and to further clarify the interaction between ghrelin secretion and insulin release. Three groups of pregnant rats were fed standard diet, high fat/high energy diet and high protein diet till lactation period. The pups of pregnant rats fed either a standard diet, high fat /high energy diet or a high protein diet were defined as C, HFE and HP group. After weaning, all the pups fed the same diet as their dams. Plasma glucose, ghrelin and insulin were analyzed at different age. Quantification of pancreas ghrelin and insulin was performed by immunohistochemical double-staining and confocal microscopy. Fasting blood glucose, plasma insulin concentrations, Homa-IR showed an increase with age and there were differences among the groups. Total plasma ghrelin concentrations decreased with age and were different among the groups. Plamsa ghrelin concentrations of three groups were negatively correlated with their glucose levels. Plasma ghrelin was negatively correlated with plasma insulin only in HFE group. Insulin secretion of HP and HFE groups and pancreatic ghrelin content of all groups decreased with age. Pancreas ghrelin content was sensitive to diet composition. Both pancreas ghrelin-positive cells and ß-cells decreased with age too. The percentage of ghrelin- positive cells was correlated with its percentage of ß-cells in all groups. Conclusively, insulin and ghrelin expression in plasam and pancreatic islets was adversely affected by long-term high fat/high energy and high protein diets.

PO2-395 Type 2 Diabetes, Insulin Resistance II

Proinsulin and the proinsulin/insulin-ratio in overweight and obese children and adolescents: relation to clinical parameters, insulin resistance and impaired glucose regulation

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In adults with type 2 diabetes mellitus (T2DM) and impaired glucose regulation (IGR) proinsulin and its ratio to insulin (PI/I-Ratio) often is elevated, possibly indicating ß-cell dysfunction. Few studies have evaluated the relationship of PI in children and adolescents at risk for T2DM.
Objective: An objective of the present study was to analyse the relationship between fasting proinsulin and relative bodyweight, puberty and sex and to assess whether proinsulin and the PI/I-Ratio, both fasting and during an oral glucose tolerance test (OGTT), differ in overweight and obese youth with or without IGR. Moreover we wanted to analyse, whether proinsulin is predictive for IGR.
Design and Setting: The study design was a cross-sectional analysis of data from children and adolescents attending our obesity-consultation at Charité-Childrens hospital.
Patients and interventions: in n = 259 children and adolsescents (48% boys, age 11.4 ±3.57 (SD), 87.6% obese, 12.4% overweight, mean BMI-SDS 2.58 ±0.65 (SD)) besides regular obesity-diagnostic (glucose, insulin, lipids, transaminases) fasting proinsulin was determined and PI/I-Ratio calculated. In n = 154 subjects at risk for T2DM an OGTT was performed with samples drawn for glucose, insulin and proinsulin at 0', 30', 60' and 120-min.
Main Outcome Measure(s): insulin, glucose, proinsulin, PI/I-Ratio, insulin resistance (HOMA-IR)
Results: Puberty (by Tanner) and relative weight (BMI-SDS) showed a linear relationship to fasting proinsulin-levels (p < 0,001 for Tanner I vs. II-V; p = 0.04 and p = 0.026 for BMI-SDS <2 and 2-2.5 and 2-2.5 to >2.5, respectively). There were no significant differences in fasting-proinsulin regarding sex or immigration background (p > 0.05). Subjects with insulin resistance (R-HOMA > 95th, n = 140) had higher fasting-proinsulin than those without (p < 0.001), with no significant difference in fasting PI/I-Ratio (p > 0,05). Subjects with IGR (n = 35) showed higher fasting proinsulin (p = 0.001) and PI/I-Ratio, both fasting and at 30-min during OGTT, than those with normal glucose regulation (p = 0.049, p = 0.014, resp.). *Conclusions:* Children and adolescents with IGR have

disproportionately elevated proinsulin, both fasting and during acute glucose stimulation in OGTT, which could indicate ß-cell dysfunction.

PO2-396 Type 2 Diabetes, Insulin Resistance II

Long-term effects of catch-up growth and early nutritional intervention on the adiponectin level and insulin resistance in rats born small for gestational age

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Objective To observe the long-term effect of catch-up growth and early nutrition on the adiponectin level and insulin resistance of SGA rats. **Methods** A no-catch-up growth SGA(SGA-NCU) and catch-up growth SGA models were built. All pups were fed with normal diet from the 4th to 12th weeks of age. On the other hand, various litters of 3 weeks SGA-NCU rats were randomly divided into SGA high-lipid group (SGA-HL) and SGA non-high-lipid group (SGA-NHL). The SGA-HL rats were fed with high lipid diet and the SGA-NHL with normal diet from the 4th to 24th weeks of age. At the end of the experiment, the glucose tolerance test and insulin release test were performed. The glucose, insulin and adiponectin were detected. **Results** [circ1]At the age of 12th weeks, the length of SGA-CU was not significantly different, compared with the AGA rats, and the weigh and BMI of SGA-CU were significantly higher than those of SGA-NCU. the HOMA-IR of SGA-CU was higher than that of AGA and SGA-NCU. The adiponectin level was lower significantly than those of AGA and SGA-NCU. The outcomes of glucose tolerance test showed that the glucose levels of 30 and 60min were higher in SGA-CU than that in SGA-NCU and AGA groups. There was no significant difference in the insulin levels among SGA-CU, AGA and SGA-NCU groups. [circ2]After 21-weeks nutrition diet, the weight, BMI and HOMA-IR of SGA-HL were significantly higher than those of SGA-NHL. The adiponectin level of SGA-HL was higher significantly not only than that of AGA, but also than that of SGA-NHL. The glucose tolerance test indicated that the glucose levels of 0, 30 and 60min were higher significantly in SGA-HL and SGA-NHL than those in AGA. While, there was no significant difference between SGA-HL and SGA-NHL. The insulin release test demonstrated that the insulin level of 30 min was higher significantly in SGA-HL than that in SGA-NHL. Conclusions Catch-up growth and early nutrition will have a long-tern effect on the adiponectin and insulin resistance of SGA.

PO2-397 Type 2 Diabetes, Insulin Resistance II

KS is a model for childhood insulin resistance and metabolic syndrome

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Background: Klinefelter syndrome (KS), 47,XXY, is associated with an extra X chromosome and testosterone deficiency. The risk for metabolic syndrome in adult males with KS is increased and is associated with abdominal obesity. Increased body fat mass has also been noted in boys with KS.
Objective: Investigate risk factors for metabolic syndrome in boys with KS including waist circumference, lipid, glucose and insulin measurements.
Design/Methods: 63 boys with KS, ages 4-12 years, and 24 control boys had auxologic (height, weight, body mass index [BMI], waist circumference [WC]) and blood pressure (BP) measurements and completed a questionnaire about physical activity. The KS boys also had measurement of lipids, fasting blood glucose [FBG] and insulin. HOMA was calculated as a measure of insulin resistance. We evaluated the frequency of elevated LDL (≥110 mg/dl), total cholesterol (≥170 mg/dl), and HOMA (≥2.5), as well as features of childhood metabolic syndrome (defined as 3: fasting TG ≥100 mg/dl; HDL < 50 mg/dl; WC > 75%ile for age; systolic/diastolic BP >90%ile, and FBG≥100 mg/dl).
Results: The KS and control groups were ages 4-12 years (means: 7.8 ±2.6 vs. 8.9±2.3 yrs). Weight, BMI and WC SDS were similar. The mean daily activity time spent running was 34 minutes less in the KS vs. control groups. For lipids measured in the KS group only, 25% had elevated LDL and 30% had elevated total cholesterol levels. Of the 5 criteria for metabolic syndrome: 65% had decreased HDL levels, 9.5% had elevated TG levels, and 49% had a WC >75%ile. None had elevated BP or FBG. 6/63 (9.5%) had fasting insulin levels greater than 20 uU/ml and 15/63 (24%) had a HOMA consistent with insulin resistance. Three (5%) KS boys met the three criteria required for diagnosis of metabolic syndrome (low HDL, increased WC, elevated TG), and 23/63 (37%) met two criteria for metabolic syndrome.
Conclusions: Truncal obesity seen in adults with KS is also present during childhood. In boys as young as 4-12 years with KS, 24% had insulin resistance, 37% met two criteria for metabolic syndrome, and 5% had the metabolic syndrome. In addition, one third of the boys had elevated total cholesterol and LDL levels. Potential mediators may include genetic factors related to the extra X chromosome, reduced running type activity, and/or testosterone deficiency in childhood. We conclude that the karyotype 47,XXY may serve as an important genetic model for childhood-onset metabolic syndrome and dyslipidemia.

LB-PO2-001 Late Breaking Submissions

Normal spermatogenesis in familial hypogonadism with mutation of the beta subunit of luteinizing hormone

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Rare natural mutations abolishing bioactivity of the beta subunit of luteinizing hormone (LHβ) have been reported. Patients displayed azoospermia, absent or rare immature Leydig cells on testicular biopsy and responded partially to prolonged choriogonadotropin treatment. This supports the current belief that initiation of normal spermatogenesis requires the physiological action of luteinizing hormone (LH), inducing full differentiation of steroidogenic Leydig cells producing large amounts of intratesticular testosterone (ITT). We report here a proband from a consanguineous family presenting hypogonadism, undetectable LH and very low serum testosterone concentrations (0.48 ng per milliliter), whereas his affected sister displayed normal puberty, secondary amenorrhea and infertility. Both carried a novel ^{10}His-Pro-Ile^{12} deletion in the LHβ-encoding gene. Surprisingly, the proband displayed complete spermatogenesis with normal numbers of spermatozoa. Rare mature Leydig cells expressing various steroidogenic enzymes and Sertoli cells expressing anti-Müllerian hormone were detected in his testicular biopsy by immunocytochemistry. Consistent with these findings, ITT concentration was 20.2 ng per gram of tissue, one eighteenth that of the age-matched control. The mutated LH had weak residual function *in vitro*. Thus, in this case, low levels of LH activity, generating a markedly reduced population of steroidogenic Leydig cells synthesising small amounts of ITT was sufficient to induce and maintain complete and quantitatively normal spermatogenesis, going against current views on normal onset and maintenance of spermatogenesis in humans.

LB-PO2-002 Late Breaking Submissions

Clinical and hormonal profile of polycystic ovary syndrome in adolescent girls with or without type 1 diabetes mellitus – a pilot study

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It is believed that in women with type 1 diabetes mellitus (T1DM) nonphysiologic insulin replacement therapy and hyperglycemia may affect pituitary-ovarian axis and ovarian function leading to menstrual disturbances and polycystic ovary syndrome (PCOS).
The aim of our study was to compare the clinical and hormonal profile of adolescent girls with T1DM with non-diabetic controls.
We analyzed clinical data and hormonal - basal and triptorelin-stimulated (Dipherelin 0.1 mg, s.c.) characteristics of 24 adolescent girls with well

controlled on intensive insulin treatment T1DM (mean HbA1c for the last 12 months 6.95±0.66%). Six of them met the criteria of PCOS (T1DM-PCOS group) and were compared to 17 adolescent girls with PCOS without T1DM (PCOS group). The remaining 18 were compared to 10 healthy adolescent girls with regular menses (control group).
The hirsutism score was significantly lower in T1DM-PCOS group than in PCOS group (2.0±1.0 vs. 8.0±5.0, p=0.04). There were no significant differences in hormonal profile between T1DM-PCOS group and PCOS group except significantly lower basal and stimulated DHEAS level in girls with T1DM-PCOS compared to girls with PCOS (162.0±58.0 mg/dl vs. 250.0±42.6 mg/dl, p=0.009; 175.6±72,4 mg/dl vs. 303.0±94.5 mg/dl, p=0.008, respectively). In T1DM group compared to control group menarche occurred 12 months later (13.0±1.1 vs. 12.0±0.9 year, p=0.03). Moreover significantly lower basal LH, basal and stimulated DHEAS level as well as LH to FSH ratio were observed in T1DM group compared to control group (2.3±1.4 mIU/ml vs. 5.0±6.0 mIU/ml, p=0.03; 146.0±58.0 mg/dl vs. 229.0±27.2 mg/dl, p=0.02; 174.9±67.4 mg/dl vs. 256.1±66.8 mg/dl, p=0.01; 0.48±0.38 vs. 0.93±0.73, p=0.02, respectively).
It is concluded that the hormonal profile of adolescent girls with T1DM meeting the criteria of PCOS seems not to be very different from the profile of their peers with PCOS non suffering from T1DM. However delayed menarche as well as some hormonal disturbances can occur in adolescent girls with T1DM despite intensive insulin treatment and good metabolic control. Supported by MNiSW *N407 015 32/0403.*

LB-PO2-003 Late Breaking Submissions

Single sample during gonadotropin stimulation test is adequate for the diagnosis of precocious puberty

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Objective: To simplify the diagnosis of precocious puberty by evaluating the stimulated LH levels during GnRH stimulation test.
Method: GnRH stimulation test was performed in 314 girls with findings suggestive of early puberty. Blood samples were collected before, and 20, 40, 60, 90 minutes after intravenous administration of 100 mcg GnRH. LH levels were measured by ARCHITECT System (chemiluminescent microparticle immunassay, Abbott Diagnostics). Diagnostic cut-off of peak LH for precocious puberty was considered to be 5 IU/L.
Results: Mean chronologic age was 7.8 ± 1.5 years, mean height age 8.8 ± 1.8, and mean skeletal age 9.5 ± 1.5 years. Mean levels of LH, as well as the percentage of LH above the cut-off (5 IU/L) and "peaking LH" at each time point are shown on Table 1.

Table 1. LH data during GnRH stimulation test in 314 girls.

	0	20	40	60	90
LH level (IU/L) (mean ± SD)	0.51 ± 0.8	7.3 ± 10.0*	7.9 ± 9.9	6.8 ± 8.2*	5.1 ± 6.0
"Peaking LH" frequency (%)		19.1	71.0	9.2	0.6
Frequency of LH > 5 IU/L (%)		82.2	98.0	83.6	60.5

* p> 0.99 20 versus 60 minutes post injection

During the test, the samples at 40 minutes post injection had the highest frequency of "peaking LH". The mean LH levels at 40 minutes was also higher than all the other time points. Comparison of mean LH levels showed significant difference between different time points (p< 0.001), except 20 and 60 minutes post injection which showed similar mean LH. Using 5 IU/L as cut-off, the level of LH at 40 minutes post injection was diagnostic in 98.4 of the patients with precocious puberty and all prepubertal patients.
Conclusion: The blood sample obtained at 40 minutes after GnRH injection is highly sensitive (98 %) and specific (100%) for diagnosis of precocious puberty. Measurement of LH level at 40 minutes post injection during the test provides adequate information for diagnosis of precocious puberty.

LB-PO2-004 Late Breaking Submissions

Bisphenol-A levels were higher in girls with precocious puberty than in controls

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Backgound: Bisphenol-A (BPA) is small estrogenic monomer used to line metal cans and an additive in other type of plastic, such as polyvinyl chloride (PVC). Exposure to BPA may affect sexual maturation by weak estrogenic activity. However, large scaled studies regarding the effect of BPA exposure on the precocious puberty in children are limited.
Objective: This study aimed to compare the levels of BPA in Korean girls with idiopathic precocious puberty (IPP) and control groups.
Method: Anthropometry, bone age x-ray and GnRH-stimulation test were conducted in 219 girls with IPP and 112 girls with normal controls. Serum BPA and urinary BPA levels were also analyzed by gas chromatography/mass spectrometry method.
Result: The basal plasma estradiol level [21.0±20.1 (IPP) vs. 11.9±3.9 pg/ml (control), p < 0.0001] and the peak LH (lutenizing Hormone) response to LHRH stimulation [17.2±19.0 (IPP) vs. 4.4±3.0 mIU/ml (control), p<0.0001] were significantly higher in girls with IPP than control. Serum BPA level was significantly higher in girls with IPP than control groups (7.8±6.4 vs. 5.8±3.5, p=0.03). Serum BPA level was weakly correlated with body weight (r=0.167, p =0.05) but not with total fat amount. Serum BPA level was correlated with serum estradiol level (r= 0.205, p=0.03) and peak LH level (r= 0.407, p= 0.0001).
Conclusion; BPA, a ubiquitous endocrine disrupting chemical may be associated with the development of IPP.

	Precocious Puberty(n=112)	Control(n=219)	p-value
Chronological age (yr)	9.0±1.3	9.1±1.3	NS
Bone age (yr)	10.4 ±1.4	9.3±1.2	<.0001
Body mass index (kg/m²)	18.3±2.5	18.0±3.0	0.06
Estradiol (pg/ml)	21.0±20.1	11.9±3.9	<.0001
Peak LH (mIU/ml)	17.2±19.0	4.4±3.0	<.0001
Peak FSH (mIU/ml)	12.1±4.8	11.4±4.3	NS
Serum BPA (ng/ml)	7.8±6.4	5.8±3.5	0.03
Urine BPA (ng/ml)	2.5±1.2	2.4±1.0	NS

LB-PO2-005 Late Breaking Submissions

Inhibin-B levels higher among non-Hispanic black than Mexican-American and non-Hispanic white prepubertal and pubertal boys

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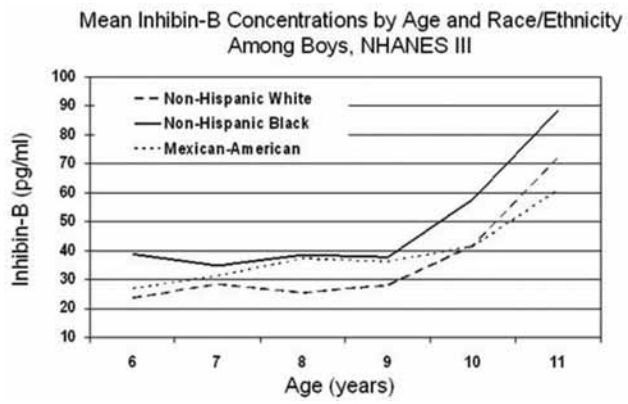
Aim: Describe inhibin-B levels in a large, nationally-representative sample of 6-11 year-old boys based on age, Tanner stage and race/ethnicity. **Methods:** Inhibin-B (pg/mL) was measured using Diagnostic Systems ELISA assay and testosterone (T) by Bio-Quant assay in N=774 samples in NHANES III, 1988-1994.Genital and pubic hair Tanner staging is available in boys ≥ 8 years of age. Analysis of variance and linear regressions using weighted models were used to compare hormone concentrations by age, Tanner stage and race/ethnicity. For both inhibin-B and T, proposed cut-off levels were assessed to determine if a value could discriminate onset of puberty (i.e.,Tanner stage 1 to 2). **Results:** Inhibin-B levels increased significantly with increasing age (p<0.0001), genital stage (p<0.0001) and pubic hair stage (p<0.0001), with progressive increases from stages 1 to 2, and from 2 to 3 or greater. Compared across all ages for non-Hispanic White (NHW), non-Hispanic Black (NHB), and Mexican-American (MA) boys, inhibin-B was significantly higher overall in NHB (p<0.01). Among Tanner stage 1 boys, there was a significant effect of race/ethnicity (p=0.02) with NHB having the highest levels, but no significant effect of age. Conversely, among those with Tanner stage ≥2, there was no significant effect of race/ethnicity, but there was a significant effect of age

(p=0.01).



This contrasts with T, where concentrations increased significantly with both age (p<0.0001) and pubic hair stage (p<0.0001), although not for genital stage (p=0.13), and there were no statistically significant racial/ethnic differences at any age. No cut-off level was found to discriminate the onset of puberty.
Conclusions: This first report of hormone measurements in a nationally-representative sample of peripubertal U.S. boys found significant racial/ethnic differences in inhibin-B concentrations coincidental with the earlier transition to Tanner genital stage 2 in NHANES III NHB boys, although the significance or related factors are unclear. These data further suggest that inhibin-B and T levels alone appear to poorly discern the onset of puberty as determined by physical examinations.

LB-PO2-006 Late Breaking Submissions

Pediatric endocrine outreach: principles and practice

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Introduction: The Mountain West region of the United States has one pediatric endocrinologist for every 200,000 children, compared to 1:45,000 in the Northeast and 1:90,000 for the United States as a whole (ABP-2008). In part, this reflects the sparse population distribution in this region as well as the relatively great distances between population centers. The Rocky Mountain region served comprises 500,000 mi.^{2} (~1.4million km^2), or an area equivalent to Norway, Sweden, Finland, Iceland and Denmark combined.
Aims: 1. To meet the needs of children with endocrine disorders who live in remote areas and 2. to provide endocrine continuing medical education (CME) for primary care providers and their staffs in the region.
Methods: 1. Establish a network of outreach clinics in the Rocky Mountain region that significantly reduce the travel distances for children and their families.
2. Develop endocrine testing methods that can be performed at these clinics.
3. Establish endocrine CME schedules with local health care providers.
Results: The outreach clinics were established beginning in 1995, when 35 children were seen. In 2008, over 1000 visits were recorded in these clinics-the majority of these (74%) requiring endocrine evaluation and/or intervention, in contrast to 60% at our home institution. The entire gamut of endocrinologic disorders were represented at these clinics, including many children with endocrinologic complications of chronic illness (MEN2A, BMT, CNS tumors, septo-optic dysplasia) and genetic disorders (Turner, Klinefelter, Prader-Willi).
Conclusions: 1. Children with complex or more routine endocrinologic disorders who live in remote areas can be served by pediatric endocrinologists in outreach clinics. 2. Endocrine testing can be done in these locations, sparing the patients and their families long travel distances. 3. The ratio of "pathology:non-pathology" is greater in these clinics compared to those at our tertiary care hospital, thus making them excellent sites for the training of endocrine fellows. 4. Endocrine CME can be made available to local health care providers, thereby enhancing detection of potential endocrinologic disorders in their patients.

LB-PO2-007 Late Breaking Submissions

Areas punctuation of the self esteem in obese and not obese children and adolescents

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Objective: it identify if differences exist in the areas punctuacion of self esteem in obese and not obese.
Material and Methods
This investigation is about comparative character. 100 subjects conformed the sample classified in two groups: a group of 50 obese children and adolescents (Group I) they beloyend to Hospital Nacional Docente Madre Niño San Bartolome and another of not obese (group II –50) The age range went from 8 to 15 years.
The gathering data was based on the application of the Inventory of Self-Esteem of Stanley Coopersmith. The condition of obesity it was defined as the index of corporal mass (IMC)>de 95, using the approaches pointed out by the (OMS) respectively. Statistical analysis was done with T-test to compare means from independent samples.
Results
The results didn't show differences statistically relevant between obesity and self-esteem (p=0.562). However it was a significant difference among and the control group children and adolescent in the Pair of sub. Area (p=0.00229).

Difference Between Means of the Global Esteem in the Inventory of Cooper Smith Areas, in Children and Adolescents According for Studies Groups Obese and not Obese of the Hospital Nacional Docente Madre Niño San Bartolome

Areas	Obese Mean	Obese Std. Deviation	Not Obese Mean	Not Obese Std. Deviation	p
General Punctuation	15.86	4.555	16.98	3.614	0.1763
Self	4.18	1.90	4.47	1.725	0.4262
Parent's Home	3.53	1.226	3.37	1.1999	0.5110
School	52.12	26.909	43.65	27.013	0.1195
Pair	4.35	1.568	5.02	1.319	0.0229

According Inventory of Cooper Smith Areas it was significant differences among and the control group children and adolescent in the Pair of sub area (p=0.0229).

Inventory of Self Esteem of Stanley Coopersmith. According to sex it was significant difference in the self Area (p=0.0156) (table 2) and general punctuation (p=0.0343) of the inventory.

Diferences Between Means of the Global Esteem Punctuation in the Inventory of Cooper Smith Areas in Children and Adolescents According to Sex for Studies Groups Obese and not Obese of the Hospital Nacional Docente Madre Niño San Batolomé

Areas	Male Mean	Male Std. Deviation	Female Mean	Female Std. Deviation	p
General Punctuation	17.25	3.975	15.51	4.128	0.0343
Self	4.74	1.734	3.87	1.801	0.0156
Parent´s Home	3.60	1.214	3.28	1.192	0.1866
School	44.19	27.007	51.87	27.042	0.1585
Pair	4.79	1.472	4.57	1.514	0.4631

In the inventory of Cooper Smith Areas, according to sex it was significant difference in the self area (p=0.0156) and general punctuation (p=0.0343) of the Inventory.

Conclusion
It was not evidence that allows establish that the obesity influences in the self-esteem.
Key words: obesity, Obese Children and Adolescents, self-esteem.

LB-PO2-008 Late Breaking Submissions

Prevalence of childhood obesity is levelling off in Germany

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Background: Prevalence of overweight and obese German children and adolescents was investigated between 2000 and 2008. Special emphasis was given to gender differences and special age groups: preschoolers 4-8 years, prepubertal children 8-12 years and pubertal children 12–16 years.
Subjects and Methods: BMI values from the computerized database CrescNet were analysed, focusing on body mass index (BMI) data above the 90th centile (P90, overweight) as well as the 97th centile (P97, obesity). The cross-sectional assessment was based upon 78.039 single values from German children and adolescents aged 4 - 16 years (40.379 males, 37.660 females) from screening and/or consulting visits. Body mass index was calculated from standardized measurements of body weight and height entered into the CrescNet database. Individual percentiles were estimated according to German reference data sets. Prevalence values were compared during 4-year-intervals, i.e. between 2000 and 2004 as well as between 2004 and 2008.
Results: Between 2000 and 2004, prevalence rates for overweight and obesity significantly increased in boys aged 8–16 years ($p<0.005$) and in girls aged 12–16 years ($p<0.005$). The increase in younger boys aged 4-8 years as well as in girls 4-12 years did not reach statistical significance.
Between 2004 and 2008, there was a signficiant decrease in overweight and obesity prevalence in boys aged 4–12 years ($p<0.005$) and in girls aged 4–8 years ($p<0.005$). In girls 8–16 years, overweight prevalence stagnated, whereas it continued to increase in boys aged 12-16 years.
Conclusion: This cross-sectional study performed in a large cohort of German children and adolescents reveals that there was a significant increase in childhood overweight and obesity for most age groups in both, boys and girls, between 2000 and 2004. Although overweight and obesity prevalence continued to increase in boys aged 12–16 years between 2004 and 2008, it significantly decreased or levelled off in boys 4- 12 years and in girls 4 -16 years. These data might suggest that intervention programs targeting to prevent childhood obesity may have had benefical effects on the obesity prevalence in German children and adolescents. Alternatively, a new balance between factors favouring obesity and those favouring leanness may have been reached in recent years in the German population.

LB-PO2-009 Late Breaking Submissions

Effect of aerobic training program on the obesity and insulin resistance in young girls with polycystic ovary syndrome

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Background: polycystic ovary syndrome (Pcos) is an endocrine disease which is characterized by unovulation, hirsutism, acne, hyperandrogenism, impaired glucose tolerance, insulin resistance and obesity. Lifestyle modifications are successfully employed to treat obese and over weight girls with Pcos.
Objectives: To evaluate the effect of aerobic training program on the obesity and insulin resistance in young girls with Pcos.
Methods: 20 obese Pcos patients underwent a 12-week aerobic training program. Anthropometric parameters, metabolic and hormonal profiles were assessed at base line and after 12-week aerobic training program.
Results: After 12-week aerobic training program showed significant improvements in BW, WC, FAT%, Vo2max, but changes in BMI, WHR, Fasting glucose, Fasting insulin, and IR were not significant.
Conclusion: A aerobic training program improves some of the anthropometric parameters, metabolic and hormonal profiles.

LB-PO2-010 Late Breaking Submissions

Effects of a high visceral/subcutaneous ratio on lipoprotein subclass particle size and concentration in obese adolescents with normal glucose tolerance

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Background: The role of adipose tissue distribution in insulin sensitivity and metabolic risk extends beyond abdominal fat. In a previous study, we found that an imbalance between visceral (VAT) and subcutaneous fat (SAT) depots in obese adolescents is associated with severe metabolic complications. In this current study we examined the role of VAT/SAT ratio, independent of total body adiposity, on lipoprotein composition.
Materials and Methods: In a multiethnic group (22 white, 18 Hispanic, 9 black) of forty-nine adolescents (mean age 15.3±0.33; 17 males and 32 females) with normal glucose tolerance, abdominal fat distribution and hepatic fat fraction (HFF) were measured by magnetic resonance imaging (MRI). The study population was further stratified into tertiles based on the VAT/SAT ratio. Fasting lipoprotein particle number and size were determined using NMR spectroscopy.
Results: There were no differences is age, BMI z-score and fat free mass across tertiles. As the proportion of VAT/SAT ratio increased across tertiles the lipoprotein profile worsened and the percentage of hepatic fat content increased. Of note, large VLDL, Chylomicrons and very small LDL lipoproteins increased ($P<0.02$) while large HDL decreased ($P<0.01$) across tertiles of VAT/SAT. These relationships did not change after adjustment for age, sex and race.
Conclusions: In obese adolescents with normal glucose tolerance, a high VAT/SAT ratio is associated with hepatic steatosis and marked alteration in lipoprotein size particle. The high proportion of visceral fat and relatively low abdominal subcutaneous fat, hepatic steatosis together with this proatherogenic profile may set the stage for future development of carbohydrate intolerance and cardiovascular disease in this age group.

LB-PO2-011 Late Breaking Submissions

Comparison of serum magnesium levels and dietary magnesium intake in overweight and lean children and its association with components of metabolic syndrome

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Background: The role of micronutrients like magnesium and its deficiency in childhood obesity is poorly studied.
Objective: To compare the serum magnesium and dietary intake of magnesium in overweight north Indian children with matched lean controls and to assess whether lower serum magnesium levels if any, is inversely correlated with fasting serum insulin levels and other components of metabolic syndrome.
Design: Cross sectional comparative study.
Universe: Tertiary level north Indian referral hospital
Subjects and controls: Overweight children (n=55) aged 4-18 yrs and controls (n=53) were enrolled between July 2007 and March 2009.
Primary outcome measure: Serum magnesium levels and dietary magnesium intake in overweight children and their controls.
Results: The baseline characteristics were comparable in both the groups. The serum magnesium levels were significantly lower in obese. (obese : 2.12 $\pm$ 0.33 mg/dl vs lean : 2.56 $\pm$ 0.24 mg/dl, $P<0.001$).No difference in dietary intake of magnesium (adjusted for calorie intake) was seen between the groups. (Obese : 0.23$\pm$ 0.05 mg/kcal vs lean : 0.24 $\pm$ 0.05 mg/kcal; P value = 0.71).

Comparison of clinical and biochemical characteristics of the lean and overweight groups			
	obese	lean	P value
N	55	53	
Sex(M/F)	44/11	37/16	
Age(years)	10.6+/-2.7	10.2+/-2.1	0.36
BMI(Kg/m2)	24.8+/-5.0	14.8+/-2.2	<0.001
Systolic BP(mm of Hg)	118+/-16.8	102+/-10.6	<0.001
Diastolic BP(mm of Hg)	78+/-12.1	68+/-7.0	<0.001
Se Magnesium(mg/dl)	2.12+/-0.33	2.56+/-0.24	<0.001
Dietary Mg(mg/Kcal)	0.23+/-0.05	0.24+/-0.05	0.71
Fasting insulin mean(IQR))	6.45(4.13-11.9)	3.192(2.22-5.63)	<0.001

Serum magnesium was inversely correlated with BMI, systolic BP, diastolic BP, waist circumference and fasting insulin levels.

Correlation of serum magnesium with components of metabolic syndrome		
	rho	P value
BMI	-0.57	<0.01
Fasting insulin	-0.28	<0.005
systolic BP	-0.36	<0.01
Diast Bp	-0.31	<0.01

Conclusion: Serum magnesium is significantly lower in overweight children compared to lean controls and an inverse correlation was found between serum magnesium and systolic BP, diastolic BP, WC and fasting insulin levels.

LB-PO2-012 Late Breaking Submissions

Does pancreatic immunoreactivity of glucagon-like peptide and its receptor differ between infants with hyperinsulinism of infancy and control infants?

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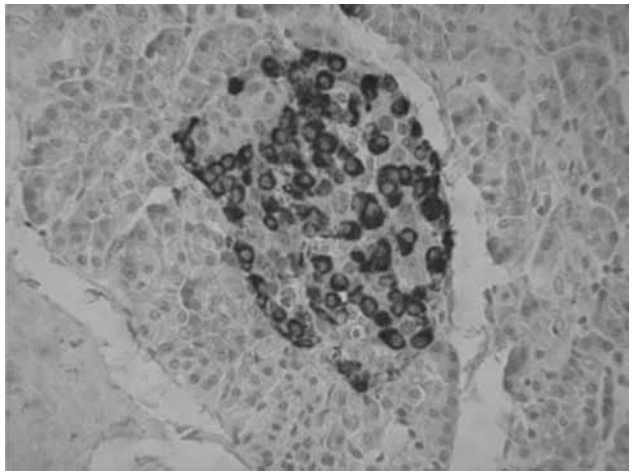
The incretin Glucagon Like Peptide 1 (GLP1) promotes beta cell proliferation and augments insulin secretion, acting on the beta cell GLP1 receptor (GLP1R). Hyperinsulinism of infancy (HI) associated with ABCC8 mutations is characterised by dysregulated insulin secretion and abnormal pancreatic histology with loss of organisation of islet structure.
We investigated the distribution of GLP1 species and the GLP1R in pancreas from infants with HI who had undergone pancreatectomy compared with controls.
Pancreatic GLP1 and GLP1R immunoreactivity were visualised using immunohistochemistry with Abcam ab22625 (which may also detect proglucagon and glucagon) and ab13181 antibodies (www.abcam.com). Sections including at least 6 - 10 islets from up to 6 HI and 7 control infants were examined. Control pancreatic tissue was obtained using specimens from infants who had died from causes unrelated to pancreatic disease. The cross sectional proportion of the islet area with positive immunoreactivity was estimated using ImageJ software (http://rsb.info.nih.gov/ij/). Mean proportions for HI and control sections were compared using a t-test.
Immunoreactivity for GLP1 in HI infants showed qualitatively abnormal distribution, with positive cells being scattered throughout the islets (figure 1) and interspersed with exocrine tissue, in contrast to organised distribution around the periphery of the islets in control sections.



GLP1 immunoreactive cells occupied more islet area in HI than control islets (mean(sd) 42(3)% vs 34(3)%, p=0.04). Immunoreactivity for GLP1R within islets was similar in HI and control sections. Numerous GLP1R positive cells were seen scattered through acinar tissue in HI sections but not in control sections.
GLP1, acting on its receptor, is a potent pancreatic growth factor. Abnormal distribution of GLP1 species and GLP1R likely contribute to the histological abnormalities seen in pancreas from HI infants. Moreover, abnormal receptor distribution which interferes with normal cross-talk between endocrine cells is likely to disrupt incretin action, contributing to intractable dysregulation of insulin secretion.

LB-PO2-013 Late Breaking Submissions

Response to ACTH stimulation takes longer in asphyxiated piglets reoxygenated with room air and is sub-optimal in those reoxygenated with pure oxygen

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Introduction Little is known about the effects of hypoxia-reoxygenation on the hypothalamo-pituitary-adrenal axis. Using a swine model of neonatal hypoxia-reoxygenation, we aimed to evaluate the effects of hypoxia and reoxygenation with 21% vs. 100% oxygen on cortisol response to an ACTH challenge in newborn piglets.
Methods Thirty-five piglets (1-3d, 1.5-2.4kg) were instrumented to measure heart rate, mean arterial pressure and cardiac output. After 2h of normocapnic hypoxia (PaO_2=20-30mmHg and pH<6.95), piglets were resuscitated with 21% or 100%oxygen for 1h and then 21% oxygen for 3h. Sham-operated piglets had no hypoxia-reoxygenation (H-R). Serial plasma cortisol levels were determined after a blinded-randomized administration of ACTH (4 µg/kg, i.v.) or saline at 2h reoxygenation.
Results Hypoxia-induced cardiogenic shock recovered with resuscitation. Piglets developed hypotension similarly in 21% and 100% H-R groups during reoxygenation (*vs.* sham-operated group, *p<0.05).* Plasma cortisol levels were higher in both H-R groups than that of sham-operated piglets (*p<0.05*) at 2h of reoxygenation after hypoxia. The response to ACTH was delayed in H-R groups with the maximum increase at 120 min post-ACTH administration (*vs.* 30-60 min post-ACTH for sham-operated piglets). Plasma cortisol levels increased significantly post-ACTH administration in 21% H-R and sham-operated piglets (115±88% and 126±38% at 120 min, respectively, *p<0.05 vs.* pre-ACTH baselines), but not in 100% H-R piglets (51±27%).
Conclusions In asphyxiated piglets with cardiogenic shock, the cortisol response to ACTH took 2h to mount while it took 30-60min in shams. Although the clinical significance of cortisol response to ACTH in H-R critically ill piglets is uncertain, our data suggest that a 2h observation period instead of the tradional 1h post-ACTH should be used before diagnosing adrenal insuficiency in critically-ill newborn piglets. The absence of doubling of cortisol post-ACTH in piglets reoxygenated with 100% oxygen is also of uncertain clinical

significance, but inclines toward the use of room air in neonatal resuscitation.
Keywords: ACTH challenge, cortisol, hypoxia-reoxygenation, piglets, oxygen.

LB-PO2-014 Late Breaking Submissions

Regulation of pancreatic beta cell mass by prolactin signaling: implications for gestational diabetes and neonatal glucose intolerance

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In late gestation the production of insulin in the mother and fetus increase markedly, reflecting increases in beta cell mass and glucose-stimulated insulin secretion (GSIS). Induction of beta cell insulin production protects against gestational diabetes and neonatal glucose intolerance and is therefore essential for maternal and neonatal well-being. The factors controlling beta cell mass and insulin production in pregnancy have not been elucidated, but studies from this and other laboratories suggest a critical role for the lactogenic hormones of the placenta (placental lactogen, PL) and pituitary gland (prolactin, PRL). PRL and PL stimulate beta cell replication and GSIS and inhibit beta cell apoptosis; conversely, targeted deletion of the PRL receptor reduces beta cell mass, GSIS, and glucose tolerance in pregnant and non-pregnant mice. Here we used microarray assays, Q-PCR, Western blot analysis, and pharmacologic assays to explore potential mechanisms by which the lactogens increase beta cell mass. We found that treatment of insulinoma cells with PRL stimulated increases in cyclin D2 mRNA and protein, which are essential for beta cell development in postnatal life. PRL also induced phosphorylation of erk1/2, which increases cell replication and inhibits apoptosis, and p70S6 kinase-1, which promotes protein synthesis and increases beta cell size. The expression of IGFBPs 3 and 5 were increased. Targeted knockdown of the PRL receptor by adenoviral-mediated siRNA reduced cyclin D2 mRNA and phospho S6K-1 and increased the level of p21, which inhibits cell cycle progression. PRL stimulated at 2.5-fold increase in beta cell thymidine incorporation; this effect was attenuated by inhibitors of Jak2/STAT5 (AG490), MAPK (U0126 and PD098059) and mTOR/S6K-1 signaling (Rapamycin) but not by Wortmannin, an inhibitor of PI 3-kinase. Our studies identify diverse pathways by which the lactogens increase beta cell mass during pregnancy and perinatal life and suggest possible targets for prevention of gestational diabetes and neonatal glucose intolerance.

LB-PO2-015 Late Breaking Submissions

Neonatal diabetes and Wolcott-Rallison syndrome: clinical and genetic studies in a Brazilian patient with a novel mutation in the EIF2AK3 gene

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Wolcott-Rallison syndrome (WRS-OMIM 226980) is a rare, autosomal recessive disorder characterized by permanent neonatal diabetes mellitus, epiphyseal dysplasia and other multisystemic clinical manifestations such as osteopenia, hepatic and renal dysfunction and central hypothyroidism among others. To date, only around 40 cases of WRS have been described. Mutations in gene encoding the eucariyotic translation initiation factor 2-α kinase 3 (EIF2AK3 also called PERK) were shown to cause WRS. EIF2AK3 is a major physiological effector of the unfolded protein response (UPR) following endoplasmic reticulum (ER) stress. **Clinical report and methods**: A 8 1/2 year-old Brazilian boy, born from first-degree cousins, was diagnosed with diabetes mellitus at the age of two months and since then has been treated with insulin. The anti-insulin, IA2 and GAD autoantibodies were negative. At that time, central hypothyroidism was also identified. At the age of 2 years, he fractured the tibia after a fall. Bone X-ray demonstrated spondiloepiphiseal dysplasia and diffuse osteopenia. Clinical diagnosis of WRS was established. At the age of 6 years he had an episode of acute hepatic dysfunction with spontaneous normalization of clinical and laboratory abnormalities. Hepatitis A, B and C were excluded. He has a history of developmental delay and mild mental retardation. He presented frequent episodes of pneumonia and was diagnosed with IgA deficiency. His clinical examination disclosed low stature with height of 95 cm (<5 percentile) and body weight of 15kg (< 5 percentile). Sequencing of the EIF2AK3 gene in genomic DNA extracted from peripheral lymphocytes demonstrated a homozygous C>T replacement at base pair c.1192, generating a stop codon at position 398 (Q398*). Both of his parents were found to be heterozygous for the mutation. **Conclusions**: This is the first case of WRS reported in Brazil. The clinical phenotype associated with the syndrome can be variable, but a combination of infancy-onset diabetes mellitus, multiple epiphyseal dysplasia, osteopenia, mental retardation or developmental delay, and hepatic and renal dysfunction is the mainstay of diagnosis.

LB-PO2-016 Late Breaking Submissions

Beckwith-Wiedemann syndrome: importance of genetic and molecular diagnose

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INTRODUCTION: Most of cases of BWS are sporadic (85-90%) and autosomal dominant inheritance (10-15%).
Incidence is 1/13.700. More risk exists in twin brothers and when assisted reproductive techniques are used.
BWS results from alteration in expression or function of imprinted genes at chromosome 11p15.5. Most of cases result from epimutations in 2 imprinting centres IC1and IC2.
Genectic defects are classified in: Cytogenetics (1-2%):Duplication of paternal origin, inversion or traslocation of maternal origin.Genetics: Paternal uniparental disomy (20%), mutation of CDKN1C gen (5%). Epigenetics:Hipermethylation H19gen (10%) and hipomethylation gen KCNQ10T1 (55-60%)
The risk of tumors is 7,5-10% but molecular advances and fenotype and genotype correlations allow us to distinguish 2 groups of risk with different prognosis and follow up. Epimutations of IC2 has lower tumoral risk (1-5%) and epimutation of IC1 has higher tumoral risk (25-45%) for Wilms tumor and other embryonal tumors.
Practice guideline for tumor screening recommendations have been created before molecular and genetic advances and the same protocols are being used for different genetic diagnosis when the tumoral risk is quite different.
PATIENTS: 4 cases of SBW which were diagnosed between 2004-2009 in our Hospital. All had hypometilation of IC2 and tumoral screening is negative at the moment.
Case 1: Preterm newborn, 2.700 g (p75) and 52 cm (p95) at 34 weeks gestation. ICSI (intracytoplasmic sperm injections) for conception. Caesarean section. Macroglossia and hemihypertrophy are evident at one month old. Nevas flammeus. Postnatal overgrowth.
Case 2: Newborn, 4100g (P95) and 54 cm (P95) at 40 weeks gestation. Onphalocele, angioma in face and ear grooves. Postnatal overgrowth.
Case 3:Newborn, 3380g (P75) and 53 cm (P90) at 38 weeks gestation. Macroglossia, nevas flammeus facial and ear grooves were observed at one month. Hypoglycemia. Left vesicoureteral reflux grade III. Postnatal overgrowth.
Case 4: Preterm newborn,1780g (P75) and 46 cm (P90) at 31 weeks gestation. Macroglossia and nevas flammeus facial. Postnatal overgrowth.
CONCLUSIONS: 1.- Incidence in our hospital is higher (1/38.000).It may be caused by higher suspicious index and the possibility to get genetic diagnosis easierly than previously.
2.- Cytogenetic and molecular diagnosis must be done not only to confirm this disorder.It is important to value the different tumoral risk which depends on genetic and molecular diagnosis.

LB-PO2-017 Late Breaking Submissions

Indian adolescent girls with PCOS are more insulin resistant and have high prevalence of glucose intolerance

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Polycystic ovary Syndrome (PCOS), a disorder of reproductive age-group women is characterized by hyperandrogenism, chronic anovulation, polycystic ovaries is associated with a barrage of endocrine and metabolic disturbances, the most important being impaired glucose intolerance and type 2 diabetes mellitus. Many studies have demonstrated higher incidence of IGT and DM in PCOS females even at younger ages. The risk of glucose intolerance in PCOS females has been shown to be similar throughout the ethnic groups although obesity and positive family history of glucose intolerance increases the probability. We aimed to study the prevalence of glucose intolerance in adolescent girls with PCOS from Indian subcontinent, an ethnically predisposed population. PCOS diagnosis was established on the basis of NICHHD 1990 and Rotterdam 2003 consensus criteria.
Analysis of plasma glucose and insulin before and 60 and 120 minutes post OGTT. Glucose intolerance was studied in 326 adolescent PCOS girls using WHO 1999 and ADA1997 criteria. Mean age of cases was 20.15±2.6 years (13-24) and MBI was 24.33±3.9 Kg/m2 (15.0-36.0) cases. Mean F-G score, serum testosterone, serum plasma glucose, insulin and LH/FSH ratios were higher and would correlate positively. OGTT results indicated that 24.3% girls had IGT and 4.5% with frank diabetes with WHO 1999 criteria. We conclude that glucose intolerance of various grades is very prevalent in PCOS girls even at younger ages and is positively correlated with number of cycles /yr, hair growth, waist circumference, serum testosterone, and OMA-IR.

LB-PO2-018 Late Breaking Submissions

Non familial Wolfram syndrome – report of three families from Indian subcontinent

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Wolfram syndrome (WS) named as Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness (DIDMOAD) syndrome is rare in general population and is believed to occur in one out of 150 patients of th Type 1 Diabetes mellitus. With the exception of a few series, the number of patients in most reports is small. We report the three cases of Wolfram with non familial are presented from North Indian muslim population where consanguinity is quite common. Our first case had behavioral disturbances and gave history of a seizure possibly related to hypoglycemia. She also had bilateral hydronephrosis and hydroureters with increased echo pattern of both kidneys. Our second case had gut atonia and malabsorption and third one had an attack of cholangitis. Around 25 % of patients have symptoms related to gastrointestinal tract, including recurring constipation and diarrhea, probably related to autonomic dysfunction. This patient also had limited joint mobility which could be because of di*a*betes mellitus or because of syndrome as such as has been reported previously.
An estimated 1 in 350 people carry the gene for W S. Two genetic routes of inheritance either autosomal recessive or mitochondrial have been identified. The mutant genes responsible for WS include WFS1gene on chromosome 4P16.1 (occurring in 90% of the patients) is inherited as autosomal recessive trait while as minority of patients may harbor WFS2 gene on chromosome 4Q22-24 or a mitochondrial genome deletion. Our cases although were not subjected to any genomic analysis had sporadic WS pointing towards a non familial occurrence of the disorder.

LB-PO2-019 Late Breaking Submissions

Insulin resistance in children born SGA after 3 years of GH treatment

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GH treatment is well accepted for children born small for gestational age (SGA) who fail catch-up growth. As GH reduces insulin sensitivity and low birth weight is associated with an increased risk for type 2 diabetes, the effect of GH on glucose metabolism is of special concern in this patient group.
We evaluated glucose tolerance and insulin sensitivity before and after one, two and three years of GH treatment in 21 (13f, 8m) SGA children. Age at baseline was 9.1±2.9 yr (5.0-14.3) when GH treatment at a dose of 33 µg/kg per day was started. At the start of GH treatment, puberty had begun in 8 (4f, 4m) patients. Oral glucose tolerance tests were performed to assess glucose tolerance and for calculation of the homeostasis model assessment (HOMA, normal <1.0) and for the insulin sensitivity index of Matsuda (ISI, normal >10).
Impaired glucose tolerance with a 120 min blood glucose level >140<200 mg/dl was found at baseline, after 1, 2 and 3 yr in 2 patients at every time point. Only one pubertal female had glucose intolerance at all 4 time points (blood glucose 140, 162, 150, 142 mg/dl). For the whole group of 21 patients the following results were obtained at baseline, after 1, 2 and 3 yr for HOMA: 0.99±0.74, 2.15±2.15 ($p<0.005$), 2.25±1.54, 2.20±1.34; the results for ISI were: 12.3±8.8, 5.87±3.28 ($p<0.001$), 6.45±5.31, 5.59±5.04. Thus, after one year of GH treatment, insulin sensitivity had decreased markedly and highly significantly in most patients. No further decline in insulin sensitivity was obsevd after 2 and 3 years. Even before GH treatment insulin sensitivity had been compromised in some children, especially in those who had entered or were entering puberty.
Careful monitoring of glucose metabolism is crucial in these predisposed subjects. Risk and benefit of GH treatment should be assessed continuously in children born SGA.

LB-PO2-020 Late Breaking Submissions

Clinical predictors of response in the first year of recombinant growth hormone treatment in short children born small for gestational age

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Introduction/Objective: Treatment with recombinant growth hormone (rGH) in short children born small for gestational age (SGA) who failed to show spontaneous catch-up growth has been well established and the outcomes are related to acknowledge clinical parameters. Recognizing the clinical predictors of the rGH therapy response is desirable, once they can optimize the management of these cases. The aim of this study was to identify clinical predictors associated with rGH-induced catch-up growth in the first year of treatment with a fixed rGH dose in a group of SGA children. **Patients and methods**: Thirty nine children born SGA, either by birth weight and/or length, who had not caught up until 2 years of age and who have been on rGH therapy for at least one year (fixed dose of 0.33 mg/Kg/week) were recruited. The clinical parameters analyzed in this study were chronological age (CA), bone age (BA), standard deviations (SD) scores of birth weight, birth length, height, weight, growth rate and target height, establishing Δ height SD score as the final outcome. **Results:** Out of the 39 children, 23 were boys, 29 were SGA for both birth weight and length, 27 were born full term and 24 were prepubertal at treatment start. Mean CA was 9,41 ± 3.54 years and mean BA was 7.61 ± 3.69 years. Mean growth rate before rGH treatment was 4.96 ± 1.16 cm/year and after one year it increased to 9.19 ± 2.05 cm/year, corresponding to a height increment of 0.67 SD score ($p<0.01$). The parameters found by multiple linear regression analysis to be statistically predictive of growth response after one year of treatment were BA and birth weight SD score. BA and birth weight SD score together explained 41% of the variability of the catch-up growth. A two-tailed *t* test showed no statistically significant difference for birth length, gestational age neither gender. Although pubertal stage was not recognized as a predictor,

the difference in Δ height SD score between the prepubertal (0.75) and pubertal (0.40) subgroups was statistically significant (p = 0.016). **Conclusion**: A fixed rGH dose of 0.33mg/kg/week promoted significant catch-up growth in these SGA children and the recognized clinical predictors of the first year response were BA and birth weight SD score, presenting the prepubertal children a better outcome.

LB-PO2-021 Late Breaking Submissions

Hypothyroidism with delayed thyroid-stimulating hormone (TSH) surge in very low birth weight (VLBW) infants: incidence and growth and developmental outcomes at 18 months corrected age

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Congenital hypothyroidism (CH) is the most common disorder detected by newborn screening (NBS) with incidence of ~1:3500-4000 in term infants. Infants with CH are often asymptomatic at birth, and delayed treatment results in poor cognitive outcome. Therefore, NBS is proven beneficial and is performed in all live birth including VLBW (< 1500 grams) and extremely low birth weight (ELBW < 1000 grams). In term infants, physiological TSH surge occurs within the first 24 hours and subsequently declines. TSH remains persistently elevated in term infants with CH at the time of initial NBS. Larson *et al*. reported the risk factors associated with a delayed TSH surge, which include VLBW infants likely due to an attenuated hypothalamic pituitary response. This observation led to the recommendation to perform repeats NBS in VLBW infants. Current recommendation varies significantly regionally with limited long-term outcome data. The increased incidence of abnormal thyroid screens detected in VLBW infants has been presumed to be transient, but not documented systematically. There is limited data in ELBW infants. Hypothesis: VLBW and ELBW infants have an increased incidence of CH due to delayed TSH surge, with the highest incidence in ELBW infants and the long term outcome is similar to control when treatment is instituted promptly. Methods: We performed retrospective review of VLBW and ELBW infants born in RI between 2000-2004 (5 Years). IRB approval was obtained. NBS data were examined in all livebirth to identify those with CH with delayed TSHsurge (TSH >15). To evaluate growth and neurodevelopmental outcomes of ELBW infants with CH and delayed TSH surge, we enrolled a group of matched controls (2:1). Results: Among the 65,000 livebirth in the State of RI over the study period. We identified 1330 VLBW infants with 660 being ELBW infants. The incidence of delayed TSH surge was 1:56 (in ELBW) and 1:330 (in VLBW). The mean age of infants diagnosed with delayed TSH surge was 21 days. Outcome at 18 months revealed lower head circumference ($p<0.05$) while weight, length, mental developmental index and psychomotor developmental index were similar to controls. All but one infant required treatment. Conclusion: we concluded that 1) incidence of CH with delayed TSH surge is higher ELBW infants.2) Most infants with delayed TSH surge have transient CH and 3) growth and neuron-developmental outcome of ELBW infants with CH and delayed TSH surge are comparable to matched controls.

PO3-001 Adrenal III

Ketoconazole treatment for Cushing' syndrome in MacCune Albright syndrome (MAS)

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Aim: ACTH independent hypercortisolism can be associated with 5% of MAS syndrome. Bilateral adrenalectomy, the currently most employed treatment, increase MAS patients morbidity. Medical treatments, such as metyrapone, can be efficient but are poorly tolerated. Our objective is to show that ketoconazole can be used as an alternative medication in this condition. *Method* : we report a 7 year-old girl presenting with severe MAS syndrome (associating fibrous dysplasia, hyperpigmented spots and precocious puberty) confirmed by the findings of *arg201cys* mutation on chromosome *20q13* in peripheral leucocytes. At age 4, she became mildly obese. Cortisol (F) levels were at the upper limit of normal without diurnal variation, contrasting with persistently low corticotropin. Except for excessive adipose tissue and decreased muscle mass for age on DEXA, the girl presented no other cushingoid feature. In the absence of patent hypercortisolism and given the fact that some cases spontaneously regress, adrenal suppressive therapy was postponed. At age 6, urine and serum levels of F dramatically increased**.** Given the long term side effects of hypercortisolism, especially on bone FD, ketoconazole treatment (2.5 mg/kg/day). was initiated *Results* : Four months later, urine and serum levels of F were very low, indicating strong adrenal suppression, which persisted at least 3 years. Liver function tests remained normal. *Conclusion*: To the best of our knowledge this is the first case of MAS-associated Cushing's syndrome treated only with low doses of ketoconazole which gave satisfactory results.

PO3-002 Adrenal III

Gender atypical behavior, sexual orientation, and quality of life in women with congenital adrenal hyperplasia correlate to CYP21A2 genotype

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Gender-atypical behavior has been described in girls and women with congenital adrenal hyperplasia (CAH) due to a *CYP21A2* deficiency. Sixty-two women with CAH and age-matched controls completed a 120 item questionnaire and a validated quality of life instrument (PGWB). Health-related, psychosexual, and psychosocial parameters were correlated to *CYP21A2* genotype. The patients were divided into four genotype groups; null, I2splice, I172N, and V281L. **Results:** The women with CAH had significantly more male dominant professions. Compared to the reported sex distribution of professions in a statistical database the mean percentage of females in the professions correlated with *CYP21A2* genotype, r=0.27, p=0.009. They reported male spare time activities more often (e.g. motor interest as main interest, rough sports). They had less often a partner and 19% stated a bi-or homosexual orientation (null 50%, I2splice 30%, I172N 5%). All these differences were more pronounced in the most severe genotype groups, with a tendency for a difference also between patients with null and I2splice mutations. Over all PGWB showed no large differences, but health-related quality of life issues were impaired among patients with null genotype only. However, the negative impact of the disease on upbringing and interpersonal relationships did not correlate with disease severity. **Conclusion:** Increased gender-atypical behavior and sexual orientaiton in women with CAH correlated to the severity of *CYP21A2* genotype. This speaks in favor of dose related effects of prenatal androgen exposure. In addition, our results underline the importance of coping strategies for psychosocial adaptation, illustrating the importance of psychological support to parents and patients.

PO3-003 Adrenal III

Surgical outcome and sexual function in women with congenital adrenal hyperplasia (CAH), clinical perspective and as perceived by the patients themselves

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Congenital adrenal hyperplasia due to a *CYP21A2* deficiency causes cortisol and aldosterone deficiency. Varying degrees of hyperandrogenism result in prenatal virilization of external genitalia in girls. Feminizing surgery is usually performed during the first year. We investigated the patient's own perception of the disease and its impact on life and correlate this to the severity of the disease (*CYP21A2* genotype), and surgical procedure used. 62 adult women with CAH and age matched controls were followed-up. The patients were divided into four genotype groups; null, I2splice, I172N, and V281L.
A composite score for sexual function (the sum of discrete parameters in a questionnaire), showed a between-groups difference (p=0.02) significant for the null genotype group only, they scored lower than the other genotype groups and the controls. Sexual function correlated to genotype, r=0.37 p=0.005. There was a significant difference for sexual function between the various operation procedures used for clitoris (p=0.01) but not for vaginoplasty (p=0.12).
The women in the null genotype group reported significantly more dissatisfaction with their sexual life than the other genotype groups.
Conclusion: The patients in the null genotype group were significantly more affected by the disease. They scored lower than the other *CYP21A2* genotype groups for sexual function and satisfaction with sexual life. In addition, they had more surgical complications, also than I2splice, possibly due to a higher degree of prenatal virilization.
This study underscore the importance of centralized surgery and psychological support to these patients and their families through childhood, adolescence and into adult life.

PO3-004 Adrenal III

P450 oxidoreductase deficiency at puberty in a 46, XY individual with Antley-Bixler syndrome

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P450 oxidoreductase (POR) plays a pivotal role in facilitating electron transfer from NADPH to microsomal cytochrome P450 enzymes including the steroidogenic enzymes 17a-hydroxylase/17, 20 lyase (CYP17A1) and 21-hydroxylase (CYP21A2). Congenital Adrenal Hyperplasia (CAH) due to POR deficiency is rare: females usually have significant ambiguous genitalia, related to intrauterine androgen excess. Virilisation does not continue and circulating androgens are low/normal. Males are often under virilised. Affected patients can have associated, predominantly craniofacial skeletal malformations, the so-called Antley-Bixler Syndrome (ABS) phenotype. An 11.9-year-old boy with ABS [height 138.2 cm (-0.5SDS), weight 42.5 kg (+0.5 SDS)] presented for an endocrine evaluation. His clinical phenotype included craniosynostosis, choanal stenosis, and radio-ulnar synostosis consistent with ABS. No genital ambiguity was noted apart from an undescended right testis that required orchidopexy. A 24-hour cortisol profile was normal; standard synacthen test demonstrated a suboptimal peak cortisol [436nmol/L (15.5µg/dl)] and elevated 17-hydroxyprogesterone (17OHP) [peak 54 nmol/L at 30 minutes]. Testosterone (2.86nmol/L), DHEAS (1.11Umol/L), and Androstenedione (1.4nmol/L) were normal, as was the peak testosterone response to a 3-day HCG test (6.69nmol/L). The bone age was delayed by 1.4 years. At 12.7 years, he had no signs of virilization but his testicular volumes were 4 and 12 mls. LHRH stimulation revealed a pubertal LH response of 13.9IU/l. Elevation of ACTH (56.4 ng/L; N 10-50) and a sub-optimal cortisol response on repeat synacthen testing [419nmol (15.2µg/dl), peak 17OHP 55nmol/L] were suggestive of mild impairment of 21-hydroxylase; hydrocortisone replacement (10mg/m^2/day) was initiated. Genetic analysis of the *POR* gene revealed compound heterozygosity for *c.830+2dupT* (splice donor site of intron 8) *c.859G>C (p.A287P).* Functional studies of the latter mutation have reported a differential inhibitory effect with decreased catalytic efficiency for CYP17A1 and near normal activity of CYP21A2. In conclusion, we present an unusual case of CAH with a mild phenotype. The suggestion of an alternative pathway for androgen production causing intrauterine virilisation with no further postnatal virilisation could be consistent with our clinical findings suggesting impaired pubertal development.

PO3-005 Adrenal III

Three unrelated cases of familial glucocorticoid deficiency: variability of phenotype and genotype

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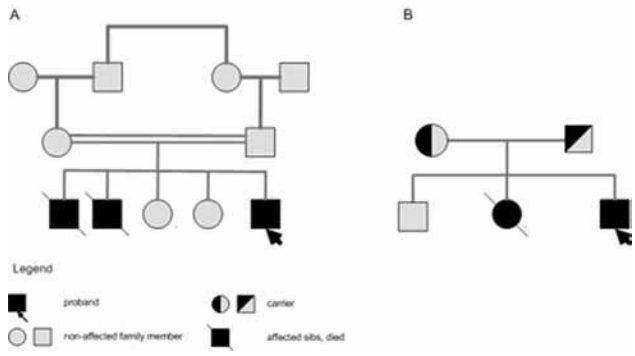
Introduction: Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder associated with clinical signs of glucocorticoid deficiency solely. Mutations in MC2R and recently disclosed MRAP genes account for approximately half of cases. Other abnormalities are not typically presented. The majority of cases manifest during neonatal period and early childhood with hyperpigmentation and severe hypoglycemia. **Objective:** We compare three unrelated cases of FGD in order to evaluate clinical variations by severity, age of presentation and diagnosis, associated symptoms and genetical findings.
Patients: Essential clinical and mutational data summarized in Table 1.

Clinical and mutational data			
	Patient#1	Patient#2	Patient#3
Sex	male	male	Female
Age of diagnosis (yrs)	14.3	14.3	1.5
Hyperpigmentation (age of onset)	11.0	From birth	From birth
Hypoglycaemia (age of onset)	None	12.4	None
Salt-wasting crisis	None	None	None
Furosemide provocation test	Normal	Normal	Normal
Height SD at diagnosis	-1.3	+1.63	+0.98
Associated hypothyroidism	No	No	Yes (5yrs)
Epilepsy	No	No	Yes (6yrs)
Parent's consanguinity	Yes	No	No
Family history**	positive	positive	Not significant
MC2R mutations	Not found	p.T152K	p.A252fsX258*, p.S256F
MRAP mutations	Not found	Not done	Not done

*Novel MC2R mutation, **Detailed in Figure 1.



A. #1 B.#2
A girl (#3) had additional symptoms. She presented with seizures at 4 on appropriate glucocorticoid supplementation which considered as associated epilepsy; at 6 low FT4 (7.4 pmol/l) and elevated TSH (9.05 mU/l), negative TPO-AB were found.
Neither MC2R mutations no MRAP mutations were found in patient #1. Interestingly, the patient #1 has a family history suggestive either for autosomal recessive or X-linked inheritance. X-linked adrenoleykogystrophy was ruled out. **Conclusion:** Other still unknown genes seem to be involved in FGD, and X-linked inheritance can not be excluded. Accompanying symptoms could

be observed compromising the appropriate diagnostic concept or provide the hypothesis of specific affect of a novel mutation on protein function or protein-protein interactions which are yet to be researched.

PO3-006 Adrenal III

Polycystic ovaries syndrome in two prepubertal girls treated by Op'DDD

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Op'DDD (ortho'para-dichloro-diphényl-dichloroéthane) is used by pediatric endocrinologists 1) to induce apopotosis of adrenal cells 2) because it has anti-glucocorticoid and anti-androgen effects through the inhibition of cholesterol conversion to pregnenolone and 11β-hydroxylase activity. In addition Op'DDD behaves as an estrogen to increase the synthesis of SHBG and CBG in vitro. We report Op'DDD side effects in two young girls 1) girl 1 (8,9 yrs) during a post-surgical remission of a feminizing corticosurrenaloma treated with Op'DDD doses of 1,6 g per day and serum Op'DDD ranging 2-9 µg/ml 2) girl 2 (11,6 years) treated for Cushing disease with Op'DDD doses of 1,3 g per day and serum level ranging 2.5-4.5 µg/ml. After 7 and 13 months of Op'DDD, both patients developed increased ovarian volume (patient 1 et 2: 61,7 cm^3) and multiple cysts. This was associated with elevated Δ4-androstenedione (girl 1: 2,86 ng/mL girl 2: 1,9 ng/mL) and oestradiol (girl 1: 30 pg/mL and girl 2: 52 pg/mL), while LH response to GnRH were 80 UI/L in girl 1,18 UI/L in girl 2). These data suggest a direct hyperstimulatory effect of Op'DDD on granulosa cells, a steroidogenic tissue where the drug is likely to have its primary effects. Direct primary effects could also be at the level of the immature gonadotrope cells. The occurrence of polycystic ovaries in Op'DDD treated adults has not been reported to our knowledge, although it may have been observed. This abstract should stimulate the reporting and analysis of this potential side effect of Op'DDD.

PO3-007 Adrenal III

Adrenal function and cortisol binding globulin in children with thalassemia

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Background: "Clinical" adrenal insufficiency (AI) is rarely presented in patients with thalassemia. However, a significant prevalence of "biochemical" AI, ranged 30-50%, has been reported. Total cortisol (TC) level has routinely been used in assessing adrenal function. In healthy individuals, 80% of measured TC is bound to cortisol binding globulin (CBG) with a small fraction bound to albumin and less than 10% in the active free form. Change in CBG could affect measured TC level. CBG level in thalassemics may be reduced as a result of hepatic hemochromatosis, leading to misleadingly low TC level.
Objective: To evaluate adrenal function of thalassemics by using free cortisol index (FCI), a ratio of TC to CBG, in comparison with TC level.
Methods: Fifty-six children with thalassemia (34 males), mean (SD) age of 10.2 (3.7) years, were recruited. Forty-four healthy children (17 males), mean (SD) age of 7.7 (3.2) years served as controls. One microgram cosyntropin (ACTH) test was performed in all enrolled children. Serum TC and CBG levels were measured. Data are presented in mean (SD).
Results: Forty-five patients (80.4%) had ß-thalassemia/E disease. Intermediate severity was found in 80.4% of patients. Peak TC level in thalassemics was 15.2 (4.0) mcg/dL which was significantly less than that of the controls (18.9 (3.1) mcg/dL). Peak FCI in thalassemics was also less than that of the controls [3.4 (0.8) and 4.2 (1.2) mcg/mg, respectively]. CBG level was not different between thalassemics and controls [45.2 (11.0) and 47.0 (8.6) mg/L, respectively]. Using an internationally accepted peak TC cut-off value of 18 mcg/dL resulted in a frequency of AI in our patients at 73.2%.
Conclusions: Serum CBG of thalassemics was comparable to that of the healthy children. FCI was not superior to TC in detecting AI in thalassemics. Relatively high frequency of AI in children with thalassemia was not a matter of reduced CBG. This may, however, indicate a "true" subclinical partial AI in these patients.

PO3-008 Adrenal III

Spectrum of 21-hydroxylase (CYP21) gene mutation and genotype-phenotype correlation in patients with congenital adrenal hyperplasia (CAH 21OHD) – Indian study

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Prediction of phenotype based on genotype is helpful in neonatal anticipation of salt waste, management and preventing salt wasting crisis. A study of the spectrum of CYP21 gene mutation and the genotype-phenotype correlation in patients with CAH 21OHD in an Indian cohort was done.
106 alleles from 53 (32 F; 46 SW) patients with CAH 21OHD belonging to 49 families were studied. Fragment analysis and multi step minisequencing was employed for rapid mutation analysis. The patients were phenotypically characterized as salt wasters (SW) or non-salt wasters (NSW). Genotypically they were grouped into three groups based on the predicted residual activity of steroid 21hydroxylase enzyme. Group A depicting mutation causing absence of any enzyme activity, Group B having less than 5% enzyme activity and Group C where the enzyme activity was undetermined.
Correlation of genotype with phenotype, biochemical control and replacement dose of hydrocortisone was studied by student T test, Analysis of variance (ANOVA), Kruskal-Wallis Test, Mann-Whitney Test when appropriate.
Percentage distribution of CYP21 gene abnormalities seen was as follows: IN2 28.7; Q318X 20; deletion17.7; I172N 11; R356W 4.4; cluster 1; not characterized 19. Three novel mutations, F305V, R354C and an insertion of 9 bases in exon 2 (codon 70) were found in three of these cases. Deletion, R356W, IN2, Q318X and cluster mutation were detected more frequently in SW phenotype while I172N and IN2 were found in NSW phenotype. Genotype was not always concordant with phenotype. Homozygous IN2 mutation showed an overlap with 85% being SW and 15% NSW phenotype.
The frequency of CYP21 gene mutations are different in this Indian cohort from other reported world literature. The frequency of mutations not characterized was comparable with other series. This information is useful to delineate appropriate strategies for prenatal diagnosis and expectant therapeutic measures in this particular population.

PO3-009 Adrenal III

Report of 7 patients with lipoid adrenal hyperplasia and their genetic study

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Lipoid adrenal hyperplasia (LAH) is a rare disorder associated with severe impairment of all steroidogenesis. In this study, clinical and genetic evaluations of 7 patients with LAH are reported. Electrolytes were measured by flame photometer, and hormones by radioimmunoassay. Results: Seven patients from 4 families had LAH. All were the product of consanguineous marriages of first cousins and had female phenotype. Genetic study of two siblings showed mutation of L157P in exon 5 of the StAR gene. The in vitro study showed that this mutation abolishes StAR activity. The first child of this family presented with hyperpigmentation and imbalanced electrolytes concomitant with gastroenteritis at 14 months of age. At entry he was 9 yr and 10 months old (karyotype, 46, XY). The second child of the family (46,XX) was brought only due to hyperpigmentation and positive family history at 3 months of age. Her serum cortisol level was 5.6 µg/dL. There were 2 female siblings in another family, that one of their male relative was affected to the same disorder. In these three patients,

presentation was at 15 days to 1.9 month of age and their general condition was critical, with poor feeding, vomiting, diarrhea, dehydration, hyper pigmentation and lethargy. Their serum cortisol level was between 0.3 and 2.4 µg/dL. Their genetic study showed IVS6-2delA in the splice acceptor site of intron 6. This mutation should disturb the splicing. Two other male patients from two separate families also presented with poor feeding, diarrhea, vomiting and lethargy at 3 – 5 months of age, but genetic study was not performed for them. Although the testes were not palpated in male patients at birth but were palpated at 1 - 1.4 years of age in 3 patients, and at 9yr and 10 months in one patient who came late. Pathologic examination of testes showed normal seminiferous tubules, spermatogonia, sertoli and Leydig cells. All of the patients had low serum Na (115 – 123 mEq/L) and high serum K (7.6 – 8.8 mEq/L) with high ACTH > 1500 pg/mL and plasma rennin activity >23 ng/ml/hr. Low 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, androstenedione and testosterone were detected in all of them. One girl with L157P mutation had breast budding at 9.9 yr and menstruation at 12.83 yr with irregular menstrual bleeding until last visit at 16 yr of age. Conclusion: Lipoid adrenal hyperplasia has wide spectrum clinical manifestations that may be due to different mutations.

PO3-010 Adrenal III

20 years of clinical, hormonal and imaging evolution in a 46XX patient with congenital lipoid adrenal hyperplasia (CLAH) due to a new mutation of the steroidogenic acute regulatory protein (StAR)

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Congenital lipoid adrenal hyperplasia (CLAH) is a severe disorder characterized by early impairing of all adrenal and gonadal steroidogenesis, leading to precoce adrenal failure and male sex reversal. The most common cause of CLAH is mutation in the gene of the steroidogenic acute regulatory (StAR) protein.
Objectives: We report here the clinical, hormonal and imaging evolution on 20 years of a 46XX patient with a new homozygous 719delC shift mutation in exon 6 of the StAR protein that (probably) leads to a 35 amino acids longer protein. Data were retrospectively collected from patient file.
Results: This patient whose one 46XY " sister " died at 4 months of acute adrenal failure, presented with typical salt-losing crisis at 10 days of life and responded immediately to replacement therapy. Imaging did not show any lipid deposits in adrenals or gonads. She had a normal mental and physical evolution, except a prolonged enuresia and an early overweight. She had a beginning of spontaneous puberty development at 12 years, then estro-progestativ treatment was institued at 13 years. This therapy was interrupted for 2 months because of a doubt on an ovulation aspect on MRI, and development of life-threating ovarian cysts made us start it again. Cerebral MRI was performed that showed previously reported white matter abnormalities. The surprising fact in this case is the absence of back regulation of ACTH and plasmatic renin activity (PRA) despite the use of major dose of hydrocortisone and fludrocortisone. Moreover, progressiv apparition of facio-troncular obesity in a context of permanent hunger is a strong argument in favor of the patient therapy observance.
Conclusion: This report, as a long-term follow-up of a patient presenting CLAH due to StAR gene shift mutation, illustrates perfectly the expanded tissue distribution of the protein and the " 2 hits " hypothesis that suggests initially left of StAR independent steroidogenesis before complete disruption of steroidogenesis secondary to local accumulation of cholesterol esters.

PO3-011 Adrenal III

Reverse-hybridization teststrips for detection of common CYP21A2 mutations in dried blood spots from newborns with elevated 17-OH progesterone

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Background and Objective: The average incidence of classical CAH is about 1 in 15,000 births worldwide, and more than 90% are caused by mutations in the CYP21A2 gene encoding steroid 21-hydroxylase. In the majority, low/abolished residual enzymatic activity leads to a severe clinical phenotype (salt-wasting CAH) due to aldosterone deficiency. Newborn screening programs based on 17-hydroxyprogesterone (17-OHP) levels have been introduced in various countries to identify affected babies prior to developing life-threatening salt-wasting crisis. Since neonatal 17-OHP screening has a considerable false positive recall rate, causing a substantial economical burden and emotional stress for parents, concurrent genetic testing for CYP21A2 defects to corroborate or exclude CAH diagnosis would be desireable. We have therefore developed a reverse-hybridization assay for rapid simultaneous analysis of common CYP21A2 mutations from dried blood spots. **Methods:** The StripAssay, applicable for use in dried blood spots, works as follows: The entire CYP21A2 gene is amplified in two overlapping fragments using PCR primers that will not co-amplify the highly homologous pseudogene. Biotinylated amplicons are hybridized under exactly defined stringency to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for the following 11 mutations: P30L, IVS-2 A/C>G, 8 bp del (exon 3), I172N, I236N/V237E/M239K ("cluster"), V281L, F306 insT, Q318X, R356W, P453S and R483P. Specifically bound PCR products are detected using enzymatic colour reaction. The entire procedure from DNA extraction to the interpretation of results takes less than 8 hours. **Results and Conclusions:** The new CAH StripAssay was validated in a series of DNA samples from known CYP21A2 genotypes. Automated instrumentation and use of a scanner-based software tool (StripAssay Evaluator) for recording and interpreting results may further contribute to making the StripAssay a useful tool in CAH newborn screening programs.

PO3-012 Adrenal III

Low-dose dexamethasone therapy of infants and children with virilizing congenital adrenal hyperplasia

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OBJECTIVE: To assess the growth and control of adrenal androgen secretion in children with virilizing congenital adrenal hyperplasia (CAH) treated with dexamethasone. METHOD: We examined doses used, control of adrenal androgen secretion, growth and skeletal maturation of 8 children with CAH treated with dexamethasone beginning in infancy. RESULTS: 3 boys and 5 girls with classical CAH (17OHP at diagnosis >20,000 ng/dl) were treated with dexamethasone beginning at diagnosis (<10 days of age). Patients were also treated with fludrocortisone and sodium chloride. The average medication dose was 0.28 + 0.015 mg/m2; all doses were given in the morning using a dosing syringe to administer a 0.1 mg/ml elixir. The children were treated for 5.5 + 1.1 years over which time the change in bone age to chronological age ratio (ΔBA/ΔCA) was 0.9 + 0.06. Most recent height Z' scores were +0.5 + 0.2, and body mass index (BMI) scores were 18 + 0.2. Late afternoon levels of 17hydroxyprogesterone, androstenedione, and testosterone were 780 + 238 ng/dl (23.4 + 7 nmol/L), 42 + 10 ng/dl (1.4 + 0.3 nmol/L) and 11.5 + 3 ng/dl; (0.4 + 0.1 nmol/L), respectively. CONCLUSIONS: These observations show that low doses of dexamethasone can be used to effectively treat CAH beginning in infancy.

PO3-013 Adrenal III

Two novel mutations in *PDE8B*, a cAMP phosphodiasterase highly expressed in the adrenal cortex, in a cohort of patients with adrenal tumors

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Genetic aberrations in the cAMP signaling pathway have been found to play a role in many types of adrenal tumors (ADTs). Our group previously identified mutations of a phosphodiesterase (PDE) gene, *PDE11A*, in patients with micronodular adrenocortical hyperplasia (MAH). A genome-wide SNP genotyping study in individuals with adrenocortical hyperplasia leading to Cushing's syndrome that was not caused by known defects indicated that, other the 2q31-33 locus (*PDE11A*), a locus on 5q14, harboring another PDE, *PDE8B*, was likely to contain a disease-related gene (*Nat Genet 2006;38:794-800*). A single base substitution in *PDE8B* that resulted in a proline-to-histidine change in an evolutionarily-conserved residue of the protein (c.914A>T/p.H305P) was then identified in a female pediatric patient with Cushing syndrome due to MAH (*N Engl J Med. 2008;358:750-2*). We now studied 20 patients with secreting and non-secreting adrenal tumors for sequence alterations of the *PDE8B* gene and studied PDE8B's expression in the adrenal cortex and its lesions. Two, previously not-reported *PDE8B* sequence variants were identified in two patients with secreting adrenal tumors; the first, a splice site mutation which leads to an estimated 20% decreased acceptor site activity of exon 14 (c1365-5 g/a), and the second, a single base substitution, that led to a valine-to-isoleucine change in exon 18 (c.2089 G>A/p.V697I). *In vitro* studies are on-going to estimate the effect of these genetic defects on PDE8B enzymatic function. Immunohistohemistry (IHC) studies showed an increase of PDE8B expression in the adrenal cortex of patients with adrenal lesions due to other defects of the cAMP-signaling pathway. In conclusion, we found two novel *PDE8B* sequence variations with potential impact on the ability of PDE8B to bind and/or degrade cAMP, and evidence of a counter-regulatory role of this PDE in cAMP signaling in adrenalocortical lesions. These data support a significant role of PDE8B in adrenal pathophysiology and/or tumorigenesis.

PO3-014 Adrenal III

Glucocorticoid receptor gene polymorphisms and their relation with glucocorticoid sensitivity and obesity in patients with congenital adrenal hyperplasia

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Background and aim: Congenital adrenal hyperplasia(CAH) is an inherited defect in one of the enzymatic steps required for the cortisol biosynthesis. The individual variations of glucocorticoid(GC) sensitivity can modify phenotype and respond to GC therapy. In normal population, variation in GC sensitivity can be influenced by the glucocorticoid receptor(GR) gene polymorphisms. We investigated relation of 3 polymorphisms (N363S, BclI and ER22/23EK) with GC sensitivity and phenotype in patients with CAH.
Patients and methods: We genotyped 146 (94 with 46,XX and 52 with 46,XY) patients [103 classic, 15 nonclassic 21-hydroxylase deficiency (21-0HD), 28 patients 11β-hydroxylase deficiency(11β-OHD)] and 124(56F, 68M) healthy children. The carriers and non-carriers for 3 polymorphisms were compared with respect to intrauterine virilizationprader stage and serum 17-hydroxyprogesterone levels at diagnosis and obesity, hydrocortisone(HC) dose, and insulin resistance during investigation.
Results: There was no significant difference in allele frequency for 3 polymorphisms between both patients and controls and boys and girls. Carriers and non-carriers of N363S and ER22/23EK were not different for compared parameters. Bcl1 carrier 46,XX patients have less virilized in intrauterine period(p=0.001). BclI carriers of classic 21-OHD and 11β-OHD needed lower dose of HC than non-carriers (p=0.001); however this difference was not observed in patients with nonclassic 21-OHD.
Conclusion: In patients with CAH, BclI polymorphism is associated with decreased intrauterine virilization and lower GC dosage, probably by increasing GC sensitivity in hypothalamo-pituitary-adrenal axis. Prenatal detection of this polymorphism and modification of prenatal dexamethasone therapy might prevent side effect of high dose of dexamethasone for both mother and fetus.

PO3-015 Adrenal III

A novel point mutation in the ligand-binding domain of the human glucocorticoid receptor gene causing primary generalized glucocorticoid resistance

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Background: Primary Generalized Glucocorticoid Resistance is a rare genetic disorder characterized by partial, generalized target-tissue insensitivity to glucocorticoids. The clinical spectrum of the condition is broad, ranging from completely asymptomatic to severe cases of hyperandrogenism and/or mineralocorticoid excess, without evidence of hypercortisolism. The molecular basis of the condition has been ascribed to mutations in the human glucocorticoid receptor (hGR) gene, which impair the molecular mechanisms of hGR action, thereby altering tissue sensitivity to glucocorticoids. We have identified three new cases of primary Generalized Glucocorticoid Resistance caused by a novel hGR mutation in the ligand-binding domain of the receptor.
Patients and Methods: A 70 year-old man presented with bilateral adrenal hyperplasia detected during melanoma surveillance. He was otherwise asymptomatic and had no clinical manifestations suggestive of Cushing's syndrome. Endocrinologic evaluation revealed elevated 08:00h serum cortisol concentrations (661 nmol/L), increased urinary free cortisol (UFC) excretion (507-749 nmol/day; normal range: 100-380), and resistance of the hypothalamic-pituitary-adrenal (HPA) axis to dexamethasone suppression. A pituitary magnetic resonance imaging scan was normal. His two daughters, aged 41 and 47 years, presented with mild hirsutism, and were also noted to have increased 24-hour UFC excretion (687 nmol/day and 611 nmol/day, respectively) and resistance of the HPA axis to dexamethasone suppression. DNA was extracted from peripheral lymphocytes and the entire coding region of the hGR gene was amplified and sequenced. A single, heterozygous nucleotide (T ® G) substitution was identified at nucleotide position 1724, resulting in valine (Val, GTG) to glycine (Gly, GGG) substitution at amino acid position 575 in the ligand-binding domain (exon 5) of the receptor. Molecular studies to determine the mechanisms through which the mutant receptor hGRalphaV575G impairs glucocorticoid signal transduction are currently underway.
Conclusions: We describe three new cases of primary Generalized Glucocorticoid Resistance caused by a novel, heterozygous hGR gene mutation. The location of this mutation in the ligand-binding domain of the receptor may predict impaired affinity for the ligand, while manifestation of the disease at the heterozygote state may indicate a dominant negative effect of the mutant receptor upon the wild-type receptor.

PO3-016 Adrenal III

Steroid secretion in young patients with hypertension

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Objective: The aim of this study was to investigate the features of adrenal steroid synthesis in young people with arterial hypertension.
Design and Methods: We examined 62 patients (15-20 years old) with hypertension: 40 patients were overweight (BMI 32.3 ± 0.8 Kg/m^2) and 22 were normal (BMI 23.2 ± 0.6 Kg/m^2) Control group included 15 healthy subjects (15-20 years old). All patients underwent hormonal evaluation: circadian rhythm of plasma cortisol and ACTH, low-dose (2mg) dexamethasone suppression test (DST) and corticosteroid subgroup analysis, including measurement of blood levels of cortisol (F), cortisone (E), corticosterone (B), 11-deoxycorticosterone (DOC), 11-deoxycortisol (S) and urinary excretion of free cortisol (UFF) and free cortisone (UFE) by high-performance liquid chromatography.
Results: Among 40 patients with obesity and hypertension, hormonal work-up revealed high baseline cortisol in 30%, ACTH in 27.5% and disturbed cortisol circadian rhythm in 22.5%. Six patients (15%) had serum cortisol concentrations above 60 nmol/L after 2mg DST.
The hypertensive patients with obesity exhibited significantly higher concentrations of F, B, UFF and UFE as compared with a healthy population, however the majority demonstrated normal indices of F/E (95%), UFF/UFE (95%) and suppression of UFF after DST (87.5%). Five patients (12.5%) were diagnosed to have subclinical Cushing's disease, based on a nonsuprresed UFF after 2 mg dexamethasone.
Some patients with arterial hypertension and normal weight showed increased concentrations of S (25%), lower excretion of free cortisol (UFF) (22.5%) and increased UFF/UFE ratio. Stimulation with corticotropin in 4 patients showed an exaggeration of 11-deoxycortisol concentration (23.5 ± 4.0 ng/ml), lowered level of B, and decreased F/S (8.9 ± 3.4) and F/E ratio as compared with a healthy volunteers.
Conclusions: These data illustrated that the majority of the young people with arterial hypertension and obesity have "functional hypercortisolism". However, some patients had biochemical abnormalities indicating subclinical corticosteroid excess. Among young hypertensive patients without obesity there is a group of patients with, probably, partial enzymatic defect that characterized by blunted response to corticotrophin. These findings support the hypothesis that activation and abnormalities in the hypothalamic-pituitary-adrenal axis are associated with high occurrence of arterial hypertension in young people.

PO3-017 Adrenal III

The short and the sweaty: a case of familial pheochromocytoma and growth hormone gene mutation

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A 12 year old boy presented with tachycardia, hypertension, sweating and flushing. Ultrasound and MRI revealed bilateral adrenal masses. Urinary epinephrine and norepinephrine levels were elevated. Bilateral adrenalectomy demonstrated pathology consistent with pheochromocytoma.
Two weeks later, a 5 year old first cousin presented with symptoms and was confirmed to have a right sided adrenal pheo. The cousin's 8 year old brother was diagnosed with a right adrenal pheo 3 months later. Mothers of the three boys subsequently had screening and were found to have adrenal masses.
The boy's parents are first cousins and herald from the Azores, Portugal. One of the 12 year old boy's sisters had a history of poor growth in the 1980's and was treated unsuccessfully with cadaveric growth hormone (final adult height 112 cm) with the development of anti-GH antibodies. Another sister has short stature (final adult height 135 cm) but refused GH testing or therapy because of failure of therapy in her sister. This sister was diagnosed 7 years ago with bilateral adrenal pheo at age 9. There are numerous family members with height < 3rd %ile but no history of other malignancies associated with the multiple endocrine neoplasia syndromes, nor symptoms to suggest a syndromic form of pheo. The question as to whether these two rare conditions were genetically linked was raised.
Genetic testing in the 'index' case revealed heterozygosity for a previously reported mutation in the von-Hippel Lindau (*VHL*) gene (Ser65A1a) on chromosome 3p. All family members with a pheo had the *VHL* S65A mutation but no other features of VHL syndrome, suggesting a dominant inheritance of VHL type 2c. The gene product, pVHL is a tumor suppressor protein. Dysfunctional pVHL leads to elevated levels of hypoxia-inducible factor 1 which induces transcription of growth factors involved in tumor formation.
Preliminary results demonstrate substitution of exons 3 and 4 of the *GH1* gene with exons 3 and 4 from the *CSH1* (chorionic somatomammotropin) gene, both on chromosome 17q and seems to be inherited in an autosomal recessive pattern. Assays to determine the functionality of the mutant proteins are under investigation.
Given that, in this family, pheo and GHD do not segregate perfectly with one another and that the *VHL and GH1* genes are unrelated and on separate chromosomes, we believe that the appearance of these two conditions in these families represents two genetically unrelated and extremely rare events.

PO3-018 Adrenal III

Serum dehydroepiandrosterone sulfate (DHEAS) levels and pubarche in children with Prader-Willi syndrome before and during growth hormone (GH) treatment

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Background:
Premature adrenarche has been reported in children with PWS and is characterized by activation of adrenal androgen production and increase in serum DHEAS.
Excessive weight gain might be a trigger for adrenarche as obesity is associated with a higher incidence of premature adrenarche. It has been suggested that this is the result of hyperinsulinism and increased serum IGF-I levels. Although children with PWS have an increased fat percentage, they have low insulin and IGF-I levels.
Objective:
To evaluate DHEAS levels in PWS children to find (1) if they are higher compared to their peers, (2) if there are differences in gender, genetic background, and before and during GH treatment, (3) if there are associations with IGF-I levels, insulin resistance, lipid profile, BMI, body composition and premature pubarche.
Design/Patients:
Serum levels of DHEAS, IGF-I, insulin, glucose, and lipid profile were assessed in 75 (45 boys) PWS children in the age of 8 mths to 16 yrs before start of GH treatment and in 86 (47 boys) PWS children after one or two years of GH. Baseline DHEAS levels were compared to a control group of 353 (201 boys) age-matched normal statured children in the age of 1 mth to 16 yrs. Body compostion and fat distribution were measured by Dual Energy X-ray Absorptiometry.
Results:
PWS children above 6 years of age had significantly higher median (iqr)

DHEAS levels than controls (resp. 3.01 (1.67-5.58) and 0.49 (1.1-2.32) µmol/l). Before the age of 6 no differences were found.
No differences were found between PWS boys and girls, and between uniparental disomy (UPD) and deletion. Children with an imprinting center defect (ICD) had significantly higher DHEAS than with deletion or UPD.
GH treatment had no effect on DHEAS levels.
After correction for age, DHEAS levels were significantly correlated with BMI, IGF-I and IGF-BP3.
Premature pubarche was found in only 1 PWS girl (age 7.14 yrs, DHEAS 0.93 µmol/l).
Conclusions:
DHEAS levels are higher than normal in PWS children above the age of 6 years, but did not result in premature pubarche.
Children with ICD have higher DHEAS levels.
DHEAS levels were associated with BMI, IGF-I and IGF-BP3 levels but GH treatment had no effect.

PO3-019 Adrenal III

Iatrogenic adrenal cortical failure in asthmatic children periodically treated with oral glucocorticoids

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Introduction: Glucocorticoids (GC) are widely used for treating bronchial asthma inflammation status. In spite of the current use of inhaled therapy, children are frequently seen in emergency rooms where they receive oral GC. The wide variability in individual responses to GC administration is well known and we are often unable to detect adreno-cortical failure. Objective: To evaluate the frequency of iatrogenic adreno-cortical failure in children with bronchial asthma treated with GC. Methods: As part of a prospective survey on childhood asthma children have been submitted to morning plasma cortisol evaluation (between 7 and 8:00h, chemiluminescent assay) under basal conditions, without any medication. Results: We identified 96 out of 141 children who needed oral GC in the last 12 months before the beginning of inhaled GC. Reduced morning cortisol plasma levels (<5.0 ug/dl) were found in 7 (7.3%) of those children. They had a median age of 1 year (0.3-4.6) and had received oral prednisone for a median period of 5 days. Their median cortisol plasma level was 2.6 (1.6-4.9) ug/dl while the non-suppressed children presented a median of 12 (5.2-25.8) ug/dl ($p<0.001$). Conclusion: Short courses of oral GC caused a variable degree of asymptomatic adrenal cortex suppression, mainly in the youngest children. We should be on the alert to the occurrence of potential dangerous events in these children. An evaluation of adrenal cortex function, as obtained with morning serial cortisol levels, would be useful to allow early recognition and even intervention over adrenal suppression.

PO3-020 Adrenal III

Evaluating an *in vivo* test of 21-hydroxylase activity for detecting or excluding heterozygosity for congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is an autosomal recessive disease with an incidence of about 1:15,000. The frequency of heterozygous carriers of *CYP21A2* mutations is about 1:55. As a prenatal therapy with dexamethasone to prevent virilisation is established for 21OHD caused by severe mutations, the knowledge of the genotype of partners of heterozygous carriers is crucial. As molecular genetic analysis can easily detect mutations but since it is not possible to exclude mutations, e.g. in intronic regions, to 100%, a reliable hormonal test distinguishing heterozygous carriers from wild type (WT) individuals would be extremely helpful.
We re-investigated the discriminative power of the ratio of plasma 17-hydroxyprogesterone (17OHP) over 11-deoxycorticosterone (DOC) 60 min after 250 µg ACTH *iv* in differentiating heterozygous carriers of 21OHD from WT individuals. A total of 50 heterozygotes (age 3-45 yrs, both sexes) with a genetically established *CYP21A2* haplotype and 36 controls (age 0.75-38 yrs, both sexes) in whom an active *CYP21A2* mutation was firmly excluded by multiplex minisequencing, direct sequencing, southern blotting and multiple ligation product analysis were all subjected to a standardized ACTH (Synacthen) test (08-10 AM, fasted, early follicular phase in cycling females). Plasma 17OHP and DOC were measured by multisteroid analysis (RIA after extraction and HPLC).
17OHP/DOC ratios after ACTH stimulation ranged between 0.24-74.3 (median 5.1) in the controls and 12.4-500 (median 30.3) in heterozygotes. Thus a completely distinguishing cutoff of 12 reported earlier using HLA haplotyping was not confirmed. However, a ratio <12 excluded *CYP21A2* heterozygosity with a positive predictive value (PPV) of 100%. The PPV to carry heterozygous *CYP21A2* mutations with a ratio >12 was 94%.
In conclusion, the 17OHP/DOC ratio after ACTH stimulation was not able to distinguish heterozygous carriers of *CYP21A2* mutations from WT individuals completely. However, a ratio <12 allowed to exclude heterozygosity in 100% of cases. Therefore, genetic testing is not necessary in these individuals. Our data stress the value of specific steroid analysis after ACTH stimulation for genetic counseling not only in families at risk for 21OHD but also in the general population.

PO3-021 Adrenal III

Management of pregnancies at risk of 21-hydroxylase or 11-hydroxylase deficiency using fetal sex determination in maternal serum proposed in France since 2002: how to limit prenatal exposure to dexamethasone

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Prenatal treatment by dexamethasone (DEX) is proposed since 1980 to prevent genital virilization in girls affected with classical form of 21 or 11-hydroxylase deficiency. DEX efficiency depends on term of start, posology and preservation until delivery in case of affected girl. As DEX remains controversial regarding possible side effects on treated fetuses, we report the French experience based on fetal sex determination in maternal serum (SRY test) to avoid treatment of males. The following protocol was proposed since 2002: early datation of gestation by ultrasonography, SRY test between 4 and 5.5 weeks of gestation (WG) with aim to start DEX before 6 WG if negative; confirmation of early negative tests at 8 WG and prenatal diagnosis on chorionic villi. In case of positive SRY test, prenatal diagnosis on amniotic fluid or management at birth were discussed. There were 160 fetuses with SRY test, 84 males and 76 females. Delay of menstruations was inaccurate for datation in 20 % of cases, underlying the necessity of ultrasonography. Sensitivity of an early SRY test was confirmed by karyotype and at birth but it has to be performed after 4 WG to avoid false-negative cases. Positive SRY test prevented treatment for 49 males, early realized for 29, and it allowed to stop DEX for others. Prenatal diagnosis was realized for only 31 males. Regarding females, 13 were affected with 21 or 11-hydroxylase deficiency, all prenatally diagnosed, only one was aborted. DEX was efficient for 8 girls, started not later than 6 WG. Four girls presented with posterior labial fusion but no clitoromegaly, requiring simple repairing

surgery. There were several explanations for partial efficiency: start at 7 WG, bad maternal compliance, error in datation, role of DEX maternal metabolism. Maternal tolerance was correct on the condition to respect low calorie and poor salt diet; bad side effects were described for 2 mothers in a particular context: gestationnel diabetes and arteriel hypertension in an obese woman, temporary acute pancreatitis in a woman with previous cholecystectomy. Treated fetuses didn't present any malformation at birth and mensurations were correct. Thus, SRY test is usefull to avoid DEX in males; respect of this protocol, in particular DEX efficiency in preventing virilization in girls, implies a very closed collaboration between clinicians and biologists. Multicentric follow-up studies of treated children will be essential to precise DEX safety.

PO3-022 Adrenal III

Anti-Müllerian hormone in prepubertal girls and boys with premature adrenarche

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Premature adrenarche (PA) has been connected with increased risk of developing polycystic ovary syndrome (PCOS). Circulating anti-Müllerian hormone (AMH) concentrations are higher in women with PCOS and in their prepubertal daughters than in the respective normal populations, probably due to larger ovarian follicular mass. The aim of this study was to determine whether serum AMH concentrations are altered in prepubertal children with PA.
The study group comprised 61 (52 girls/9 boys) prepubertal children with PA and 57 (48/9) prepubertal age-matched control children, with a median age of 7.4 years. Serum AMH, FSH, LH, insulin, DHEAS and androstenedione concentrations were analyzed by specific immunoassays (ELISA, FIA, RIA), HOMA-IR was calculated and a GnRH-test (Relefact© 3.5 µg/kg) performed. Girls with PA had lower serum AMH concentrations than the control girls (2.65 vs. 3.43 ng/ml, P=0.035; Independent samples t-test). There was a similar difference between the PA and control boys (10.10 vs. 12.14 ng/ml, P=0.038; Mann-Whitney test). Serum AMH concentrations did not correlate significantly with age in either girls or boys (|r|<0.04, P≥0.88 for both; Pearson correlation test). In the pooled group of PA and control girls, there was an inverse relationship between AMH concentration and BMI SDS (r=-0.23, P=0.019), and between AMH and androstenedione concentration (r=-0.22, P=0.03). Accordingly, the differences in AMH concentrations between the study groups remained significant after adjustment for age in the univariate linear model (P=0.035 in girls; P=0.042 in boys) but vanished between the PA and control girls when adjusted for BMI SDS (P=0.16). Serum AMH concentration did not correlate significantly with HOMA-IR, fasting serum insulin, stimulated LH or FSH concentration. Baseline LH concentration was below the sensitivity of the assay (<0.1 IU/l) in most of the subjects. There was a trend towards an inverse relationship between serum AMH and DHEAS concentration in boys (r=-0.42, P=0.09). Serum AMH concentration was negatively correlated with DHEAS (r=-0.32, P=0.028) and baseline FSH concentration (r=-0.47, P=0.001) only among the control girls.
Unlike the prepubertal daughters of PCOS women, our prepubertal children with PA had lower AMH concentrations than their controls. The difference was explained by the higher BMI SDS in the PA girls. Thus, girls with PA do not seem to have PCOS related abnormalities in ovarian function at prepubertal age.

PO3-023 Adrenal III

Primary pigmented nodular adrenocortical disease in pediatric patients

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During childhood, Primary Pigmented Nodular Adrenocortical Disease (PPNAD) is a rare cause of ACTH-independent Cushing's syndrome. About 80% patients with PPNAD suffer from the autosomal dominant multiple neoplasia called Carney complex (CNC). We report 4 cases of PPNAD including 3 CNC observed in children over a period of 30 years.
Initial presentation combined obesity, hypertension and ACTH-independent hypercortisolism. The dexamethasone test induced paradoxical elevation of urinary free cortisol. The clinical description of the 4 cases are in table 1.

table 1

	1	2	3	4
Age at first signs (years)	2.5	3	13	2
Sex	F	F	F	M
Cyclic hypercortisolism	Yes	Yes	No	No
Growth	Normal	Normal	Slow down	Slow down
CNC (during childhood)	Yes (cardiac myxoma)	Yes (none)	Yes (myxoid tumors, ovarian cyst)	No
Adrenal CT imaging	Normal	Bilateral hyperplasia	Mild hyperplasia	Irregular outline
iodo-cholesterol scintigraphy	Bilateral uptake	Not done	Not done	Bilateral uptake
Initial treatment	Ketoconazole, Op'DDD	None	Op'DDD	Op'DDD
Final treatment	Bilateral adrenalectomy (12 y)	Unilateral adrenalectomy (15 y)	Op'DDD	Bilateral adrenalectomy (4.5 y)
Duration of follow-up (years)	9.25	35	14	4.5
Genetic mutations	PRKAR1A (578delTG)	PRKAR1A (p1255fsX268/g763-764delAT)	PRKAR1A (124C>T exon2)	PDE11A gene variant

PPNAD is uncommon in children aged less than 4 years, however, 3 out of the 4 patients were younger. These patients showed various clinical as well as various responses to treatment. Growth was either normal or slow whether hypercortisolism was cyclic or permanent. Adrenal hyperplasia could be missing at CT imaging while bilateral adrenal uptake was typically found by iodocholesterol scintigraphy. Three of these patients were submitted firstly to a medical treatment. Subsequently to side effects and/or poor efficiency, adrenalectomy was indicated, more over it allowed to establish the histology of PPNAD. The 3 children with CNC had a mutation in the PRKAR1A gene. For the patient suffering from an isolated PPNAD, an atypical variant in the PDE11A gene had been identified. PRKAR1A gene codes for the regulatory R1A subunit of protein kinase A and PDE11A gene for the phosphodiesterase 11A4, both are involved in the cAMP signaling pathways in adrenal cells.
PPNAD is a rare but severe disease in children. Diagnosis of PPNAD is not easy and must be evoked even in young children before 4 years. Furthermore, features of CNC must be collected and genetic analysis of the PRKAR1A gene be made.

PO3-024 Adrenal III

New treatment option in Cushing syndrome caused by renal tumour in childhood

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Ectopic production of adrenocorticotropin (ACTH) and/or corticotropin-releasing hormone (CRH) by a malignant renal tumour in children is a rare cause of Cushing syndrome (CS). We present a 3-year-old girl with a palpable mass in the right abdomen as presenting symptom. Additionally she had clinically and biochemically ectopic CS. Radiological imaging showed a mass suspicious for a Wilms' tumour (WT). Consequently, CS due to ectopic production of ACTH and/or CRH by the WT was diagnosed. Preoperative chemotherapy was started before tumournephrectomy was performed. This in contrary to the other, few, cases that have been described in literature. One week after the first course an extensive decline of both ACTH and cortisol was monitored. After four weeks of chemotherapy tumournephrectomy was performed. Immunohistochemistry

of the nephroblastoma revealed no positive staining for ACTH and dubious presence of CRH. Hence more tests will be performed on the histological material before a final conclusion can be drawn. So far, we postulate that either the amount of CRH producing cells decreased after the start of chemotherapy, resulting in decline of ACTH and cortisol, or that the tumour harboured both CRH and ACTH producing cells of which the latter disappeared already by chemotherapy. In conclusion, we describe the first pediatric patient with ectopic CS due to a WT who received preoperative chemotherapy leading to a satisfying endocrine and oncologic response.

PO3-025 Adrenal III

Pseudoprecocious puberty in a 2 ½-year-old boy caused by a functioning adrenocortical oncocytoma

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Introduction: Adrenocortical oncocytoma has been reported in approximately 50 cases so far. The age range was 6 to 77 years, female to male predominance (2.5:1), and in the majority of cases the tumour was non-functioning.
Case report and results: A 2 ½ -year-old boy was presented because of pubarche and aggressive behaviour. We found Tanner stages B3, P2, G3 and bone age advanced to nearly 5 years. In plasma, corticosterone, androstenedione and total testosterone were elevated, whereas catecholamines, cortisol, and renin were normal. Ultrasound examination demonstrated a left sided adrenal tumour, approximately 3 cm in diameter; magnetic resonance imaging (MRI) confirmed the adrenal mass, isointense to spleen parenchyma, sized approx. 3.6 x 2.7 x 3.9 cm, with sharp boundaries. MRI scans validate of T1-weighted as well as T2-weighted sequences.
Laparoscopic adrenalectomy was performed. The tumour mass had a soft consistence and a brownish appearance. Histopathology was suggestive of adrenocortical oncocytoma, and the diagnosis was confirmed by an independent reference lab. No signs of malignancy were found. After surgery, corticosterone, androstenedione and total testosterone returned to prepubertal levels and the behaviour disturbance slowly regressed.
Conclusions: Apparently, oncocytoma may occur as early as in the third year of life, and be functional leading to pseudoprecocious puberty. Although functioning adrenal tumors are rarer than late-onset AGS, this observation underlines the role of adrenal sonography in the differential diagnosis of pseudoprecocious puberty.

PO3-026 Adrenal III

DAX-1 gene mutation in X-linked congenital adrenal hypoplasia. One case report

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Introduction: Congenital Adrenal Hypoplasia (CAH) is a rare disorder and cause of primary adrenal insufficiency in childhood. X-linked CAH is due to a mutation in, or deletion of, the *DAX-1 gene. The DAX-1* gene is located in the chromosome bands Xp21.3-Xp21.2. It occurs more commonly in males, is associated with total or partial gonadotropin deficiency and affects 1/12,500 births.
Case Report. A 20 year old male patient, was assessed at one month of age, in the Pediatric Endocrinology Department of the Children´s Hospital "J.M. de Los Ríos" Caracas, Venezuela, due to dehydration, hyponatremia, hyperkalemia, and metabolic acidosis without hypoglycemia. He presented hyperpigmentation and normal male external genitalia. His parents were not consanguineous, and his older brother had died of unknown cause in his first week of life. Lab exams:
17 OHP: 2,3 ng/ml (VN: 0,2-1,6 ng/ml), Aldosterone: 0 pg/ml (VN:35-300), Androstenedione: 2,1 ng/ml, Testosterone: 2,0 ng/dl (VN: 0,5-501), DHEA-S: 23 µg/dl (VN: 6,4-47),Cortisol: 15,5 µg/dl (VN: 5-20), Sodium: 122 mEq/l, Potasium: 5,46 mEq/l.
Abdominal Computed Tomography Scan reported a small left adrenal gland. Primary adrenal insufficiency is diagnosed and glucocorticoid treatment is indicated.
The patient made irregular visits since diagnosis, with long intervals without medical control. When assessed at 16 years old, he had a tall stature, absence of pubertal signs and a bone age of 13 years and 6 months. GnRH-stimulated LH levels were compatible with a prepubertal pattern and Hypogonadotropic Hypogonadism was confirmed. Treatment with testosterone enanthate was initiated. Molecular analysis was performed and the sequencing revealed a single nucleotide deletion in exon 2 c544delG that resulted in a frameshift and a stop codon at 263 residue. Therefore this mutation is predicted to result in a truncated protein with disrupted carboxy-terminal.
Conclusion: This case confirms the association of adrenal insufficiency and hypogonadotropic hypogonadism. The determination of *Dax-1* gene mutation in X-linked CAH was done in the Endocrinology Department of "JP Garrrahan" Hospital in Argentina and is our first case with a genetic test. We emphasize the value of genetic testing in boys with primary adrenal insufficiency and suspected X-linked CAH.

PO3-027 Adrenal III

No correlation of androgen receptor CAG and GGN repeat polymorphisms with the degree of genital virilization in females with 21-hydroxylase deficiency

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In 21-hydroxylase (*CYP21A2*) deficiency (21OHD) the degree of *in vitro* enzymatic function allows for classification into mutation groups (Null, A, B, C). The adrenal phenotype strongly correlates with residual CYP21A2 activity and allows for prediction of disease severity. However, the degree of external virilization only weakly correlates with the *CYP21A2* mutation groups suggesting the importance of genetic modifiers.
Objective: To investigate the influence of the polymorphic CAG and GGn repeats of the androgen receptor gene on the degree of genital virilization in 21OHD females.
Design and patients: Analysis of *CYP21A2* genotype, degree of genital virilization according to Prader and determination of X-weighted biallelic means of CAG and GGn repeat lengths in 205 21OHD females.
Outcome measurements: Correlation of AR CAG and GGn repeat lengths with Prader stages using nested logistic regression analysis.
Results: Mutation groups Null and A show significantly higher degrees of genital virilization compared with groups B and C ($p<0.01$). However, Prader stages varied strikingly within *CYP21A2* mutation groups. Null: Prader I-V (median IV); A: Prader I-V (median IV); B: Prader I-V (median III); C: 0-III (median I). GGn repeat lengths did not differ between marginally (Prader 0-I), intermediately (Prader II-III) and highly (Prader IV-V) virilized patients (OR 0.98 [0.71-1.35, 95% CI, $p = 0.89$]). In contrast, patients with Prader II-III and Prader IV-V tended to have shorter CAG repeat lengths compared with Prader 0-I. Odds ratio per CAG decrement did not reach statistical significance (OR 0.82 [0.65-1.02, 95% CI, $p = 0.07$]).
Conclusion: Neither CAG nor GGn repeat length are statistically significant modifiers of external genital phenotype. Inter-individual differences of prenatal

steroid biosynthesis or local factors of genital androgen sensitivity other than the AR are more likely determining the phenotypic variability observed in genital virilization in 21OHD females.

PO3-028 Adrenal III

IL1RAPL gene deletion associated with mental retardation in patient with complex glycerol kinase deficiency

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Contiguous gene deletion syndrome is caused by a segmental chromosomal deletion. The example of such a disease is complex glycerol kinase deficiency (GKD) caused by a contiguous deletion of genes in Xp21 involving glycerol kinase locus together with adrenal hypoplasia congenita (AHC) and/or Duchenne muscular dystrophy (DMD). In their close neighbourhood one of genes responsible for mental retardation - *IL1RAPL* (interleukin-1 receptor accessory protein-like) is located. We report on a case of 2-year-old patient with this rare complex deletion. To our knowledge there are only few patients diagnosed with such a deletion.

Case report: A male infant at the age of 5 weeks was admitted to hospital due to salt losing syndrome. The diagnosis of primary adrenal insufficiency was confirmed by documentation of elevated plasma ACTH level, subnormal peak cortisol level after ACTH stimulation, low aldosterone level in the face of hyperreninemia and analysis of urine steroid profile. His condition improved after starting glucocorticoid replacement. However his development was significantly delayed. At the age of 2 his height was 75 cm, weight 8.3 kg, he couldn't stand or walk. Elevation of muscle enzymes and triglycerides in plasma was shown. GKD was confirmed by elevated urinary glycerol concentration. Genetic studies revealed complete deletion of *DAX-1 and GK* genes and the C-terminal region of *DMD* gene. Contiguous gene syndrom was diagnosed. With respect to mental retardation *IL1RAPL* gene examination was performed, confirming its deletion.

Conclusion: Boys with congenital adrenal hypoplasia should be examined regarding DMD and improper glycerol metabolism. Mental retardation in such a condition can be caused by *IL1RAPL* gene deletion therefore its assessment is also important.

PO3-029 Adrenal III

Growth hormone-releasing peptide-2 (GHRP2) test is clinically useful for evaluation of pituitary-adrenal axis in children

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[Background] The hypothalamo-pituitary-adrenal (HPA) function is currently assessed by insulin-tolerance test (ITT), corticotropin-releasing factor (CRF) test, and low dose ACTH test in children. ITT is a standard test, though severe hypoglycemia may occur. GHRP2 has been developed as a potent stimulator of GH secretion from pituitary. Recently, it is known that GHRP2 also stimulates pituitary ACTH secretion.

[Aims] To investigate the clinical usefulness of GHRP2 test in evaluating HPA axis in children.

[Subjects & Methods] 53 children (1 ~ 19 years old) were studied. 37 were with short stature (SS); 29 were idiopathic SS (ISS) and 8 were with isolated GHD. 13 patients were suffered from intracranial tumor (ICT), 1 was with congenital combined pituitary hormone deficiency (CPHD), and 2 were diagnosed as congenital adrenal hypoplasia.

ACTH and cortisol secretion were determined by both CRF test and GHRP-2 test in each subject. The subjects were examined at bed rest after overnight fast. CRF was administered intravenously at a dose of 1.5 μg/kg BW (max 100 μg). The blood sampling was obtained at -30, 0, 30, 60, 90, and 120 min. GHRP2 was administered in the same condition at a dose of 2 μg/kg BW (max 100μg). The blood was obtained at 0, 15, 30, 60 min. Plasma ACTH and serum cortisol levels were determined by IRMA and RIA, respectively.

[Results] No adverse events were observed. In 37 subjects diagnosed with HPA axis, peak ACTH and cortisol levels after GHRP2 test ranged 15.7 ~ 142 pg/ml (median 31.3 pg/ml) and 13.8 ~48.3 μg/dl (median 23.8 μg/dl), respectively. Peak ACTH after GHRP2 and CRF did not show significant correlation, while peak cortisol levels correlated significantly between these two tests (r=0.41, P=0.0135). In two patients with primary adrenal failure, serum cortisol did not show any increase after GHRP2 injection, while ACTH increased to 2240 and 261 pg/ml. Among 13 ICT patients with secondary adrenal insufficiency, ACTH and cortisol were undetectable. Three showed exaggerated ACTH response with relatively low cortisol levels; they developed adrenal insufficiency during follow-up.

[Conclusions] GHRP2 is a safe and favourable test for children. The diagnostic value of GHRP2 in HPA axis is comparable to CRF test. Furthermore, it may be useful to evaluate progressive dysfunction of HPA axis especially among those with organic intracranial lesions.

PO3-030 Adrenal III

Glucocorticoid steroidogenesis and metabolism in anorexia nervosa

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Background: Anorexia Nervosa (AN) is associated with 'hyperactive' HPA axis: hypercortisolism associated with increased CRH levels but normal ACTH levels, free and total testosterone are low, while DHEA and DHEAS are reported low or normal.

Hypothesis: AN is associated with dysregulation of steroidogenesis enzymes. We aimed to study the activity of adrenal and glucocorticoid modulating enzymes in AN patients using a quantitative gas chromatography mass-spectrometry (GC-MS) method.

Patients and Methods: Six AN patients during the acute phase of the disease and with secondary amenorrhea, age 14 – 28 y, 19.6 ± 4.9 y, BMI 17.5 ± 1.5 kg/m2, were compared to age matched controls, BMI 25.9 ± 3.2 kg/m2. GC-MS was used to profile 31 urinary steroid metabolites, and the product/substrate ratios were calculated to estimate enzymes activity.

Results: All patients had hypercortisolemia, with 8 am cortisol 854 ± 130 nmol/l (mean±SD, normal 170-540 nmol/l). On the other hand, the sum of urinary cortisol metabolites was low in AN (2008 ± 635) as compared to controls (4668 ± 1797 mcg/l, p<0.019). While 17-OHase and 21-OHase activities were similar to controls, 3b-HSD activity was high in AN patients (34.3 ± 22.6 vs.3.5 ± 1.6 mcg/l, p<0.01). The pregnenolone metabolite pregnanediol was low in AN (3.8 ± 8.6 vs. 61.3 ± 50.7 mcg/l, p<0.04). 11bHSD-1 activity was high in AN (0.023 ± 0.009) as compared to controls (0.015 ± 0.03, p<0.0005).

Conclusions: High serum cortisol in AN is associated with low urinary glucocorticoid metabolites.Despite their low BMI, which normally correlates positively with 11bHSD1, AN patients demonstrate high enzyme activity. The serum/urine discrepancy may be due to enhanced 11bHSD-1 and 3bHSD activities.

PO3-031 Adrenal III

Adrenal cortisol secretion capacity in children and adolescents with Crohn's disease

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Introduction: The hypothalamic-pituitary-adrenal (HPA) axis may modify the inflammatory process of Crohn's disease (CD). Previous studies have partly

attributed the flare of several inflammatory diseases including CD to dysregulation of the HPA axis, with a subsequent failure to contain the inflammatory activity. Some of the reports were biased however by a prior exposure to corticosteroids therapy.
Aims: To assess the adrenal cortisol secretion capacity in a cohort of treatment-naive children and adolescents with CD. We also aimed to assess correlations between cortisol levels and indices of inflammation severity and disease activity in these patients.
Study design: We retrieved data on CD patients aged 9.5 to 19.7 y (32 M, 20 F) that participated in a prospective study where budesonide treatment was evaluated. Basal and ACTH-stimulated cortisol levels in 52 children and adolescents with CD were compared to levels obtained in 52 age-matched controls. Correlations of cortisol levels with pediatric CD activity index (PCDAI) and C-reactive protein (CRP) as an inflammatory marker were also assessed.
Results: Both basal and stimulated cortisol levels in CD were significantly higher than in control subjects: 364 ± 173 *vs.* 290 ± 122 nmol/L (p=0.029) and 865 ± 236 *vs.* 738 ± 148 nmol/L (p<0.001), respectively. Cortisol levels were correlated with CRP but not with PCDAI. Unlike control group, stimulated cortisol levels in CD patients were not correlated with basal levels but rather with CRP (positive correlation) and age at diagnosis (negative correlation).
Conclusions: Contrary to previous reports suggesting that dysregulation of the HPA axis is implicated in the susceptibility to, and severity of CD and other chronic inflammatory diseases, we demonstrated an adequate response of this axis in pediatric CD, in proportion to the inflammation severity.

PO3-032 Bone, Calcium III

A novel mutation in the GNAS gene causing pseudohypoparathyroidism type Ia in an infant

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Introduction: Pseudohypoparathyroidism Type Ia (PHP-Ia, Albright hereditary osteodystrophy) is a rare disorder presenting usually during childhood. The clinical characteristics are short stature, obesity, mental retardation, subcutaneous calcium deposits, different skeletal abnormalitie and hypocalcemia, hyperphosphatemia with elevated PTH levels. PHP-Ia is caused by heterozygous inactivation mutations of the GNAS1 gene that encodes Gαs protein.
Subjects and methods: We describe a female patient born after an uneventfull pregnancy with a birth weight of 4400 gr. Although she was exclusively breast fed she presented at the age of 4 months because of excessive weight gain (10.6 kg). Her length was on the 96th percentile. The first workup revealed mild hypothyroidism with TSH 14.2 mIU/l and FT4 10.6 pmol/l, (normal range 10.3-19.7) Levothyroxine treatment was started. Calcium, phosphor and PTH were in the normal range. She continued to gain weight excessively while growing on the same length percentile. Psychomotor development was normal for her age. At 6 months subcutaneous calcifications appeared and were described as osteoma cutis on biopsy, consisted with the diagnosis of Albright hereditary osteodystrophy. PTH was followed serially and at the age of 1 year levels increased above the normal range.
Genetic analysis: Genomic DNA was extracted from peripheral lymphocytes, and full sequencing of the coding regions of the GNAS gene has been undertaken.
Results: The patient was found to be heterozygous for a novel mutation Nt831delG in the GNAS gene. This is a frameshift mutation creating an immediate premature stop codon: W277X. The mutation was not present in both parents and therefore is probably a de-novo mutation. At the age of 20 months the patient's PTH is 5 times the upper normal limit, with calcium and phosphor in the normal range.
Follow-up and conclusions: PHP-Ia is rarely diagnosed in infancy and can be the cause of excessive weight gain accompanied by tall stature. In addition to resistance to PTH, resistance to other G protein-coupled receptors (especially TSH receptor) can occur. A subset of infants with excessive weight gain and tall stature, who have TSH in the upper normal, with or without delayed milestones, should lead to a suspicion of PHP-Ia. Finding these rare disorders, as the novel mutation we describe, has importance for both genetic counseling and clinical follow up and treatment.

PO3-033 Bone, Calcium III

Randomised trial of physical activity intervention to improve bone health of preterm infants in the neonatal unit – results from the Glasgow women & infants' skeletal health (WISH) study

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Introduction - Immobility in preterm infants may contribute to osteopenia of prematurity. The role of passive exercise in improving bone health in these infants is unclear and requires further study.
Aim - To assess the effect of a physical activity intervention in preterm infants on bone health assessed by quantitative ultrasound
Methods - 31 infants born at <33 weeks gestation were randomised to receive range of motion flexion and extension exercises once daily for 5 days each week starting 'early'(n=15) or 'late' (when on 100kcal/kg/day enteral feeds, n=16) and continuing until term corrected gestational age (CGA) or discharge from hospital. All outcomes were compared between groups and to a group of matched historical controls. Tibial quantitative ultrasound measurement of speed of sound (SOS) was performed using the Sunlight Omnisense 7000P scanner.
Results - Tibial SOS declined significantly from birth to end of physical activity in both 'early' and 'late'groups with a median change in SOS SDS-1.1(10th and 90th centiles, -2.9, -0.4) p<0.005 and -0.8 (10th and 90th centiles, -3.8, -0.25) respectively, p <0.005.Mean SOS SDS decrease in the historical controls was -1.58 (10th and 90th centiles, -2.2, -0.85), this was not significantly different from the intervention groups. Weight gain and head growth did not show a significant difference between groups or between study infants and controls. No infant was reported to have sustained a fracture. Length of hospital stay was not significantly different between groups. There was no significant increase in sepsis rate, retinopathy of prematurity, or chronic lung disease in study infants. At 5 months CGA (n, 25) no infant had a skeletal deformity or fracture, and the rate of adverse neurodevelopmental outcomes was not increased.
Conclusion - The physical activity intervention in this study did not confer any benefits on the bone health outcome measure studied and was not associated with any adverse effects.

PO3-034 Bone, Calcium III

The Glasgow women & infants' skeletal health (WISH) study – maternal bone health during pregnancy and its relationship to newborn bone health

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Introduction - It is possible that the maternal environment plays a vital role in influencing the infants bone health.
Aim - To investigate the factors which may influence maternal bone health in pregnancy and their interaction with the bone health of the offspring.
Methods - 188 women were recruited at their antenatal booking visit and had measurement of serum bone biochemistry and radial speed of sound (SOS). Repeat measurements were performed immediately after delivery. Infants of these mothers had a single tibial quantitative ultrasound measurement of SOS at a mean age of 2 days (SD,1d) after birth. The measurements were performed by a single operator using the Sunlight Omnisense 7000P scanner.
Results – Median SOS SDS antenatally was 0.0 (IQR, -0.6, 1.0.) There was a significant negative correlation with SOS SDS and BMI, r -0.2, p<0.05.

Antenatal median SOS SDS was -0.5 and 0.2 in smokers and non smokers, respectively, p<0.05. Median change in SOS SDS across pregnancy was 0 (NS). In a subgroup of women of Asian origin (n, 5) there was a substantially larger decrease in SOS SDS across pregnancy, median -1.8, p<0.05. Offspring (n, 125) had a median SOS SDS of -0.4 (IQR -0.9, 1.0). There was no relationship between mother and infant SOS SDS. SOS SDS was significantly lower in infants of smokers, median -0.75, compared to non smokers, median -0.3, p<0.05. Race, deprivation scores, maternal BMI, season of first trimester, and maternal hypertension had no significant effect on infant SOS. In the women who had Vitamin D levels measured, 83/140 (59%) women were Vit D sufficient (Vit D3 >55nmol/l) at booking and 8/42 (19%) sufficient at delivery. In 14/91 women Vit D insufficiency was associated with a raised PTH. Median Vit D level at booking and delivery were 66nmol/l and 26nmol/l, p<0.05. Median maternal postnatal Vit D and umbilical cord Vit D levels, available in 42 mothers and offspring were 26nmol/l and 23nmol/l, and showed a positive association (r,0.7, p<0.005). Obese women had lower vit D levels antenatally, median 46nmol/l, compared to median of 70nmol/l in thse with a normal BMI, P<0.05. Women of Asian origin had significantly lower Vit D levels at all stages of pregnancy.

Conclusion - Bone strength assessed by radial SOS does not significantly change across pregnancy. Smoking and obesity may adversely affect women's bone health and that of her offspring. Vit D deficiency is common at the end of pregnancy.

PO3-035 Bone, Calcium III

Teriparatide treatment in a young adult with hypophosphatasia

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Introduction: Hypophosphatasia is an inheritable disorder characterized by defective bone mineralisation and impaired activity of the tissue-non-specific alkaline phosphatase (TNSALP), leading to low serum levels and activity of alkaline phosphatase. The disease is divided into five subclasses: perinatale, infantile, childhood, adult type and odontohypophosphatasia. The disease is due to mutations in the *TNSALP* gene localized on the chromosome 1p36.1-34. So far there is no recognized efficient treatment of hypophosphatasia. Affected adults manifest osteomalacia with bone pain, pseudofractures and slowly healing fractures. Reduction of pain and fracture repair duration has been demonstrated in some adult patients under treatment with teriparatide (TPTD), a recombinant human 1-34 PTH. We present the case of a young adult with infantile hypophosphatasia treated one year with TPTD for severe bone pain.
Case report: A girl born SGA (birth length: 45 cm) present at birth with chest, limbs deformities, and very low levels of serum alkaline phosphatase which led to the diagnosis of infantile hypophosphatasia. She was found double heterozygous with two *TNSALP* mutations inherited from her parents. Both were heterozygous, had low levels of AP but were asymptomatic. When she was 17 years, she was confined to wheelchair because of severe bone pain mainly affecting knees and back. Non steroidal anti inflammatory treatment was ineffective to reduce pain. TPTD treatment was initiated with 20mg subcutaneous injected daily during one year.
Results: TPTD treatment provided benefit on bone mineral density (BMD): total body BMD increased from 0.861 to 0.886 gr/cm^2, and lumbar spin BMD increased from 0.924 to 0.981 gr/cm^2. However, TPTD was ineffective to reduce bone pain and the treatment was stopped after one year.
Conclusion: TPTD was ineffective to reduce bone pain in our patient. Chronic pain can be a major complication of hypophosphatasia, with highly incomplete efficiency of the usual analgesic treatments.

PO3-036 Bone, Calcium III

SHOX gene molecular analysis in a series of 113 French patients with a clinical phenotype of Leri-Weill dyschondrosteosis

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Background and aims: haploinsufficiency of the SHOX gene causes variable degrees of short stature and is responsible for mesomelic growth retardation with Madelung deformity in Leri-Weill dyschondrosteosis (LWD). 113 patients with short stature associated with certain bone deformities and dysmorphic signs suggesting a LWD phenotype were recruited for SHOX gene defects analysis. We retrospectively assessed clinical, auxological and radiological features of the individuals identified with SHOX mutations or deletions.
Patients: the study participants that were screened for deletions or mutations in the SHOX gene presented with at least 3 of the following criteria:
- Height < -2 SDS percentile
- Clinical LWD phenotype
- Radiological LWD phenotype
- Familial history of short stature or Madelung deformity in 1st or 2nd degree relatives

Results: SHOX gene abnormalities were identified in 22/113 (14F) patients (20%). In these patients, leg length (F: < -3 SDS in 58%; M: < -3 SDS in 28%), sitting height, and sitting height/height ratio (F: > +1 SDS in 100% and > +2.5 SDS in 57%; M: > +1 SDS in 85.7% and > +2.5 SDS in 57%) charts had great diagnostic value for indicating the disproportionate short stature. Dysmorphic signs typically observed in Turner syndrome were frequent including hyperconvex nails. Patients with SHOX deficiency had a significantly improved height under treatment with GH (n=19) (mean, - 1.32 SDS *vs*. 2.21 SDS in the entire group before treatment) and final height was documented in 5 cases.
Conclusion: this one-center study shows a high rate of detection of SHOX defects and underlines the importance of developing efficient scoring systems for identifying the most appropriate patients for SHOX testing.

PO3-037 Bone, Calcium III

Normalization of cortical bone density in children and adolescents with hyperthyroidism treated with anti-thyroid medication

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Introduction: We have previously reported low cortical bone density (BD) in children and adolescents with untreated hyperthyroidism by using computed tomography (CT). It is unknown whether this abnormality can be corrected by achieving euthyroidism.
Objective: To determine whether normalization of thyroid function improves/normalizes the decreased cortical BD observed in patients with hyperthyroidism.
Study design: Anthropometrics and 3-dimensional CT bone measurements including vertebral cross sectional area (CSA) and BD (cancellous bone) and mid-shaft femoral CSA, cortical bone area (CBA), and BD (cortical bone) of 15 children and adolescents after one and two years of treatment with anti-thyroid medication were reviewed and compared to their pre-treatment values.
Results: The one -year post-treatment height SDS was significantly decreased ($P < 0.001$), while the BMI SDS and Tanner stage were significantly increased ($P < 0.01$ *and* $P < 0.001$, respectively). After adjusting for age, height, weight and Tanner stage, we noted a significant increase in cortical BD in all patients after one year (15/15) ($P = 0.026$). Normalization in all tested patients (10/15) was demonstrated after 2 years of anti-thyroid treatment. There were no significant changes in the other cancellous or cortical bone parameters.

Conclusion: Achievement of euthyroidism by anti-thyroid medication significantly improves cortical BD after one year and completely normalizes it after 2 years of treatment.

PO3-038 Bone, Calcium III

Bone mineral density in Korean children: correlations with clinical growth parameters

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Purpose: Since the mean height of Korean children has been increased recently, new version of growth chart was made in 2007. Nevertheless, the reference value on bone mineral density (BMD) of normal children has not been established yet. The aims of this study were to obtain mean values of BMD in normal children and investigated correlation between BMD and their relating factors. **Methods**: 136 normal children from 6 to 14 years old were included, who were 70 boys and 66 girls. BMD was measured at lumbar spine and proximal femur according to sex and age by dual energy x-ray absorptiometry (DEXA: discovery A, Hologic, MA, USA, 2004). We also assessed the relating factors such as height, weight, BMI, bone ALP, IGF-I, testosterone and estradiol levels, and then analyzed their correlation with BMD by Pearson's correlation and linear regression analysis. **Results**: The result showed a trend of an increase in BMD according to the age in both boys and girls. The mean value of BMD showed the greatest increase during age of 10 to 11 in girls and 12 to 13 in boys. According to pubertal stage, a sudden increase of BMD in both boys and girls occurred during the overt puberty. Bone age and IGF-I level were demonstrated the most significant correlation with BMD of lumbar spine (bone age, R^2=0.65; IGF-I, R^2=0.44). The mean values of BMD of this study were similar to the previous studies about BMD of Korean children which carried out in 1990's. **Conclusions**: This study showed that bone age and IGF-I level were indicated the best correlation with BMD among the relating factors and the mean values of BMD in Korean children had no remarkable change during last 10 years.

Keywords: bone mineral density, bone age, IGF-I, Korean children

PO3-039 Bone, Calcium III

Soluble form of klotho in serum correlates with serum levels of hormones involved in phosphate homeostasis

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Klotho is a single-pass transmembrane protein and acts as a cofactor for fibroblast growth factor-23 (FGF23), to participate in the regulation of phosphate homeostasis and vitamin D biosynthesis. Although the expression of Klotho is limited to some tissues such as distal tubules in kidney, parathyroid gland, and choroid plexus, the soluble form is detectable in blood and cerebrospinal fluid. However, neither the function nor the regulatory mechanisms of soluble Klotho is known to date. Therefore, in the current study, we investigated relation of the levels of serum soluble Klotho with other factors involved in phosphate homeostasis in humans. Cord blood from healthy newborns with normal body weight whose gestational ages were 37 w 0 d to 41 w 6 d (N = 23), and serum from healthy adults aged 23 to 50 y (N = 35) were collected after informed consent was obtained. Using these samples, we measured the levels of soluble Klotho, FGF23, phosphate (Pi), intact parathyroid hormone (i-PTH), and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Soluble Klotho and FGF23 levels were measured by ELISA kits (Kyowa Hakko-Kirin, Gunma, for soluble Klotho; Kainos, Tokyo, for FGF23 measurement). Interestingly, serum level of soluble Klotho was significantly elevated in cord blood samples (3,125.7 ± 1,148.0 pg/ml) compared to the value in healthy adults (594.0 ± 168.5 pg/ml, p<0.01). In contrast, serum level of FGF23 was significantly lower in cord blood samples (4.57 ± 6.14 pg/ml) compared to the value in healthy adults (40.9 ± 16.6 pg/ml, p<0.01). The level of soluble Klotho correlated negatively with those of FGF23 and i-PTH (r = -0.698, p<0.01, and r = -0.571, p<0.01, respectively), and positively with that of Pi (r = 0.786, p<0.01). On the other hand, the level of FGF23 correlated positively with that of i-PTH (r = 0.481, p<0.01), and negatively with that of Pi (r = -0.599, p<0.01). We found no obvious correlation between 1,25$(OH)_2$D level and soluble Klotho level or FGF23 level. Limitations of this study are relatively small number of subjects and no data included from neonates and children. The results in the present study suggest that the production of soluble Klotho might be regulated negatively by the levels of FGF23 and i-PTH, and positively by that of Pi. In addition, soluble Klotho may function as a humoral regulator of phosphate homeostasis, or may be an indicator of function of hormones involved in phosphate homeostasis.

PO3-040 Bone, Calcium III

Insulin resistance in patients with vitamin D hypervitaminosis

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Several studies suggest a link between serum 25-hydroxyvitamin D (25(OH) D) and metabolic risk factors. However, the relation between 25(OH)D and IR-HOMA is unclear. We investigate the relation between serum 25(OH)D, calcium (Ca), parathyroid hormone (PTH), phosphorus (P) levels and IR-HOMA in 14 patients with vitamin D hypervitaminosis. Three cases (21.4%) had insulin resistance. While mean serum calcium level was significantly higher (p<0.01), mean serum phosphorus level was significantly lower (p<0.05) in cases with insulin resistance. However, there was no correlation between serum insulin levels and 25(OH)D, PTH, Ca, P.
It is possible that elevated Ca and decreased P levels in patients with vitamin D hypervitaminosis are involved in pathogenesis of the insulin resistance.

PO3-041 Bone, Calcium III

Bone mineral density by DXA in children with Hurler syndrome

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Purpose. To estimate the prevalence of low bone mineral density (BMD) by dual energy x-ray absorptiometry (DXA), evaluate methods for Z-score adjustment due to small body size, and begin to identify risk factors for low BMD, in children with Hurler syndrome after hematopoietic cell transplantation (HCT).
Methods. Retrospective chart review focusing on bone health parameters and risk factors for low BMD was conducted in pediatric HCT recipients with Hurler syndrome at the University of Minnesota. Total body minus head (TBMH) and lumbar spine (L1-4) BMD and Z-scores were determined by DXA using chronologic-age and gender matched reference groups. Z-scores were adjusted for bone age, height age, and an estimation of volumetric L1-4 BMD Z-score.
Results. Eighteen children with Hurler syndrome, ages 5.1-18.3 years, had DXA data from the last 5 years. 20% had low TBMH BMD (Z-score < -2) and 40% had low L1-4 BMD. Adjusted for bone age, 38% had low TBMH BMD and 42% had low L1 – L4 BMD. Adjustment for height age 1) decreased the prevalence of low TBMH BMD to 0% and of low L1 – L4 BMD to 8% and 2) resulted in comparing 6 pubertal children to prepubertal children. BMD Z-scores worsened with time since HCT and with decreasing BMI and percent body fat.
Conclusions. In children with Hurler syndrome who were treated with HCT at a young age, there appears to be an increased prevalence of low BMD, which lessens with height age adjustment. Worsening low BMD with time since HCT suggests continuing metabolic bone disease in these children in spite of HCT. The impact of these findings on fracture risk or skeletal deformities is unknown. This chart review, reiterates the challenges of BMD assessment in children in general, and particularly in children with Hurler syndrome.

PO3-042 Bone, Calcium III

Pseudohypoparathyroidism type Ia and pseudopseudohypoparathyroidism

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Pseudohypoparathyroidism (PHP) is a disease associated with a characteristic phenotype known as Albright hereditary osteodystrophy (AHO) and multiple hormone resistance. The AHO phenotype includes short stature, round facies, obesity, brachydactyly and cutaneous calcifications. Systemic manifestations can include mental retardation, cataract and calcifications in the basal ganglia. Pseudopseudohypoparathyroidism (PPHP) is a term describing patients with isolated AHO features, who have normal calcium metabolism and parathormone (PTH) levels. PHP type Ia and PPHP are autosomal-dominant inherited disorders, which can occur in the same family. In the case of maternal transmission, the children develop PHP type Ia. If the mutation is derived from the father (paternal transmission), the children develop PPHP. Both PHP and PPHP are caused by heterozygous inactivating mutations of the exons of the GNAS gene, which is located on the long arm of chromosome 20 and contains 13 exons. This gene encodes the α-subunit of the stimulatory guanine nucleotide-binding protein and downstream of many different G protein-coupled hormone receptors. Therefore, the PHP type Ia is often associated with resistance to hormones that act via Gs-coupled receptors, such as thyroid stimulating hormone (TSH) and gonadotropins

Case report:

We demonstrate two families with this disease. The affected members were the mother and her child. Categorization of patients to PHP type Ia or to PPHP was based on the results of physical and endocrinologic examination. The mothers had PPHP with typical morphological criteria (multiple cutaneous calcifications, brachydactyly) and a normal PTH level. Their children with PHP type Ia had clinical AHO features and biochemical resistance to PHT, TSH and gonadotropins. In one affected patient cutaneous calcifications have already appeared at the fifth week of life.

Conclusion:

The diagnosis in both families was confirmed by the determination of GNAS gene mutation. In the first family GNAS gene mutation is located in exon 10, E268X (c.802G>T), in the second family gene mutation is in exon 1, Q35X (c.103C>T).

PO3-043 Bone, Calcium III

Smaller and weaker metacarpal bones in the obese children

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Background. It is debated whether weight excess exerts a positive effect on bone. In fact although many but not all authors describe an increased BMD and a more favourable geometry of the long bones, an increased frequency of fractures at wrist is also reported.

Aim. To evaluate the effect of weight excess on the metacarpal bones, a non-weight bearing site.

Patients and methods. 69 overweight/obese children (43m,26f), median age 11.5 years (6.5-18.9) with a BMI >85 centile were selected for the study. Digitalized X-rays (Dicom files) taken for the assessment of bone age were evaluated and the following parameters were calculated at the level of the 2nd metacarpal bone: outer (D) and inner (d) diameter, cortical area (CA), endocortical area (EA), metacarpal index (MI) and bone strength (BBRI). Bone quality was also assessed by ultrasonography and expressed as sound of speed (SOS) and bone transmission time (BTT). The results have been evaluated according to bone age and expressed as SDS compared to a group of 483 control children of normal weight and height, matched for age and sex.

Results. Males: D –0.5±0.86 ($p<0.000$), d –0.22±0.82 ($p<0.05$), CA –0.55±0.66 ($p<0.0001$), EA –0.26±0.78 ($p<0.05$), MI –0.02±0.74 (NS), SOS –1.56 ± 1.38 ($p<0.0001$) and BTT –1.33 ± 1.25 ($p<0.0001$). BBRI (474 ± 164 vs 495 ± 174 mm^3;$p<0.0001$) was lower than in controls.

Females: D –1.08±1.03 ($p<0.0001$), d –0.40±0.85 ($p<0.025$), CA –0.87±0.75 ($p<0.0001$), EA –0.49±0.72 ($p<0.0025$), MI 0.22±0.78 (NS), SOS –1.04 ± 1.03 ($p<0.0001$) and BTT –0.83 ± 1.16 ($p<0.0025$). BBRI (374 ± 108 vs 649 ± 318 mm^3;$p<00001$) was lower than in controls.

Conclusion. Obese children show an unfavourable geometry at the metacarpal bone characterized by smaller bones with a normal cortical thickness. This results in a worse bone quality and reduced bone strength, which could explain the higher frequency of wrist fractures in these children.

PO3-044 Bone, Calcium III

Evaluation of regional bone mineral density after zoledronic acid treatment in a child with spinal cord injury

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Introduction Spinal cord injury (SCI) is associated with disuse osteopenia. Bisphosphonates have been increasingly used to manage childhood osteoporosis and disorders of bone fragility however, the use of Bisphosphonates for management of osteopenia after childhood SCI remains controversial.

Aim To evaluate regional changes in bone mineral density (BMD) using Peripheral Quantitative Computer Tomography (pQCT) after bisphosphonate treatment in a child with SCI and paraplegia.

Methods pQCT of the non dominant radius and tibia, at the 4% and 65% sites was performed pre and post 12 months Zoledronic acid treatment. Results are presented as age, sex matched z-scores. The 9yr old female patient had paraplegia due to a congenital dysplastic spine at L1. She presented after sustaining a second lower limb fracture; 1st fracture at 4 yrs (R distal femur) and 2nd fracture at 7 yrs (L supramalleolar). Initial dose of Zoledronic acid was 0.0125mg/kg and subsequent doses 0.025mg/kg; given at 3 monthly intervals over 12 months. Intravenous Zoledronic acid was infused over 30 minutes in 50 ml of normal saline.

Results In the unaffected upper limb, radius 4% site: total vBMD improved after bisphosphonate treatment, z-score 0.20 (pre) and 3.01 (post), this was reflected by a significant increase in trabecular vBMD, z-score -0.21 (pre) and 2.09 (post). There was no change in trabecular vBMD of the lower limb z-score -2.76 (pre) and -2.96 (post). Cortical vBMD and total bone cross-sectional area of radius and tibia was unchanged.

Conclusions Regional reduction in bone mass was more severe in the lower limbs where bone strength was also reduced. Preservation of upper limb function in this patient with paraplegia highlighted these regional changes and supported the concept of the bone-muscle unit.

Following bisphosphonate therapy, the radial metaphyseal total and trabecular volumetric bone mineral density increased. No treatment effect was seen in the trabecular vBMD of the affected lower limbs indicating that Zoledronic acid was unable to maintain primary tibial trabecular bone formed during the 12 months of treatment. Cortical vBMD was unchanged in the upper and lower limbs confirming the major effect of bisphosphonates on trabecular bone.

A combination of biomechanical stimulation and bisphosphonate therapy may provide a greater stimulus to the development and subsequent preservation of trabecular and cortical bone mass in children with SCI. Further large studies are required.

PO3-045 Bone, Calcium III

Bone mineral density in children and adolescents with Fanconi anemia

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Background: Concern that low bone mineral density (BMD) may be common in Fanconi Anemia (FA), even in childhood and before hematopoietic cell transplant (HCT), has been raised by two recent studies. Giri (2007) reported low BMD in 12 of 13 adults. Petryk (2006) reported decline in BMD (similar to that seen in other children without FA) during one year after HCT.
Objective: To assess whether low BMD occurs in children and adolescents with FA.
Method: 31 patients with FA, participating in an IRB-approved clinical database, had DXA evaluation of lumbar spine BMD for clinical surveillance. 15 had no history of HCT and 16 had prior HCT. BMD was normalized for age, gender, and ethnicity (BMD Z-score). BMD Z-score was also adjusted for height age. Data were collected for height SD, bone age, pubertal stage, glucocorticoid or androgen therapy, and duration since HCT. Mean duration after HCT was 5.7y (median 4.2y, range 1-18y). Androgens were used in 4 patients, 1 who later underwent HCT. Glucocorticoids were used briefly in most post-HCT patients, prolonged in 4 patients.
Results: Mean (range).

	N (gender)	Age (y)	N Pubertal	Height SD	BMD Z score	BMD Z for Height Age
No HCT	15 (12 girls)	11.7 (3.8, 18.9)	6 girls, 1 boy	-1.3 (-3, +1.3)	-0.3 (-2.5, +1.8)	+0.9 (-0.7, +2.9)
Prior HCT	16 (9 girls)	13.4 (6.1, 23.0)	5 girls, 5 boys	-2.1 (-5.3, +0.7)	-1.1 (-3.5, +1.3)	+0.3 (-2.2, +3.5)

Height-adjusted BMD tended to be lower in HCT patients, but this difference was not statistically significant (p=0.23). Height-adjusted BMD Z-score was below -2.0 in a single child who received prolonged glucocorticoid therapy after HCT.
Conclusion: Children and adolescents with FA have normal BMD prior to and after HCT, when DXA results are adjusted for stature. Individual results may be influenced by HCT status (which may reflect severity of FA phenotype), gonadal function and prolonged glucocorticoid therapy.

PO3-046 Bone, Calcium III

Rickets in adolescence – which laboratory parameter are most sensitive?

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The most common cause of acquired rickets is vitamin D- deficiency. Vitamin D requirements are high at times of rapid growth during infancy or puberty. Dietary and cultural peculiarities as well as skin colour due to ethnicity are risk factors for vitamin D- deficiency, particularly in Non-caucasian children.
We evaluated the following parameters in 40 children and adolescents aged 10 to 18 years with proven vitamin-D-deficiency (25-hydroxyvitamin D < 10 ng/ml) during the last 6 years: parathyroid hormone, alkaline phophatase, calcium, phosphate and creatinine related calcium excretion in urine (available in 38 patients).
35 of 40 patients were of asian, african or arabic descent, 5 patients were Caucasians.
In 39 of 40 children or adolescents the PTH was elevated (maximum 424 pg/ml – normal range 15- 55 pg/ml), in comparison the AP was increased only in 26 of 40 affected persons. In 13 of 40 patients we found low calcium, only in 2 of 40 low phosphate. The creatinine related calcium excretion in urine as a marker of renal calcium saving was below the reference range in 9 of 38 affected patients.
We conclude that measurement of PTH is the most sensitive parameter to discriminate relevant vitamin D- deficiency. In contrast, alkaline phophatase has a poor sensitivity and values in the normal range do not exclude rickets in children or adolescents. Also in Caucasian adolescents vitamin D- deficiency can occur.

PO3-047 Bone, Calcium III

A novel mutation in the vitamin D receptor gene in a Korean girl with vitamin D-dependent rickets type II

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Purpose: We discovered a case of Vitamin D-dependent rickets type II (VDDR-II) in a three-year old Korean girl. This is the first report of a unique mutation in the vitamin D receptor (VDR) gene in Korea.
Subjects & **Methods:** We extracted a small amount of mRNA and genomic DNA from the blood of the patient and her parents. Their cDNA sequencing analyses were done with 3 primer sets including whole coding regions of cDNA. Their genomic DNA sequencing analyses were done with 11 primer sets including whole coding regions and non-cording regions, including exon/intron splice sites of genomic DNA.
Results: She had a short stature, curved forearms and legs but didn't have any type of alopecia. Her initial serum level of calcium (7.2 mg/dL) was below normal, the levels of phosphorus (3.3 mg/dL) and 25-OH Vitamin D (31.5 ng/mL) were within normal ranges, but those of alkaline phosphatase (1545 IU/L), 1,25-(OH)2 Vitamin D3 (165 pg/mL) and parathyroid hormone (1197 pg/mL) were above normal. The X-ray findings of her wrists and ankles were compatible with rickets. In molecular studies, we found a 719 C-to-T transition (Ile146Thr) in Exon 4 of her and her father's VDR gene, and a 754 C-to-T transition (Arg154Cys) in Exon 5 of her and her mother's one. In this familial study, we concluded that the girl has two compound heterozygous mutations in her VDR gene which caused VDDR-II.

PO3-048 Bone, Calcium III

Paediatric DXA interpretation – applying international guidelines

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Background
Paediatric dual x-ray absorptiometry (DXA) is most commonly used to measure bone mineral density (BMD). BMD results are presented as an age-matched Z-score and are used to determine treatment needs and outcomes with pharmacologic agents such as bisphosphonates, sex hormones and growth hormone. Current international best practice advises that BMD results be adjusted for patient height. A number of adjustment methods have been postulated. Apparent bone mineral density (BMAD) uses vertebral width to mathematically approximate vertebral volume and thus height.
Methods
The BMD results of 66 children scanned over a 2 year period using a single GE Lunar Prodigy DXA machine were reviewed. The patients studied either belonged to a small for gestational age (SGA) study group (n=19) or had cystic fibrosis (n=47). BMAD was calculated for each patient and compared to a published set of age- and sex-matched reference data. The original Z-score (calculated from BMD) and the height-adjusted Z-score (Zh) (calculated from BMAD) were compared by applying Student's t-test.
Results
Zh-scores differed significantly from Z-scores in both groups studied. In the SGA group, the mean Z-score was -1.1 and the mean Zh-score was 0.1; p=0.000. In the CF group, the mean Z-score was -1.3 and the mean Zh-score was -0.4; p=0.002. Overall, the mean Z-score was -1.1 and the mean Zh-score

was -0.2; p=0.000.

Conclusion

International guidelines state that paediatric DXA results should be modified for height to avoid misdiagnosis of osteopenia or osteoporosis. Available software for paediatric DXA interpretation using the GE Lunar Prodigy machine does not adjust for height. We have shown that adjusting BMD for patient height using BMAD significantly alters results and moves many patients from below the mean to the normal range, therefore potentially altering treatment plans. A software programme to make the required modifications is under development locally.

PO3-049 Bone, Calcium III

Mucolipidosis II presenting as neonatal hyperparathyroidism

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Introduction: Mucolipidosis type II (ML II), a disorder of lysosomal enzyme transport, includes mucopolysaccharidosis type I (Hurler syndrome)-like features and dysostosis multiplex, usually apparent after 6 mo. of age. We describe here a ML II case presenting as neonatal hyperparathyroidism.

Case report: He was born at 34 ws gestation following an uneventful twin pregnancy. Mother was a 25-year-old G2 P1A0 woman who had one healthy daughter. Birthweight was 1.730 kg (10-25th centile), birth length of 43 cm (25th centile) and a OFC of 29,5 cm (10th centile). The left leg was bent and foreshortened. X-rays showed generalised osteopenia, irregular and osteopenic ribs. Femuri and tibiae were angulated and fracture of distal tibial epiphisys. There was an appearance of ''bone cloaked in bone'' and expanded metaphyses. Serum calcium was 8,7 mg/dl mmol/l (reference range 8,45 mg/dl) and the ionised calcium 4,53 mg/dl (reference range 3,8- 4,9 mg/dl). The phosphate was 5,08 mg/dl (reference range 2,7-4,5 mg/dl), and the ALP was 1042 U/l (reference range 40-129 IU/l). Serum PTH was also elevated, measuring 260 pg/ml (reference range 14-65 pg/ml). Serum 1,25-dihydroxyvitamin (vit D3) was 103 pg/ml (reference range14-65 pg/ml). Renal function parameters and calciuria were into normal range. He was treated with vitamin D and calcium supplementation. His metabolic bone disease improved biochemically. At 1,5 months he suffered a septic shock and died. The diagnosis of ML II had been confirmed by assay of enzymes in serum: alphafucosidase 148,50 nmol/min per mL (12,99 nmol/min/mL in control), alphamanosidase 72,28 nmol/min per mL (0,48 nmol/min/mL in control), total β hexosaminidase 513 nmol/min per ml (48 nmol/min per mL in control). Enzyme activities in fibroblasts showed a drastic reduction in substrate hydrolysed compared to controls. The presenting feature of our patient was bone disease with increased serum PTH and ALP activity, but normal serum calcium concentrations.

Conclusions: Recognising ML II as a possible cause of neonatal hyperparathyroidism is important because the overall prognosis is determined by the underlying lysosomal disorder. Neonatal hyperparathyroidism in ML II is severe, transient, and probably secondary to impaired placental calcium transport. Clinicians should be aware that when a fetus or infant presents with shortened and bowed limbs and osteopenia, hyperparathyroidism secondary to severe ML II is a diagnostic possibility.

PO3-050 Bone, Calcium III

Novel treatment for neonatal severe hyperparathyroidism: a new use for cinacalcet

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Introduction: Neonatal severe hyperparathyroidism (NSHPT) is a rare condition caused by an inactivating mutation of the calcium-sensing receptor (CaSR). This results in an increase in the set-point for maintaining normal serum calcium, with subsequent hypercalcemia and hyperparathyroidism (HPT). Most cases are caused by homozygous mutations, inherited from parents with the heterozygous form of the disease, familial hypocalciuric hypercalcemia (FHH). Classically, NSHPT is characterized by failure-to-thrive, respiratory distress, and fractures. A less severe form of NSHPT has been reported in infants with a heterozygous mutation of the CaSR born to normocalcemic mothers. The usual treatment for NSHPT is parathyroidectomy. Cinacalcet, a calcimimetic agent approved for treatment of secondary HPT, has been used in adults with FHH. Herein, we present the case of an infant with NSHPT, successfully treated with Cinacalcet. Case: A female infant born at 35-wk gestation presented at 1 wk of age with decreased oral intake, elevated serum levels of ionized Ca (iCa) 6.7 mg/dL (4.5-5.3), PTH 663 pg/mL (10-71), Mg 2.4 mg/dL (1.6-2.3), and alk phos 355 U/L (80-270); normal Phos 4.8 mg/dL (4.5-6.7); and undetectable urine Ca:Cr ratio <0.05 mg/mg. A skeletal survey showed diffuse osteopenia with trabecular changes, but no fractures. Treatment with IV fluids and furosemide was initiated, but hypercalcemia, HPT, and low urine Ca persisted. Pamidronate 0.5 mg/kg IV was given at 2 wk of age resulting in hypocalcemia (iCa 3.7 mg/dL) and further elevation in PTH (1261 pg/mL) after 4 d. Hypercalcemia reoccurred within 1 wk. At 3 wk of age, she was given an oral dose of Cinacalcet (20 mg/m^2). Prior to the dose, her PTH was 1091 pg/mL; at 2 hr, 97.7 pg/mL, and, at 24 hr, 568 pg/mL. Serum iCa pre-treatment was 6.5 mg/dL, reached a nadir of 4.8 mg/dL at 10 hr, and then rose to 5.4 mg/dL at 24 hr. Cinacalcet (20 mg/m^2 PO q24 hr) was continued and her iCa remained normal (4.6 – 5.1 mg/dL) for 6 d prior to discharge. The parents' serum calcium levels were normal. Conclusion: We present the case of an infant with a less severe form of NSHPT born to unaffected parents. We presume that she has a heterozygous form of disease, presenting as NSHPT secondary to maternal normocalcemia. In the short term, Cinacalcet decreased PTH secretion and normalized serum calcium levels, preventing the need for immediate parathyroidectomy, and with no side effects to date.

PO3-051 Bone, Calcium III

A prospective study of fibroblast growth factor-23 in relation to markers of bone turnover and bone mineral density in children with chronic kidney disease

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Fibroblast growth factor-23 (FGF-23) is a novel regulator of phosphate metabolism. Children with chronic kidney disease (CKD) are at risk of developing bone and mineral problems. This prospective study over 2 years investigated the development of bone mass and bone turnover in relation to serum fibroblast growth factor-23 (FGF-23) in children with CKD. Thirteen patients, 4–15 years, were included with a median corrected glomerular filtration rate of 39 (range 12–74) mL/min/1.73 m^2. The median serum FGF-23 levels for the investigated children with CKD were, at baseline, 127 RU/mL, and at follow-up 70 RU/mL. Two patients had very high FGF-23 values (1333 and 1700 RU/mL) and a characteristic feature for these patients was that they both had end-stage renal disease. No obvious correlation with age or puberty was found for FGF-23. FGF-23 was inversely correlated with GFR, thus, the lower GFR the higher FGF-23. FGF-23 was positively correlated to PTH. No correlation was found between bone markers and FGF-23 except for osteocalcin. Total body BMD was not correlated at baseline or follow-up, however, the lumber spine Z-score correlated with FGF-23 at baseline. This study contributes to the understanding of the role of FGF-23 in the pathogenesis of pediatric mineral bone disorder in pediatric patients with CKD.

PO3-052 Bone, Calcium III

KDEL ER-retention signal is essential for prolyl 3-hydroxylase 1; a novel compound heterozygous mutations in *LEPRE1* causes osteogenesis imperfecta type III

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[Introduction] Ostegenesis imperfecta(OI) is characterized by fragile bones and caused, in majority of cases, by heterozygous mutations in *COL1A1 or COL1A2*. Recently, leucine and proline enriched proteoglycan1(*LEPRE1*) and cartilage-associated protein(*CRTAP)* have been shown to be the responsible genes of lethal recessive form of OI. *LEPRE1* encodes prolyl 3-hydroxylase 1(P3H1), which provides the enzymatic activity for the modification in endoplasmic reticulum(ER) and has KDEL ER-retention signal at the carboxyl terminus. Here we present a sibling with OI type III due to a compound heterozygous mutations in *LEPRE1*.

[Case report] Patient 1 was a 3-yr-old girl from nonconsanguineous healthy Japanese couple. She was born with multiple fractures at 35w gestation by caesarian section. Birth weight and length were blow 3rd percentile. Patient 2 was the couple's next child and electively terminated. Both patients were diagnosed as OI radiologically.

[Materials and Methods] All coding exons and surrounding intronic regions of *LEPRE1* were amplified from genomic DNA by PCR, followed by direct sequencing. Total RNA was extracted from skin fibroblasts of patient 2 and cDNA of *LEPRE1* was amplified by PCR, followed by direct sequencing. Real-time quantitative PCR was performed using cDNA.

[Results] Identification of *LEPRE1* mutations; Both patients were found to be a compound heterozygote for two frameshift mutations(c.484delG p.A162LfsX22 and c.2155dupC p.E719RfsX11). Their father carried c.484delG and mother did c.2155dupC. These mutations were not found in 100 control alleles. No mutations were found in *CRTAP, PPIB, and COL1A1*. RNA analysis and real-time PCR of *LEPRE1*; Only the allele with c.2155 dupC was successfully amplified and sequenced. Real-Time PCR revealed that the transcript of patient 2 was about one-half the control level.

[Discussion] Our study shows the KDEL ER-retention signal is essential for P3H1 *in vivo* for the first time. All *LEPRE1* mutations previously reported results in premature stop codon and mRNA of them are destroyed by the process of nonsense mediated decay (NMD). From the results of RNA analysis, c.484delG allelic variant leads to NMD, whereas c.2155dupC dose not. The product from c.2155dupC allelic variant can not stay in ER because it lacks KDEL ER-retension signal, while other functional domains are intact.

PO3-053 Bone, Calcium III

The effect of alendronate treatment on glucocorticoid induced osteoporosis in children

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OBJECTIVE: Glucocorticoid-induced Osteoporosis (GIOP) is the most popular type of osteoporosis in childhood. And efficacy and safety of the use of oral bisphosphonate treatment have been established in adult area. But few randomized control trials have been published yet and its efficacy and safety are still controversial in growing children. In this study, we evaluated the effect of an oral bisphosphonate (alendronate) on bone mass in children under long-term glucocorticoid treatment. METHODS: Fifty six children aged 5 to 18 years under long-term (over 6months) glucocorticoid treatment (mean daily dose of over 15 mg of predonisolone) were randomly assigned into 2 groups; 30 children(Group A) were treated with alfacalcidol (0.5 mcg/day)and alendronate (5 mg/day), and 26 (Group B) were treated with only alfacalcidol as a control for 2 years. We measured lumbar bone mineral density (LBMD) and metabolic bone markers (NTx and Bone specific ALP) every 6months. RESULTS: Three of group B withdrew before the study medication, thus 53 were used in analysis (30 in group A and 23 in group B). At baseline LBMD was not different between groups. At 24 month, there were significant difference in percent change from baseline between the groups (27.2 ± 17.6% vs 6.9 ± 8.4%, p=0.0007). The Z-scores of LBMD in group A were significantly greater than those in group B (-0.1 vs -1.8 SD). Urinary excretion of NTx decreased by 33.7 % at 6 month while it increased by 9.6 % in group B. Height Z-scores were not different between the groups throughout the study. One of 30 in group A and one of 23 in group B showed new vertebral fractures during the study. No severe adverse events were observed during the study. CONCLUSION: Alendronate treatment in pediatric GIOP showed significant increase in bone mineral density and kept their bone mass. But we failed to show any significant effects on fracture. This may be due to the small scale of the study or less severity of the GIOP, because the major underlying disease in the study was nephritic syndrome. Thus we conclude that alendronate treatment effectively and safely keep or increased bone loss in GIOP but its effect on fracture prevention is still unclear.

PO3-054 Bone, Calcium III

Novel cause of severe hypocalcemia in an adolescent male

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MN is a 15 yr old M who presented to his MD with a 4 wk history of pain, numbness, and tingling in his arms with some discomfort in his legs. Parents thought this was due to a repetitive motion injury since he played computer/video games extensively. He also noticed periodic jerky movements in his feet and that his face twitched if he hit his cheek with his finger. Noting a low Ca, his MD sent him to the ER.

Dietary hx showed he ate primarily meats/desserts. He avoided dairy products and ate few fruits and vegetables. He intermittently took vitamin supplements. He tried not to spend much time outside.

PE: Thin, Caucasian M in NAD; VS-nl; exam remarkable for positive Chvostek sign, brisk reflexes, clonus, mild bowing of his legs and negative Trousseau sign.

Initial lab data: Ionized Ca 1.57mEq/L; Phos 6.0mg/dl; Alk phos 557 U/L; urinary Ca<2mg/dl.; urinary Phos 18.6mg/dl; Mg 1.7mEq/L; LDH 259 U/L; lipase 199U/L; renal fn nl. QT interval markedly prolonged at 0.5.

Hospital Course: He was transferred to the PICU and treated with IV calcium, ergocalciferol and calcitriol. His calcium very slowly stabilized. Several days later his initial PTH came back at 801pg/ml and his initial 25,Vit D was undetectable at <6ng/ml with a 1,25Vit D of 78pg/ml.

His calcium took 5 days to normalize. Calcitriol was stopped after the initial 25VitD and PTH levels were back. His hospital course was otherwise uneventful. He went home on oral Ca and Vit D.

Initial /Subsequent Lab Data

Date	ionized Ca (nl 2.4 - 2.76 mEq/L))	Ca (nl 8.4-10.2mg/dL))	Phos (nl 4.5-5.5 ng/dL)	25, Vit D (nl 25-80 ng/dL)	1,25 Vit D (nl 18-64 pg/mL)	PTH (nl 10-65 pg/mL)
3/14/09	1.57	6.9	6.0	<6.0	78	801
3/15	1.86					
3/17	2.26		7.0			
3/18	2.31	9.7	5.5	41		71
3/19	2.38		6.7			
3/24	2.46	9.4	5.7	76	179	204
4/10		9.9	5.4	53	184	34

Discussion: This appears to be the first reported case of severe hypocalcemia caused by the lifestyle choice of playing computer/video games so extensively that the child had such limited sunlight exposure that, when combined with limited dietary Vit D intake, his calcium levels went so low that he had potential tetany. Compensatory abnormalities in PTH and phosphorous levels were also apparent.

MN proudly stated that he chose this lifestyle to become a master at the computer game World of Warfare (abbreviated WOW) which is played by millions around the world. We suggest this is the first reported case of "WOW Rickets".

PO3-055 Bone, Calcium III

Methylation defects of exon A/B of GNAS can be associated with all subgroups of pseudohypoparathyroidism type I

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Background: Pseudohypoparathyroidism (PHP), defined by end-organ resistance toward parathyroid hormone, is historically classified into subgroups based on the presence of further clinical abnormalities, Gsalpha protein activity in erythrocyte membranes, molecular genetic findings and epigenetic alterations. Individuals with PHPIa present with features of Albright hereditary Osteodystrophy (AHO) and deficiency of Gsalpha, mainly caused by mutations of the GNAS gene. PHPIc patients also present with AHO signs, but Gsalpha activity is normal. PHPIb patients lack AHO features and present normal Gsalpha activity, but show mutations in regulatory elements of GNAS, leading to a loss of methylation on maternal Exon A/B. Recently, a loss of maternal exon A/B methylation has also been described in some patients with the clinical diagnosis of PHPIa. Methods: We investigated the methylation pattern of a CpG island 3´ of Exon A/B by bisulfid conversion and EpiTectTM sequencing (Qiagen) in 25 PHPIa, 8 PPHP, and 2 PHPIc patients. These patients, initially characterized by clinical AHO signs and Gsalpha activity measurement, were without a mutation in exons 1-13 of GNAS. Two PHPIb patients were used as positive controls. Results: We found a loss of Exon A/B methylation in 4 patients with PHPIa, one with PHPIc and our positive controls. We did not find a correlation between Gsalpha activity and methylation deficiency. Conclusions: Exon A/B methylation deficiency may be a modulator of Gsalpha activity and thus contribute to AHO as well as to all forms of PHP I.

PO3-056 Bone, Calcium III

Odontohypophosphatasia associated with a missesnse mutation of the tissue-nonspecific alkaline phosphatase (TNSALP) gene

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Background: Hypophosphatasia is a disorder of bone mineralization caused by a deficiency of tissue-nonspecific alkaline phosphatase and is categorized into perinatal, infantile, childhood, and adult forms. Odontohypophosphatasia is the mildest form of the disease in which biochemical and dental changes (usually premature exfoliation) are not accompanied by skeletal manifestations. While the mode of inheritance of severe forms of this disease is autosomal recessive, the mode of inheritance of less severe forms, including odontohypophosphatasia, is unclear. We describe a case of a young child with odontohypophosphatasia who has a genetic mutation which has only been previously described with the severe perinatal form.
Clinical case: A 2-year-old girl who developed primary teeth at the age of 6 months presented to us with the loss of a mandibular central incisor at 14 months of age without a history of trauma. She had stable growth with a height at the 50-75th percentile. She had no history of fractures or bone pain and on physical exam did not have any skeletal deformities including bowing of her legs.
Initial evaluation revealed a low alkaline phosphatase (ALPL) of 38 U/L. Serum calcium, phosphorus, and Vitamin D levels were within normal limits. Dental examination revealed bone loss around the roots of the adjacent mandibular and maxillary incisors. Left knee and wrist X-rays were negative for any abnormality or rachitic changes. Vitamin B6 level was elevated at >100 ng/mL (normal range 5-30 ng/mL), which is consistent with hypophosphatasia. DNA sequencing of the TNSALP gene revealed a heterozygous mutation in exon 4 (c.212G>A), converting arginine for histidine (Connective Tissue Gene Tests). The patient's mother was also found to have the same mutation in conjunction with a low ALPL of 18 U/L and a history of poor dentition. The father did not have any mutations. Our subject continued to lose teeth despite careful dental follow-up, and by the age of 27 months had already lost a total of 4 primary teeth.
Conclusion: A previous report by Taillandier, et. al., noted this same change as one of two TNSALP mutations in a patient with severe perinatal hypophosphatasia. To our knowledge, this is the first reported case of odontohypophosphatasia due to the heterozygous c.212G>A mutation. This case may represent the phenotypic variability of this particular mutation and further studies will be necessary to elucidate the exact phenotype associated with this mutation.

PO3-057 Bone, Calcium III

Bone disease in thalassemia: the diverse role of ineffective erythropoiesis and iron overload – lessons from mouse models

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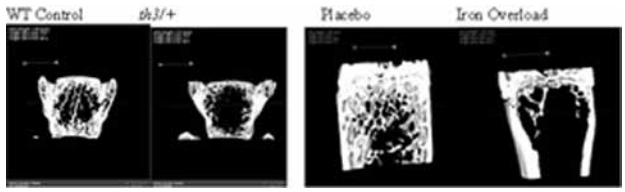
Background: Low bone mass is a frequent problems in children and adults with thalassemia. Both ineffective erythropoiesis and iron overaload are implicated in its pathogenegis, however, their exact role is unclear. To better understand the role of erythropoiesis on the bone, we studied the *th3/+* mouse model of beta thalassemia. Since this mouse is not iron overloaded, we created a model of iron overload (IO) by treating WT BL6 mice with iron dextran (1g/kg/week IM x 2mo). This resulted in increased liver and spleen iron content in the treated animals compared to placebo.
Methods: *Th3/+* and IO mice and their controls (n≥6 per group) were evaluated. Trabecular and cortical bone changes were studied by micro-CT. Bone turnover was assessed by i) histomorphometry, ii) staining for TRAP (specific to osteoclasts) and procollagen I (specific to osteoblasts) iii) RT-PCR on bone extracts for genes involved in bone turnover. Serum levels of TNFa and IGF1 were measured.
Results: Compared to controls i) both *th3/+* and IO had trabecular and cortical bone changes by microCT (Figure 1). ii) Bone resorption was decreased in the *th3/+* mouse and increased in the IO. iii) Serum TNFa was increased in both *th3/+* and IO animals. TNFa concentrations were higher in the IO animals. iv) Serum IFG1 was decreased in *th3/+* animals and unchanged in IO.
Conclusions: Iron overload in mice results in significant bone abnormalities associated with increased bone resorption and high serum TNFa concentrations. Ineffective erythropoiesis of thalassemia in *th3/+* mice also results in bone abnormalities. However, the bone changes in the *th3/+* mouse are associated decreased resorption and decreased serum IGF1 concentration. These results suggest that both iron overload and ineffective erythropoiesis are involved in the development of osteoporosis in thalassemia through different mechanisms. Studies of iron overload in the thalassemia mouse are underway.



PO3-058 Bone, Calcium III

Primary care providers' knowledge and clinical practices in screening and prevention of low bone mass in children and the impact of a practice-based pilot educational program: bone bank

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Pediatric primary care providers (PCPs) may not uniformly recognize the value of supporting their young patients to maximize their bone mass during their bone-forming years. Achieving an adequate peak bone mass is known to minimize risks and delay the onset of symptomatic osteoporosis later on in life.
To assess the knowledge, counseling, and referral activities of Connecticut's (CT) PCPs in the screening and prevention of low bone mass in children, a multiple choice questionnaire was sent to 500 PCPs, with a response rate of 38

% (n=190). 78.4 % (n=149) screened calcium (Ca) intake, whereas only 32.1 % (n=61) assessed vitamin D intake. 91% of PCPs assessed type/frequency of exercise. PCPs were asked to correctly identify the recommended daily dietary Ca intake in children ages 4 -8 years and 9-18 years, with correct response rates of 42.6% and 55.8 % respectively. Only 27.4% (n=52) of respondents were aware of the high risk groups of children for low bone mass that require referral to an endocrinologist for evaluation.
These results indicate a need for educational programs for PCPs to improve their knowledge and practice activities to enhance bone health in youth. Recent evidence indicates that educating PCPs within their practice setting has a greater impact than traditional CME lectures.
We therefore invited those PCPs with an interest in learning more about bone health to participate in a practice-based CME pilot: the "Bone Bank." 9 PCPs entered the pilot which included a follow up post CME survey.100% of participating PCPs were satisfied with the format of the Bone Bank CME and found the educational materials useful. There was a trend towards more consistent assessment of Ca intake (78.4% pre versus 100% post) and of Vitamin D intake (32.1% pre versus 66.7% post pilot). The correct knowledge of the recommended daily dietary Ca intake improved to 78% for both age groups. The increase in awareness of need for endocrine referral for targeted high risk groups was statistically significant with an increase to 78% (p=0. 005).No improvement in the assessment of exercise frequency/type was observed. Although our sample of CT PCPs acknowledge osteoporosis prevention as an important pediatric issue, their knowledge of bone health preventive measures and referral guidelines is suboptimal. Practice-based educational activities such as the "Bone Bank" has the potential to improve knowledge and impact practice behavior as it relates to bone health in youth.

PO3-059 Bone, Calcium III

Hearing assessment in a cohort of children with osteogenesis imperfecta who have been treated with bisphosphonates

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Background: Osteogenesis imperfecta (OI), an inherited disorder of Type 1 collagens is complicated by progressive hearing loss. The natural history of hearing loss in OI is significantly altered by bisphosphonate treatment. There has been no previous systematic documentation of possible effects of bisphosphonates on hearing function in OI.
Of most concern and chronicity in adults with OI is hearing loss, with consequent social isolation. Reduced hearing in the first decade hinders speech and language development. Increasing second decade loss has a pervasive negative impact on learning and psychosocial development. Prevalence of hearing loss in OI is reported as 34 -78% in all age groups, with progressive loss, usually beginning in the 2nd to 4th decade. Hearing loss is reported at 11-31% in children aged 4 – 9.
Aims: to evaluate hearing function of a cohort of mainly paediatric patients with type III or IV OI, treated with bisphosphonate for more than 2 years.
Methods: observational study undertaking hearing assessments for all patients or retrieving results if they had been performed in the 12 months preceding the study.
Results: 44 subjects had audiological assessment, 81.8% aged <20 years. No child or adolescent < 19 years had persistent hearing loss. 3 subjects aged 20 – 29 years had significant hearing loss but these had only commenced treatment with bisphosphonate at ages 9-18 years.
Conclusions: Bisphosphonate treatment of severe OI reduces the risk of hearing loss in childhood and adolescence.

PO3-060 Bone, Calcium III

Hearing loss in osteogenesis imperfecta is reduced after bisphosphonate treatment

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Background: Osteogenesis imperfecta (OI), an inherited disorder of Type 1 collagens is complicated by progressive hearing loss. The natural history of hearing loss in OI is significantly altered by bisphosphonate treatment. There has been no previous systematic documentation of possible effects of bisphosphonates on hearing function in OI.
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PO3-061 Disorders of Sexual Differentiation (DSD) II

How could the identification of heterozygous *NR5A1* mutations in 46, XY DSD patients, their mothers, having or not primary ovarian insufficiency, but also in their fathers, be explained?

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During the last 10 years, mutations of *NR5A1* have been identified in an increasing number of patients with isolated 46, XY DSD. Our initial strategy to study *NR5A1* was to screen a cohort of 46, XY DSD patients (119) with testicular dysgenesis attested by low stimulated testosterone and AMH<200 pmol/L. Twenty height heterozygous mutations have been identified in a group of 30 patients (28 families) with severe DSD ranging from complete female phenotype (3) to severe hypospadias with micropenis. As one of the two affected children from the same family had hormonal pattern at birth compatible with PAIS (normal stimulated testosterone and normal AMH), *NR5A1* has been sequenced in 57 "PAIS" patients with normal testicular functions and no *AR* mutation. No mutation has been identified in this group. The parents were studied when possible (23 families) especially since, in our family with two affected children, the mother was heterozygous for the *NR5A1* mutation. In 7 families the mothers were heterozygous. All had normal puberty and menarche, and spontaneous pregnancies without any treatment between 20 and 37 years old. These clinical data thus suggested normal ovarian development and function. However, two of these mothers, aged of 28 and 30 years, had hormonal pattern suggesting primary ovarian insufficiency, attested by elevated gonadotropins and low AMH. Moreover, we identified a *NR5A1* mutation in another woman, with sporadic primary ovarian insufficiency. Taken together, these data

suggested that heterozygous mutations of *NR5A1* impair more testis than ovary and that SF1 is essential for maintenance of the testicular but also the ovarian functions. Nevertheless, a recent and unexpected result questioned the role of *NR5A1* haploinsufficiency. Indeed, we found two fathers heterozygous for the mutations of their 46, XY DSD children, one with the nonsense mutation, p.Q316X, the other with the p.G123A and the p.P129L missense mutations, the latest being *in vitro* deleterious. Could these results be explained by a variable penetrance, and, if not, is *NR5A1* haploinsufficiency really enough to cause 46, XY DSD and primary ovarian insufficiency?

PO3-062 Disorders of Sexual Differentiation (DSD) II

45,X/46,XY post-pubertal patients born with ambiguous genitalia: clinical characteristics, outcome and genetic specificities in a series of 54 patients

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Gender assignment is a difficult problem in 45,X/46,XY neonates born with ambiguous genitalia, considering the paucity of large clinical studies. We report a retrospective study of 54 patients with 45,X/46,XY mixed gonadal dysgenesis associated with ambiguous genitalia. Mean age at study was 25 ± 1.3 years. 33 patients were raised as girls and 21 as boys. At birth, 73 % presented with asymmetrical external genitalia, 92 % with perineoscrotal hypospadias (versus penile hypospadias), and 84 % with one visible orifice. Mean genital tubercle was 21± 1.5 mm length. This phenotype was associated with the presence of a hemi-uterus on the streak gonad side and a controlateral dysgenetic testis in the majority of the cases. Forty-eight percent of the patients were born with an in utero growth retardation and 73 % developed features of Turner syndrome (dysmorphy, kidney and cardiac malformations in majority). Puberty was initially spontaneous in all except three boys. A testosterone treatment had to be added in 4 other patients during puberty, while the others actually have a decrease in inhibin B and testosterone levels and an increase in FSH levels. Two boys developed testicular cancer. Puberty was hormonally induced in all girls. The mean pubertal peak was 11.5 ± 1.6 cm (boys), and 11.8 ± 1 cm (girls). Final height was not statistically different between boys and girls (156.9 ± 2 vs 154.1±1.3 cm) and was independent of growth hormone treatment and parents' height. Finally, both boys and girls had gone through the same average number of genital surgeries (3.5± 0.4 vs 3.1± 0.2, respectively).
Actually, half of the patients are satisfied with their physical appearance and only 4 patients (1 man and 3 women) among the patients of more than 20 years old (34 patients, mean age 30 ± 1.5 years), have a stable affective life. No child has been born from this cohort of patients yet.
The prospective analysis of the Y chromosome in 39 patients revealed an abnormal structure or a microdeletion in the AZF region in 59 % of the cases.
This first large cohort study of post pubertal 45,X/46,XY mixed gonadal dysgenetic patients brings new insights into the clinical evolution and the genetic origin of this disorder and might therefore help clinicians in sex decision at birth.

PO3-063 Disorders of Sexual Differentiation (DSD) II

Psychological follow-up of adolescent and young adult offspring from CAH-risk pregnancies treated with dexamethasone

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Rationale: Females affected with classical congenital adrenal hyperplasia (CAH) are born with variably masculinized genitalia and behavior, and therefore at increased risk of assignment to the male gender, genital surgery, and later gender change to male. Dexamethasone (DEX) treatment of CAH-risk pregnancies has been introduced to reduce genital masculinization, but is currently highly controversial because high-dose animal studies suggest potential adverse side effects on brain development and memory (plus blood pressure and glucose regulation), and some small-scale human studies have suggested that prenatal DEX may affect temperament and induce behavior and cognitive problems.
Objective: To compare prenatally DEX-exposed and -unexposed adolescents and young adults in regard to gender outcome, behavior problems, educational outcome, and neuropsychological function.
Method: Participants were recruited from two regional clinics providing or consulting on such treatment, one each in the Eastern US and in central France. A battery of 6 standard questionnaires (in English or French, respectively) was administered by mail. A subset of participants (U.S. only) underwent a battery of 3 neuropsychological tests. Statistical analysis involved comparison of DEX-exposed and -unexposed participants by multiple regression controlling for age. (The samples did not differ in parental educational level.)
Results: N=37 DEX-exposed (16 males, 21 females; 12 CAH) and 30 DEX-unexposed individuals (16 males, 14 females; 20 CAH) completed questionnaires, and N=7 DEX-exposed and N=7 DEX-unexposed individuals underwent testing to date. Gender outcomes showed 5 significant differences (inconsistent in direction) among 48 comparisons (separately performed for CAH and nonCAH participants, and females and males) on 12 scales. DEX-exposed and -unexposed participants did not differ on any of the 8 behavior problem scales or 3 summary scales of the problem-behavior questionnaire, nor in educational outcome. There was also no difference or even statistical trend indicating a neuropsychological deficit on the 6 test scales in the DEX-exposed group.
Conclusion: The data from this long-term follow-up study do not show a suppression of behavioral masculinization by nor any adverse psychological consequences of prenatal DEX exposure. However, additional samples are desirable for more definitive conclusions.

PO3-064 Disorders of Sexual Differentiation (DSD) II

Two novel androgen receptor gene mutations in two families with complete androgen insensitivity syndrome

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[Background] Mutations in androgen receptor (AR) gene can manifest a wide range of clinical phenotypes, demonstrating different degree of androgen insensitivity. Complete androgen insensitivity syndrome (CAIS) is the most severe form, presenting complete female external genitalia and female psycho-sexual orientation, although subjects with CAIS have a 46, XY karyotype, testes and

normal-to-elevated plasma level of testosterone. AR protein harbors three major domains, which are the N-terminal transactivation domain (NTD) encoded by exon1, the DNA-binding domain (DBD) encoded by exon2 and 3, and the C-terminal ligand-binding domain (LBD). Until now more than 300 different AR gene mutations have been described. More than half of the AR mutations are located in the LBD, about 20% are located in the DBD, and less than 15% are found in the NTD although it covers a major part of the AR. We identified two novel AR gene mutations in two families with CAIS.
[Family 1] The sisters presented with amenorrhea at 17 years and 15 years of age, respectively. They had normal female external genitalia. Older sister had past history of operation for right inguinal hernia. Tanner stage were B5, PH3 in older sister, and B4, PH2 in younger sister, respectively. Karyotype was 46, XY and serum testosterone concentration was normal male level in both. Magnetic resonance imaging (MRI) revealed a testis at left side of pelvic cavity in older sister and testes at both side of pelvic cavity in younger sister.
[Family 2] The 16 year-old-female presented with amenorrhea as a chief complaint despite of normal breast development. She was born with normal female external genitalia, and operation of bilateral inguinal hernia was performed at 1 year of age. She exhibited slight clitoromegaly. Karyotype was 46, XY and serum testosterone concentration was normal male level. MRI revealed bilateral testes in inguinal region.
[AR gene analysis] DNA sequencing identified the two novel hemizygous mutations: c.1307_1343del, p.F437Vfs29X of exon1 in family 1, and c.1710C>A, p.H570Q of exon2 in family 2.
[Discussion] The novel deletion identified in family 1 leads to a frameshift with loss of the DBD and LBD, thus this predicted truncated AR protein can induce loss of function. Another new mutation of p.H570Q is located in Zn finger domain important for DNA binding, thus can be considered to be a disease-causing mutation.

PO3-065 Disorders of Sexual Differentiation (DSD) II

Long-term psychological gender evaluation of 4 XY individuals with complete androgen insensitivity syndrome

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Introduction: It is known that the concepts of sex and gender is crucial with CAIS patients because the term sex usually is based on physical attributes, while the concept of gender is based on an individual's self-concept and self-identification as well as the role that an individual assumes in the society. Previous findings described that most patients with complete androgen insensitivity syndrome (CAIS) have a female gender. This may be due, in part, to the patient's role assignment. The gender expression have been scarcely studied during adolescence and adult age considering their doubts about sex attributes. The aim of this study was to evaluate psychological outcomes and gender development in 4 women with CAIS after at least 2 years of intensive psychotherapy.
Patients and methods: Four women aged 17–22 yrs have been evaluated for any psychological outcome and sexual function. Outcome measures included quality of life (psychological general well-being and emotional representations of illness), gender-related psychological characteristics (gender identity, sexual orientation, and gender role behaviour in childhood and adulthood), character traits that show sex differences. Intensive psychotherapy were developed for two years.
Results: In two of our patients the sense of identification as female was not conform to their sexual orientation. All patients but one have never been involved with boyfriend and were sexually non-active. Only one experienced orgasm through clitoral stimulation while the experience of penetration was limited because painful. Emotional reactions indicated that they had suffered from being misinformed about CAIS in their childhood. General well-being of the patients is impaired, even after psychotherapy. The patients show a high fear for long life consequences of their illness and adaptation process is on going. Psychological intervention may have a beneficial effects supporting the evolutive process of these patients and may contributes to ameliorate their self-esteem and their social competencies.
Conclusions: CAIS patients need to achieve their own gender despite any constrain for sex assignment and genital adjustment. Psychological support of parents and intensive psychotherapy from infancy to adulthood is necessary.

PO3-066 Disorders of Sexual Differentiation (DSD) II

A new luteinizing hormone receptor inactivating mutation with only a micropenis

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Background
Inactivating mutation in the luteinizing hormone receptor (LHR) is a rare autosomal recessive disorder causing mostly 46 XY Disorders of Sex Development (DSD) with female phenotype. Less severe LHR mutations caused milder phenotypes. Only 5 cases of micropenis have been described in the literature due to LHR mutations (1,2,3).We describe a newborn with a micropenis whom resistance to continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone revealed the diagnosis of LHR mutation.
Clinical Case
The patient was a 3 months old boy, born to consanguineous parents. Penile length was 10 mm at birth with normal urethral opening and intra-scrotal testis. Basal testosterone was < 0.25 nmol/l before and after hCG stimulation. Basal LH and FSH were 2.6 UI/l and 5 UI/l. Serum anti-Müllerian hormone (AMH) was 1202 pmol/l and inhibin B 177 pg/ml. Because he was considered as congenital hypogonadotropic hypogonadism he received a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone (4). Mean testicular volume increased from 1 ml to 2 ml and penile length remained < 15 mm. Inhibin B value increased up to 1270 pg/ml, AMH to 1830 pmol/l and testosterone to 0.42 nmol/l. This minimal testosterone response and normal inhibin B/AMH suggested a resistance to the infused LH due to a LHR mutation.
Molecular study
Direct genomic sequencing of the complete LHR gene conding region revealed a new homozygote substitution in exon 11 yielding a Phe630Ser change in the intracellular domain of the receptor.
Conclusion
Inactivating mutation in the LHR can caused only a micropenis, and is probably underestimated.Congenital hypogonadotropic hypogonadism diagnosis can be worn by mistake and the continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone can be a diagnostic test, to diagnose LHR mutations, probably underestimated.
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PO3-067 Disorders of Sexual Differentiation (DSD) II

Functional and structural characterization of seven novel mutations in the *CYP11B1* gene – four mutations associated with non-classic and three mutations causing classic 11β-hydroxylase deficiency

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Steroid 11β-hydroxylase (CYP11B1) deficiency (11OHD) is the second most common form of congenital adrenal hyperplasia (CAH). Cases of non-classic 11OHD are rare compared with the incidence of non-classic 21-hydroxylase deficiency. To date, only three *CYP11B1* mutations associated with mild 11β-hydroxylase deficiency have been described. We have analyzed the functional consequences of seven novel *CYP11B1* mutations (p.M88I, p.W116G, p.P159L, p.A165D, p.K254_A259del, p.R366C, p.T401A). These mutations were found in three patients with classic 11OHD, two patients with non-classic 11OHD and two heterozygous carriers for *CYP11B1* mutations. The functional studies employed a COS7 cell *in vitro* expression system comparing wild-type (WT) and mutant CYP11B1 activity. Mutants were examined in a computational three-dimensional model of the CYP11B1 protein. The activity assays showed that all mutations (p.W116G, p.A165D, p.K254_A259del) found in patients with classic 11OHD retain only 0-3% of 11β-hydroxylase activity relative to wild-type. The mutations detected in patients with non-classic 11OHD showed partial functional impairment with one patient being homozygous (p.P159L; 40% of WT) and the other patient compound heterozygous for a novel mild p.M88I (70% of WT) and the known severe p.R383Q mutation. The two mutations detected in heterozygous carriers, p.R366C and p.T401A, also reduced CYP11B1 activity by 38 and 60%, respectively. The *in silico* analysis using our three-dimensional CYP11B1 protein model showed that the mutant p.W116G affects the substrate access channel and p.K254_A259del leads to a major structural change. No obvious severe impairment was detected analyzing the p.A165D mutation. All mutations resulting in mild CYP11B1 functional impairment had only minor effects on structure consistent with the *in vitro and in vivo* findings. The results of the functional analysis allow for the classification of novel *CYP11B1* mutations as causative for classic and non-classic 11OHD, respectively. Four partially inactivating mutations are predicted to result in non-classic 11OHD. These findings more than double the number of mild CYP11B1 mutations previously described as associated with non-classic 11OHD. Our data are important to predict phenotypic expression and provide important information for clinical and genetic counselling in 11OHD.

PO3-068 Disorders of Sexual Differentiation (DSD) II

Combined *in vitro and in silico* analysis of 21-hydroxylase gene mutations is a valuable tool for genetic counselling – analysis of six novel *CYP21A2* sequence variants

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Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency (21OHD) is the commonest inborn error in steroid biosynthesis. It is caused by mutations in the 21-hydroxylase gene (*CYP21A2*), one of the most polymorphic human genes. A good genotype-phenotype correlation exists allowing for a prediction of the expressed adrenal phenotype, with the less severe mutation in compound heterozygous individuals commonly determining the clinical phenotype. Functional *in vitro* analysis of novel *CYP21A2* variations is of paramount importance to allow for correct molecular diagnosis and genetic counselling. We performed the functional and structural analysis of six novel *CYP21A2* variants (c.69G>T, p.W22C; c.553G>A, p.D184N; c.597A>T, p.L198F; c.917T>G, p.V305G; c.931C>T, p.H310N; c.1331C>A, p.T443N). p.H310N was identified in one individual suspected to suffer from 21OHD. p.L198F and p.V305G were found in 2 men related to patients with 21OHD and, the sequence variants p.W22C, p.D184N and p.T443N were identified in 3 individuals from a random sample of the general population from Northwest Spain. The characterisation of these novel variants was performed by yeast microsomal co-expression of wild-type and mutant CYP21A2 and wild-type P450 oxidoreductase and a computational, three-dimensional CYP21A2 protein model. p.W22C and p.D184N reduced wild-type activity to 70-80%, while p.L198F and p.V305G showed enzyme activities similar to the wild-type. Finally, p.H310N and p.T443N reduced 21-hydroxylase activity to 65-70% for the conversion of the two natural substrates. The *in silico* analysis was consistent with the *in vitro* findings. *In vitro* expression analysis revealed that p.L198F and p.V305G are rare allelic variants not associated with a 21OHD phenotype. The activity of variants p.W22C and p.D184N are in a borderline area, which has been associated by others previously with non-classic CAH. Residual enzyme activities of p.H310N and p.T443N are compatible with non-classic 21OHD if homozygous or compound heterozygous. It is unlikely that either of the variants is associated with the expression of a severe classic form of CAH (salt wasting/ simple virilising). Thus, our findings emphasise the importance of CYP21A2 *in vitro* analyses in the molecular diagnosis of 21OHD to facilitate appropriate genetic counselling.

PO3-069 Disorders of Sexual Differentiation (DSD) II

Vanishing testis: a new clinical expression of SF1 gene mutation

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Vanishing testis syndrome encompasses a spectrum of clinical expressions that share a neonatal male phenotype. The steroidogenic factor-1 (SF1) gene regulates multiple genes involved in testis development. Since mutation of this gene has been reported to be associated with various degrees of testis dysgenesis, we hypothesized that vanishing testis could be due to an SF1 gene mutation.

We analysed the SF1 gene in 6 boys with vanishing testis and identified a mutation in a 3-year-old boy referred to our paediatric endocrine unit for bilateral cryptorchidism. Clinical examination revealed a small inguinal testis on both sides, which was confirmed by ultrasonography (length of 11 and 13 mm for the right and left testis, respectively). Bilateral orchidopexy was performed. At 6 years, only the right testis was palpable in the scrotum. A new ultrasonography confirmed the absence of the left testis and the presence of a very small (6 mm) right testis in the scrotum. The karyotype was 46,XY. Hormonal investigations showed basal plasma FSH and LH levels, respectively, at 11UI/l (N<0.5 UI/l) and 0.8 UI/l (N< 0.5 UI/l). Plasma inhibin B and AMH levels were very low <6 pg/ml and 0.5 pmol/l, respectively. HCG stimulation testing did not evidence any testosterone (T) variation (T= 0.2 ng/ml). These investigations suggest some degree of testicular regression secondary to orchidopexy or to testicular dysgenesis.

The sequence of the SF1 gene revealed an R114Q mutation. This gene substitution was not found in 50 control boys, thus excluding an SF1 gene polymorphism. The type of substitution, along with the sequence alignment, strongly suggests the involvement of this mutation in the patient phenotype. Functional analysis is in progress.

In conclusion, we report the first case of vanishing testis due to a new heterozygous SF1 gene mutation. Our data clearly demonstrate that the SF1 gene is involved in both foetal testis determination and postnatal testis maintenance.

PO3-070 Disorders of Sexual Differentiation (DSD) II

Two hypospadias cases in a family with translocation involving chromosomes X and 21

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Background: Robertsonian translocations or reciprocal apparently balanced translocation especially involving autosomes are described in the literature associated with hypospadias with or without other malformation.
Aim: To report a translocation between chromosomes X and 21 in two related children with disorder of sexual development (DSD).
Material and methods: Clinical examination, cytogenetic and hormonal analysis were recorded in our index case, a boy with DSD identified at birth and his uncle with hypospadias.
Results: The index case is the first child of a consanguineous couple. He was born after a normal vaginal delivery, with a birth weight of 3100 g, and a birth length of 50 cm. Clinical examination revealed posterior hypospadias, micropenis, bifid scrotum and palpable bilateral gonad in the inguinal area. Laboratory results at 2 weeks old, with no medication - LH: 2.6 mUI/L, FSH: 0.84 mUI/L, testosterone: 4.38 nmol/L and 17 OH Progesterone: 4.84 nmol/L. Chromosome analysis revealed 46,XY,t(X;21)(p11;q11). At 3 months old, hCG stimulation test had been performed (1500 UI/m2, on day 1 and 3) and the basal results were FSH: 1.6 mUI/L, LH: 3.7 mUI/L, testosterone: 2.74 nmol/L. On day 3, testosterone was 11.41 nmol/L and on day 6, testosterone: 10.82 nmol/L. At 12 months old, orchidopexy and surgical correction of the hypospadias have been performed.
The mother of this boy showed at cytogenetic analysis the same chromosomal rearrangement but no endocrine abnormalities or congenital malformations were found. Her brother (the uncle of our proband), at the first endocrine evaluation at the age of ten years, had penoscrotal hypospadias and undergone surgery for this malformation. His weight was 38 kg, height 149 cm, Tanner stage for pubic hair I and he had a bilateral testicular volume of 6 ml. Before surgery, his hormonal values were: LH: 2.04 mUI/L, FSH: 3.39 mUI/L and testosterone: 2.168 nmol/L. His karyotype was: 46,XY,t(X;21)(p11;q11).
Discussion and conclusion: Although no such association has been reported so far, we conclude that the DSD in these two cases is associated with the translocation (X;21). A detailed molecular characterization of the rearrangement described in our case report is required. This may help identifying the genes and mechanisms behind the aberrant development of the urogenital systems.

PO3-071 Disorders of Sexual Differentiation (DSD) II

A new SF1/NR5A1 mutation, that impairs anti-mullerian hormone (AMH) gene transactivation: transmission from a normal father to his son affected by a disorder of sex development (DSD), mosaicism at play

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Background: SF1/NR5A1gene mutations have first been described in adrenal insufficiency (AI) combined with XY sex reversal, then in XY DSD patients without AI and very recently as a cause ovarian premature failure.
Aim: to understand the possible inheritance from a normal father to his son of a SF1/NR5A1 gene mutation and delineate further the molecular mechanism by which this mutation led to XY DSD.
Patient: The child was born from two non consanguinous parents, with a DSD: scrotal fold were symmetric, well developed with two gonads that could be palpated in the scrotal folds. Penis was buried and angled but of normal length with posterior hypospadias. No mullerian derivatives were documented by ultrasound nor by genitography. No AMH blood levels were available in the neonatal period but a hCG test allowed the testosterone to increase to 2ng/ml, a subnormal response. Surgery was performed to treat the hypospadias and did not visualized mullerian derivatives. Subsequently puberty developed spontaneously at the normal age with a raise in testosterone levels and a normal LH. However FSH levels was above the upper limit of the normal range with a decreased inhibine B level showing the Sertoli cells lesion. During puberty an ACTH test ruled out AI. His father has normal genitalia and fathered a second normal female child.
Molecular studies: Genetic analysis permitted to identify a new SF1/NR5A1 mutation p.Arg281Pro (c.842G>C). This mutation was not found in 100 control DNAs. Arginine residue in position 281 is highly conserved between species, suggesting an important role for this amino-acid. In vitro functional studies showed that the p.Arg281Pro mutant lost its ability to activate the AMH transcription by 90%. These in vitro data confirm that this SF1 gene mutation drastically alters the Sertoli cell functions. This mutation was found at low copy number in the father DNA isolated from peripheral blood leukocytes, suggesting mosaicism. Sperm could not be analyzed.
Conclusion: We described a new heterozygous SF1/NR5A1 mutation, which altered the transactivation ability of the AMH gene. However, the XY DSD child did not had any mullerian derivatives suggesting that AMH was secreted in a normal way during in utero development. Thereafter specially in the pubertal years, the Sertoli cell lesion became more apparent but no adrenal insufficiency was documented.

PO3-072 Disorders of Sexual Differentiation (DSD) II

The 316-404 region of the SF1 ligand binding domain is critical for its transcriptional activity: evidence from two new mutations in 46, XY patients with opposite male and female phenotype

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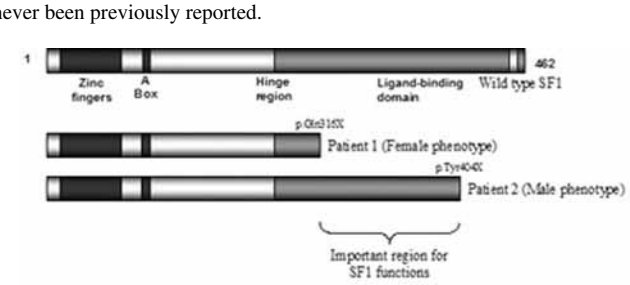
SF1 is a key regulator of many genes involved in testes development and migration (AMH, StAR, Cyp11a1, Cyp19, INSL3, etc.). In the last few years, several SF1 mutations have been reported with clinical expression ranging from anorchia to sex reversal with adrenal insufficiency. The structure/function of SF1 remains unknown. We had the opportunity to study two patients with opposite male and female phenotypes and SF1 premature stop codons.
The first patient was a 15-month-old female referred for isolated clitoromegaly (clitoris length: 1.5 cm). Subtle palpation revealed a gonad in the left labia and another in the right inguinal position. Pelvic sonography showed an absence of uterus. The karyotype was 46,XY and confirmed the sex reversal. Basal plasma testosterone (T) was low (0.24 ng/ml) and low response was observed at HCG stimulation testing (1.6 ng/ml). The AMH level was normal (43 ng/ml).
The second child was referred for perineoscrotal hypospadias with bifid scrotum. Basal plasma T level was low (0.29 ng/ml) and low response to the HCG stimulation test was observed (1.12 ng/ml). The diagnosis of testis dysgenesis was raised and a testicular biopsy showed few tubules and no spermatogonia cells.
Genetic analysis revealed a c.946C>T substitution leading to a premature stop codon (p.Gln316X) for patient 1 and a c.1212C>A substitution creating a stop codon at position 404 (p.Tyr404X) for patient 2. These two mutations have never been previously reported.



For patient 1 with female phenotype, the stop codon was responsible for abolishing SF1 function. For patient 2 with male phenotype and insufficient virilisation, the stop codon located near the C terminal domain permitted some SF1 activity

Taken together these data show the importance of the 88 amino acids between Gln316 and Tyr404. This SF1 region probably interacts with crucial co-factors for its activity.

PO3-073 Disorders of Sexual Differentiation (DSD) II

A novel mutation of SOX9 gene: gonadal dysgenesis without bone dysplasia

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In many cases SOX9 mutations are responsible for 46XY disorders of sex development (DSD) and campomelic dysplasia with severe skeletal malformations.

Case report: We reported the case of Dorian who presented a DSD with persistent right Müllerian duct. He was a first child born after 38-weeks pregnancy, without growth defect (birth height: 48 cm). There was no familial history of genital surgery, sterility or short stature. The clinical examination was done at 20 months: his height was 108.5 cm (+2 SDS), target height was 180 cm (+1 SDS). His genital development was male, with the followings: the penile length was 38 x 10 mm, the penis was bent but erectile, the testes were in the inguinal canal (1 ml). There was a balanic hypospadias. Hormone exploration noted a low AMH level (48 ng/ml, normal range 50-120 ng/ml) and low testosterone response after stimulation by 3 doses of 1500 IU bHCG (testosterone peak: 0.98 ng/ml). The search for associated malformations was negative. The renal function, the ultrasound kidney and heart exploration were normal. We did not note any skeletal abnormalitie. The surgical exploration found a persistent Müllerian duct in the right side, with a Müllerian residue and a small vas deferens. The left vas deferens was normal without Müllerian structure. The testes were macroscopically normal. The karyotype was 46XY. The clinical picture directed more towards a gonadal dysgenesis than a defect of the AMH gene or its receptor genes. The search for SF1 and TW1 mutations was negative. A missense mutation was identified in the SOX9 gene sequence C296G> S99N mutation located in the exon 1.

Conclusion: To our knowledge, this is a new description of a SOX9 gene mutation associated with DSD, but without skeletal abnormalities. Unlike SRY mutations that cluster within the HMG box, SOX9 mutations occur throughout the ORF. The S99N mutation located outside the HMG box but within a phosphorylation site, seems deleterious more for sex differentiation than for bone development.

PO3-074 Disorders of Sexual Differentiation (DSD) II

AMH determination is essential for the management of 46,XY DSD patients

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AMH is useful for evaluating 46,XY DSD patients. Nevertheless, normal AMH values have been determined in small group of subjects and mostly after 2 months of life without longitudinal study. Recently, as prenatal diagnosis and progress in fetal sonography have permitted detection of external genital abnormalities during pregnancy, investigation of DSD should be done at birth in order to make an early diagnosis and choice of sex rearing. Using a new AMH/MIS enzyme immunoassay kit (Coulter Beckman, Immunotech) available from april 2003, we report our experience of AMH determination at day1 and during the minipuberty (15^{th}-90^{th} days) for the management of 46,XY DSD. These results were compared with normal values obtained in a cohort of 110 male neonates without abnormalities of external genitalia (no micropenis, palpable gonads, no hypospadias) and detectable diseases. AMH values were determined in each neonate three times around 15^{th}day, 3^{th} and 9^{th}months of life and were expressed in pmol/L as following: mean±1SD (value range): day13-20, 882±335 (286-2116); 2,8-5,1 months, 1816±577 (704-3250); 8,5-9,8 months, 1736±616 (639-4364). In this longitudinal study, a significant increase of AMH was observed for each neonate between 15^{th} day and 3^{th} month of life ($p<0,001$) and slight but no significant decrease after minipuberty (9^{th}month). Moreover all other controls have lower AMH values in the first days of life than at 15^{th} day. In our DSD patients, four groups could be distinguished. Group 1 with low value (< 50, often undetectable), no increase during minipuberty but high gonadotrophins corresponds to vanishing testis or gonadal dysgenesis. Group 2 with normal AMH>250 at birth and an increase to normal value at minipuberty corresponds after further investigations (basal/hCG-stimulated testosterone, gonadotrophins) to 46,XY DSD patients with normal Leydig cell function. Group 3 with low value at birth (100-250 pmol/L) but an increase to normal value at minipuberty could be considered as group 2. Group 4 with intermediate values at birth (50-250 pmol/L) remaining under normal values later corresponds to patients with a testicular dysgenesis (increased FSH, low hCG-stimulated testosterone). Our strategy for early sequencing was dependant of these results : AR or steroid enzyme genes in groups 2-3; SF1 & WT1 in group 4; firstly SRY, duplication of Dax-1 & Wnt-4 in group 1. In conclusion, AMH appears essential in the first weeks of the life to manage 46,XY DSD.

PO3-075 Disorders of Sexual Differentiation (DSD) II

Quantitative analysis of the long term outcomes of intellectual, psychological, sexual functioning and of the well-being in a cohort of XY disorder of sexual development (DSD) patients raised as girls, treated in a single institution

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Few studies have addressed the question of the long term outcomes including well-being in DSD patients. Our study, aimed to assess cognitive, psychic functioning, sexual behavior and quality of life in a cohort of XY DSD raised as girls, treated in a single institution.

Subjects and methods : 15 patients aged 18 to 41 with a 46 XY DSD, whose gender was assigned as female, and treatment included genital surgery and raised as girls, were recruited. The protocol included a gynecological examination, a semi-directive interview, a WAIS III-R intelligence test, two projective tests (Rorschach, TAT), the WHO quality of life 26 and a questionnaire of sexual behavior to be compared to normative data from the same French population.

Results : The global cognitive abilities of the cohort are in the lower half of the normal range (average IQ = 90). The assessment given by the women to their quality of life, from a psychological point of view (57.5%) and as concerns social relationships (56.4%), was significantly lower than that of the reference population (RP) ($p = <0.05$). Six of the fifteen women had never had sexual relations; a proportion much higher than average among women of their age (40% versus 5.2%) ($p= <0.05$). Homosexual attraction (38.3%) and genital sexuality (30%) are more frequent than in the RP (respectively 6.5% and 1.9%) ($p = <0.05$). The question of sexuality was addressed as a medical issue (27.7%, vs 5.2% for the RP) ($p= <0.05$). Despite medically normal external genitalia, interviews underlined 1) a great deal of confusion between the perceived anomalous genitalia and sterility 2) a vagueness in the ability to name the disorder, to recall the information that was indeed given (inhibition) 3) fear and anxieties for the sexual and love life 4) a feeling of secrecy and guilt 5) a family and social isolation.

Conclusions : The quantitative analysis as well as interviews revealed the women's dissatisfaction with emotional and social life. We underscored the fact that these XY DSD patients are in great psychic pain; even if they do not always express it verbally, they clearly suffer from depression, along with more or less severe mood disorders (despair, anxiety, anguish, suicidal thoughts). Their quality of life is greatly altered, their sexual and love life inhibited; they

are not subjectively satisfied with the surgery (despite satisfactory gynecological examinations) and with the knowledge of their disorder.
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PO3-076 Disorders of Sexual Differentiation (DSD) II

Undescendet testes and micropenis due to a new mutation in exon 6a of the luteinizing hormone receptor

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Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals. Inactivating mutations of the human luteinizing hormone receptor (LHR) lead to decreased response of Leydig cells to LH. 2007 an addional Exon 6A, a primate-specific bona fide exon, within the LHCGR gene was detected by us. It displays composite characteristics of an internal/terminal exon and possesses stop codons triggering nonsense-mediated LHCGR mRNA decay (NMD). Transcripts including exon 6A result in an intracellular, truncated LHCGR protein of 209 amino acids. Functional studies in two families with 46,XX-girls revealed a dramatic increase in the expression of the mutated internal exon 6A, thereby preventing the transcription of the normal LHR transcript. We have now studied a family with a boys suspected to have LCH.
At birth, the phenotypically boy (unrelated parents) presented with undescendent testis and a micropenis. 46,XY was found. At the age of 5 week testosterone levels were low with 0.042 nmol/l (nl 0.1 –0.6 nmol/l).
PCR followed by sequencing of the LH receptor gene of all exons resulted in the identification of a new heterozygous mutation in exon 11 (I415T) which interrupt cAMP production in the boy. A second heterozygous mutation A580G was found in exon 6a.
In conclusion, we present the first patient with mild LCH with mutations in a new exon of the LH receptor gene that redefines the genomic organisation of LHCGR gene and indicates new regulatory pathways of receptor regulation.

PO3-077 Disorders of Sexual Differentiation (DSD) II

Denys-Drash syndrome mimicking a salt wasting crisis in a newborn with ambiguous genitalia: a case report

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Denys-Drash syndrome (DDS) is a rare disorder characterized by pseudohermaphroditism, nephropathy and Wilms' tumor. Here we present an infant with ambiguous genitalia that initially appeared to have findings consistent with congenital adrenal hyperplasia (CAH), but was eventually found to have DDS
Case presentation: The patient was a full term newborn with ambiguous genitalia, who presented at 19 days of life to the emergency department (ED), after an incidental serum metabolic panel obtained during the evaluation of frequent vomiting showed hyponatremia, hyperkalemia, hypochloremia, hypoglycemia, relatively low bicarbonate, a BUN level of 21 mg/dL and a serum creatinine of 4.2 mg/dL. He had a known normal male karyotype (46, XY) and an abdominal/pelvic ultrasound with no evidence of gonads at the time of presentation. His initial exam did not show dehydration, sepsis or ill appearance. His phallus was 1.5 cm in length with hooded foreskin and hypospadias with the urethral opening at its base.
Because of the association of the ambiguous genitalia and the initial laboratory findings, CAH was suspected. The infant was treated aggressively with intravenous fluids and steroids and admitted to the PICU for close monitoring. By knowing the karyotype, the potential CAH etiologies were narrowed to 3 possibilities; 17-alpha-hydroxylase deficiency, 3-beta-hydroxysteroid dehydrogenase deficiency, and lipoid hyperplasia. However his adrenal androgens were eventually reported as being within normal limits.
On his second PICU day, the patient became oliguric and edematous, with no response noted to appropriate fluid management. He was found to have worsening renal function, which eventually required peritoneal dialysis. A repeat abdominal and pelvic ultrasound showed gonads in the inguinal canals bilaterally and hyperechogenic kidneys without evidence of renal artery stenosis. A renal biopsy later demonstrated severe diffuse mesangial sclerosis. At this point the combination of male pseudo-hermaphroditism and renal failure was considered to be consistent with DDS, despite of the initial apparent salt wasting crisis.
Discussion: The initial management of infants with ambiguous genitalia includes the exclusion of CAH. When patients in apparent salt wasting crisis fail to respond to usual therapies for CAH and demonstrate evolving renal failure, less common causes of ambiguous genitalia must be considered including DDS.

PO3-078 Disorders of Sexual Differentiation (DSD) II

Assessing health-related quality of life (HRQoL) in disorders of sex development (DSD): phase I – item generation

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Background: Disorders of sex development (DSD) are congenital conditions in which chromosomal, gonadal, or anatomic sex development is atypical. Only anecdotal, retrospective reports exist regarding the experiences of children with DSD and their families, with no concurrently collected data on the specific stressors children and families face, nor on their processes of adaptation.
Objective: The objective of this study is to develop validated parent-proxy report and parent self-report HRQoL questionnaires that focus on issues specific to, and shared by young patients (newborn to 6 years) with DSD and their families, which are not otherwise covered by generic HRQoL measures.
Methods: In-person or phone focus groups were conducted with pediatric urologists (2 groups, n=7), pediatric endocrinologists (2 groups, n=10), DSD-experienced mental health providers (1 group, n=5); parents of affected children (3 groups, n=11); and patient advocates (2 group, n=4). Sampling of content domains was judged to be complete when later focus group responses showed redundancies (ie, "saturation"). Transcripts were coded by two independent raters. Content was categorized as either child or parent/family-centered, followed by a grouping of content according to themes.
Results: **Child-centered themes** included: *physical functioning*, *medications and procedures*, *gender concerns/body image*, *emotional functioning*, and *social functioning*. **Family/Parent-centered themes** included: *role functioning/family activities*, *future worries*, *medical management*, *gender concerns, disclosure*, *anatomy & voiding*, *appearance*, *decision making*, *communication/information*, *diagnosis*, *emotional functioning*, and *social functioning*. The frequency with which DSD-related situations/reactions were mentioned, within and across focus groups, was used to prioritize content for questionnaire item development. A breakdown of content by stakeholder status will be presented.
Discussion: This project represents the first attempt at characterizing subjective experiences of young patients and families to a DSD. The success of the provisional questionnaires in comprehensively capturing situations and reactions are currently being assessed through cognitive interviews in an independent parent sample. The project will conclude with the establishment of the instruments' psychometric properties (ie, reliability and validity) in a large national sample of parents of affected children.

PO3-079 Disorders of Sexual Differentiation (DSD) II

Parents' stories of their child's DSD, their construction of harmony and the implications for clinical practice

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Method: A qualitative doctoral study used narrative inquiry to explored 15 parents' experiences of their child's DSD and genital surgeries. At the time of data

collection all of the children were under the age of eleven and had experienced reconstructive genital surgeries.
Analysis: In-depth narrative interviews explored parents' stories and narrative analysis resulted in three key stone stories comprising of aggregate and foundational stories.
Results: Interpretation from parents' stories consistently illustrated three core elements; shock, protection and anxiety. By learning to balance these core elements parents were able to work towards a goal of harmony in relation to their child's DSD. The interplay between these elements was important in helping parents live their lives and understand their experiences.
Conclusion: Professionals have a critical role in supporting parents following the birth of a baby with DSD. An understanding of the core issues parents' experience allows for the development of realistic and achievable goals for both parents and professionals. In seeking harmony the parents are constantly engaging with and accommodating the events that have disrupted their lives. Professionals have the opportunity and duty to build strong foundations of information sharing, to provide ongoing consistent support, check out understanding and facilitate parent involvement in decision making. Professionals' appreciation of the protective nature of genital surgery and its impact on bonding needs to be considered within the context of any management approach. An organized and reflective MDT approach to the management of DSD for both parents and children is crucial. Ongoing educational updates for professionals from research evidence are vital. Together parents of a child with DSD and professionals can actively engage in complex political and legal debates in relation to DSD management.

PO3-080 Disorders of Sexual Differentiation (DSD) II

A novel mutation in 5α-steroid reductase 2 deficiency (CD 65 pro-ALA) in a Turkish family

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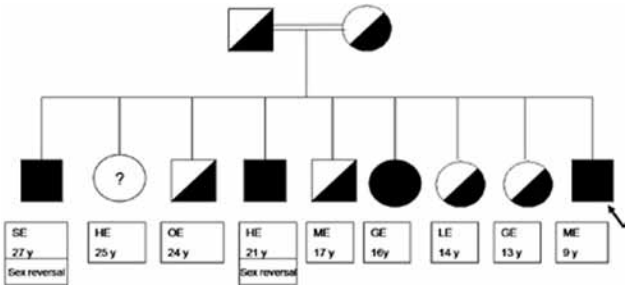
We describe the molecular and clinical characteristics of a related Turkish family with 5αSR2 deficiency who had a novel homozygote mutation of 5α-steroid reductase 2 gene.
This patient (9.7 years old) was born with predominantly female phenotype except clitoral-like phallus and bilateral inguinal masses, and he was raised as a girl. The genotype of patient was 46, XY, and had no mullerian structures on pelvic ultrasonography. Hormonal evaluation revealed high basal testosterone level and normal basal testosterone to dihydrotestosterone ratio. Family history revealed that his two siblings who were raised as girl and had sex reversal at the age of puberty, both of them underwent a change gender role from female to male with a history of psychiatric readjustment period.
5αSR2 gene analysis was performed to ten members of this family with direct DNA sequencing of exon 1 of the gene which revealed a novel homozygous point mutation of 5αSR2 gene at codon 65 a proline for alanine substitution. The mutation was also analysed with restriction enzyme, which abolished Cfr42I restriction site. Molecular characteristics of the ten members of this family are shown in the figure.



Previously, we reported the molecular characteristics of six 46,XY patients from unrelated Turkish families with 5αSR2 deficiency. Five of them had homozygous mutation of the 5αSR2 gene. Detected homozygous mutations were Leu55Gln in exon 1 in three of them, delta Met157 in exon 3 in one of them, and in another one splice junction abnormality. One individual was found to be a compound heterozygous two different mutations, Leu55Gln in exon 1 and Arg171Ser in exon 3.
In Conclusion: The mutation presented here is a novel homozygote mutation which was not defined in 5αSR2 deficient patients previously. Although basal testosterone to dihydrotestosterone ratio was normal, molecular analysis helped to differentiated 5αSR2 deficiency from partial androgen resistance in our reported patient.

PO3-081 Disorders of Sexual Differentiation (DSD) II

Anorchia as a primary triggering factor of suffering and severe mental imbalance

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INTRODUCTION Anorchia is the absence of both testes at birth in a phenotypically normal XY male. The therapy, most of the times, is mainly directed to hormonal replacement and the psychological aspects are often forgotten.
METHOD It is a qualitative research using phenomenology as its philosophical method and theoretical referential. The study was held in the Genetic-Endocrinology Clinic of Dr. Cesar Cals General Hospital. The patients were all of male gender with average age of 20 years. The patients were submitted to an interview for data collection. The leading question was: how were their lives since the childhood without testicles? The interviews were conducted from January to February, 2009 by the same examiner. The analytical method was the discourse analysis. The criterion for completion of the interview was saturation of the answers.
RESULTS Among the initial findings, we point out the following psychological impacts: the insecurity, low self-esteem, the feelings of inferiority, excessive body shame, self-depreciation, the difficulty for developing interpersonal relationships (mainly sexual), social isolation, the introversion, reduced self-confidence; negative, pessimistic and distorted thoughts and also, the anger and bitterness of the belated discovery of the disease. The interviewees referred that after starting treatment there was an improvement of such feelings, and that they have high expectation for cure. All the patients stressed that their family were very dysfunctional. On one side there is an absent father and on the other, a neglectful relationship of symbiosis with the mother. It is a family environment full of absences or deprivation that can cause psychological disorders or personality traits such as insecurity and low self-esteem. The search for a religion, with emphasis on Protestantism and the use of psychoactive substances, such as alcohol, were generally referred by the interviewees as coping strategies or mechanisms for relief of the anxiety caused by psychological distress.
CONCLUSION We conclude that the individual with Anorchia often shows intense mental suffering. The psychological effects are varied and intense and can cause serious problems for public health such as the increase in the rates of mood disorders (depression) and/or anxiety disorders (social phobia). Deeper investigation on the subject is suggested aiming at health policies for these individuals in order to improve their quality of life.

PO3-082 Disorders of Sexual Differentiation (DSD) II

Inguinal hernia as a presenting feature in a phenotypic female revealed a 46XY disorder of sexual differentiation (DSD) due to a novel SF1 gene mutation

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Presumed inguinal hernia in a phenotypic female is a common presenting signs of 46 XY disorder of sexual differentiation (DSD) in infancy and childhood. Androgen insensitivity syndrome (AIS) is the most common identifiable cause of 46 XY DSD present with inguinal hernia (about 40 %).
Objective: To identify the molecular basis of inguinal hernia in a 46 XY DSD with normal adrenal function.
Design/Methods: index case is a 4 year old phenotypic female, presented for

endocrine evaluation after gonads were identified during suspected inguinal hernia repair. On physical examination she is found to have no clitoromegaly or urogenitalia sinus. Her FSH = 7.2 mIU/mL, LH= <0.3 mIU/mL, and testosterone = <10 ng/dL. Further hormonal analysis revealed AMH =28 ng/ml, Inhibin B= 41 ng/dl. Karyotype found to be 46, XY. There was no testosterone response after stimulation with human chorionic gonadotropin but appropriate adrenal response to ACTH stimulation test (cortisol 5.1 to 31 ug/dL).
Results: Molecular analysis by direct sequencing of SF1 gene (*NR5A1*) revealed a novel heterozygous missense mutation (L361P). This gene abnormality was not identified in 100 controls, excluding a gene polymorphism. The replacement of Leucine by Proline likely leads to a abnormal SF1 function, as confirmed by the conserved Leucin in position 361 among species. In vitro analysis of the mutated SF1 is in progress. We were unable to determine the mode of inheritance because samples were not available for evaluation from other family members due to social issue.
Conclusion: Identification of a novel SF-1 mutation provides further evidence that 46 XY DSD with inguinal hernia with low testosterone response to HCG stimulation test warrant evaluation of rare genetic disruptions other then androgen receptor eg. SF-1, StAR protein mutation, inactivation mutation of LH receptor with similar phenotypic presentation.

PO3-083 Disorders of Sexual Differentiation (DSD) II

Frasier syndrome: a possible cause of end-stage renal failure in early childhood

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Diagnosis of Frasier syndrome, related to mutations of the Wilms' tumor suppressor gene WT1, is based on the association of complete sex reversal in 46XY patients with gonadal dysgenesis and steroid- resistant nephrotic syndrome with focal and segmental glomerular sclerosis (FSGS), progressing to end-stage renal failure (ESRF) during adolescence or adulthood.
We report the case of a 7 year-old patient with known male pseudo-hermaphroditism (MPH), who was admitted for ESRF and hemodialysis in emergency, with no previous history of renal disease. Kidney biopsy showed only glomerular sclerosis. The genetic analysis of the MPH, performed in early infancy, associated with bilateral gonadectomy, had revealed a mutation in intron 9 of the WT1 gene; the association of this mutation with the ESRFat the age of 7 led to the diagnosis of Frasier syndrome; There hadn't been any medical or biological, especially no kidney morphological or functional assessment follow-up after the molecular study of WT1 gene in early infancy, since the parents did not accept it; this can explain that our patient presented with ESRF and had to undergo a kidney transplantation much earlier than usual in this disease.
The interest of this case report is, beside the young age of the patient, the total lack of clinical manifestation of renal dysfunction until the age of 7, contrasting with the severity of the renal failure on admission. In the literature we did not find any data of patients with ESRF due to Frasier syndrome, occuring without previous clinical manifestations of nephrotic syndrome.
Our case report underlines the importance of a renal function follow-up even in asymptomatic patients known as having a Frasier syndrome. The molecular analysis of the WT1 gene, necessary in all cases of MPH with gonadal dysgenesis, must lead to an appropriate kidney follow-up in case of a mutation of the gene, as well as the prevention of gonadal and kidney malignant degeneration. It is the reason why our patient underwent beside the former gonadectomy, a preventive total nephrectomy; kidney transplantation was successfully recently realised.

PO3-084 Disorders of Sexual Differentiation (DSD) II

Age at diagnosis and mortality in 47,XYY persons

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Aim:
To describe the age at diagnosis and total mortality in all persons diagnosed with 47,XYY in Denmark.
Method:
Using the Danish Cytogenetic Central Registry we identified all males diagnosed with 47,XYY during 1965-2008. Hereby we identified 236 persons, whereof 38 were deceased. Approximately 2.6 million men were at risk yearly. We divided the men into three subgroups according to their karyotype. In the Statistics Denmark we identified up to 100 controls per index-person, all matched on gender and age (year and month), furthermore we retrieved date and causes of death. All controls were alive on day of diagnosis.
Statistics:
Age at diagnosis, median year of birth, and median year of diagnosis in the subgroups were analyzed using Kruskal-Wallis. Time at risk was calculated from date of birth. Total and cause specific mortality were compared in 47,XYY persons and their controls using Kaplan-Meyer plot and log-rank analysis.
Results:
We identified a reduced number of diagnosed males with 47,XYY compared to expected. Data in the three subgroups are shown with medians and ranges.

Characteristics of 47,XYY persons

Karyotype	Number of cases	Median age at diagnosis (range)	Median year of birth (range)	Median year of diagnosis (range)
47,XYY	178	17.3 (0.0-67.1)	1971.5 (1909.4-2007.3)	1988.1 (1965.7-2008.9)
46,XY /47,XYY	25	7.8 (0.0-70.7)	1980.7 (1899.8-2007.6)	1995.7 (1968.9-2007.6)
Others	35	16.1 (0.0-66.1)	1970.8 (1916.0-1998.8)	1985.7 (1966.1-2008.8)
Total	238	16.9 (0.0-70.7)	1971.9 (1899.7-2007.6)	1988.2 (1965.7-2008.9)

There were no significant differences in the three subgroups; however, age at diagnosis was surprisingly delayed. Mortality in 47,XYY persons was significantly increased with a median survival of 70.6 years, compared to 77.8 years in controls, with a log-rank analysis corresponding to p<0.0001.

PO3-085 Disorders of Sexual Differentiation (DSD) II

Functional studies of androgen receptor N-terminal domain mutations in patients with androgen insensitivity syndrome (AIS)

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Numerous androgen receptor (AR) mutations are associated with AIS. Missense mutations of the N-terminal domain (NTD) are rare compared to the other domains and the patient phenotype is less severe. We have investigated six NTD missense mutations (Q118E, and Tau1 region mutations; A157T, G214R, N233K, G246V, L270F) and two trinucleotide slippage mutations ins58L (Q58L) and del57L (L57Q) identified in mild (MAIS) and partial (PAIS) forms of AIS. The study had two aims: to understand the pathogenic role for the NTD mutations and to identify tools for investigating NTD dysfunction.
Transcriptional activity of the mutant ARs were defined *in vitro*, using GRE and Pem promoter luciferase constructs. A157T and N233K showed decreased transactivation activity for both reporter constructs. Pem promoter activation was reduced for G246V and L270F but not the GRE promoter. In contrast, Q118E exhibited normal activity for the Pem promoter but reduced activity with GRE. The differential responses for G246V, L270F and Q118E hin-

der defining a pathogenic role in AIS. Kindred analysis for Q118E revealed inheritance via the maternal grandfather. The index case had severe PAIS and was raised female, yet we have no evidence that Q118E has a pathogenic role. G214R was normal for both promoters. Further studies on G214R involving activation of the PI3/Akt pathway that induces phosphorylation of S213, did not show any defect.
Mutations A157T, N233K and G246K should be useful tools to define the molecular mechanism of NTD dysfunction. All three map close to RAP74/TFIIF binding sites located in Tau1. In terms of trinucleotide slippage mutations, we observed reduced cellular expression of ins58L relative to wild-type AR or del57L. This occurred in transiently transfected and stable cell lines. Transcriptional activity of ins58L was restored to normal when receptors were expressed at the same level following transfection with 5-fold more ins58L plasmid relative to WT or del57L. The *in vitro* studies are suggestive of a pathogenic role for ins58L. However, defining pathogenicity is complicated by inheritance from the maternal grandfather and the moderate phenotype. It is unclear if ins58L should be regarded as non-pathogenic, or if the PAIS phenotype depicts variable penetrance of a mild AR mutation. In conclusion, some NTD-located mutations identified in AIS patients appear to be pathogenic. Their study may explain mechanisms of disease.

PO3-086 Disorders of Sexual Differentiation (DSD) II

Psychosocial well-being of children with disorders of sex development (DSD)

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Children with disorders of sex development (DSD) such as congenital adrenal hyperplasia (CAH), androgen insensitivity, gonadal dysgenesis or hypospadias are exposed to specific stressors like surgery, lack of virilization in boys and over-virilization in girls. Within the clinical evaluation study of the German network DSD/Intersexuality we analyzed if the psychosocial well-being of children with DSD is impaired. Methods: We investigate the psychosocial aspects of children between four and 12 years between 2005 and 2007 in Germany, Austria and Switzerland. Health-related quality of life (HrQoL) was measured with the proxy-versions for parents and the self-assessment questionnaires for children of the KINDL-R. To screen for mental health problems we used the proxy-version for parents of the SDQ. Disease-specific aspects were collected with new parent's questionnaire and with a medical data questionnaire by the attending physician. Results: 166 children with DSD and their parents participated, 111 raised as girls, 55 raised as boys. For statistical group comparisons we used four diagnostic subgroups: girls with 46,XX & overvirilization/CAH (N=62), girls with 46,XY & partial androgen effects (N= 36), girls with 46,XY without androgen effects (N= 13), boys with lack of virilization (N= 55). HrQoL is impaired concerning emotional well-being in comparison with norm data in self and proxy reports. Older children (8-12) report lower scores compared to younger children (4-7). Self-assessments of HrQoL are worse than parent's assessments. Results from the KINDL-R and the SDQ did not reveal differences between the four diagnostic groups. The results for the SDQ did not identify high risk for mental health problems. Discussion: The impairments concerning HrQoL in children show the impact of DSD on the emotional well-being. The fact that mental health is not affected in pre-pubertal children may be explained with social support and personal resilience. The results indicate a decline of wellbeing with increasing age so that analyses with adolescents with DSD seem to be crucial.

PO3-087 Disorders of Sexual Differentiation (DSD) II

46,XX DSD, aortic stenosis, and normal gonadal function

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The term 46,XX male (46,XX testicular, disorders of sexual differentiation) describes subjects with a non-mosaic 46,XX karyotype and complete testicular determination. The vast majority of 46,XX males have Y-specific chromosomal material including the SRY gene translocated to the paternal X chromosome. The presence of the SRY gene accounts for the testis determination in these individuals. However, about 10% of 46,XX males lack SRY. The etiology in most individuals in the latter group is unknown. However some studies have indicated an increase in the prevalence of cardiac abnormalities, suggesting a contiguous gene sequence or the involvement of a single gene that plays a role in both cardiac and gonadal determination. Among subjects with SRY-negative 46,XX maleness, the incidence of abnormities of external genitalia is high, and infertility is considered inevitable. We describe a subject with 46,XX maleness, absence of SRY, cardiac abnormality, and apparent normal gonadal function. The subject was born at 3.4 kg following a pregnancy complicated by maternal type 1 diabetes and preeclampsia. The parents were unrelated, and midparental height was 168 cm. Aortic valvular stenosis was detected on the third day of life, and surgery was performed in the newborn period. Genitalia were normal. The karyotype was 46,XX, and SRY was not detected by either PCR or FISH. Developmental milestones and school performance were normal. Puberty began at approximately 13 years of age. At 14 years, serum LH (2.3 mIU/mL) and serum FSH (3.1 mIU/mL) were in the normal range. Puberty progressed normally. Adult height (173 cm) was attained at 17 years. At that time, he was at Tanner Stage V for pubic hair. Penile length (12 cm), and testis size (2.5 cm by 5 cm bilaterally) were both normal. He had minimal gynecomastia. Serum LH (4.5 mIU/mL) and FSH (4.4 mIU/mL) were both in the normal range, indicating normal Leydig cell and Sertoli cell function. The patient we present is unusual in several ways. He is an SRY-negative 46,XX male with normal external genitalia and normal puberty. Although we do not have sperm count, his hormonal profile at age 17 years does not indicate primary gonadal dysfunction. The patient also has congenital heart disease. The latter finding strengthens the previously described increased prevalence of cardiac abnormalities among subjects with 46,XX maleness in the absence of SRY.

PO3-088 Disorders of Sexual Differentiation (DSD) II

N/C interaction of the androgen receptor mutation R840H is ligand and cell type dependent

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The mutation R840H in the human androgen receptor leads to androgen insensitivity syndrome (AIS) with variable phenotypes, from partial AIS with ambiguous genitalia and male sex of rearing to complete AIS with female external genitalia and female sex of rearing.
Searching for explanations for the highly variable phenotype of the R840H mutation, we analysed the N/C interaction of the AR both in Cos1 and CHO cells with various concentrations of testosterone and DHT. The N/C interaction of R840H was strongly impaired at low DHT concentrations in both, Cos1 and CHO cells, in mammalian two hybrid assays. At higher concentrations R840H rescues the N/C interaction and surprisingly exceeds the wild type (WT) activity 3-5 times at high to supraphysiological levels. In general, WT and mutant showed stronger response to low hormone concentrations in CHO than in Cos1 cells. Switching the hormone from DHT to testosterone, a similar effect could be detected in CHO cells, but surprisingly not in Cos1 cells. When using the less potent androgen testosterone as a ligand, as expected, higher hormone concentrations are needed for maximum response in the reporter assay. In CHO cells again the mutant rescues WT activity and exceeds the WT 2-3 times at high to supraphysiological levels. Interestingly, this was not the case in Cos1 cells. Within these cells the N/C interaction of the mutant was strongly impaired compared to the WT, even at supraphysiological levels (33nM).
This study suggests that the activity of a mutant receptor might not only depend on the individual androgen production in the testis, but could be also affected by the individual cofactors supplied by a specific cell type. In addition, intracellular conversion of androgens, e.g. by 5α-reductase II, might lead to an unexpected receptor activity or silencing. These factors will modulate the phenotype of patients in a variable fashion.
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PO3-089 GH and IGF Use III

Results and potential consequences of the diagnostic re-evaluation in young adults with congenital GHD

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Aims: The apparently low frequency of confirmation of childhood-onset congenital GHD in young adults has raised questions about the appropriate criteria for diagnosing GHD in children and/or young adults. The aim of this retrospective study was to add to the issue, based on the large data collected from a single centre.
Patients and Methods: Of 518 children with congenital GHD (ESPE Code #1B.3a.ff), since 1967, 209 have reached adult height (AH). In 108 GHD was isolated and no pituitary anomaly was known (group I); in 101 GHD was combined and/or a pit. anomaly was known (group M). [median; I vs. M; * = p< 0.01] (*at GH start*): age: 8.8 vs. 9.1 [yrs]; Ht: -3.2 vs. -3.7 [SDS]; test maxGH: 6.7 vs. 4.2* [µg/L]; IGF-1: (n=95) -2.4 vs. (n=64) -3.7* [SDS]. *Retested at AH*: GH (n=134) and IGF-1 (n=153), GH+IGF (n=114; I=66; M=48); low was: maxGH < 5 (ITT), < 16.5 (Arg+GHRH) [µg/L], or basal IGF-1 < -2.0 [SDS].
Results: [I=isol, M=mult.; Lo=low; No=normal; N; % of I or M]: A) GH Lo: I (n=29; 37%); – M (n=39; 70%) – B) IGF Lo: I (n=40; 49%) – M (n=49; 72%) – C) a) GH No + IGF No: I (n=24;36%) – M (n=9;19%) – b) GH No + IGF Lo: I (n=18;27%) – M (n=6;13%) – c) GH Lo + IGF No: (n=8;12%) – M (n=8;17%) – d) GH Lo + IGF Lo: I (n=16;24%) – M (n=25; 52%). The correlations both between maxGH (standard tests) and basal IGF-1 [SDS] at diagnosis and at retest were low ($R^2 = 0.05$).
Conclusions: a) In *isolated* cong. GHD the relatively low % of confirmation in adult life suggests lower GH cut-off(s) to be used at primary diagnosis in this cohort. Retesting should be done at puberty onset. It needs to be answered, whether transient, isolated GHD or idiopathic NSD are really existing in children. b) In *combined/pit.anomaly* cong. GHD the relatively high % of non-confirmation in young adults questions the GH cut-off(s) used for defining GHD in this age group.

PO3-090 GH and IGF Use III

Growth hormone (GH) improves the height of short children with very low birth weight <1500 g (VLBW) but appropriate for gestational age (AGA) – first year results of a prospective, randomized, multicenter study in Germany

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Aims GH is an approved therapy for short children born small for gestational age (SGA) aged 4 years in Europe (35 µg/kg BW/d) and 2 years in the USA (68 µg/kg BW/d). However, there is also evidence that 5-10% of children born with birth weights below 1.500 g (VLBW) but AGA remain short[1,2]. The effects of GH on height, growth velocity and body composition in these children are unknown.
Patients and methods A controlled, randomized, multicenter study (Pfizer Pharma GmbH, A6281273) was performed. Inclusion criteria were: pre-pubertal children (age; boys 4-10 yrs, girls 4-9 yrs), height (Ht) <-2.0 SDS, height velocity (Ht vel) <0 SDS, birth weight (BW) <1500 g, GH levels to standard tests >7 µg/L. Children with severe sequelae of prematurity were excluded. Children were randomized into treatment (Genotropin®: 68 µg/kg BW/d) and non-treatment (control) groups for the first year. Study objectives were: (primary) gain in Ht (SDS) and Ht vel (SDS); (secondary) change in body composition (skinfold thickness, bone structure (pQCT) and muscle strength (grip force)) and insulin sensitivity.
Results [Treatment vs. Control; mean ± SD; * = p<0.0001; § = n.s.] - *Baseline*: N: 18 (9m/9f) vs. 15 (7m/8f) - age (yrs): 5.4 ± 1.5 vs. 5.7 ± 1.9§ - GA (wks): 27.9 ± 2.0 vs. 27.9 ± 1. 8§ - BW (g): 1056 ± 293 vs. 979 ± 202§ - BL (cm): 35.9 ± 2.7 vs. 35.6 ± 1.9 - Ht (SDS): -3.1 ± 1.1 vs. -2.7 ± 0.9§ - Ht vel (SDS): -2.3 ± 2.0 vs. -1.9 ± 1.6§.
At *12 months*: ΔHt (SDS): 1.2 ± 0.4 vs. 0.0 ± 0.3* - ΔHt vel (SDS): 6.7 ± 2.3 vs. 1.4 ± 1.7*.
Conclusions This early analysis reports the interim results of the primary endpoints of the randomised study after 12 months of treatment. There was a pronounced increase in height SDS and height velocity SDS in the GH group after 1 year, whereas there was little change in these parameters in the control group. Based on these findings, we suggest that short children born VLBW-AGA will benefit from GH similar to short children born SGA.
[1]Trebar et al., Pediatr Res 2007;62:209-14; [2]Euser et al., Horm Res 2008;70:319-28

PO3-091 GH and IGF Use III

First year growth to growth hormone (GH) in pre-pubertal children with congenital GH deficiency (cGHD) is proportional in boys and girls

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There is little exact information about the sex-dependent changes in body proportions during GH treatment in pre-pubertal children with congenital GHD.
Patients and Methods: In 144 (103 m, 4 f) children with cGHD height (Ht), sitting Ht (sHt), arm span (AS), head circumference (HC) and IGF-1 were measured strictly longitudinally during 1st yr on GH. SD scores (SDS) for anthropometrical data were based on Swiss (Prader) references; for IGF-1 local refs. (Blum). Basal data [mean (SD); male vs. female]: age: 6.9 (2.5) vs. 6.8 (2.3) [yrs]; maxGH: 7.1 (5.0) vs. 7.1 (3.3) [µg/L]; IGF-1: -2.4 (1.3) vs. -2.0 (1.2) [SDS]; GH dose: 31 (5) vs. 32 (6) [µg/kg d].
Results: [mean (SD); male vs. female; *=p<0.05)]: Basal data Ht: -2.8 (0.5) vs. -3.2 (0.8)* [SDS]; sHT: -2.8 (2.2) vs. 3.2 (1.2) * [SDS]; AS: -2.9 (0.8) vs. -3.6 (1.0) * [SDS]; HC: -1.3 (1.0) vs. -1.4 (1.3) [SDS]; delta = basal minus after 1 yr: Ht: 0.8 (0.3) vs. 0.9 (0.4) [SDS]; sHt 1.1 (2.3) vs. 0.8 (0.8) [SDS]; AS 0.9 (0.5) vs. 0.9 (0.4) [SDS]; HC: 0.3 (0.4) vs. 0.5 (0.6) [SDS]; IGF-1: 1.9 (1.3) vs. 1.6 (0.9) [SDS].
Conclusions: Girls with cGHD were relatively smaller than boys at GH start despite similar severity of GHD. Proportions (relative relationship between height, sitting height, and arm span) are normal in pre-pubertal children (boys and girls) with cGHD. On the same dose of GH, changes in height, sitting height and arm span are in the same order of magnitude and practically identical in both sexes.

PO3-092 GH and IGF Use III

Subcutaneous injections of a pegylated long-acting recombinant human growth hormone (NNC126-0083) in adults subjects with growth hormone deficiency is well tolerated

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Recombinant human Growth Hormone (rhGH) is normally administered as a daily subcutaneous injection. NNC126-0083 is a pegylated rhGH developed with the aim of reducing clearance and thereby prolonging the exposure. It has previously been demonstrated that NNC126-0083 is well tolerated in healthy volunteers with the potential for once weekly administration.

In this trial NNC126-0083 was administrated subcutaneously once weekly for three weeks to adult subjects with growth hormone deficiency (AGHD). The subjects enrolled into the trial were on GH replacement therapy, either male or female with a BMI between 18.5 to 35.0 kg/m^2, age 20 to 65 years, HbA1C≤8.0% and not on insulin treatment. Fourteen days before being randomized the AGHD subjects discontinued their GH replacement therapy. Four escalating doses of NNC126-0083 were tested; 0.01, 0.02, 0.04 and 0.08 mg protein/kg/week. And in each dose-group 8 AGHD subjects were dosed with a subcutaneous administration of NNC126-0083 (n=6) or placebo (n=2).

At each dose level the safety and tolerability of NNC126-0083 were evaluated after 1st and 3rd dosing as well as the pharmacokinetics and pharmacodynamics. Especially parameter describing the liver function (ALP, GGT, ALAT, ASAT and bilirubin) were closely followed due to the expected change in elimination of pegylated GH compared to endogenously GH as later publish by Webster et al., 2008.

The results from this multi dose trial in AGHD patients indicate that NNC126-0083 has the potential of being a safe and well tolerated and efficacious once-weekly GH compound for the treatment of growth hormone deficiency in adults.

R. Webster et al. 2008, Xenobiotica; 38(10):1340-1351

PO3-093 GH and IGF Use III

Multiple dosing of a pegylated long-acting recombinant human growth hormone in healthy subjects

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Recombinant human Growth Hormone (rhGH) is normally administered as a daily subcutaneous injection. NNC126-0083 is a 40 kD pegylated rhGH developed with the aim of reducing clearance and thereby prolonging the exposure. It has previously been demonstrated that NNC126-0083 after single dose is well tolerated in healthy volunteers with the potential for a once weekly administrated compound.

The objectives of this study were to evaluate the safety, tolerability pharmacokinetics and pharmacodynamics of multiple dose administrations of NNC126-0083 in healthy subjects.

Healthy male subjects with a Japanese passport and Japanese born parents; a BMI between 18.0 and 27.0 kg/m^2; age between 20 to 45 years, were included in the study.

Groups of 8 subjects were dosed once weekly for three weeks with a subcutaneous administration of NNC126-0083 (n=6) or placebo (n=2). The doses were escalated between the cohorts in a sequential mode starting at 0.02 mg protein/kg/week, highest dose being 0.16 mg protein/kg/week.

Blood samples for assessment of safety and pharmacodynamic response Insulin-like growth factor-I (IGF-I)and IGF binding protein-3 (IGFBP-3) were sampled up to 168 hours after first dosing and up to 240 hours after third dosing. Physical examinations, vital signs, ECG, antibodies and local tolerability at the injection site were examined as well.

The results from the multiple dose study in healthy Japanese subjects indicate that NNC126-0083 has the potential as an efficacious, safe and well tolerated once-weekly treatment for growth hormone deficiency in children and adults.

PO3-094 GH and IGF Use III

The DATAC study: a new growth database. Description of epidemiology, diagnoses and therapeutic attitude in a group of Spanish children with short stature

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Observational retrospective multicentric national study to evaluate the baseline data and main aetiologies of patients prior to rhGH therapy.

Patients and methods

Data from 38 centres included gender, chronological age (CA), height, weight, target height (TH), growth velocity (GV), pubertal stage (Tanner), IGF-I, IGFBP-3, GH after stimuli, FT4, TSH, celiac disease markers, karyotype, bone age (BA), MRI (n, normal; p, pathological), diagnoses and rhGH dosing.

Diagnostic Groups: GH deficiency (GHD); Partial or functional GHD; Small for gestational age (SGA); Turner's syndrome (TS).

Results

N=718 patients (395 males & 323 females), age range (0.5-17.0 years), including: GHD (495; CA 10.65±2.83), SGA (191; CA 7.53±3.04), TS (32; CA 7.01±3.61) (see Table). Systemic disorders were excluded and markers of coeliac disease were negative in all patients. The stimulation tests performed were as follows: Propranolol+exercise (227/31.6%), clonidine (224/31.2%), ITT (209/29.1%), others (100/13.9%).

Group (N, M%)	Height (SDS)	TH (SDS)	GV (SDS)	BA-CA (years)	IGF-I ng/mL (N)	IGFBP-3 µg//mL (N)	rhGH mg/kg/d
GHD (495, 60.8)	-2.48 ±1.09	-1.01 ±0.92	-1.87 ±2.33	-2.10 ±1.43	148.02 ±73.40 (181)*	3.16 ±3.00 (164)*	0.035 ±0.007
GHD +nMRI (217, 63.6)	-2.63 ±1.23	-1.17 ±0.81	-2.11 ±1.90	-2.47 ±1.42	145.43 ±78.14 (87)*	3.10 ±1.17 (77)*	0.032 ±0.005
GHD +pMRI (47, 63.8)	-2.81 ±0.81	-0.61 ±1.34	-2.60 ±1.37	-2.28 ±1.08	147.52 ±94.83 (14)*	3.13 ±2.13 (16)*	0.032 ±0.004
Partial GHD (231, 57.6)	-2.26 ±0.97	-0.95 ±0.87	-1.47 ±2.77	-1.71 ±1.40	150.92 ±64.28 (80)*	3.24 ±0.99 (71)*	0.038 ±0.007
SGA (191, 49.2)	-2.96 ±0.97	-1.24 ±0.82	-1.53 ±1.41	-1.99 ±1.44	124.99 ±63.81 (76)*	3.08 ±0.89 (61)*	0.040 ±0.008
TS (32)	-2.96 ±0.96	-0.73 ±0.85	-2.28 ±1.55	-1.44 ±0.96	154.30 ±60.10 (26)¤	3.12 ±0.98 (25)¤	0.047 ±0.008

* Males Tanner I; ¤ Tanner I

Summary

-A new growth database has been developed (DATAC)
-Males are predominant among patients with GHD.
-The age at diagnosis is significantly greater in patients GHD.
-No serious adverse events were reported after the stimulation tests.
-Therapy with rhGH is started at lower CA in SGA and TS groups.
-Prescribed rhGH doses are significantly higher in patients with partial GHD.

PO3-095 GH and IGF Use III

Gender differences in growth hormone (GH) responsiveness in growth hormone deficient (GHD) children and short children born small for gestational age (SGA)

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The aim was to investigate any gender differences in GH responsiveness in GHD and SGA children treated with Norditropin® for at least 2 yrs. Data from the NordiNet® International Outcome Study (IOS) (Europe) and the ANSWER® Programme (USA) were pooled for these analyses. ANCOVA were applied. **GHD:** The cohort included 1,808 children. On average, girls were younger than boys (9.1 vs. 10.6 yrs; p<0.0001) and shorter (HtSDS -2.73 vs. -2.43;p<0.0001). Girls received a lower mean GH dose than boys (40 ug/kg/d vs. 43 ug/kg/d), p<0.0001. Table 1 demonstrates changes in serum IGF-I and HtSDS after 2 years of treatment. The influence of gender on HtSDS change was not significant in multivariate analysis in contrast to age, HtSDS at baseline, GH dose and pubertal status. (table2) **SGA:** The cohort included 501 children. GH treatment was started at a mean age of 8.0 yrs in girls and 7.8 yrs in boys. Mean HtSDS at treatment start was -3.21 in girls and -3.17 in boys. Mean GH dose was 42 ug/kg/d in both genders. Mean delta IGF-I and HtSDS are shown in table 1.

Mean change in serum IGF-I and HtSDS after 2 years of GH treatment in GHD and SGA children.

Indication	Parameter	Girls: n	Girls: Delta value±SD	Boys: n	Boys: Delta value±SD	p- value*
GHD	IGF-I (ug/L)	192	266.4 ±181.5	438	250.3 ±177.1	0.298
	HtSDS	495	1.07 ±1.01	1313	1.0 ±0.65	0.043
SGA	IGF-I (ug/L)	73	247.7 ±151.2	102	201.1 ±180.5	0.040
	HtSDS	212	0.98 ±0.58	289	1.13 ±0.68	0.012

*delta value girls vs. boys

In SGA children, multivariate analysis demonstrated significant influence of gender on two year HtSDS change.

P-value for baseline characteristics and average GH dose in relation to HtSDS change from baseline to 2 years of GH treatment in GHD and SGA children.

Indication	Age	HtSDS	Average GH dose	Gender	Puberty
GHD	0.0017	<0.0001	<0.0001	0.530	<0.0001
SGA	0.0035	<0.0001	0.005	0.041	0.002

Conclusions: Better treatment outcomes in GHD and SGA children were associated with lower age, lower HtSDS at baseline, pre-pubertal status, and higher GH dose. For children with GHD, female gender was associated with a higher delta IGF-I, but similar HtSDS change in girls and boys. For children with SGA, gender is a treatment consideration because HtSDS gain was less in girls than boys.

PO3-096 GH and IGF Use III

Predictors of growth response after one year of Norditropin® treatment in growth hormone deficient (GHD) patients from the ANSWER program® and NordiNet®

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The ANSWER Program® and NordiNet® registries have been collecting information on patients treated with growth hormone (Norditropin®) in the US and Europe, respectively, since 2002. As of February 2009, over 15,000 patients have been enrolled in the registries. This analysis of the pooled data from the registries focuses on identifying predictors of height standard deviation score (HtSDS) gain in GHD patients after one year of GH treatment. A total of 3,148 patients (2297M/851F), naïve to prior GH therapy, were included in the analysis and, at baseline, mean (SD) age was 10.3 (3.7) years and HtSDS -2.46 (0.95). Over the first year of treatment, change in HtSDS (ΔHtSDS) was 0.58 (0.49), and the average GH dose was 41.5 (13.2) µg/kg/day. A linear regression model (ΔHtSDS = intercept + b1*variable 1 + b2*variable2 +….) was used to identify predictors of growth response (ΔHtSDS) at one year in the patient population. The model included baseline variables of age, gender, puberty (prepubertal or pubertal), HtSDS, as well as the average GH dose during treatment. The following variables (in descending order of contribution) were identified as significant predictors of ΔHtSDS at one year: baseline HtSDS (negatively correlated), baseline age (negatively correlated), pre-pubertal status (in favor of pre-pubertal status) and average GH dose (positively correlated) (Table). Using this model, gender was not a significant predictor of the response to GH. In conclusion, in GHD children undergoing GH therapy, low baseline HtSDS, young age, pre-pubertal status and higher GH dose are associated with a greater increase in HtSDS at one year of treatment.

Significant Predictors of HtSDS Response at Year 1

Predictor	Beta	P value	% contribution
Baseline HtSDS	-0.196	< 0.0001	49.6
Baseline age	-0.235	< 0.0001	27.8
Puberty (prepubertal=0, pubertal=1)	-0.149	< 0.0001	11.6
Average GH dose during treatment	0.042	0.0117	2.8
Gender	-0.024	0.1587	0.9

PO3-097 GH and IGF Use III

Predictors of growth response after one year of Norditropin® treatment in Turner syndrome patients from the ANSWER program® and the NordiNet®

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The ANSWER Program® and the NordiNet® have been collecting information on patients treated with growth hormone (Norditropin®) in the USA and Europe since 2002. As of February 2009, over 15,000 patients have been enrolled in the registries.This analysis of pooled data from the registries focuses on identifying predictors of height standard deviation score (HtSDS) gain in Turner Syndrome (TS) patients after one year of GH treatment. All patients were naïve to prior GH therapy and had HtSDS and age available at baseline and one year. For each height measurement, HtSDS was calculated applying population based reference data. A total of 453 patients (baseline mean±SD age 8.9±3.6 years, HtSDS -2.79±0.91) were included. HtSDS change (ΔHtSDS) at one year was 0.479±0.391, and the average GH dose during treatment was 48.7 µg/kg/day. A linear regression model (ΔHtSDS= intercept + b1*variable

1 + b2*variable2 +….) was used to identify predictors of ΔHtSDS over the first year of treatment. The model included the baseline variables of age and HtSDS, as well as the average GH dose. Age at baseline, HtSDS at baseline, and the average GH dose were significant predictors of the first year growth response (ΔHtSDS) with contributions of 68.5%, 24.8%, and 6.7%, respectively (Table). The negative beta values indicate that both HtSDS and age at baseline were inversely correlated with HtSDS outcome. In conclusion, in pediatric patients with TS undergoing GH therapy, young age and low HtSDS at baseline are associated with greater HtSDS response over the first year of treatment. Average GH dose during GH treatment is positively correlated with HtSDS outcome. These results confirm the results from other researchers (Davenport MP et al. J Clin Endocrinol Metab. 2007 92(9):3406-16) that GH treatment should be initiated as early as possible in children with TS to improve the treatment response.

Significant predictors of first year growth response (Δ HtSDS) in TS patients			
Predictor	Beta	P value	% contribution
Baseline age	-0.311	< 0.0001	68.5
Baseline HtSDS	-0.186	< 0.0001	24.8
Average GH dose	0.0957	0.0331	6.7

PO3-098 GH and IGF Use III

Growth hormone therapy (GHT) in children with short stature: results from the ANSWER program®

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Since 2002, the American Norditropin Studies: Web-Enabled Research (ANSWER) Program® has collected clinical data on patients receiving Norditropin® (somatropin rDNA origin) at the discretion of the physicians. In the US, Norditropin is currently approved for treatment of children and adults with growth hormone deficiency (GHD), and children with short stature associated with Turner syndrome (TS), Noonan syndrome, and small for gestational age (SGA). As of February 2009, clinical data were available for 7507 patients treated with GH. Part of this population was included in this longitudinal analysis for GH naïve patients with GHD, multiple pituitary hormone deficiency (MPHD), TS, SGA, or idiopathic short stature (ISS) (Table). At one year (Y1), patients in all diagnosis categories showed improvement in height standard deviation score (HtSDS) from baseline, with GHD, MPHD, and SGA having the greatest ΔHtSDS. MPHD patients received the lowest average GH dose and achieved the greatest ΔHtSDS. GH naïve GHD patients were also analyzed for correlation between ΔHtSDS at Y1 and baseline characteristics. Y1 ΔHtSDS was negatively correlated with baseline age (R= -0.352, p<0.0001), bone age (R= -0.334, p<0.0001), and IGF-I SDS (R= -0.207, p< 0.0001), and positively correlated with Y1 ΔIGF-I SDS (R= 0.184, p<0.0001). No significant gender differences in ΔHtSDS were found at Y1 in GHD patients (ΔHtSDS: 0.53±0.43 for males; 0.60±0.45 for females). In conclusion, patients with GHD, MPHD or SGA had the greatest response to GHT. In GH naïve GHD patients, ΔHtSDS showed a significant inverse correlation with the age, bone age, and IGF-I SDS at start of therapy, reinforcing the importance of beginning GHT at a younger age.

One Year Longitudinal HtSDS and GH Dose by Disease Category in GH Naïve Patients						
	HtSDS, mean (N)			GH Dose (µg/kg/d), mean (N)		
	Baseline	End of Year 1	ΔHtSDS	Baseline	End of Year 1	ΔGH dose
GHD	-2.222 (2029)	-1.675 (2029)	0.547 (2029)	46 (1887)	50 (1887)	4 (1887)
MPHD	-1.930 (152)	-1.271 (152)	0.659 (152)	40 (175)	42 (175)	2 (175)
TS	-2.619 (201)	-2.175 (201)	0.444 (201)	51 (198)	52 (198)	0 (198)
SGA	-2.624 (149)	-2.000 (149)	0.624 (149)	51 (135)	51 (135)	0 (135)
ISS	-2.325 (357)	-1.848 (357)	0.477 (357)	49 (300)	52 (300)	4 (300)

Window for Y1 data is 12±3 months. HtSDS values less than -5.0 were not included in the analyses.

PO3-099 GH and IGF Use III

Gender disproportion in children treated with growth hormone depends on country/region, diagnosis and severity of growth retardation

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Previous analyses have demonstrated a higher prevalence of boys treated with growth hormone (GH), especially in patients with growth hormone deficiency (GHD). The aim of this evaluation was to analyse factors which might influence the gender distribution in the growth hormone treated children such as indication, country or region of origin, age at treatment start or severity of growth retardation. Data from the observational studies in Europe (NordiNet®IOS), USA (ANSWER® Program) and Japan (NordiPAD®) were analysed. Children with Isolated Growth Hormone Deficiency (IGHD), Multiple Pituitary Hormone Deficiency (MPHD) and short children born small for gestational age (SGA) were selected for this analysis. All children were treated with Norditropin®. Gender distribution varied between countries/regions and indications, however the proportion of boys was higher in all patient cohorts and was highest in the IGHD cohort, especially in the US. Compared to IGHD, the proportion of treated boys was lowest amongst MPHD and SGA patients, most notable in the US for MPHD.

Gender distribution by indication and country/region, all patients.						
	IGHD		**MPHD**		**SGA**	
Country/region	**N**	**Female %**	**N**	**Female %**	N	**Female %**
Europe	1786	33.0	294	40.5	995	44.0
Japan	528	35.0	47	38.3	-	-
USA	3168	24.4	258	40.7	239	38.1

Age at treatment start did not influence gender distribution apart from the GHD children in the US where a higher proportion of treated girls was found when treatment was initiated at age <9 yrs compared to age ≥ 9 yrs. (31.7% vs. 21.7%). Gender disproportion was somewhat less pronounced in children with height SDS (HTSDS) below -3 at treatment start.

Gender distribution by indication and country/region, patients with HtSDS < -3.						
	IGHD		**MPHD**		**SGA**	
	N	**Female %**	**N**	**Female %**	**Female %**	**Female %**
Europe	731	40.8	127	42.5	620	46.6
Japan	132	43.2	19	47.4	-	-
USA	487	34.3	65	43.1	69	47.8

These results demonstrate that more boys than girls with short stature are treated with growth hormone, however the degree of the gender disproportion depends on country/region, severity of growth retardation, and diagnostic indication. The finding, that this gender disproportion is lowest in younger prepubertal patients, patients with MPHD and patients with more severe short stature may indicate a gender bias in a referral and diagnostic process.

PO3-100 GH and IGF Use III

48 months data of treatment with the rhGH Omnitrope® 5 mg/ml lyophilized formulation in growth hormone deficient children: efficacy and safety results

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INTRODUCTION:

Report of the follow-up of 48 months treatment with Omnitrope® 5mg/mL lyophilized formulation of short children with GHD given s.c. at a daily dose of 0.03 mg/kg.

METHOD:

51 previously untreated, prepubertal children with growth failure (HSDS -2, HVSDS -1) due to GH deficiency (GH peak < 10 ng/mL in 2 tests) were enrolled for treatment with Omnitrope®. Auxological and safety parameters, anti-HCP and anti-hGH antibodies were assessed during periodic visits.

RESULTS:

Expected growth velocity was maintained during Omnitrope® treatment. The change in BA/CA during 48 months treatment indicates a sustained growth potential. IGF-I and IGFBP3 levels increased to mean levels in the upper normal range. None of the patients had consistently high IGF-I levels throughout the study period. The most common AEs were general disorders and administration site conditions and the majority were mild in intensity. There were no drug-related SAEs and no withdrawals due to AEs or SAEs. Only one patient was tested positive for anti-GH antibodies without any clinical significance. The overall patient exposure covered by this study was 209.4 patient-years (mean 4.11 yrs, 1.0 to 4.6 yrs).

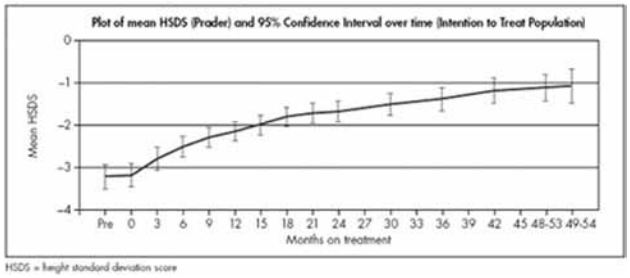
CONCLUSION:

Efficacy and safety of treatment with Omnitrope® for a period of 4 years were proven.

Efficacy results		
	Baseline	**48 months**
Patient No.	51	46
Age (yr)	7.95 ±2.57	11.97 ±2.40∗
Bone age (yr)	6.22 ±2.82	11.32 ±2.88∗
ΔBA / ΔCA		1.27 ±0.27∗
Height (cm)	111.9 ±15.5	142.8 ±14.0
HSDS	-3.2 ±1.0	-1.1 ±1.0
HV (cm/yr)	3.7 ±1.4	6.3 ±1.9
HVSDS	-2.7 ±2.0	+1.4 ±2.5
IGF-I (ng/ml)	79 ±47	260 ±115
IGFBP-3 (mg/l)	2.7 ±1.0	4.0 ±1.0

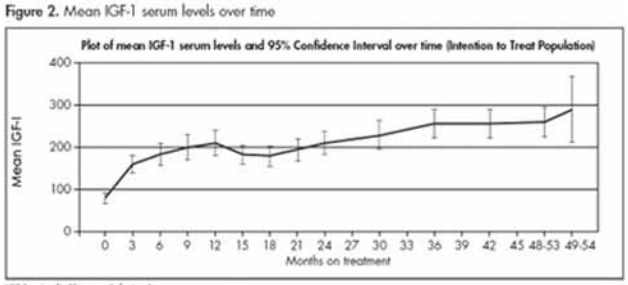
∗: n=41, : n=44

Figure 1. Maintenance of growth during treatment with Omnitrope®



HSDS = height standard deviation score

Figure 2. Mean IGF-1 serum levels over time



IGF-1 = insulin-like growth factor 1

PO3-101 GH and IGF Use III

Long-term efficacy and safety of Omnitrope® liquid in the treatment of growth retardation in growth hormone deficient children

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METHOD:

In an open-label study, 70 treatment-naïve pre-pubertal children (26 girls, 44 boys) with primary or secondary growth hormone deficiency (GHD; peak < 10 ng/ml, in 2 tests) were treated with recombinant hGH Omnitrope® liquid (daily dose of 0.03 mg/kg body weight s.c.) for an average of 44 months (up to 5 years).

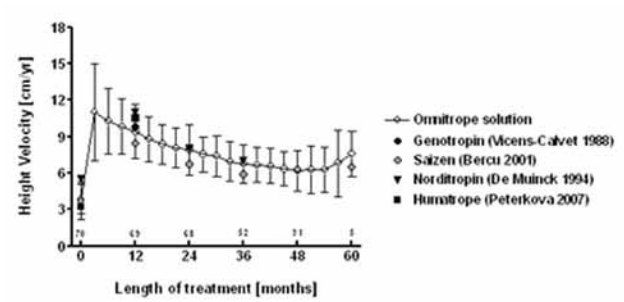
RESULTS:

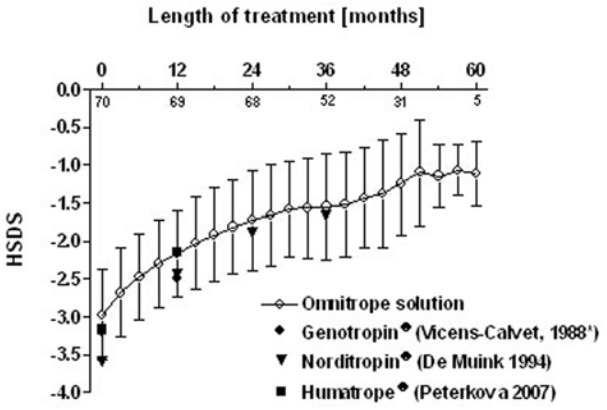
This treatment resulted in the increase of the pharmacodynamic parameters (IGF-1 and IGFBP-3), and in an excellent growth response, as illustrated with the significant increase measured for all the auxological parameters evaluated (height, HSDS, HV and HVSDS). The safety profile was shown to be satisfactory, both in regards to the reported adverse events and immunogenicity (incidence of anti-rhGH antibody similar to that reported for other GH products). There were no significant changes in laboratory safety data or vital signs. The overall exposure with Omnitrope® was 257 patient-years.

CONCLUSION:

The long-term efficacy and safety of the first rhGH biosimilar – Omnitrope® – was demonstrated.

Efficacy results		
	Baseline	**60 months (5yrs)**
age (yrs)	8.7 ± 2.4	
HSDS	-3.0 ± 0.6	1.1 ± 0.4
HV (cm/yr)	3.9 ± 1.3	7.4 ± 1.4
ΔHVSDS		4.5
IGF-1 (ng/ml)	130 ± 70	390 ± 100
IGFBP-3 (ng/ml)	2960 ± 760	3710 ± 70





PO3-102 GH and IGF Use III

The effect of twelve months rhGH treatment on upper airways of non severely obese children with Prader-Willi syndrome

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Background and aims: In a previous study we showed in 16 non-severely obese and obstructive apnoea free children with Prader-Willi Syndrome that a six weeks rhGH treatment does not significantly affect airways patency. We report here the auxological and polisomnographic results of twelve among the above children who attained 12 month of rhGH treatment.

Patients and Methods: Twelve children (9 boys, aged from 1.6 to 7.7 median 2.25 yrs) with genetically confirmed Prader-Willi syndrome were studied before and after 6 weeks and 12 months of rhGH treatment. In each of the three evaluations the patients were studied by anthropometry, one night 16 channels polysomnography and ENT examination of upper airways by flexible fibreoptic endoscope. The polysomnographic parameters considered were respiratory disturbance index (RDI), obstructive apnea index (OAI) and minimal SaO2. The Katz criteria were used for polysomnography evaluation. Tonsils hypertrophy was scored from 0 to +4 according to the Brodsky criteria and adenoid hypertrophy was classified as Wang criteria. SDS BMI was calculated according to CDC standards. Non parametric Friedman test and Fisher's exact test were used for statistical analysis.

Results: The main results are reported in the table. In particular we found a statistically significant reduction of RDI after 12 months of rhGH treatment and any reduction of upper airways patency in all but in one patient who required adenotonsillectomy. SDS BMI showed a slight, statistically unsignificant increase.

Main findings (median, min and max in brackets)				
	Before rhGH Tx	6 weeks GH TX	12 months GH TX	p
SDS-BMI	0.75 (-1.25;2.0)	0.68 (-0.75;2.0)	0.85 (-2.9;2.5)	ns
OAI	0.42 (0;1)	0.37 (0;1.7)	0.00 (0;1.2)	ns
RDI	3.37 (1.2;12.6)	4.5 (0.7;13)	1.1 (0.3;6.9)	<0.02
SaO2 min	88 (80;92)	87 (82;92)	88 (75;97)	ns

Conclusions: The significant reduction of RDI is in agreement with previous studies showing improvement of respiratory function during GH treatment of Prader-Willi children. The important adenoids and tonsils hypertrophy observed in one of our patients during medium-term GH treatment, does not prove a GH dependency of lymphatic tissue hypetrophy, however points out the importance of a close ENT follow-up in these patients.

PO3-103 GH and IGF Use III

Adult height in children with growth hormone deficiency: a randomized controlled GH dose-response trial

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CONTEXT: The dose-effect relationship of GH on adult height (AH) in naive GH deficient (GHD) patients as well as in pre-pubertal GHD children already on GH ('transfer') is not well determined, owing to absence of controlled trials until AH.

OBJECTIVE: To investigate the effect of 2 GH doses on AH in GHD.

DESIGN: A multicenter randomized controlled dose response trial.

INTERVENTION: Two doses of biosynthetic GH (Norditropin^{R}): 0.7 mg/m^2/d (~0.025 mg/kg/d) or 1.4 mg/m^2/d (~0.050 mg/kg/d).

PATIENTS: 20 'naive' and 15 'transfer' (on GH for ≥ 1 yr) pre-pubertal GHD children.

MAIN OUTCOME MEASURE: attained AH minus target height.

RESULTS: Four patients switched from GH dose because of high growth velocity or insufficient catch up growth, respectively. In the intention to treat analysis of the naive group, mean±SD AH minus target height (cm) was -5.3±6.1 and -2.2±6.9 in patients on 0.7 and 1.4 mg/m^2/d, respectively (mean±SE difference 3.1±2.9, P=0.3). In the transfer group, mean AH minus target height (cm) was -4.4±6.4 and +0.6±7.0 in patients on 0.7 and 1.4 mg/m^2/d, respectively (mean±SE difference 5.0±3.5, P=0.17). In the per protocol analysis the difference in AH minus target height between 0.7 and 1.4 mg/m^2/d in the naive patients was 3.2±3.1, P=0.32, and in the transfer group 3.4±4.3, P=0.44. Spontaneous puberty started 1.1 yr earlier in children on 1.4 compared to 0.7 mg/m^2/d (95% CI -2.1 to -0.1, P=0.04). Induction of puberty was more often delayed in transfer children on 0.7 than on 1.4 mg/ m^2/d (3 out of 8 *vs.* 0 out of 7). After correction for age of start of puberty or age at start of pubertal induction the AH minus target height was not significantly different between the two GH dosage groups in the naive patients (P=0.17), nor in the transfer patients (P=0.06).

CONCLUSIONS: In GHD, AH is 4-5 cm lower than target height on 0.7 mg GH /m^2/d (~0.025 mg/kg/d) compared to 0-2 cm on 1.4 mg/m^2/d (~0.050 mg/kg/d), but the difference did not reach statistical significance, probably due to limited numbers of patients in this study, considerable variability in growth response, and earlier spontaneous puberty and pubertal induction in the children on 1.4 mg/m^2/d.

This study was sponsored by Novo Nordisk A/S Denmark

PO3-104 GH and IGF Use III

The IGF-I and IGFBP-3 response after two years of growth hormone treatment using recommended doses differs in children with growth hormone deficiency, small-for-gestational age status, Ullrich-Turner and Prader-Willi syndrome: implications for treatment?

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Introduction: Response to growth hormone (GH) therapy varies widely. Measurements of serum IGF-I and IGFBP-3 are used to monitor treatment compliance and safety. For some diseases, it is proposed to lower GH doses if IGF-I concentrations exceed +2 standard deviations (SD). At the same time, specific GH dosage levels must be maintained to achieve catch-up growth or keep up desired metabolic effects. We hypothesized that at recommended GH doses (RD), IGF-I and IGFBP-3 responses to treatment differ between children with growth hormone deficiency (GHD), small-for-gestational age status (SGA), Ullrich-Turner syndrome (UTS) and Prader-Willi syndrome (PWS). That would include varying proportions of patients with elevated IGF-I and IGFBP-3 levels in these diagnostic groups.
Patients and Methods: 88 consecutive patients on GH therapy with RD from a single center (Essen) who had completed 2 years of treatment, were analysed. (GHD n=25, RD 0.025-0.035 mg/kg/d; SGA n=28, RD 0.035 mg/kg/d; UTS n=17, RD 0.045 mg/kg/d; PWS n=18, RD 0.035 mg/kg/d) Height was measured, and IGF-I and IGFBP-3 concentrations were determined with commercial assays (Mediagnost, Germany) at baseline and after 1 and 2 years of treatment.
Results: At start, GHD, SGA and UTS patients were shortest with mean height SDS ranging from -3.46 to -3.05, in contrast to PWS with -2.50 (P<0.05). Mean IGF-I (IGFBP-3) SDS at start of therapy ranged from -1.96 to -1.32 (-0.76 to -0.16) in SGA, UTS and PWS and was lowest in GHD with -3.26 (-2.22)(P<0.0001 for both hormones). After one year, overall IGF-I SDS had increased to -0.62 to -0.42 with no difference between groups. Similar increases were found for IGFBP-3, resulting in a significant difference between means for GHD (+0.13 SD) and PWS (+1.34 SD; P=0.008). In year 2 further increases in IGF-I SDS were noted only for the PWS group, resulting in a mean of +1.5 vs. -0.73 to +0.07 for the other groups; P=0.0002 vs. GHD, UTS). Similar changes were observed for IGFBP-3 (P=0.0015; PWS vs. GHD). In 7 of 18 PWS patients IGF-I was above +2 SD, whereas only 1 of 25 GHD patients, 3 of 28 SGA patients and 3 of 17 UTS patients exceeded +2 SD.
Conclusion: Our data shows that at 2 years of GH treatment with RD, children with PWS have increased IGF-I (IGFBP-3) to higher levels than children with GHD or UTS (GHD). Proposals to lower GH dose in response to elevated IGF-I (IGFBP-3) concentrations may conflict with the need to maintain GH doses required for metabolic effects.

PO3-105 GH and IGF Use III

Comparison of European and US treatment practices with recombinant human growth hormone (rhGH): data from the Ipsen sponsored international (iNCGS) and the Genentech sponsored national cooperative growth study (NCGS) data bases, 2004-2008

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Treatment (Rx) with rhGH of idiopathic growth hormone deficiency (IGHD), organic GHD (OGHD), and Turner syndrome (TS) has been ongoing for decades. However, comparisons of Rx practices between US and Europe are few. Patient ascertainment and physician selection may vary and thus affect outcomes. Our aim was to compare patient demographics, dose, first year Rx outcomes with NutropinAQ between iNCGS and the NCGS.
Data from patients enrolled between 22 April 2004-30 June 2008 in both registries were compared. Definitions for etiologies were standardized. Height SD scores (ht sds) were calculated from country specific reference data. First-yr growth velocity was determined for pre-pubertal patients naive to prior rhGH Rx. rhGH Rx was initiated in 9,906 US NCGS and 670 iNCGS EU patients. Gender distribution (69% and 62% male) age (mean:10.7 vs 10.3 yrs) and ht sds (mean: -2.3 vs -2.4) US vs EU at rhGH start, were similar. Average doses in US were higher than in EU [mean 0.32 + 0.08 vs 0.24 + 0.05 mg/kg/wk for all, slightly higher in TS (0.35±0.07 vs 0.28±0.07 mg/kg/wk)] reflecting differences in approved doses.

Height changes (SDS) after the first-year rhGH treatment for prepubertal patients

	NCGS n = 9,906		iNCGS n=670	
GHD	Mean ± SD	n (%)	Mean ± SD	n (%)
IGHD		4114 (41.5)		361 (53.9)
Δ ht SDS	0.7 ± 0.6	1001	0.7 ± 0.4	85
OGHD		889 (9.0)		79 (11.8)
Δ ht SDS	0.9 ± 1.1	300	0.7 ± 0.4	23
Turner syndrome		561 (5.7)		49 (7.3)
Δ ht SDS	0.6 ± 0.6	215	0.5 ± 0.4	21
Short stature (other forms)		2736 (27.6)		92 (13.7)
Δ ht SDS	0.6 ± 0.5	678	0.6 ± 0.4	25

Results in these and other relevant conditions will be presented.
The implementation of iNCGS allows comparison of Rx practices in real time between US and Europe. Preliminary data indicate that the almost 2:1 gender distribution of males to females, and starting ages and ht sds are similar. While doses differ for regulatory reasons, first year growth responses are close and consistent with a flat dose response curve in the ranges used. As the iNCGS database enlarges, further analyses could look at areas of potential differences eg. the diagnostic work-up, Rx regimens, time of stopping GH treatment, and other variables to see if cultural and health care differences influence practice or outcomes.

PO3-106 GH and IGF Use III

In girls with Turner syndrome, a fixed growth hormone dosage per m² body surface area leads to stable IGF-I SDS, good adult height gain, and less costs

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Background: There are theoretical arguments that body surface area (BSA) is superior to body weight (BW) for scaling growth hormone (GH) dosage in children. In most countries, however, GH is dosed per kg BW. In reports on clinical trials using a fixed dose/m^2 usually the ratio 1m^2=28kg is given, but this ratio varies substantially with age. **Objectives:** To analyze plasma IGF-I SDS of girls with Turner syndrome treated with a fixed GH dose/m2 and to compare the given dose by BSA with the calculated dose by BW. **Methods:** IGF-I levels were obtained from 44 girls with Turner syndrome treated with GH 1.33 mg/m^2/d s.c. until adult height. They were included in the placebo arm of a clinical trial with a total number of 129 Turner subjects on the additional effect of 2 doses of oxandrolone. At 12 years low dose estrogens were started. Subjects were grouped into three age categories by their age at start of treatment: 2.00-7.99 years (n=16), 8.00-11.99 years (n=16) and 12.00-16.00 years (n=12). IGF-I was converted to SDS for age and gender. Assuming equal efficacy of a fixed dose per kg BW, we calculated what the dose would have been if calculated per kg BW (50 µg/kg/d) for all 129 subjects, and investigated the effect of BMI and age on dose. The average cumulative GH doses by BSA and by BW per age group were calculated and compared with a paired t-test. **Results:** Mean±SD IGF-I SDS during GH therapy remained stable at +0.8±1.4. Adult height gain in the 3 age groups was 9.8±5.4 (2-7.99 yrs), 5.8±3.7 (8-11.99 yrs) and 5.0±2.8 (12-16 yrs) cm. Dosing by BW would result

in 84% of the dose per BSA at age 3, 111% at age 10 and 132% at age 16. In underweight children dosing by BSA resulted in a higher dose than dosing per BW, and vice versa for overweight children. For each age group, the cumulative GH dose was significantly greater if calculated per kg BW compared with per m^2 BSA (average for all age groups 4,346 versus 3,680 mg, $p< 0.001$), equivalent to an average cost difference of €2,250 per subject. **Conclusion:** GH dosing by BSA leads to stable IGF-I SDS levels, while GH dosing by BW leads to relatively low doses in young and/or lean children, and high doses in older and/or overweight children and adolescents. Dosing by BSA is financially beneficial, and the adult height gain of girls with Turner syndrome treated with a fixed GH dose per m^2 BSA in this and previous clinical trials appears superior to the results reported on trials that dose by BW.

PO3-107 GH and IGF Use III

Development of new in-house methods for the detection of treatment-related neutralizing antibodies against human growth hormone

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Background: Human growth hormone insensitivity (hGH-IS) is an uncommon cause of pathological growth dynamics of patients with growth hormone deficiency (GHD) treated with recombinant (r)hGH. Neutralizing antibodies against rhGH (hGH-NAb) may arise during treatment and may lead to hGH-IS in these patients. The aim of our study was to develop and compare immunological and cell-based methods for the determination of hGH-Ab.
Methods: 1) Immunoprecipitation assay with 125-Iodine- labeled pituitary-derived hGH and polyethyleneglycol solution as precipitating reagent for forming antibody-antigen complexes. 2) Immunoassay based on hGH-coated microtiterplates and biotinylated hGH as well as on SulfoTag-labeled Streptavidin for the detection by the Electrochemiluminescence Imager 2400 (MSD). 3) Nab assay based on the hGH-Nab related inhibition of proliferation of GH-receptor overexpressing BAF3 cells. For this assay patient sera were pretreated to receive the enriched IgG fraction. Signals were detected by a photometric dye measurement. hGH-Ab raised in rabbits were used as positive controls or standards for all assays. Sera of healthy hGH naïve subjects were used as negative controls. Serum samples of GHD patients treated with hGH were tested.
Results: 1) and 2): All samples of rabbit hGHAb revealed distinctly increased signals compared to the negative controls. Additionally, three sera of patients treated with hGH were also found to be antibody positive. Both methods delivered sufficient quality acceptance criteria with an intra and interassay precision below 30% for the whole measuring range. 3) In dose response curves performed with rhGH preparations we determined EC50 dose-response values of approximately 2 ng/ml. The addition of 0.5 µg/ml commercially available hGH-NAb lead to a 50% inhibition of the hGH proliferation and confirms the functionality of the Nab detection assay. The serum of a patient with GHD 1A, undiluted and up to a dilution of 1:500, completely suppressed the GH activity in our cell assay.
Conclusion: Using our immunoassays we are capable to screen sera for increased anti-hGH immunreactivity. The detection of NAb by the cell-based assay may prove the neutralizing functionality of specific hGH antibody epitopes.

PO3-108 GH and IGF Use III

The usefulness of endocrinological retesting in subjects with growth hormone deficiency following replacement therapy

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Aims: To assess the somatotropinic function in patients with isolated idiopathic GH deficiency following replacement therapy with recombinant GH and to correlate the auxological data of the patients with the results of the retesting.
Methods: The clinical-auxological data of 30 patients (7 females, 23 males) with a diagnosis of isolated idiopathic GH deficiency, were evaluated over an average of 7 years and endocrinological retesting was carried out at the end of the growth period, at least 3 months after withdrawing treatment.
Results: 87% of the patients had a normal response to the retesting (GH peak ≥ 10 µg/L in response to the tests with Arginine, Insulin or Clonidine, or ≥ 19 µg/L in response to the test with GHRH + Arginine). The gain in growth was statistically significant for all the subjects (from a mean initial height of 2.40 ± 0.60 SDS, to a mean final height of -1.14 ± 0.71 SDS). No significant differences were observed in the auxological data between the subjects who had normalised the somatotropinic function on retesting and those still with a deficiency on retesting.
Conclusion: The normal results of the endocrinological retesting in 87% of patients suggest the need to carry out retesting also before the end of the growth period in order to be able to detect the short-term transitory deficiencies or misdiagnosis and allow to withdraw treatment.

PO3-109 GH and IGF Use III

hrGH therapy may cause pathologic proteinuria

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Aim: To assess the occurrence of pathologic proteinuria (≥ 20mg/dl) in patients with isolated idiopathic GH deficiency in treatment with hunan recombinant GH (hrGH).
Methods: During follow up, protein urinary levels were measured in 137 patients (86 males and 51 females) suffering from isolated idiopathic GH deficiency and treated with hrGH since at least one year.
Results: Pathologic proteinuria was discovered in 27,7% of patients, without any significant difference between males and females. These patients had been treated with a mean dose of hrGH (0,248 ± 0,023 mg/kg/week) significantly higher (P = 0,007) than the mean dose of hrGH given to patients without proteinuria (0,238 ± 0,017 mg/kg/week); even the mean duration of therapy was significantly higher (P = 0,007) in patients with proteinuria (5,61 ± 2,54 years) than in patients without proteinuria (4,28 ± 2,53 years). Furthermore, there was a significant inverse correlation (P < 0,05) between the duration of treatment before the appearance of proteinuria and the number of times that this side effect occurred. Six months after the discontinuation of treatment the proteinuria had disappeared in every patient.
Conclusion: In a significant number of patients, treatment with hrGH causes pathologic transitory proteinuria, related with mean administered dose of hrGH and with duration of therapy.

PO3-110 GH and IGF Use III

GH treatment should be continued after normalizing height SDS in very young short children born small for gestational age (SGA): resuls of a French collaborative study

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Age at the start and GH doses influence height (H) gain during GH treatment in short SGA children.We reported that these children, treated early during childhood (2 to 4 years) with high GH doses regimen (HDR: 85µg/kg/day) reached their target height (TH) channel within 30 months of treatment. In the second part of this study, we evaluated whether GH treatment can be discontinued after reaching TH channel. At the end of HDR, 110 SGA children (CA: 5.7±1.0 years) were assigned either to continue GH treatment at a lower dose for 3 years (Group A, n= 56, 29 girls, 57µg/kg/d) or to be untreated for at least 2 years (Group B, n=54, 27 girls). In Group B, GH treatment was resumed if patients had a height loss ≥1 SD after 2 years. During follow-up, GH dosage was adjusted to maintain IGF1 levels below- 2.5 SD.

	End of HDR	Year 1	Year 2	Year 3
HV (cm/ year)				
Group A		6.8 ±1.4	5.9 ±1.3	5.8±1.2
Group B		3.5±1.0*	3.5±0.9*	5.9±1.9
H SDS				
Group A	-0.7±0.9	-0.5±1.0	-0.4±1.0	-0.3±1.0
Group B	-0.7±0.9	-1.2±0.8	-1.4±0.7	-1.3±0.8
ΔH -TH SDS				
Group A	-0.04±1.1	0.1±1.2	0.2±1.2	0.3±1.1
Group B	0.1±1.0	-0.4±1.0§	-0.7±0.9*	-0.6±1*
ΔH HDR-H SDS				
Group A	2.5±0.7	0.2±0.3	0.3±0.4	0.4±0.5
Group B	2.6±0.6	-0.5±0.2*	-0.9±0.4*	-0.8±0.4*
IGFI SDS				
Group A	2.5±2.0	2.1±1.4	1.6±1.6	0.8±1.4
Group B	2.8±1.8	-0.7±1.3*	-0.6±1.4*	0.7±1.6
GH dose (μg/kg/d)				
Group A		55±3	51±7	48±8.5
Group B				57±3

§p= 0.01, *p<0.0001comparisons between groups

In group B, height velocity (HV) decreased after GH discontinuation leading to a height loss of -0.9±0.4 SDS at Year 2. GH treatment was resumed in 32 patients (59%), after a median time of 31.5 months.16 patients (30%) continued to grow normally and remained off GH treatment. In group A, height gain was 0.4±0.5 SDS at Year 3. GH dosage was decreased once (n=21), twice (n=8) or 3 times (n=1) in 30 (53%) patients. Overall, 37 (84%) patients in Group A and 21 (48%) patients in Group B remained in their TH channel (p=0.03).In conclusion: GH treatment should be continued after normalizing HSDS in SGA children. Lower GH doses ranging from 27 to 57μg/kg/day were sufficient to maintain these children in their TH channel. However, in a subgroup of patients, GH treatment could be stopped during a 3-year period without major change in their height SD.

PO3-111 GH and IGF Use III

Long term effects of growth hormone treatment on cardiac function and structure in non GH deficient children

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Background
Growth hormone (GH) treatment is extendedly used in non-GH deficient children in order to promote linear growth. Growth hormone excess on the other hand is known to have adverse effects on the heart. The long term effect of GH treatment on cardiac function in such patients has not been widely evaluated.
Aim
To investigate the effect of GH on cardiac structure, and function, in 2 groups of patients - Idiopathic Short Stature (ISS) and Intrauterine Growth Retardation (IUGR), who received GH treatment for 3 years.
Patients and Methods
Twenty three children, 16 with ISS (7 males, 9 females) with a mean age of 10.9±1.9 y, and 7 with IUGR (3 males, 4 females) with a mean age: 6.8±2.8 y, were studied. All children had a peak GH response, either to glucagon or to clonidine provocation test, more than 10 ng/ml. All subjects were prepubertal or at the beginning of puberty on the initiation of treatment (Tanner stage 1-2). Anthropometric measurements and echocardiograms were performed at baseline, 6 months, 1, 2 and 3 years after initiation of GH therapy (0.3 -0.5 mg/kg/wk).
Results
After 3 years of treatment height SD score increased significantly in both groups (ISS and IUGR: -2.5±0.2 to -2.0± 0.3 SDS, p=0.001 and -2.9±0.5 to -2.1± 0.8, p=0.018 respectively).The Left Ventricular Mass (LVM) increased in both treated groups. However no such difference was observed after indexing LVM for body surface area (BSA). The indexed LVM in the ISS group changed from 59±8 to 57±10 g/m^2 (p>0.05) and in the IUGR group from 53±9 to 57±7 g/m^2, (p>0.05). Indices of systolic performance (Ejection Fraction and left ventricular Shortening Fraction) were normal at baseline and did not change significantly with GH therapy. Lastly both Left ventricular posterior wall and interventricular septal thickness showed no significant change.
Conclusions
Three years of GH treatment in children with ISS and IUGR did not have any adverse effects on cardiac function and structure. A tendency towards increased left ventricular mass may reflect the increase in lean body mass during treatment.

PO3-112 GH and IGF Use III

Final height and body composition in growth hormone-treated short Greek children born small for gestational age

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Background: It is generally recognized that children born small-for-gestational age (SGA) have a 5-7 times higher risk of short stature than children born at normal size. These children remain not only shorter but also have lower body mass index (BMI) than children who were born appropriate for gestation age.
Aim: To assess retrospectively the effects of GH therapy on final height and body composition in short SGA Greek children.
Methods: Thirty eight children (22 males, 16 females with mean age just before the initiation of therapy 9.4 ± 3.4 years) were included. Thirteen of them were GH deficient. The inclusion criteria were: (1) birth weight standard deviation score (SDS) below -2 for gestation age and (2) height SDS below -2. All children were treated with recombinant hGH at a dose of 0,4mg/kg per day. Children's height and weight at the initiation, after 6 months, 1 year and at the end of the treatment were recorded and serum glucose levels were measured. Target height (TH), predicted adult height (PAD), bone age (BA), height velocity (HV) and final height (FH) were also calculated and expressed as SDS.
Results: In the whole group, HV and HSDS for chronological age increased significantly after 6 months, one year and at the end of GH treatment (p<0.05). A significant increase in the mean final HSDS was also established in the group of children whose chronological age differed from bone age more than 2 years at the initiation of the treatment (p=0.05). Younger subjects also tended to gain better final height. No significant difference however was found in HSDS and HV between GH and non-GH deficient short children. Finally, GH treatment resulted in a significant increase of BMI compared to baseline (p<0.04) without any adverse effects on glucose levels.
Conclusions: Our data show that long term GH treatment in short SGA born children either GH deficient or not leads to a better final height and an improved BMI. The greater the difference is between chronological and bone age, as well as the younger the child is at the initiation of treatment, the better seems to be the response.

PO3-113 GH and IGF Use III

Response to growth hormone therpapy in two patients with severe vitamin D resistant rickets

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Introduction :
Hereditary vitamin D-resistant rickets is a very rare form of rickets, with fewer than 50 known affected kindreds. It is an autosomal recessive disorder due to end-organ resistance to calcitriol usually caused by mutations in the gene encoding the vitamin D receptor (VDRL). The clinical spectrum varies widely. Treatment involves a therapeutic trial of calcitriol and calcium supplementation.
Clinical cases:
We report a 10-year-old boy who was diagnosed at the age of 14 months with a very severe form of vitamin D resistant hypocalcemic rickets. The diagnosis was confirmed by a homozygous mutation in the VDRLgene. His disease has led to severe rickets, alopecia, short stature, profound limb and thorax deformities with secondary chronic respiratory insufficiency, repeated fractures, severe

muscle weakness and delayed motor development. At the age of 4 years bone densitometry showed severe osteoporosis with an age adjusted z-score of the lumbar spine of -5.06SD. Treatment with oral high doses of calcitriol, phosphor- and calcium has been given: neither biochemical nor clinical response could be demonstrated. Therapy was extended by administration of growth hormone and biphosphonates. During the latter treatment his muscle strength, motor development, respiratory function and bone mass improved; bone densitometry showed an age adjusted z-score of the lumbar spine of +2SD at the age of 9 years.

Our second patient is a 5-year-old girl who was diagnosed at the age of 1.5 years with a very severe form of vitamin D resistant hypocalcemic rickets. A homozygote mutation in exon 3 of the VDRLgene was found. She developed severe osteoporosis with severe growth retardation (<3rd centile, growth velocity of 0.25cm/month), pulmonary problems due to thorax deformities, limb deformities, fractures and alopecia. Supplementation of calcitriol, calcium and phosphor has been given without any biochemical and clinical response. Since the age of 4.5 years treatment with growth hormone has been given. During the latter treatment she shows a growth velocity of 0.6cm/month.

Conclusion:

In the patients described who suffer from a very severe form of vitamin D resistant hypocalcemic rickets, the administration of growth hormone has led to clinical improvement of the muscle strength, a declined rate of fractures and an acceleration of growth based on a better mineralization of the bones. In our opinion growth hormone should be added in the treatment of this severe bone disease.

PO3-114 GH and IGF Use III

Growth hormone (GH) treatment to idiopathic short stature (ISS) children increased peripheral thyroid hormone receptor (TR) expression

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Introduction: ISS is defined as a condition in which the height of an individual is more than 2 SD score (SDS) below the mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities. Several clinical trials demonstrated beneficial effects of GH treatment *(J Clin Endocrinol Metab 93:4210,2008).* In turn, the major systemic hormones that influence growth during childhood include not only GH/ insulin-like growth factor I (IGF-I) but also thyroid hormones (TH). The impact of TH action at tissue level is determined by TH availability as well as by TR expression at tissue level. We have reported *(Clin Endocrinol 67:629,2007)* that GH treatment to Turner Syndrome patients impaired peripheral TH action. The aim of this study was to assess the effect of GH treatment on peripheral TR expression in ISS patients.

Patients & Methods: Twenty ISS patients (growth velocity <2 SD and normal GH secretion after pharmacological provocation tests), 10-14 y/o, were analyzed previous and after 6 and 12 months of GH therapy (50µg/kg/day). All patients were euthyroid. Peripheral blood mononuclear cells (PBMC) were obtained (Fycoll-Hipaque), total RNA extracted (Trizol) and TR mRNA levels determined by Real-time RT-PCR. In turn, thyroid function was tested by thyrotropin, total thyroxine, free thyroxine and total triiodothyronine serum levels and the biochemical response to GH treatment by osteocalcin (OC), β-crosslaps (B-CL) and IGF-I serum levels (measured by electrochemiluminescence except for IGF-I that was determined by a immunoradiometric assay).

Results: As depicted in Table 1, results achieved showed a significant increase in TR mRNA expression in PBMC after both 6 and 12 months of GH treatment (ANOVA-Student-Neuman-Keuls, *p<0.05 vs pre GH treatment sample). In turn, no changes in thyroid function tests and significant increases in IGF-I, OC and β-CL were registered.

Table 1

	6 months	12 months
fold change in TR mRNA-level (after/before GH treatment) (mean±SD)	1.41 ±1.15*	1.90 ±1.61*

Conclusions: Results indicate that GH treatment to ISS patients enhanced the peripheral expression of TR. It is not possible to conclude with surety whether results of TR expression in PBMC are predictive of the thyroid status in the whole organism. Nevertheless, the pivotal role of TH action for normal growth points out the importance of analyzing the evolution of thyroid signaling during GH treatment to ISS patients.

PO3-115 GH and IGF Use III

Genomic markers improve the prediction of short-term IGF-I response to growth hormone (GH) in girls with Turner syndrome (TS): the PREDICT study

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Background: The PREDICT Phase IV study is examining the relationships between conventional biomarkers and genomic markers after 4 weeks of GH therapy in GH treatment-naïve prepubertal children with GHD or TS.

Objective: To identify factors that best predict IGF-I SDS level after 4 weeks of recombinant human GH therapy in girls with TS using PREDICT data.

Methods: Akaike's Information Criteria for multivariate model selection were used to identify the factors best predicting IGF-I SDS level at Week 4 in 149 girls with TS (intention-to-treat population). Potential covariates included birth, auxological, treatment and parental characteristics, and baseline biomarkers. The best-fit model was then applied in subgroup analyses by selected genetic markers (single nucleotide polymorphisms; SNPs) in 123 girls with TS to evaluate their impact on IGF-I SDS level prediction.

Results: The best-fit model identified included the following predictive factors:

- IGF-I SDS at baseline
- Fasting triglycerides at baseline (mmol/L)
- Weight SDS at birth
- Weight SDS at baseline
- Thyroid hormone treatment
- Mean GH dose (mg/kg/day)

The coefficients for these factors were positive, except for fasting triglycerides at baseline, weight SDS at birth and thyroid hormone treatment, which were negative. These factors accounted for 47% of the variability in IGF-I SDS level at week 4 (N=124). When subgroup analyses were carried out, the absence of a SNP in PIK3CB (N=91) or in CDK4 (SNP A) (N=70) or (SNP B) (N=100) increased the adjusted R^2 to 57%, 57% and 56%, respectively. The absence of karyotype i(Xq) inversions (N=94) increased the adjusted R^2 to 62%.

Conclusions: The PREDICT study provides evidence that adding selected genomic markers to conventional predictive factors in a best-fit model improves the prediction of IGF-I SDS level at week 4 in girls with TS, stressing that pharmacogenomic research is key to understanding responsiveness to GH. How specific karyotypes and these genes relate to GH action deserves additional research.

PO3-116 GH and IGF Use III

Oxandrolone improves height velocity and BMI in patients with cystic fibrosis

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Background: Patients with cystic fibrosis (CF) often have poor weight and height gain, which is a risk factor for poor pulmonary function, morbidity, and mortality. Multiple agents have been used to stimulate appetite and growth in patients with CF, but their utility has been limited due to side effects (such as adrenal suppression with megestrol) or cost (such as growth hormone). Oxandrolone is a weak androgen that stimulates appetite and promotes growth. Since oxandrolone cannot be aromatized to estrogen, advancement of the bone age is minimized. To our knowledge, there are no reports in the literature of the use of oxandrolone in patients with CF.

Objective: To evaluate the effectiveness of oxandrolone in improving the nutritional status of pediatric patients with cystic fibrosis.

Methods: We conducted a retrospective analysis of patients with cystic fibrosis treated with oxandrolone. Medical records were reviewed to determine height z score, height velocity (HV), BMI z score, and weight velocity (WV). Additional information obtained included age, Tanner stage, pulmonary function, liver function enzyme levels, and any reported adverse events. Data were compared before (pre-Tx) and after (Tx) initiation of oxandrolone using a paired t test.

Results: 5 subjects (3 boys and 2 girls), ages 8.5-14.5 years, were treated with 2.5 mg of oxandrolone daily for 8-38 months. All subjects had pancreatic insufficiency. At the start of oxandrolone therapy, four subjects had Tanner 1 genitalia or breasts, and one male patient had Tanner 4 genitalia. After 8-12 months of treatment, there was a statistically significant improvement in HV (pre-Tx = 5.3 ± 1.4 cm/yr, Tx = 8.3 ± 1.2 cm/yr, $p < 0.01$) and BMI z score (pre-Tx = -0.61 ± 1.04, Tx = -0.30 ± 0.86, $p = 0.02$). Both height z score (pre-Tx = -1.64 ± 0.63, Tx = -1.30 ± 0.49, $p = 0.057$) and WV (pre-Tx = 4.2 ± 3.7 kg/yr, Tx 6.8 ± 1.0 kg/yr, $p = 0.072$) showed beneficial trends which did not reach statistical significance. There was no significant change in pulmonary function or liver function, and no adverse events were reported. Neither female subject experienced hirsutism, clitoromegaly, or any evidence of hyperandrogenism.

Conclusions: In this small retrospective study, oxandrolone improved the height velocity and BMI z score in patients with CF. Larger studies are needed to determine if oxandrolone is an effective, safe, and affordable option to stimulate appetite and promote growth in patients with CF.

PO3-117 GH and IGF Use III

Posttraumatic brain injury with hypothalamic and pituitary hormone insufficiency

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Posttraumatic brain injuries including endocrine deficiencies of pituitary axes are mainly reported as case reports, prospective studies and systematic investigation are rare. The neuronal localization of such injuries is discussed contradictorily.

We report a case of an 8 y old girl suffering from mental retardation and disturbed midline brain development. MRI had shown a missing pituitary stalk and a normal situated anterior pituitary gland. During the first three years of life no endocrine disturbances occurred. At the age of three years a severe head and brain trauma happened.

We present the endocrine course and therapy of a combined hypopituitarism over the last 5 years. The thyroid axis showed initially a low fT3 syndrome and recovered to normal function shortly after the injury. TRH testing revealed normal findings. The growth hormone axis remained severely impaired, documented through markedly reduced growth velocity and growth hormone secretion in stimulation tests. The steroid axis developed a partial insufficiency with recurrently reduced cortisol excretion but normal CRH and ACTH stimulation tests. These findings represent a hypothalamic localized injury of the steroid axis. One year after the traumatic brain injury we started to give her steroids in case of infections and the duration of recovery was shortened clearly. Stimulation tests of the gonadotrophic axis revealed normal levels of LH and FSH after GnRH administration. Nevertheless, the spontaneous course of puberty has to be estimated later. The regulation of water was disturbed immediately after the accident which is still persisting as partial central diabetes insipidus. As the posterior pituitary - due to the missing stalk - is located ectopically the injury of the ADH secretion is localized in the hypothalamic region.

Taken together, our patient presents a case of combined posttraumatic hypopituitarism, in which the site of the injury is hypothalamic as well as pituitary. Posttraumatic hormone deficiencies have to be taken in account in the follow up investigations of children after severe head and brain injuries or concussions.

PO3-118 GH and IGF Use III

Can the phenotype of septo-optic dysplasia at presentation be used to predict the severity of associated hormonal abnormalities, developmental delay, obesity, sleep and behavioural disorders?

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Background: Septo-optic dysplasia (SOD) is a phenotypically variable disorder in which associated hormonal abnormalities, developmental delay, obesity, sleep and behavioural disorders can evolve over time. There is very little reported on the presentation and prevalence of these associated problems.

Aim: To define the phenotype and evolution of SOD and isolated optic nerve hypoplasia (ONH) in a large cohort of patients.

Methods: A retrospective case-note review of all patients with SOD and ONH registered at our Institution was undertaken. SOD was defined as the presence of ONH plus one or both of the following; (i) pituitary hormone abnormalities and (ii) midline brain defects. Data including age, sex, MRI brain appearances, level of visual impairment, hormonal insufficiencies, neurodevelopment and sleep pattern were recorded.

Results: 147 children with SOD and 26 with ONH were identified. Mean age at follow up was 9.97 years (SD 6.13years) with a mean age at diagnosis of 1.84 years (SD 2.6years). The most common presenting complaint was concern regarding vision (63%). 39% had no hormonal insufficiencies at presentation, in 30% of these children hormonal insufficiencies evolved during follow up. 38% reported abnormal sleep patterns, 59% of these individuals received treatment with melatonin of whom 53% reported no improvement in symptoms. 46% had normal neurocognition, 19% mild, 16% moderate and 19% severe developmental delay. 30% of patients had a diagnosis of autistic spectrum disorder. Children with isolated ONH identified on neuroimaging were significantly less likely to develop hormonal, obesity, sleep or developmental problems than those with additional midline abnormalities ($p<0.01$).

Conclusion: Examining the phenotype in detail at presentation and relating it to the evolution of pituitary hormonal insufficiencies and the severity of problems associated with SOD may allow us to better counsel parents. By identifying those children most at risk of developing hormonal insufficiencies we can ensure that we do not miss the evolution of potentially life-threatening pituitary hormone insufficiencies by arranging regular assessment of pituitary hormone production in these individuals. This study also highlights the significant non-hormonal problems that children with SOD have and the need for developing more targeted intervention strategies (for example in sleep where many of these children are resistant to conventional approaches) in this cohort of patients.

PO3-119 GH and IGF Use III

The IGF-I generation test revisited: how does IGF-I and IGFBP-3 increase predict responsiveness to therapy in children under rhGH treatment?

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With the availability of recombinant IGF-I as a therapy for children with severe IGF-I deficiency the so called IGF-I generation test has regained interest for

clinical endocrinologists. It holds the premise to early identify those patients with severe IGF-I deficiency who will show decreased growth response (GR) to GH treatment.
In order to assess the validity of IGF-I and IGFBP-3 increments under GH-therapy to predict GR in rhGH treated children we evaluated IGF-I and IGFBP-3 levels before and 1 week, 1 month or 3 months after start of GH therapy (0,025 -0,035 mg/kg/day) and correlated them to the observed height gain after 12 months treatment.
We examined 649 patients (404 boys and 245 girls) of our Growth Clinic who received GH therapy due to GHD.
If we consider height SDS after 1 year of treatment – height SDS at start of treatment as responsiveness to rhGH treatment (GR), following correlations (R^2) of GR with the listed parameters were obtained:

Parameter	R^2	Slope	P-Value	n
IGF-I bef. therapy (SDS)	0,04223	-0,03653	1,9 e-03	226
ΔIGF-I 1 week (SDS)	0,005106	-0,02128	6,11 e-01	53
ΔIGF-I 1 month (SDS)	0,03878	0,05747	1,31 e-02	158
IGFBP-3 bef. therapy (SDS)	0,07038	-0,05130	5,10 e-04	168
ΔIGFBP-3 1 month (SDS)	0,00071	0,00873	7,94 e-01	98
Δheight 3 months (SDS)	0,5565	0,48892	< 2,0 e-16	639

The values show that both IGF-I and IGFBP-3 measurements at 1 or 3 months are poor predictors of responsiveness to treatment (auxological measurements are better).
If IGF-I measurements after 1 week or 3 months of treatment are correlated with those after 12 months:

Parameter	R^2	slope	P-Value	n
IGF-I 1 week (SDS)	0,6249	0,93237	2,45 e-16	71
IGF-I 3 months (SDS)	0,6591	0,97689	< 2,0 e-16	236

Our data indicate that IGF-I increases per se are bad predictors of GR as there are many children that grow well despite persistingly low IGF-I values. However, as Δheight SDS after 3 months treatment well predicts later growth and IGF-I values after 3 months (and probably after 1 week - small n!) well predict IGF-I values after 1 year of treatment, it seems possible to identify a subgroup of patients at risk for poor GR and permanently low IGF-I levels by combining auxological measurements after 3 months of treatment with the results of an IGF-I generation test.

PO3-120 GH and IGF Use III

Case report: preliminary use of recombinant IGF1 in a case of secondary IGF1 deficiency due to hepatic disease (Alagille syndrome)

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Recombinant human IGF1 (rhIGF1) has been administered to short children in cases of genetic or presumedly genetic primary IGF1 deficiency, but to our knowledge no written publication has been devoted to a secondary IGF1 deficiency due to hepatic disease. Here we report a case with such a deficiency due to Alagille illness, an autosomal dominant genetic condition including neonatal biliary ductus paucity leading to hepatic insufficiency.
MB, a girl born from apparently healthy non consanguineous parents was referred at the age of 21 months for failing to thrive after an IUGR, anicteric cholestasis, high transaminases but normal hepatic cell funtion. Liver biopsy showed hypoplasia but no paucity of biliary ductules and no fibrosis. Despite the absence of characteristic ocular embryotoxon, vertebral butterfly wing X-Ray aspect and cardiac abnormalities at ultrasound, Alagille syndrome diagnosis was retained according to Jag 1, exon 6, gene mutation found in this patient and in her mother.
She was refered to the pediatric endocrinology unit at the age 13 for a growth delay of -3.5 SDS. Puberty was rated stage 2 according to Tanner. No other clinical or biological signof hepatic insufficiency was seen than a IGF1 plasma level lower than -3SD (0.56 U/L), but with a normal GH response and a normal IGF BP3 level. After 6 months of rhIGF1 (Increlex), 80 µg/d in 2 sc injections, her growth velocity increased from 4cm/y to extrapolated 12 cm/y. No adverse sign was noticed (hypoglycemia, deterioration in hepatic function or signs of inflammation, tonsil hypertrophy, cardiac, hepatic nor splenic hypertrophy at ultrasound).
In conclusion, rhIGF1 therapy merits to be considered in selected cases of secondary IGF1 insufficiency of hepatic origin.

PO3-121 GH and IGF Use III

Recombinant human growth hormone Improves linear growth in children with inflammatory bowel disease: results of a randomised controlled trial

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Background:Despite optimal gastrointestinal management, some children with inflammatory bowel disease(IBD) may have growth retardation. The role of rhGH in these children is unclear.
Design:Randomised controlled trial of rhGH(0.067mg/kg/day) over a 6 month period. Primary outcome measure: height velocity (HV).
Subjects:22 children with IBD and HtSDS<-2 or HtSDS<-1 and HVSDS<-1. Eleven(9M) were in the control group (C) and eleven(9M) in the treatment group(Rx). All children had Crohn's Disease (CD) except 2 in the C group who had ulcerative colitis (UC).
Methods:HtSDS, HV, HVSDS were compared between in Rx and C at baseline(T0) and 6 months(T6). HVSDS was adjusted for Tanner stage(TS) for girls≥11yrs and boys≥12yrs. Disease severity including CRP, ESR, albumin(Alb), haemoglobin(Hb) and cumulative dose of Prednisolone were collected at T0 and T6. Glucose homeostasis was assessed by fasting glucose, insulin, c-peptide and HBA1C at T0 and T6. All data are expressed as median(10^{th},90^{th}).
Results:CA at T0 was 14.7yrs(9.3,16.2) & 13.7(9.1,15.5);median CA-BA at T0 was 1.7yrs(-0.3,3.6) & 1.7yrs(-0.7,4.1) for Rx and C. TS at T0, in Rx were TS1-2; TS2-5; TS3-2; TS4-2. In C, they were, TS1-3;TS2-6;TS3-1;TS4-1. By T6, pubertal progress was noted in 5/11 & 3/11 of Rx and C groups. HtSDS at T0 was in Rx and C: -2.8(-4.1,-1.5) & -1.8(-2.7,-1.3,(p=0.001). Change in HtSDS at T6 in Rx and C was significantly different: 0.3(0.1,0.8) & -0.1(-0.3,0.3),p<0.0001. HV at T0 was similar in Rx and C: 5.0cm/yr(0.8,8.8) & 3.8cm/yr(1.6,6.5), and so was HVSDS: -3.1(-6.0,4.4) & -2.4(-6.2,1.8). HV at T6 in Rx and C was 10.8cm/yr(6.1,14.3) & 3.5cm/yr(2.0,9.3),p<0.0001. HVSDS at T6 in Rx and C was 3.2(-0.4,16.4) & -2.0(-6.3,4.9),p=0.0001. CRP, ESR, Alb, Hb and cumulative prednisolone dose were similar between the Rx and C group at T0 and T6. 2 children in each group had clinical relapse during the study period. ΔBA/ΔCA was similar in the two groups at T6. At T6, in Rx and C, fasting insulin, was 7.0mU/L(2.1,15.7) & 3.8mU/L(2.1,6.6),p=0.04; C-peptide was 0.7nmol/L(0.4,1.2) & 0.3nmol/L(0.2,0.8),p=0.002,and HOMA index was 1.5(0.3,3.7) & 0.3(0.2,0.8),p=0.05. Fasting glucose and HBA1C, were similar in both groups at T6.
Conclusion:rhGH at a dose of 0.067mg/kg/day in children with IBD and growth retardation can increase HV by over 100% without excessive skeletal maturation. Therapy is associated with a reduction in insulin sensitivity but no overt abnormality of glucose tolerance.

PO3-122 GH and IGF Use III

Improvement in growth of children with Crohn's disease (CD) following anti-TNFα therapy can be independent of pubertal progress and reduction in glucocorticoid (GC) therapy

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Introduction Therapy with anti-TNFα therapy such as Infliximab (IFX) may improve growth in children with CD but the extent of improvement in growth and the underlying mechanisms are unclear.
Aim A retrospective study of growth, puberty and disease activity over the 6 months prior (T-6) to starting infliximab (IFX),at baseline (T0) and for the following 6 months (T+6) in CD. Results are expressed as median (10^{th},90^{th}).
Subjects & **Methods** The growth and treatment details of 29 children (M:17) who were started on IFX at an age of 13.1yr(10.0,15.7) were reviewed. In 20 children, puberty data were also available at all time points. Data on disease markers (CRP, ESR, Albumin), total Alkaline Phosphatase (ALP) and a physician global assessment were also collected.
Results 22/29(76%) of cases demonstrated a clinical response to IFX treatment. Overall, height velocity(HV) increased from 3.6cm/yr(0.4,7.8) at T0 to 5.5cm/yr(2.1,9.2) at T+6(p=0.003). HV also increased in the subgroup of children who had remained prepubertal from 4.5cm/yr(0.4,8) to 5.5cm/yr (3.3,8.4)(p=0.05). In the subgroup of 12 children who had a reduction (n,2) or cessation in GC (n,10), HV increased from 1.6cm/yr(0.3,8.3) at T0 to 5.6cm/yr(2.2,9.2) at T+6 (NS), whereas those children who did not receive GC over the 12 months had an increase from 3.7cm/yr(0.6,6.5) to 6.4cm/yr (2.9,9.0) (p=0.04). HV at T0 and T+6 showed a significant association with the average ALP over the prior 6 months. In IFX responders HV increased from 1.9cm/yr(0.3,7.1) to 6cm/yr(2.3,9.1) (p=0.003) and in the non-responders, HV remained static at 4.3cm/yr(2.5,8.6) at T0 and 3.0cm/yr (2.0,11.3)(NS) at T+6. HV did not show any association with individual markers of disease activity. 10/29 children(33%) had a reduction in Ht SDS after starting IFX and 7 of these 10 children had also been growing poorly before starting IFX.
Conclusion Clinical response to IFX therapy is associated with an improvement in linear growth in children with CD. This increase is also seen in prepubertal and GC naïve children and cannot solely be attributed to a change in these factors. Approximately one third of children continue to grow poorly after starting IFX and may require other forms of therapy to imporve their growth.

PO3-123 GH and IGF Use III

Factors affecting growth response to growth hormone treatment in prepubertal patients with chronic renal failure

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The purpose of this study was to evaluate the factors affecting growth-promoting effect to recombinant human growth hormone (rhGH) treatment in growth-retarded prepubertal patients with chronic renal failure (CRF).
The study included 38 prepubertal patients with CRF whose glomerular filtration rate is less than 60 ml/min per 1.73 m^2 calculated by Schwartz equation, and height is less than -2 standard deviation score (SDS) or height velocity (HV) less than 4 cm/yr. All patients were treated with rhGH up to three years. Among patients, 22 were on conservative treatment, and 16 were on dialysis at baseline. All patients were treated with rhGH for one year, 23 for two years, and 11 for three years. Height SDS was -3.0±1.7 at baseline, 2.4±1.6 in the first year, -2.0±1.5 in the second year, -1.8±1.3 in the third year (P=0.068). Difference in height SDS was -0.4±0.6 at baseline, 0.6±0.5 in the first year, 0.3±0.5 in the second year, 0.2±0.2 in the third year (P<0.001). HV was 4.2±1.8 cm/yr at baseline, 7.9±2.8 cm/yr in the first year, 6.8±1.6 cm/yr in the second year, 5.8±1.5 cm/yr in the third year (P<0.001).
In the first year of rhGH treatment, height SDS was positively correlated with height SDS at baseline and the interval between CRF diagnosis and baseline; difference in height SDS was correlated with the interval between CRF diagnosis and rhGH treatment positively, and height SDS at baseline inversely; HV was positively correlated with the interval between CRF diagnosis and rhGH treatment and small for gestational age. In the second year of treatment, height SDS was positively correlated with height SDS at baseline and the interval between CRF diagnosis and rhGH treatment; difference in height SDS was positively correlated with bone age at baseline; HV was positively correlated with the interval between CRF diagnosis and rhGH treatment.
In conservatively treated patients, predicted adult height (PAH) SDS was -1.1±0.8 before rhGH treatment and -0.1±1.5 after rhGH treatment (P=0.003); in patients on dialysis, PAH SDS before and after rhGH treatment was -1.8±2.3 and -1.4±1.8, respectively (P=0.083). However, PAH SDS of all subjects was increased significantly from -1.5±1.7 to -0.8±1.8 after rhGH treatment (P<0.001).
In conclusion, rhGH treatment in CRF patients significantly increased difference in height SDS, HV and PAH SDS in the short term. Long-term follow-up is necessary to validate the effect of rhGH to the final height.

PO3-124 GH and IGF Use III

Growth responses to growth hormone therapy in chilldren with growth failure, who showed normal growth hormone response to stimulation tests

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Purpose: This study was aimed to investigate the clinical characteristics and response to growth hormone(GH) therapy in children with growth failure, who showed normal GH responses to GH stimulation tests(GHST).
Methods: The study included 39 patients with growth velocity of <4 cm/year and normal GHST result. Clinical characteristics of patients were analyzed through the retrospective review of medical records.
Results: Eleven(28.2%) were born as small for gestational age(SGA) and 28(71.8%) as appropriate for gestational age(AGA). In the SGA group, the age and the height standard deviation score(SDS) measured at their first visit were significantly low. Sixteen patients(41.0%) were treated with GH and six of 23 without GH therapy were followed for 1 year after GHST. Growth velocity was 7.9±2.6 cm/year in GH-treated patients and 3.6±0.8 cm/year in patients without GH therapy, which was statistically significant(P=0.03).

Comparison between Patients Received Growth Hormone Therapy and Followed without Growth Hormone Therapy*

	GH treated (n=16)	GH untreated (n=6)	P value
GV at GHST (cm/yr)	3.2±0.5	3.3±0.3	0.629
GV after 1 year of follow-up (cm/yr)	7.8±1.6	3.6±0.7	<0.001
GV difference (cm/yr)	4.7±1.6	0.3±0.7	<0.001
Height SDS at the start	-3.5±1.9	-4.3±3.1	>0.999
Height SDS after 1 year of follow-up	-3.2±1.9	-4.5±3.1	0.461
Height SDS difference	0.3±0.3	-0.2±0.3	0.002

*In GH-treated patients, height and height SDS are measured at the start of GH treatment and l year after GH treatment, whereas in patients without GH treatment, height and height SDS are measured at the time of GHST and 1 year after follow-up.

In the GH therapy group, growth velocity and difference in height SDS during the treatment increased significantly(growth velocity, P<0.001; height SDS, P<0.001). Growth velocity increased significantly after 1 year of GH therapy in SGA and AGA group (SGA, P=0.043; AGA, P=0.003). The level of Insulin-like growth factor-I was significantly lower in the group received GH therapy with height SDS <-3 than ≥3 (P=0.023).
Conclusion: In children with growth failure and normal GHST, growth velocity increased significantly by short-term GH therapy. The assessment of long-

term effects of GH therapy is necessary. Moreover, further studies should be considered to evaluate GH-IGF-I axis due to the possibility of GH insensitivity syndrome.

PO3-125 GH and IGF Use III

Long-term efficacy of growth hormone in Japanese short children born small for gestational age

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European and US data show improved childhood growth and adult height following GH treatment in short children born small for gestational age (SGA). The long-term efficacy of GH treatment in Japanese short children born SGA has not been published. This report examines the growth response after up to 4-yr GH (Norditropin® SimpleXx®, Novo Nordisk, Denmark) treatment in short Japanese SGA children. In a multicentre, double-blind, parallel group trial short Japanese children (60% male; age, 3.0-8.9; HSDS, -4.83 – -1.87) were randomised to a GH (0.033mg/kg/d, n=33; 0.067mg/kg/d, n=33) or untreated (n=20) group for 52 wk. At 52 wk GH-treated patients continued treatment at the same dose up to 208 wk. Untreated patients were randomised to GH (0.033mg/kg/d, n=10; 0.067mg/kg/d, n=10) for 156 wk. A dose-dependent increase in HSDS for chronological age (CA) from baseline was observed (Table). After 208 wk treatment the increase in mean HSDS was significantly greater in the higher dose group (estimated treatment difference [95% CI], 0.84 [0.59, 1.09], p<0.01). After 156 wk of treatment there was also a significantly greater gain in HSDS in the higher dose group (0.73 [0.039, 1.42], p<0.05). A dose-dependent increase in HVSDS for CA was observed with a waning effect after the first yr. After 208 wk mean (SD) HVSDS for CA was 0.28 (1.33) in the 0.033mg/kg/d group and 1.47 (1.87) in the 0.067mg/kg/d group. For groups untreated in yr 1 mean HVSDS for CA after 156 wk was 1.76 (2.10) and 2.35 (1.31) in the 0.033 and 0.067mg/kg/d groups, respectively. Treatment with GH was safe and well tolerated. Most adverse events were mild/moderate in severity. Levels of HbA1c increased during active treatment but were within the normal range.

In summary, results from this trial confirm the long-term efficacy of GH in short Japanese children born SGA. The observed increase in HSDS was dose-dependent over the duration of the trial and comparable to results in a preceding European study.

HSDS for CA by treatment group				
	208 wk		No treatment in yr 1 (156 wk)	
	0.033mg/kg/d	0.067mg/kg/d	0.033mg/kg/d	0.067mg/kg/d
	(n=30)	(n=29)	(n=7)	(n=8)
Baseline	-3.00 (0.61)	-2.83 (0.62)	-2.96 (0.67)	-2.75 (0.35)
156 wk			-2.06 (0.75)	-0.97 (0.67)
208 wk	-1.92 (0.78)	-0.92 (0.71)		
Change from baseline	1.08 (0.41)	1.91 (0.52)	0.90 (0.45)	1.78 (0.58)

Mean data (±SD)

PO3-126 GH and IGF Use III

The role of IGFBP-3 on high glucose induced apoptosis in proximal tubular epithelial cells

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Purpose : Insulin-like growth factor binding protein-3(IGFBP-3), the major circulating carrier protein for IGFs, also acts as a potent antiproliferative agent, by blocking cell cycle and inducing apoptosis in various cell types. Recently, it was reported that IGFBP-3 mediates high glucose induced apoptosis in mesangial cells and podocytes. Tubular cells are a primary target of hyperglycemia, and tubulointerstitial pathology, rather than glomerular pathology, correlates with renal dysfunction in diabetic nephropathy. This study was performed to investigate the role of IGFBP-3 on high glucose induced apoptosis in proximal tubular epithelial cells (PTEC).

Methods : IGFBP-3 expression in a PTEC line (LLC-PK1 cells) was measured by western blot and real time PCR, in low glucose (5.5mM) or high glucose (30mM) media. Apoptosis was quantified by Annexin V flow cytometry and DNA fragmentation ELISA. To investigate the role of IGFBP-3 on PTEC apoptosis, IGFBP-3 overexpressed PTEC line was established by transfection of IGFBP-3 cDNA.

Results: IGFBP-3 mRNA expression was significantly increased in high glucose media compared to low glucose media (P<0.01). High glucose media also augmented IGFBP-3 protein expression. Exposure to high glucose media for 72 hours significantly increased PTEC apoptosis (P<0.05). PTEC apoptosis was partially inhibited by adding SI RNA to IGFBP-3. IGFBP-3 overexpression increased PTEC apoptosis, which was also decreased by SI RNA to IGFBP-3.

Conclusion : Our results suggest that increased IGFBP-3 expression by high glucose may mediate PTEC apoptosis. Further investigation is required to verify the mechanism of IGFBP-3 induced apoptosis, and the role of IGFBP-3 in the development of diabetic nephropathy.

PO3-127 GH and IGF Use III

IGF-I generation test and first year response to GH

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Introduction

The IGF-I generation test can be used in the diagnostic work up of short children with low IGF-I levels (<-2 SD) and a high max. GH peak during the stimulation test, in order to assess GH sensitivity. An increase of IGF-ISDS >1 after administration of a low dose GH is suggestive for an abnormal GH molecule, while increase of IGF-I on a high dose suggests GH insensitivity.

Objective

We evaluated the growth response during 1 year GH treatment in patients with an increase of IGF-ISDS >1 during the IGF-I generation test.

Patients/methods

IGF-I generation tests were performed according to the protocol in Table 1. Inclusion criteria were heightSDS < -2.5, max. stimulated GH > 30 mU/L and IGF-ISDS < -2.

Table 1. IGF-I generation test

	GH dose	Biochemical evaluation
Week 1	0.7 mg/m2/day	IGF-I + IGFBP3 at day 0+8
Wash out period		
Week 2	1.4 mg/m2/day	IGF-I + IGFBP3 at day 0+8
Wash out period		
Week 3	2.8 mg/m2/day	IGF-I + IGFBP3 at day 0+8

Response criterion: an increase in IGF-I > 1 SD on day 8

Results

Baseline characteristics and results after 1-year GH treatment of 51 patients are shown in Table 2. Patients are subgrouped according to the GH dose which stimulated IGF-ISDS > 1.

In group 3 GH treatment was started with 1.4 mg/m2/day, lower than the dose of 2.8 mg/m2 with which IGF-ISDS increased in the test, but with a similar effect on growth response as in group 2.

Table 2. Characteristics and results of 1 year GH treatment

	Group 1: 0.7 mg/m2	Group 2: 1.4 mg/m2	Group 3: 2.8 mg/m2	
N	31	12	8	
Male/Female	12/19	6/6	3/5	
Age at start GH	8.4 (±2.9)	7.6 (±2.7)	8.7 (±3.8)	ns
HeightSDS at start GH	-3.3 (±0.7)	-3.1 (±0.6)	-3.4 (±0.9)	ns
TH-SDS	-0.4 (±1.2)	-0.1 (±1.1)	-0.6 (±0.9)	ns
TH-SDS - heightSDS at start	2.9 (±1.1)	3.2 (±0.8)	3.1 (±1.1)	ns
max. GH, mU/L*	67 (56-86)	98 (67-142)	70 (48-83)	p= 0.007
GH dose at start (mg/m²/day)*	***0.75 (0.5-0.9)***	***1.4 (0.8-1.5)***	***1.4 (1.3-1.4)***	***p= 0.000***
delta heightSDS after 1 yr	***0.6 (± 0.31)***	***0.73 (± 0.34)***	***0.82 (± 0.16)***	ns

Mean (±SD) *Median (IQR)

Conclusions

Patients with GH insensitivity (group 2 and 3) show a moderate growth response in 1 year with 1.4 mg/m2/day.

Growth response in group 3 is achieved with a lower dose than in the IGF-I generation test. This suggests that 1 week GH in the generation test is not long enough.

Further investigation and long term follow up are needed.

PO3-128 GH and IGF Use III

Growth hormone treatment children with connective tissue diseases and growth failure

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The interest of this research was focused on opportunities of growth hormone (rGH) treatment in children with connective tissue diseases (CTD), who have growth failure (GF).

Materials and methods: Seven children in the age of 5 to16 years old (y.o.) (3 boys and 4 girls) with CTD (6 children with juvenile rheumatoid arthritis (JRA) and 1 girl with systemic lupus erythematosus (SLE)) were examined. Average age of a disease debut - 3.6 y.o. Average duration of disease - 5.5 years. A height, weight, body mass index (BMI) and somatotropic function (GH, including nighttime secretion, simulative tests, IGF-1), bone age were estimated. All children were treated with rGH at dose - 0.05 U/kg as a daily SC injection during 3 months and 6 months interval, 2 treatment courses.

Results: Before treatment all patients had a GF, (-)3.0±1.2 SDS. Somatotropic insufficiency was not reveled in any child. All children were treated with glucocorticoid (GC) at onset of the disease. Earlier debut of disease and the beginning of GC treatment (from 1-2 y.o.) were associated with more expressed GF (3.5-4.4 SDS).GC treatment started in children older than 5-6 y.o. was characterized by smaller GF. Also CTD severity and GC dose influences a degree of GF (the higher GH dose causes more significant growth failure). During the 1st course of rGH treatment (2.5 months) in all children the height increase an 2.5±1.4 cm was noted. Within 3 months after the 1st course the height increase was 1.5±0.6 cm. Growth rate was enlarged on the average by 4 cm/years. But within the next 6 months, without rGH treatment, growth rate decreased the same before start rGH treatment. Acceleration of the bones maturing during rGH treatment was not observed, that improved the prognosis of final height of a child. By the end of treatment a GF decreased from (-) 3.0±1.2 SDS to (-) 2.65± 1.1 SDS. No adverse events were observed. During rGH treatment an increases of weight was not observed, with tendency to decrease one. Results of the research had shown a useful of rGH treatment at children with CTD with and growth failure. Acceleration of growth rate is a good psychological factor and improves medical effect and social adaptation of children.

PO3-129 GH and IGF Use III

User acceptance of the easypod™ growth hormone (GH) auto-injection device: survey results from 655 children and their parents

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Background: easypod™ is the first electronic auto-injector for GH (Saizen®, Merck Serono) administration.

Aims: To assess patient acceptance of easypod™ and effect on adherence to recombinant human growth hormone (r-hGH) therapy through a large-scale survey study in 14 countries (Europe and Latin America).

Methods: Study subjects were children using easypod™ for ≤3 months and their parents. After institutional approvals and appropriate consent/assent procedures, the survey was administered by the nurse or investigator. Children and their parents were asked to rate specific easypod™ features on 5-point scales. Self-reported adherence to r-hGH therapy was also recorded, with those who reported missing ≤2 injections per month (92% of injections given) considered to be adherent to treatment. Adherence was also assessed by downloading the electronically recorded injection history from each easypod™ device. The dose log diary was locked for patient/parent access during the 3-month study period.

Results: A total of 655 children using easypod™ were surveyed (median [range] age: 11 [1–18] years; 58% were boys); 77% were naïve to r-hGH therapy. 41% were responsible for their own injections (median [range] age 13 [5–18] years), parents were responsible in 52% of cases (median [range] age 9 [1–16] years) and 6% involved shared responsibility. easypod™ was rated as easy/very easy to use by ≥80% (ease of use; preparation of the injection; changing cartridge and needle; holding and gripping the device). The injection was considered to be of short duration by 84%, as silent/almost silent by 82% and as painless/less painful than expected by 59%. In addition, 90% of children considered easypod™ comfortable/very comfortable to use and 91% wanted to continue to use it. For reported adherence, 52% of children missed ≥1 injection over the 3-month period, mainly due to forgetfulness (46% of reasons given). Overall, the electronically recorded adherence showed that 85% of children/parents performed ≥92% injections. Adherence was significantly higher in treatment-naïve children (87%) than in those previously treated (79%) (p=0.0252). Concordance between reported and recorded adherence was 82%.

Conclusions: Children and parents reported that easypod™ was easy to use. Most adhered to therapy and wanted to continue using the device. These results demonstrate patient acceptance and clinical utility of an electronic injection device for daily recording and adminstration of r-hGH.

PO3-130 GH and IGF Use III

Severity of short stature and not GH peak at diagnosis is the major determinant of growth response to GH therapy in short subjects without severe GH deficiency

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A diagnosis of GH deficiency (GHD) is still considered the gold standard for GH therapy in many European countries. Aim of the present multicentre study was to retrospectively analyze growth response to GH therapy and factors related to good response of 2 groups of short subjects with different GH secretion. Inclusion criteria for both groups, besides short stature, were absence of puberty, no other diseases affecting height (Ht) and treatment to adult Ht with same GH therapy schedule (7 mg/m2/wk or 0.20-25 mg/kg/wk). Specific criteria for group 1 (n.76, 47 M and 29 F) was non severe GHD (peak 3-10 µg/L), whereas for group 2 (n.33, 21 M and 12 F) normal GH secretion (peak > 10 µg/L) with IGF-1 levels <-1 SDS responsive (50% increase) to IGF-1 generation test. Therapy duration was similar in the 2 groups: 77 mo in group 1 and 69 mo in group 2. At diagnosis (group 1 vs 2) Ht-SDS was different (-2.6± 0.8 vs -3.0±0.8; p=0.03), while age (9.7±2.5 yrs vs 9.2±2.5), target Ht-SDS (-1.3±0.8 vs -1.5±0.9), bone age delay (-2.0± 0.9 vs -2.3±1.0), IGF-1 SDS (-1.7±1.3 vs -2.2±1.1) were similar.

Results.

Results at adult Ht

	Adult Ht SDS	Adult Ht-initial Ht SDS	Target Ht-adult Ht SDS
group 1	-1.3± 0.7	1.3± 0.7	0±0.8
group 2	-1.4± 0.8	1.1± 0.9	0±1.1

There were no differences between males and females. The percentage of subjects with a Ht gain SDS <0.5 (15 vs 9%) and >1 SDS (63 vs 79%) was similar between the 2 groups. By examining the parameters affecting Ht gain, the subjects showed similar characteristics not associated to the group of treatment, i.e. significant negative correlation with statural deficit at diagnosis (r=-0.49; p=0.0001), age at diagnosis (r=-0.20; p=0.05) and positive with duration of therapy (r=0.28; p=0.007). GH peak at diagnosis was not related to Ht gain in both groups. In the whole group and in the 2 separate groups, among Ht SDS at diagnosis, age at diagnosis and therapy duration, multiple regression analysis identified Ht deficit at diagnosis as the only parameter influencing Ht gain (R2=0.42 in the whole group, R2=0.36 in group 1, R2=0.49 in group 2).

Conclusions. Short subjects with normal GH secretion but low IGF-1 levels showed a similar growth response to GH therapy as short subjects with non severe GHD. In our subjects with short stature and different type of GH secretion, degree of short stature at diagnosis seemed the major determinant for height gain during treatment.

PO3-131 Gonads and Puberty III

Fetal ovarian cyst: to improve the neonatal management

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Management of fetal ovarian cyst is controversial, the major complication is torsion leading to the loss of the ovary but neonatal surgery is not always necessary.

The aim is to evaluate the ovarian outcome with multidisciplinary neonatal management.

Population 36 ovarian cysts were diagnosed in 33 patients at the 3rd trimester of gestation. These cysts were fluid or simple appearance in 26 cases, bleeding or complex appearance in 4 cases, bilateral in 3 patients.

Results : In unilateral simple cysts, the mean diameter was 43.4 ± 19 mm (range 30 – 102 mm), the disappearance of the cyst occurred on average in 2 months. The ovarian recovery with follicular ovary is effective in 21 cases (80%), homogenous ovary in 3 cases and one cyst persists with a hyperechoic shell. The antenatal detection showed 4 cases of complex unilateral cysts, there was no surgery for these too old cysts. Their mean diameter was at 54.5 ± 10 mm (range 40 – 63 mm), tumor markers were negative. The U.S. controls had never viewed the ovarian follicles. In 2 cases of bilateral cysts, one evolved with gestational diabetes and vanished at birth, the 2d case disappeared 2 months later. In these 2 cases normal follicular ovaries were visualized in 3 month. In the 3rd case, the surgery has helped puncture the right fluid cyst and a oophorectomy for left ovarian torsion. The search for Mc Cune Albright was negative in peripheral blood and right follicles were seen at the 2nd month of US monitoring. In all cases the levels of estradiol and antimullérian hormone (AMH) were in the standards for age.

Conclusion: The risk benefit balance is not in favor of neonatal systematic surgery. Simple cysts regress spontaneously in 1st trimester of life, at most one puncture is indicated if the diameter is> 40 mm, the ovary is functional. In the case of complex cyst, surgery is unnecessary, the ovary is lost probably due to old torsion. Nevertheless, the family is always warned that neonatal surgery may be indicated if there is recent deterioration of ultrasound images. We emphasize the need for multidisciplinary care in neonatal period.

PO3-132 Gonads and Puberty III

Impaired exercise adaptation in adolescents with craniopharyngioma

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Background- Adolescents with craniopharyngioma usually have hypopituitarism. Despite adequate pituitary hormone substitution, they often complain of fatigue. Our aim was to objective this lack of resistance by measuring their aerobic ability during an exercise test.

Methods- Twelve adolescents (4 F, 8 M) with craniopharyngioma (cases) and twenty-four healthy adolescents (controls, 8 F, 16 M) matched for sex, age and pubertal stage underwent an exercise test on cycloergometer. Maximal oxygen upake (VO2 max), maximal heart rate (MHR) and systolic and diastolic blood pressure (SBP and DBP) were measured during exercise.

Results- Mean age was 15,7 ± 2,6 yrs, mean height was 0,3 ± 1.1 SDS, and mean BMI was 0,8 ± 1,9 SDS, with no difference between cases and controls. Exercise tests were maximal for each subject and respiratory quotient was > 1 in cases and controls with no difference. Vital capacity was comparable between groups. VO2 max was 1644 ± 547 ml/min in cases vs. 2533 ± 630 ml/min in controls (p<0.0001) and was also reduced in cases vs controls when compared with theoretical values (67,7 ± 13 % vs 104,5 ± 18,3 %, p<0.0001). MHR was 178 ± 13.3 /min vs 189 ± 9.8 /min (p<0.02, cases vs. controls) and theoretical MHR was 88,9 ± 6,6 % vs 94,6 ± 4,2 %, (p<0.02). Resting SBP and resting DBP were comparable between groups, but maximal SBP was 153 ± 23 mmHg vs. 186 ± 29 mmHg (p<0.006, cases vs. controls).

Conclusion- Despite adequate pituitary hormone substitution, adolescents with craniopharyngioma have a marked impaired exercise adaptation compared to healthy adolescents.

PO3-133 Gonads and Puberty III

Norethisterone induced hepatic adenomas can led to life-threatening hemorrage in girls with inherited bleeding disorders

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Introduction: Adolescents and women with inherited bleeding disorders are particularly at risk of bleeding complications during menstruation. In severe bleeding disorders menorrhagia, since menarche, are of particular severity, requiring hospitalization and transfusion for acute or chronic anaemia. Morbidity includes significant decreases in the quality of life, higher incidence of school and work absenteeism. **Aim of the study**: To report on the risk of haemorrhage in adolescents and young women with inherited bleeding disorders treated by norethisterone for bleeding complications during menstruation. **Patients and methods:** We identified 4 cases of adolescents and young women with hepatic adenomas (HA) who were previously and continuously treated by norethisterone. **Results** are summarized in following table: etiology of inherited bleeding disorder, posology and duration of norethisterone treatment, chronological age (CA) at diagnosis of HA, clinical manifestations of HA, number of lesions, treatment of HA and evolution of HA.

table 1

Cases	1	2	3	4
Etiology	Bernard Soulier	Glanzmann	Glanzmann	Glanzmann
Posology(mg/d)	20	10-20	20	10
Duration(yrs)	13	1,5	2,5	10
CA(yrs)	24	14	18	22
Clinical manifestations	Haemorragic shock Haemoperitoneum	Routine hepatic US	Routine hepatic US	Haemorragic shock Haemoperitoneum
Number of lesions	1 HA	6 HA	>20 HA	>6 HA
Treatment	Left hepatectomy	No	No	Embolisation
Evolution	Normal liver by US	Normal liver after 2 yrs by US	Normal liver after 1,5 yrs by US	50%regression after 6 months

Conclusions: We described 4 young women who developed hepatic adenomas and life threatening haemorrhage in 2 while using norethisterone. We believe that is advisable not to use 10 mg to 20 mg norethisterone in a continuous way to treat menorrhagia in adolescents and young women with bleeding disorders. If others nortestosterone derivatives are needed, this should be done with close monitoring of the appearance of HA by ultrasound. The treatment of menorrhagia in adolescents and young women should be individualized and monitored closely by a team composed of a gynaecologist and a haematologist.

PO3-134 Gonads and Puberty III

High prevalence but different hormonal profiles of hirsutism, menstrual disturbance and polycystic ovarian syndrome among obese (OB) or type 1 diabetic adolescents (DM)

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Background: Prevalence and predisposing factors of hyperandrogenism (HA) are not well established among OB and DM adolescents.
Aims: To define in DM and OB adolescents puberty characteristics, prevalence and factors of HA, menstrual cycle disturbances (MCD) and polycystic ovary syndrome (PCOS).
Patients: We collected data on 96 OB (mean body mass index (BMI) 6.2+/-2.2 SD), 78 DM, and 251 healthy adolescents from 12 to 17 years old.
Results: Age of puberty and pubarche were in the normal range in both group, but significantly earlier in OB than DM. Age of menarche was significantly earlier in OB (11.9+/-1.16 years) and later in DM (12.7+/-1.2 years) than in controls (12.4+/-1.3 years). 36% of the OB had an hirsutism (H), with a significantly earlier age of pubarche and puberty, higher testosterone (T), Δ4 androstenedione (Δ4), SDHA and free androgen index (FAI) levels compared to the OB without H. 42% of the OB had MCD, higher than 20% in the control (p=0.01); with a significantly longer obesity duration, higher abdominal perimeter (AP) and insulin peak compared to the OB without MCD. In the OB group there was a positive correlation between BMI, AP, HOMA-IR and FAI. 21% of the DM had an H, with a significantly longer DM duration, higher AP without obesity, Δ4, SDHA and FAI levels compared to the DM without H. 44% of the DM had MCD, with a significantly later age of menarche, higher T, Δ4, LH and FAI levels compared to the DM without MCD. In the DM group there was a positive correlation between BMI (<2DS), HbA1c and FAI. High prevalence of PCOS was found in both groups, 38% in DM and 29% in OB. Biological profile of DM and OB with H were significantly different: DM had a higher and elevated levels of Δ4 (2.6+/-1.63 ng/ml), T (0.62 +/-0.38 ng/ml) associated with a higher and normal levels of SHBG (47+/-18.7 nmol/l) than the OB who had normal levels of Δ4 (1.75+/-0.83ng/ml), T (0.41 +/-0.21 ng/ml) with decreased levels of SHBG (26.4+/-19.9 nmol/l) and similar FAI. We found similar differences between DM and OB with MCD and PCOS.
Conclusions: H, MCD and PCOS prevalence is increased in our cohort of DM and OB adolescents. The HA is linked to the decreased SHBG levels in OB and the increased androgens secretion in DM.

PO3-135 Gonads and Puberty III

Increased perinatal intracranial pressure and early brainstem dysfunction predict early puberty in boys with myelomeningocele

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Background: Children with myelomeningocele (MMC) run an increased risk of developing early or precocious puberty (E/PP). In girls an incidence up to around 50% has been reported, while in boys there is a variation between 10-20 %. The cause is unclear. In a previous study, we observed an association between increased intracranial pressure during the perinatal period and early puberty in girls with MMC.
Objective: To examine risk factors for E/PP in boys with MMC.
Methods: We identified the boys born 1970-92 and treated for MMC at the Folke Bernadotte Home, at the University Children's Hospital, Uppsala. Out of 43 subjects possible to evaluate, we obtained permission from 38 to be included in the study. We examined the medical records retrospectively with regard to clinical signs and data about growth and development. Early puberty was defined as Tanner stage(s) PH2/G2 before the age of 10 years and 2 months. Precocious puberty was defined as appearance of the above sign before 9 years of age.
Results: Of the 38 boys in the study population, 8 (21 %) had E/PP. There was a significant association between increased perinatal intracranial pressure and early puberty, and also between early brainstem dysfunction (Chiari II) and early puberty. Multivariate regression analysis showed early Chiari II symptoms to have the highest explanatory value with regard to the occurrence of early puberty.
Conclusion: Signs of increased intracranial pressure perinatally as well as symptoms of brainstem dysfunction early in life are predictors of early or precocious pubertal development in boys with MMC. It is important to inform the parents of these risk factors and to monitor growth and development of the boys in order not to miss signs of early puberty. Arm span can be useful when measuring the linear growth of MMC patients.

PO3-136 Gonads and Puberty III

Clinical and hormonal aspects of puberty in boys with cryptorchidism

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There is evidence that in adult men with treated cryptorchidism the testicular cells function is impaired. Aim: to study the clinical and hormonal features in pubertal boys who underwent orchidopexy. Methods: assessment of growth and puberty; tests of serum gonadothropins and testosteron level; GnRH- test; gonadal ultrasound; transrectal ultrasound in boys over 14 y.o.
Results: 31 boys who underwent orchidopexy were examined. 15 of them had unilateral cryptorchidism, 16 - had bilateral cryptorchidism. Control group – 30 healthy boys. The boys have been monitored for 2 years till they got 14- 15 and put into two groups: 1 - pubertal boys (24); 2 - the boys with delayed puberty (7). Group 1. Testicular volume in descended testis -10,8±2,3 ml, in contralateral testis 16,4±1,5 ml, p< 0,05 and in healthy boys -17,2 ±1,8 ml. Testicular sonography showed the deficiency of testicular blood supply on the side of the orchidopexy. Transrectal ultrasound showed prostate hypoplasia - 7,3±1,2 ml compared to healthy boys - 11,3±1,4 ml (M.Pikov, E.Philipova, 2006). Hormonal tests was normal. Group 2. Patients had Tanner stage G1 with testes <4ml Transrectal ultrasound showed prostate involution – 1,5±0,43 ml. The hormonal test results showed low basal levels of gonadotropins and response to GnRH stimulation – 5,8 ± 0,2 units/l in 5 boys - Hypogonadotropic Hypogonadism; high level of gonadotropins and low serum level of testosterone in 2 boys - Hypergonadotropin Hypogonadism. Conclusions: 1) delay of puberty was found in 7 of 31 boys with the history of cryptorchidism; 2) deficient testicular blood supply was observed in descended testis; 3) prostate hypoplasia – the in pubertal boys with the history of cryptorchidism and it's involution in the patients with pubertal delay.

PO3-137 Gonads and Puberty III

Central precocious puberty in a male with chronic renal failure and hyperprolactinemia

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Chronic renal failure and/or hyperprolactinemia usually cause hypogonadotropic hypogonadism and delayed puberty. We describe a case of persistent central precocious puberty in the setting of chronic renal failure and hyperprolactinemia. A male, born at 37 weeks gestation, birth weight 7 lb 8 oz., developed end stage renal disease due to diffuse mesangial sclerosis at 2 months of age and was started on continuous ambulatory peritoneal dialysis. At 17 months he developed hair at the base of the phallus. There was no history of exogenous androgen exposure. His medications included metoclopramide, enalapril, famotidine, amlodipine, clonidine, and epogen. Physical exam was negative for hirsutism, acne, gynecomastia or galactorrhea. He had Tanner II pubic hair, and a stretched phallic length of 5 x 1.7cm with bilateral hydroceles. On ultrasound his testes measured 2 x 0.7 x 1.4cm and 2 x 0.5 x 1cm, consistent with Tanner I stage. His weight was 13.6 kg and length 83 cm. At 23 months, bone age was 32 months (>2 SD advanced for age). Laboratory testing (table 1) showed a pubertal LH (3.3mIU/ml) but a low FSH (1.27 mIU/ml), pubertal testosterone (130 ng/dl), and a markedly elevated prolactin (439 ug/L).

Test	Pre-Pubertal/Age Appropriate Values	23 Months of Age	29 Months of Age	Post-Transplant/ Baseline Leuprolide Stimulation Test	60 Minutes Post-Leuprolide
LH	<0.3 mIU/ml	3.3	2.6	0.28	6.3
FSH	<3 mIU/ml	1.27		1.06	3.83
Testosterone	< 5 ng/dl	130	199	4	
Andros tenedione	5 – 51 ng/dl	193			
17 - Hydroxy-pro-gesterone	4-115 ng/dl	15			
DHEA-S	< 5-57 ug/dl	39			
Prolactin	3.3-19.7 ug/l	438.6	280.7	3.3	
TSH	0.7-5.7 mIU/l	2.33			
Free T4	13–26 pmol/l	16			
Other		Creatinine 5.1 mg/dl			

17-hydroxyprogesterone and DHEA-S were normal for age and pre-adrenarchal. Brain MRI showed a normal hypothalamus and pituitary. He received a deceased donor renal transplant. Subsequently his prolactin fell to a low normal level and an unstimulated LH and testosterone were in the pre-pubertal range. However, a leuprolide stimulation test four months post-tranplant showed a pubertal LH response of 6.3 mIU/ml at 60 minutes. Only 2 similar cases have been described for male children with chronic renal failure and hyperprolactinemia who developed transient central precocious puberty with biochemical reversal occurring after renal transplantation. Our patient appears to have persistent central precocious puberty despite normalization of prolactin levels after renal transplant.

PO3-138 Gonads and Puberty III

Practical aspects of the use of histrelin subdermal implant for the therapy of central precocious puberty: review of 16 cases

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Background: After the original multicenter trial confirmed the efficacy and safety of the histrelin subdermal impant (HSI) for the therapy of central precocious puberty (CPP)[1] and the commercial availability of the product in 2007, pediatric endocrinologists were given an additional therapeutic tool. However, the practical aspects of the use of HSI in the pediatric endocrinology clinic out of the research setting cannot to be ignored. HSI requires a small surgical procedure that in the original study was performed by pediatric surgeons, but that can be done by the pediatric endocrinologist if desired.
METHODS: The medical records of patients diagnosed with CPP from August 2007 till today were reviewed. 17 patients were identified, out of which 16 received HSI after independently choosing this therapy over intramuscular GnRH(a) analog injections.
Results: The average age at the time of diagnosis of CPP was 6y 3 m. The group was 12.5 % Hispanic, 31.25% African American and 56.25 % Caucasian. There was only 1 male. 68 % of patients were diagnosed with idiopathic CPP. Of the17 patients, 14 elected HSI and 2 switched from intramuscular GnRHa to HSI. The procedure was performed by the pediatric endocrinologist under local anesthesia in the outpatient clinic procedure room. One patient with severe neurological disorder required conscious sedation with the assistance of a general pediatrician. The average length of the procedure for all patients was approximately 15 minutes. 2 patients have had removal and reinsertion of HSI, which was perceived as longer and more difficult. 15 patients were considered to have appropriate LH-sex steroid suppression, except for a partial response in a female with a tuber cinereum hamartoma. No significant adverse events have been reported.
Conclusions: HSI is a relatively easy procedure that can be done successfully in the pediatric endocrine clinic, provided trained personnel and proper space are available to the endocrinologist. The opportunity to present patients with treatment alternatives for CPP enriches the overall endocrine practice experience. It is possible that one of the factors for the selection of HSI over monthly GnRHa injections in our institution is the large geographical referral area covered and the difficulty of some families to return frequently for care.

PO3-139 Gonads and Puberty III

Kallmann syndrome: a clinically and genetically heterogeneous disease

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Introduction: The Kallmann syndrome is characterized by hypogonadotropic hypogonadism with anosmia or hyposmia. It is a clinically and genetically heterogeneous disease. The commonest mutations are in KAL1 (X chromosome-linked recessive inheritance) and FGFR1 gens (autosomal dominant inheritance)
Case 1: A 6 years 11 months-old boy with E320X mutation in KAL1 gen. Family history: Two uncles with this syndrome and carrier mother. Personal data: Right multicystic kidney. Small penis with good response in HCG test. Karyotype 46,XY normal. Physical exploration: height 128.7 cm (+0.45 SD), weight 30.75 Kg (+0.56 SD), synkinesia, hypoplasic scrotum without testes, penis 4.2 cm. Complementary studies: FSH 0.3 U/L, testosterone <0.12 ng/mL, ultrasound: testes in inguinal canal (1.5 y 0.7 cm), magnetic resonance: anomalous encephalic tissue in anterior cranial fossae without olfactory bulbs hypoplasia. Evolution: orchidopexia. Currently (8 years 3 months): Height 134.5 cm (+0.17 SD), weight 40.1 Kg (+0.96 SD), teste 1 ml, penis 4.5 cm.
Case 2: A 12 years 5 months-old girl with delayed puberty and decreased growth velocity. Personal data: anosmia, macroamylase. Physical exploration: height 143.5 cm (-1.45DS), weight 39.4 Kg (-0.59DS), S1P1A1. Complementary studies: bone age: 12 years, pelvic ultrasound: prepuberal, gonadotropins and estradiol: prepuberal, Karyotype: 46XX normal, magnetic resonance: olfactory furrows hypoplasia and lack of olfactory tracts, genes: KAL1, FGFR1, PROK2 y PROKR2 normals. Evolution: induction of puberty with conjugated estrogens. Menarche at 15 years 11 months. Currently (16 years 1 month): complete sexual development, height 161 cm (-0.24 DS), weight 50.5 Kg (-0.58 DS). Substitute treatment with estrogens and progestins.
Case 3: A 18 years old boy with delayed puberty. Personal data: hyposmia. Physical exploration: Height 175.6 cm (-0.13 SD), weight 70 Kg (-0.12SD), left teste 2,5 cc, right cryptorchidism, micropenis and gynecomastia. Complementary studies: bone age: 15 years, ultrasound: right dystrophic teste in inguinal canal, gonadotropins and testosterona: prepuberal, prolactin 4 ng/cc, Karyotype: 46 XY normal, magnetic resonance: normal, genetic: normal Xp22.3 (FISH); KAL1, PROK2 and PROKR2, FGFR1 in study.
Comments: Most of the cases are sporadic and mutations of genes exist for discovering yet. The precocious diagnosis is important to induce the puberty, to initiate the substitute treatment and to treat the possible complications.

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Uterine artery pulsatility index: an adjunctive method in monitoring therapy of precocious puberty?

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Objective: to evaluate uterine artery pulsatility index (IP) in monitoring hormonal therapy of girls with central precocious puberty (CPP).
Materials and methods: 24 girls between 5 and 9 years old with diagnosis of CPP underwent transabdominal pelvic ultrasonography (US) before and during therapy with LHRH analogue (LHRHa). US scans were performed using a convex and microconvex 5/8 MHz transducer by the same investigator, experienced in this matter. Uterine and ovarian dimensions and right uterine artery doppler flow were measured and PI was calculated.
Results: US performed before LHRHa therapy showed a greater uterine (mean length 44 mm), ovarian (mean volume 1,5 ml) size in all girls. The PI mean value was 3,5. All PI values inversely correlated with uterine dimensions. After 6 months with LHRHa therapy higher PI values were detected in all girls (100%; PI mean value 7,4); a reduction of uterine and ovarian size was observed in 21 girls (87%)(mean uterine length 40 mm, mean ovarian volume 0,8 ml).
Conclusions: PI can be an adjunctive method in monitoring the effectiveness of therapy in CPP. Variations of PI inversely correlate with uterine and ovarian size, and they seem to be expression of oestrogenic hormonal activity, with lower values during pubertal development and higher values due to the hormonal inhibition during LHRHa therapy.

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Delayed puberty can mask severe pathology

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Background
Pubertal development is the result of increasing discharge of the hypothalamic gonadotropin releasing hormone (GnRH), which stimulates the pituitary to release gonadotropins and in turn gonadal activity. Delayed puberty is defined as the onset of puberty after the age of 13 in girls and 14 in boys and often, is an extreme variant of normal pubertal timing. Pathologic causes of delayed puberty can be severe. The induction of puberty is indicated in patients who do not undergo spontaneous development.
Aim
We propose to describe clinical, endocrinological and radiological features of 4 patients with delayed puberty.
Patient 1. A 14-year-old female was consulted due to absence of breast development. Her personal history showed a psychomotor and mental retardation,

microcephaly, ataxia and growth retardation. MRI of the brain at 10 years old showed a hypoplasia of the cerebellum, and a normal pituitary gland. Her growth had always been below the third centile and at the age of 14 she presented a Tanner stage I. The gonadotropin hormones were elevated: LH 35 and FSH 149 UI/l. The pelvic ultrasound did not show the right ovary. The karyotype and audiometry were normal.
At present cerebellar ataxia and hypergonadotropic hypogonadism has been diagnosed.
Patient 2. A 17- year-old male was consulted due to growth retardation and an absence of secondary sexual characteristics. Tanner pubertal stage was G1P2A1. LH and FSH levels were 1.75 and 8.8 UI/l. MRI of the brain revealed a craniopharyngioma and the patient underwent neurosurgical intervention.
Patient 3. A 15-year-old female was consulted due to absence of breast development. She had female external genitalia. The hormonal tests showed a hypergonadotropic hypogonadism: LH 40 and FSH 100 UI/l. The pelvic ultrasound examination showed a hypoplastic uterus and the right ovary could not be identified. Her karyotype was 46,XY. A diagnosis of Swyer syndrome was made.
Patient 4. A 14-year-old female was consulted due to absence of breast development. She presented a Tanner stage I. Her personal history revealed anosmia. Serum FSH and LH were low. The MRI of the brain showed that the olfactory tracts and bulbs were absent. She was diagnosed with Kallman syndrome.
Conclusions:The clinical history, laboratory and neuroradiologic assessment were relevant to the diagnosis. Early diagnosis of a hypogonadism cause is crucial to rule out severe pathologies and for optimal clinical and therapeutic management.

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Comparison of the effects of letrozol with oxandrolone upon height growth and puberty of children with constitutional delay of growth and puberty

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Background: Constitutional delay of growth and puberty (CDGP) is one of the most common problems in pediatrics. It causes a lot of psychological and social disorder in families.
The aim of this study is to compare the effect of oral letrozol with that of oxandrolone in promoting height growth and bone age of children with CDGP. HSDS, WSDS, BMI, SMR, bone age and serum IGF-1 levels were used as milestones of improvement.
Methods: This 5 year- clinical trial was performed on 85 CDGP Iranian boys (14-15 years old), referred to outpatient pediatric endocrine clinic of Mofid Children's Hospital in Tehran. After registration of personal identities and taking blood, urine and stool specimens to rule out probable underlying diseases and interfering factors, children were randomly divided into three groups including: a control group, taking placebo medication, those treated with oxandrolone and those treated with letrozol, for a two year duration period in each case. Data were transferred to SPSS program where the results were analyzed by paired T-, ANOVA, Tukey, Wilkakson and Cruscal-Valis tests.
Findings: There was no significant differences between the treated groups in HSDS (p=0.56) (table 1), WSDS (p=0.08) and BMI (p=0.51).

Table2- HSDS changes in study groups

Study group	HSDS before	HSDS after	Difference	p value
Control	-2.59(0.28)*	-2.60(0.3)	-0.01(0.12)	0.587
Oxandrolone	-2.72(0.36)	-2.27(0.47)	0.44(0.32)	<0.0001
Letrozol	-2.61(0.25)	-2.17(0.41)	0.44(0.29)	<0.0001

*Mean (SD)

On the other hand, there were significant differences in SMR (p<0.001) and bone age (p<0.001) between the groups.

table2- Changes of bone age in study groups

Study group	Bone age before	Bone age after	Difference	p value
Control	11.7(0.43)*	12.2(0.36)	0.45(0.30)	<0.0001
Oxandrolone	11.9(0.67)	14.1(0.59)	2.15(0.53)	<0.0001
Letrozol	12.1(0.8)	13.2(1.0)	1.1(0.33)	<0.0001

*Mean (SD)

Eventually, no significant difference was found in serum IGF-1 levels between the two treated groups (p=0.98).
Conclusion: letrozol can be used instead of oxandrolone in CDGP teenage boys with the same efficacy in linear growth and less adverse effects of advancing bone age and pubertal stage.

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Hypothalamo-pituitary system in girls' hyperprolactinemia

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Pathogenesis of hyperprolactinemia (HPRL) is mediated by tonic dophaminergic inhibiting control of prolactin (PRL) secretion. Continuous stimulation of PRL secretion leads first to hyperplasia of prolactotrophic factors followed by development of micro- and macroadenoma (prolactinoma) of pituitary. Major criterion for identification of hyperprolactinemia is detection of baseline PRL level and brain X-ray, CT-scan or MRT to study the pituitary.
While the HPRL in females of reproductive age is mediated mainly (about 70%) by pituitary tumor, only 15% of females at puberty develop HPRL caused by pituitary adenoma.
Our research is focused on study of hypothalamo-pituitary system in girls aged 14-20 that suffer from primary or secondary amenorrhea and express HPRL.
Girls (86) aged 14-20 years suffering from HPRL were studied and treated at the Children Gynecology Department of Yerevan center of family planning.
Functional HPRL (average 57.75±4.1 ng/ml) was found in 73 patients.
CT-scan showed pituitary microadenoma in 7 patients and macroadenoma in 6 patients.
X-ray analysis found macroadenomas (more than 1 cm in diameter) expressing in local or total osteoporosis of *Sella turcica*, rough internal contours along with unchanged cranial arc bones. Microadenomas less than 1 cm in diameter were detected by CT-scan.
Four girls with macroadenoma were operated on, and 2 girls with same pathology were treated with *Dostinex* (Pfizer).
Noteworthy, 16 and 18 years old 2 girls with macroadenomas who were treated conservatively (parents refused from operation) applied for the absence of mammary glands and menstrual cycle (average age for menarche in our region is 11.8 years). Ultrasound examination revealed expressed sexual infantilism resembling that in dysgenesis of gonad: uterine body as a steak, endometrium – single line, both ovaries were 2.1×0.2 cm in size, no follicle development; secondary sexual traits $Ma_0Ax_0P_iMe$ abs. PRL in both cases exceeded the higher normal limit 10 folds (195.0 and 220.0 ng/ml, respectively). CT-scan showed intra- and subsellar pituitary macroadenomas. Both patients were prescribed Dostinex (1 tablet × 2 per week) during 6-8 months. Repeated tomography showed twice as reduced tumor size.
In conclusion, it is of best to recommend compulsory detection of PRL in all the girls suffering from primary or secondary amenorrhea as well as perform CT-scan examination for on-time diagnosis of pituitary tumors and perfect treatment of the patients.

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A case of hypogonadotropic hypogonadism with diabetes insipidus

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Introduction: Hypogonadotropic hypogonadism is defined as a permanent absence of spontaneous pubertal development due to lack of serum gonadotrapin production or action. The deficiency may be isolated or associated with combined pituitary hormone deficiencies. Acquired gonadotropin deficiency may be due to intracranial trauma, tumors, surgery or radiotherapy. On the other hand, central diabetes insipidus (DI) is considered an indicator of serious underlying

disease in children. Important causes of childhood central DI include tumors and malformations of the central nervous system, histiocytosis and insults to the hypothalamic-pituitary region by surgery, radiation and trauma. Although there is a similarity between both diseases' causes, there are not many reports about female hypogonadotrapic hypogonadism with central DI.
Case report: A 13-year-old girl had polyuria and polydipsia for 2 months. She came to our hospital and underwent a thorough examination. A MRI of the hypothalamic - pituitary area revealed no hyperintense signal of the posterior pituitary. With water deprivation test, we diagnosed central DI, and we started long acting vasopressin analog dDAVP. A brain MRI was repeated after 3 months, and it revealed thickening of the pituitary stalk. There was no clear evidence of LCH, germinoma, or sarcoidosis. We decided simple observation, and the thickening of the pituitary was not changed after that. However 15 months after the DI occurrence, she had secondary amenorrhea. Her luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol were decreased, but other anterior pituitary disfunctions were not observed. We started replacement therapy with conjugated estrogens and the thickening of the pituitary was gradually decreased 3 months after the replacement therapy. Although we did not perform pituitary biopsy, her clinical course resembles lymphocytic infundibuloneurohypophysitis (LIN).
Conclusions: There are some reports about hypogonadotropic hypogonadism with diabetes insipidus, but many of them are male or due to neurosarcoidosis in adults. This is a rare case of female hypogonadotropic hypogonadism with diabetes insipidus.

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Ovarian and uterine dimensions by ultrasound in the evaluation of precocious puberty: comparison with leuprolide stimulation tests

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Purpose: The evaluation of subtle precocious puberty in females includes GnRH stimulation along with assessment of growth velocity, bone age and gonadal and uterine size and morphology. We previously presented data showing that Leuprolide stimulation test (L-stim) is a reliable predictor of onset and progression of puberty. This studys objective was to compare L-stim results with pelvic sonography in the assessment of female precocious puberty.
Methods and Materials: Retrospective chart review of patients (pts) who had L-stim and pelvic sonogram in 2004-2008. Pts with hypothalamic, pituitary or gonadal disorders were excluded. L-stim: FSH and LH (ICMA) measured at 0, 60 and 120 min after 20mcg/kg of Leuprolide s.c. E2 (extracted RIA, sensitivity 5 pg/ml) measured at baseline and 24 hrs (Esoterix Lab,CA). We used baseline LH 0.3 IU/L and/or estradiol 1.0 ng/dl and peak LH 5.0 IU/L and/or estradiol 5.0 ng/dl as hormonal criteria for pubertal (PUB) or prepubertal (PRE) response. FSH levels overlapped widely between PRE and PUB. All sonograms were reviewed by single radiologist. Average (R+L/2) ovarian volume (AOV), uterine length (UL), uterine volume (UV), uterine configuration (UC) and endometrial stripe thickness (ES) were assessed.
Results: Data on 41 pts were analyzed. Among 22 patients with PRE, 4 (18%) had an AOV ≥ 2cc, 4 (18%) had a UL of ≥4 cm, 2 (9%) had a UV of ≥4 cc; only 1 (5%) had a pubertal UC and an ES > 1.5 mm. Among 19 patients with PUB, 11 (58%) had an AOV ≥ 2cc, 6 (32%) had a UL of ≥4 cm, 7 (37%) had a UV of ≥4 cc, 4 (21%) had a pubertal UC and 3 (16%) an ES > 1.5 mm. Compared to pts with PRE, pts with PUB had significantly larger AOV (p=0.01) and UV (p=0.01). Peak LH and peak estradiol were significantly correlated with AOV and UV.
Conclusions: Peak LH and estradiol values obtained with L-stim significantly correlate with ovarian and uterine volumes. Because of substantial variability, we recommend caution in the interpretation of data. Careful, long term clinical follow up is necessary to validate L-stim and sonographic findings.

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Adequate free testosterone levels in female-to-male transsexual adolescents treated with intramuscular testosterone-esters

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At the VU University Medical Center gender identity clinic for children and adolescents, the only gender identity clinic in the Netherlands, approximately 115 adolescents with a gender identity disorder (GID or transsexualism) are currently eligible for treatment with GnRH analogues (Decapeptyl-CR®) to delay puberty. Puberty delaying treatment aims to provide adolescent transsexuals with time for a balanced decision regarding sex reassignment, starting with cross-sex hormones at age 16. Puberty of the desired sex in female-to-male transsexual adolescents is induced through administration of an intramuscular testosterone-esters depot according to the Dutch protocol.
To evaluate this protocol, salivary testosterone levels between injections were measured as a practical alternative for sequential measurements of serum testosterone.
Two 19 year old female-to-male adolescents treated with intramuscular testosterone-esters depots (Sustanon® 250 mg) every four weeks collected saliva at home. The first collection took place prior to injection, other samples were collected 12h, 24h, 48h and 72h after injection and subsequently every two days for 4 weeks. Free testosterone levels were determined by using a sensitive specific isotope dilution-liquid chromatography-tandem mass spectrometry based method.
Similar testosterone profiles were seen in both patients. As a result of the injection, testosterone levels quickly increased to supra-physiological levels. Nine days after injection, the concentrations decreased to relatively low levels but were still within the reference range. Reference values were determined in 14 healthy male subjects (140-420 pmol/L).

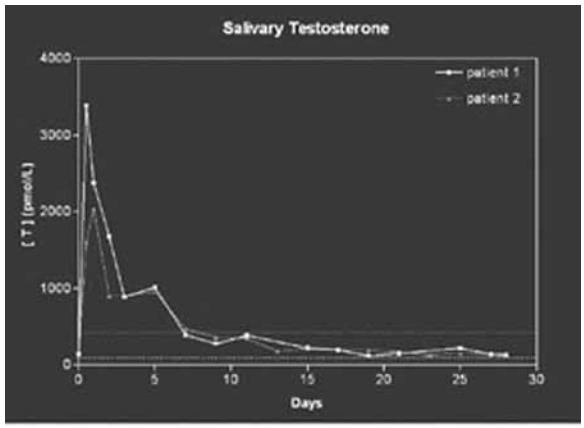

Fig. 1 free testosterone levels (pmol/L) in-between two subsequent testosterone-depot injections in two female-to-male adolescents.
Treating female-to-male adolescent transsexual with 250 mg Sustanon® every four weeks seems to be an adequate way to reach free testosterone levels within the reference range.

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Effect of treatment with cyproterone acetate on uterine bleeding at the beginning of GnRH analogue therapy in girls with idiopathic central precocious puberty

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Background. The flare up effect of GnRH analogues may cause transient uterine bleeding in girls affected with idiopathic central precocious puberty (ICPP).
Aims. To assess the incidence of endometrial bleeding and verify whether pretreatment with cyproterone acetate could counteract it.
Methods. 54 girls affected by ICPP were divided into 2 groups. The first group (30 girls) was treated with triptorelin (3.75 mg, i.m. injection) every 28 days. The second group (24 girls) was treated with cyproterone acetate and triptorelin: cyproterone acetate (50 mg/m^2) was administered every day for 8 weeks; triptorelin (3.75 mg) was commenced 4 weeks after starting the cyproterone, then the i.m. injection of triptorelin was repeated every 28 days.
Results. 8 of 54 girls (15%) had mild withdrawal bleeding. There were no differences between incidence in group 1 or group 2. Girls with pubertal uterus at pelvic ultrasound had higher incidence of uterine bleeding than girls with infantile uterus (25% vs 7%), but this difference was not significant.
Conclusion. Coadministration of cyproterone acetate and GnRH analogues does not significantly decrease the incidence of uterine bleeding.

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Longitudinal measures of growth and pubertal development in Russian boys

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Background
A temporal trend of earlier pubertal onset and increased obesity has raised concerns regarding the potential role of environmental factors. To investigate these concerns, we collected longitudinal anthropometric data and measures of pubertal status for boys in the small industrial town of Chapaevsk, Russia.
Methods
From 5/2003 to 5/2005, we recruited 499 boys comprising 85% of all 8- to 9-year old boys enrolled in the Chapaevsk national health system for baseline and annual follow-up visits (year 1 = 470; year 2 = 448; and year 3= 438). Pubertal status was assessed by Tanner staging of pubic hair (P) and genitalia (G) and measurement of testicular volume (TV) using a Prader orchidometer by OS. Pubertal onset was defined three ways: G2 or greater, P2 or greater, or TV > 3 ml of either testis. Descriptive statistics were computed using SAS (SAS Institute, Cary, NC). Age-adjusted, standardized z-scores for height and BMI were constructed using the WHO Growth Standards (http://www.who.int/growthref/en/).
Results
A higher percentage of boys had puberty onset based on genitalia (G2) than based on testicular volume (TV) or pubic hair (P2), particularly at younger ages. The height and BMI Z-scores suggest that Chapaevsk boys were slightly taller and thinner than boys included in the WHO standards.

Table 1. Onset of puberty and measures of growth

Measurement	Age at visit (years)				
	8 (N=310)	9 (N=476)	10 (N=451)	11 (N=445)	12 (N=171)
TV>3ml	37 (12%)	106 (22%)	168 (37%)	254 (57%)	141 (82%)
G2 or higher	68 (22%)	209 (44%)	273 (61%)	332 (75%)	153 (89%)
P2 or higher	11 (4%)	46 (10%)	63 (14%)	85 (19%)	67 (39%)
Growth (Mean, SD)					
Height, cm	128.6 (6.0)	133.5 (6.2)	138.9 (6.5)	143.7 (6.9)	148.5 (7.4)
Height Z-score	0.19 (1.04)	0.12 (1.03)	0.14 (1.01)	0.07 (1.02)	-0.13 (1.05)
Weight, kg	26.6 (5.6)	29.2 (6.4)	32.8 (7.5)	36.2 (8.9)	39.4 (9.8)
BMI, kg/m2	16.0 (2.3)	16.3 (2.5)	16.9 (2.8)	17.4 (3.2)	17.7 (3.3)
BMI Z-score	-0.08 (1.32)	-0.12 (1.28)	-0.05 (1.29)	-0.13 (1.37)	-0.28 (1.36)

Conclusions
From our longitudinal cohort study we generated reference data for growth and pubertal onset for Russian boys from Chapaevsk, Russia. We found substantial differences in rates of puberty onset in this cohort according to pubic hair development, genital maturation, and testicular volume.

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Bicalutamide and letrozole treatment of precocious puberty due to elevated levels of human chorionic gonadotropin (hCG)

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Elevated serum hCG levels can cause gonadotropin-independent precocious puberty in boys. We report a 6 ½ year-old boy with a history of growth acceleration, penile and testicular enlargement, body odor, pubic hair and aggression which developed over a 6-8 month period. This patient had transient elevation of serum and CSF hCG levels, yet a tumor has not been identified.
METHODS/CASE: Physical examination revealed Tanner 3 pubic hair, testicles 6 ml and stretched penile length 10cm. Bone age was advanced to 9y with elevated testosterone (771ng/ml) and suppressed gonadotropin levels. His serum β-hCG was initially elevated (113 IUnits/L) with normal serum alpha-fetoprotein. When imaging of the chest, abdomen, pelvis and testes with ultrasound, CT, MRI and PET did not identify a germ cell tumor, an MRI of the brain was performed. This showed a 4 x 6 mm pineal cyst with no evidence of intracranial tumor. Simultaneous serum and CSF hCG measurements done within three weeks of presentation were 19 and 21 IUnits/L, respectively, suggesting intracranial tumor. However, four serial MRI scans over the next 9mo failed to identify tumor, and the pineal cyst appeared to naturally involute. After imaging, bicalutamide plus letrozole was started to ameliorate end-organ effects of precocious puberty. Virilization and behavioral symptoms stabilized, and testicular size decreased. Serum testosterone levels, with intermittent spikes, gradually returned to normal. Therapy was well tolerated, and both serum and CSF hCG levels returned to normal. Bone maturation advanced by 2y within 9m, then stabilized. Treatment was discontinued after 11m because testes no longer appeared to be actively stimulated. Subsequently, the patient experienced onset of central precocious puberty, necessitating the use of a GnRH agonist.
CONCLUSION: This is the first reported use of bicalutamide plus letrozole for therapy of precocious puberty caused by excess hCG. If the source of hCG cannot be found and removed, the combination of an aromatase inhibitor and androgen receptor blocker can be used to interrupt peripheral precocious puberty. Even after interrupting peripheral androgen effect, prior testosterone exposure can lead to further advancement in bone maturation during the subsequent 6-9 months.

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The auxological and hormonal differences in girls with atypical thelarche and central precocious puberty

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Background

Atypical thelarche (AT) is defined as premature thelarche associated with signs of systemic estrogen effects without progression to complete puberty. Although early recognition of central precocious puberty (CPP) is essential, it may be difficult to distinguish CPP from AT at the beginning point of breast development in girls. We compared anthropomorphic measures and hormone levels in girls with AT and CPP and tried to find out factors discriminating CPP from AT.

Methods

We analyzed clinical and laboratory data of 62 girls (mean age, 7.32±1.03 years) with precocious breast development from May 2004 to May 2008 at Seoul St. Mary's Hospital. CPP was diagnosed if peak LH levels were >6.9 IU/L after GnRH stimulation test. A multiple logistic regression analysis and the area under the receiver operating characteristics curve (AUC) were used to analyse the prediction capacity of variable factors to diagnose CPP.

Result

We did not find any difference in chronological age, bone age, height-SDS, weight-SDS, mid-parental height and predicted adult height between AT and CPP. But girls with AT had a lower degree of breast maturation than girls with CPP (P=0.001) at the time of first hospital visit. The basal LH levels (2.4±1.1 vs. 1.7±0.6 IU/L, P =0.001), IGF-I (471.0±192.1 vs. 370.8±146.8 μg/L, P =0.049) and the peak LH levels (12.7±8.2 vs. 3.9±1.3 IU/L, P <0.001) in GnRH-stimulation test in girls with CPP were higher than AT. The basal FSH, estradiol, IGFBP-3 and the peak FSH levels in GnRH-stimulation test showed no differences between AT and CPP. A multivariate logistic regression model including the basal LH levels and IGF-I levels revealed a strong relation of the basal LH levels to CPP [OR : 2.7, 95% confidence interval(CI): 1.1-6.7, P =0.035]. The AUC for basal LH levels showed prediction capacity of basal LH levels to diagnose CPP [AUC: 0.70, 95% CI: 0.57-0.81, P <0.01]. The statistically ideal cut-off value of basal LH levels to discriminate CPP from AT was 2.66 IU/L (sensitivity 40%, specificity 98%) and the clinically meaningful cut-off value was 1.86 IU/L (sensitivity 70%, specifity 62%).

Conclusion

The basal LH levels and IGF-I were elevated in girls with CPP compared to those with AT and the basal LH levels above 1.86 IU/L in patients with precocious breast development favor the diagnosis of CPP rather than AT. These results suggest that the basal LH level could be an useful parameter predicting CPP.

PO3-151 Gonads and Puberty III

Ovarian Sertoli-Leydig cell tumor (SLCT) in an adolescent girl: usefulness of immunohistochemical markers

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Sertoli-Leydig cell tumors are rare sex cord-stromal neoplasms comprising less than 0,5% of ovarian tumors. Frequently reported as a solid mass, most of them occur in second to fourth decade of life. Little is known about the outcome in pediatric patients. We report a 13 year-old girl with a history of arrest of breast development which had started 2 years before consultation. No attained menarche. Over the previous year, deepening of the voice, increase of body hair and acne were observed. Physical examination showed moderated inflammatory acne and severe hirsutism (Ferriman&Gallway score 20). Pubic hair was Tanner VI. She had clitoromegaly and atrophic breasts Tanner IV**.** A firm mass of 10 cm diam was found on the lower right side of the abdomen. Ultrasonography (USG) showed a 95.6 x 62.9 mm mixed solid and cystic mass in the right ovary with irregular edges showing many cystic images, the biggest diam was 58.8mm. Computed tomography (CT) confirmed the presence of the cystic/solid mass and ruled out disease in the adrenal glands, thorax and abdomen.

Laboratory	Total testosterone ng/ml (0.06-0.82)	Androstenedione ng/ml (0.3-3.3)	17 hydroxy progesterone ng/ml (0.4-2.0)	SHBG nmol/l (15-114)	Inhibine B pg/ml (5-200)
Before surgery	10.47	7.9	9.7	19.8	277
Post surgery	0.6	1.4	1.9	31.25	43

SDHEA, αfetoprotein, βHCG, anti-müllerian hormone (AMH) were normal. LH was 3,1mUI/ml, FSH 0.9 mUI/ml. Karyotype 46 XX.Salpingoophorectomy was performed. Microscopically, the tumor showed variable-sized masses of spindle and polyedric cells with dense stroma between them and isolated primary follicles. Immunolocalization of αinhibin subunit (normally expressed by Leydig and Sertoli cells), AMH (specific of Sertoli-granulosa cells) and the 3βHSD were detected. Forkhead box L2 (FoxL2) expression was focally seen in cytoplasmic location. Sertoli-Leydig cell tumor with intermediate differentiation was diagnosed. After surgery, breast became trophic and menarche was achieved at day 25. Acne improved and laboratory normalized. Six month post surgery, body hair had thinned out. USG and MRI were normal. We present a SLCT with unusual cystic appearance in an adolescent girl confirmed by multiple immunohistochemical studies. As the scanty cytoplasmatic FoxL2 localization was previously suggested as an indicator of poor prognosis in sex-cord stromal tumors, follow-up should be especially close in this case.

PO3-152 Gonads and Puberty III

The novel GnRH agonist (Histrelin) implant is safely and effectively implanted under local anesthesia in the office setting

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Backround: Recently, a novel GnRH agonist therapy, histrelin, has been approved by the FDA for treatment of central precocious puberty. This is a subcutaneous implant that supresses the pituitary gonadal axis for one year. In the pivotal studies, the majority of centers participating placed the implant under either concious sedation or general anesthesia.

Aims: To determine if the implant could be placed under local anesthesia in the out patient setting

Methods: The pediatric endocrinologist, in conjunction with the parents determined which patients would be suitable for local anesthesia. The procedure was performed in the surgical office by a pediatric surgeon. Distraction therapy was performed by a child life specialist. Topical anesthetic cream was applied for 30 mins while the procedure was explained by the play therapist. 1% lidocaine with epinepherine was injected at the site and a 3-4 mm incision was made in the skin through which the implant was placed. The implantation procedure took 10 mins.

Results: 60 patients had an initial implant performed. Age range 5.5 -13.5 years. 47 (78%) were female. 25 (42%) patients had been previously treated with other GnRH analogues and 58% were naive to treatment. 46 (77%) were performed under local. All tolerated the minor procedure very well with out having to convert to general anesthesia. Of the first 24 patients who had completed one year of histrelin, all were determined to need ongoing GnRH therapy. One of these 24 patients chose to switch to depot leuprolide injection as the parent felt the implant was not working, however biochemical and clinical data confirmed she was completely suppressed at one year. The remaining 23 (96%) elected to re-implant histrelin. Of these 23, 7 had been done under GA the first time and 3 (42%) of the 7 switched to local for the implant explant procedure. No patient required radiographic localization of the implant to assist in the explantation procedure. Of the 83 total implants done, all proceeded simply with out problems during or after the procedure.

Conclusion: The new Histrelin implant is safely and cost effectively placed under local anesthesia. The majority of patients who needed GnRH treatment for an other year chose to have the implant over other therapeutic options.

PO3-153 Gonads and Puberty III

Low incidence of cryptorchidism in Iceland: a two year prospective study

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Introduction

Epidemiological studies suggest that the incidence of congenital cryptorchidism has increased in many countries. Of special interest are the results from a collaborative study where a much higher incidence, 9,0% was found in Danish boys compared with 2,4% in Finnish boys (1). The aim of our study was to assess the incidence of cryptorchidism in Icelandic boys at birth.

Material and methods

This was a prospective study and the cohort included all boys born at the Department of Obstetrics Landspitali University Hospital during 2 years Oct. 1st 2006 – Sept. 30th 2008. Approximately 70% of all newborn infants in Iceland are delivered at that institution. The boys were examined at birth, five days after birth and follow-up examinations were performed at the age of 3 and 6 months. Preterm boys were examined at discharge from the neonatal intensive care unit. Testicular position was assessed by a standardized technique. All subtypes of congenital cryptorchidism were included, but retractile testes were considered normal. The examiners are all experienced neonatologists.

Results

During the study period 3391 boys were examined. The median birth weight was 3647,5 grams (range 2775 – 4770). Median gestational age was 39 weeks (33 – 42). At the first examination a total of 36 boys were diagnosed with cryptorcidism. The incidence of cryptorhidism in our cohort is therefore 1,06 %. One boy had bilateral undescended testicles. The examination at 6 months of age revealed that in 6 boys (16,6%) the testicles had spontaneously descended. The remaining boys were referred to pediatric surgeons according to a recent Nordic concensus (2).

Conclusion

The incidence of congenital cryptorchidism is low in Iceland compared to what has been reported recently from other Nordic countries. Further studies are needed to explain the geographical difference in incidence of congenital chryptorchidism.

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The Lancet 2004; 363: 1264
(2) E Martin Ritzén et. al. Nordic concensus on treatment of undescended testis. Acta Pediatrica 2007;96: 638-43

PO3-154 Gonads and Puberty III

Anemia and thrombocytopenia with extreme splenomegaly in a girl with McCune-Albright syndrome (MAS)

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Objective: McCune-Albright syndrome is well known by the classical triad of polyostotic fibrous dysplasia (FD), *café-au-lait* spots, and endocrine dysfunctions, mainly precocious puberty (PP). There is some data that affection of the liver and heart may worsen the overall good prognosis of MAS. We present a case report of extraordinary severe manifestations of FD leading to bone marrow dysfunction.

Case Report: Our patient (female, 5 years of age) showed failure to thrive and prolonged icterus at the age of five weeks. *Café-au-lait* spots and periodic vaginal bleedings were reported since birth. Since the first fracture at the age of 15 months, more than ten fractures occurred. X-rays, CT scans, and MRI showed polyostotic FD of the axial and craniofacial skeleton. In addition, hepatomegaly with elevated liver enzymes persisted since birth. Liver function tests were normal, a liver biopsy showed an *Arg201Cys* mutation of the *GNAS1* gene. Medical therapy of PP with tamoxifen at the age of three months was stopped since liver enzymes increased. The bone age (BP) is significantly advanced (+4 years). Therapy of FD with pamidronate was performed for 1 year from the age of three years without improving FD. Splenomegaly was observed at the age of two years. The spleen continuously enlarged during the following years down to the small pelvis and displacing other neighboured organs. The blood count showed elevated reticulocytes, promyelocytes, myelocytes, and blast cells. A puncture of bone marrow revealed an almost vanished erythropoiesis and myelopoiesis, but no evidence for malignancy. Thus, the diagnosis of extramedullary erythropoiesis due to the severe FD was made. Our patient needed transfusion of erythrocytes every 4-6 months, and one transfusion of thrombocytes every 6-12 months. During the last two years recurred episodes of systemic inflammatory response syndrome occurred which were treated by intravenous antibiotics.

Conclusion: This is a rare case of severe manifestation of McCune-Albright syndrome that shows affection of the hematopoietic system secondary to polyostotic FD.

PO3-155 Gonads and Puberty III

Precocious anticipated puberty in international adopted girls: to treat or not to treat?

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Precocious anticipated puberty (PP) in international adopted girls raises the question if to treat or not. Until now we have based the decision on the predicted adult height (PAH) and on the presence of psychological risks. Usually we consider treatment when PAH is below the 10th percentile (155–7 cm) or if there is a fall at a follow-up visit. A previous preliminary report (2007 ESPE Meeting) indicated a trend, with respect to the initial prediction, for a slight increase of adult height (AH) with treatment and a similar slight decrease without treatment. This study, on a larger group of girls, evaluated if other growth parameters were helpful in making a decision.

Forty-five adopted girls (age 14–29 yrs) were recalled as they reached AH, 7 failed to participate in the study. Seventeen (group A) were treated with a GnRH analogue starting at mean age 7.44±1.22 yrs for 4.27±1.30 yrs. Initial PAH (Bayley-Pinneau) was 155.2±5.3 cm. with an AH of 158.5±5.8 cm ($p<0.005$) and a gain of 3.3±4.7 cm. Menarche occurred at a mean age of 11.3±1.9 yrs. Fourteen 17 girls increased initial predicted height, 2 of the 3 who did not reach PAH had menarche at 8 yrs before starting therapy.

Twenty one (group B) were not treated, in these PP was diagnosed at 7.6±1.1 yrs, menarche occurred at 10.30±1.57 yrs. Initial PAH was 159.2±6.1 cm. with an AH of 156.4±5.7 cm ($p<0.005$) and a loss of 2.8±4.6 cm. Sixteen 21girls decreased the initial PAH.

Initial mean PAHs were different in the 2 groups ($p<0.05$) and the differences (AH-PAH) in the 2 series were also significant ($p<0.001$), while AH was not different. In treated girls AH correlated significantly with age at adoption (R: -0.34; $p<0.05$), and age at menarche (R: 0.38; $p<0.05$), while no correlations were found with age at onset of thelarche, age at starting and length of therapy, initial PAH. The gain in AH was higher in the girls with lower BA at onset of therapy (below 10 yr; $p<0.05$).

In conclusion, therapy was useful to obtain normal AH. Younger age at adoption and lower bone age at the beginning of treatment indicate a better height outcome, suggesting that delaying the decision to treat could affect negatively final height. The correlation, in treated girls, of AH with age of menarche, stresses further the importance to delay puberty.

PO3-156 Gonads and Puberty III

Girl with Turner syndrome with a mosaic karyotype 47XXX/45XO and a large paraovarian cyst

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Aim: To report a rare case of a girl with Turner syndrome and karyotype 47XXX/45XO

CASE PRESENTATION: The girl presented at the age of 11 5/12 yrs for evaluation of short stature and delayed puberty. At the initial examination dysmorphic features consistent with Turner's syndrome were recognized (triangular face, small lower jaw, high arched palate, clinodactyly). The karyotype was 47XXX/45XO (89%/11%). Baseline FSH and LH were 8.99 mIU/ml and 0.49 mIU/ml and after stimulation with LHRH 25.48 mIU/ml and 7.28 mIU/ml respectively. At the age of 12 9/12 yrs FSH and LH were 12.99 mIU/ml and 1.66 mIU/ml. Thyroid function tests were normal and thyroid antibodies were not detected. Pelvic sonogram revealed a small uterus and small ovaries. Kidney ultasound was normal and cardiologic evaluation was normal as well.

CLINICAL COURSE: At the age of 11 9/12 yrs., growth hormone therapy was initiated with good response, growth rate of 7 cm/yr. At the age of 13 yrs having a bone age of 12-12 6/12 yrs, small dose of conjugated estrogens were added to the treatment regime. Annual growth velocity was 6.5 cm the 2nd yr, 6.5 cm the 3rd year and 3.4 the 4th year and final height 155.8 cm exactly at the target height. OGTT was nl and IGF-I at the desirable level. At the age of 15 yrs she was placed on cyclic estrogen/progesterone substitution and she menstruated. She had a pelvic sonogram annually. At the age of 15 3/12 yrs a cyst with maximum diameter of 6 cm arising most likely from the left ovary was found, which was not present at the follow-up sonogram 6 weeks later. Six months later, at the pelvic sonogram, a large cyst with a maximum diameter of 15.9 cm was appreciated. a-FP, CEA-125, and HCG were low. She was operated and a large paraovarian cyst was removed. In the wall of the cyst was incorporated the left salpinx and gonad. The histologic diagnosis was serous cystadenoma.

Discussion: Mosaic karyotype 47XXX/45XO is a rare cause of gonadal dysgenesis. The diagnosis is possible to be delayed until adulthood as it is possible to have normal ovarian function at puberty. To our knowledge there is no other report in the literature of serous cystadenoma in association with this karyotype. It is important to obtain a karyotype in all girls with height below third percentile and a careful appreciation of dysmorphic features. Furthermore, pelvic sonogram should be obtained during therapy.

PO3-157 Gonads and Puberty III

Constitutional delay of growth and puberty predicts minor psychiatric morbidity in adolescence

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Background: Constitutional delay of growth and puberty (CDGP), a variant of the normal spectrum of pubertal timing, is characterized by spontaneous albeit later than average pubertal maturation. Based on clinical experience, it may be accompanied by considerable psychological distress in adolescence. Some data also suggest life-long consequences and adult psychopathology.

Objectives: We tested the hypothesis that delayed puberty is related to elevated risk of psychiatric morbidity.

Methods: We obtained cross-sectional data of 38 adolescents aged less than 18 years (24 boys, 14 girls) and 193 adults aged more than 18 years (101 men, 92 women) from a population of families of patients investigated for CDGP in university clinics in Finland. CDGP in family members was objectively defined by use of growth charts (growth spurt taking place 2 SD beyond the mean). This CDGP criterion was met in 58% of adolescents and 63% of adults. Psychiatric health was questioned by validated 12-item version of the General Health Questionnaire (GHQ) measuring depression and social dysfunction.

Results: We found a statistically significant association between CDGP and minor psychiatric morbidity among adolescents. Adolescents with CDGP had higher GHQ-score than those with average pubertal timing: mean 1.88 (95% CI 1.74 - 2.10) versus 1.66 (95% CI 1.50 - 1.82), p = 0.04. The association was robust to adjustments for age, gender, birth weight and length, target height, and treatment for delayed puberty. Among adults, similar association was not found.

Conclusions: This study provides evidence that CDGP is an important predictor of psychological problems in adolescence. Psychiatric morbidity needs to be recognized, and adequate support offered in boys and girls investigated for delayed pubertal growth and maturation. Studies in longitudinal cohorts are required to reassure that psychological problems do not reflect into later adult life.

PO3-158 Gonads and Puberty III

Hypogonadotropic hypogonadism, anosmia and limb abnormalities due to a 122 Kb microdeletion in chromosome 14

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Kallmann syndrome consists of congenital hypogonadotropic hypogonadism and anosmia. Several distinct forms have been described due to mutations in different genes, including X-linked Kal1 (Xp22.3), and autosomal dominant KAL2 (FGFR1 gene on 8p11.2), KAL3 (PROKR2 gene on 20p13), KAL4 (PROK2 gene on 3p21.1), KAL5 (CHD7 gene on 8q12.1); KAL6 (FGF8 gene on 10q24), and others. The cases described is a 46 year-old male of Jewish Ashkenazi descent who presented with clinical manifestations of Kallmann syndrome (hypogonadotropic hypogonadism and ansomia), as well as minor limb defects (bilateral absent thumbs and short arms), and mild dysmorphic features. He was otherwise healthy and of normal intelligence. He had undergone surgery for bilateral cryptorchidism as a child. As an adult he had undergone treatment with testosterone and chorionic gonadotropin, resulting in slight alleviation of severe azoospermia.

Chromosomal analysis was normal however chromosomal microarray analysis (CMA) performed by the Medical Genetic Laboratories at Baylor Collage of Medicine (Houston, TX) revealed a *de novo* 122 Kb deletion in the peri-centromeric region of chromosome 14q. Fluorescence in situ hybridization (FISH) demonstrated this deletion in 6/20 (30%) of the cells, confirming a mosaic state for this microdeletion.

This case presents a new form of Kallmann syndrome with limb defects caused by a mosaic microdeletion in chromosome 14q. Based on the genetic composition of the microdeleted region, possible genetic mechanisms for this phenomenon, are discussed

PO3-159 Gonads and Puberty III

Effect of endocrine distruptors on oxidant system of the testicular tissue in rats

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Introduction and Purpose: 4-Cholorophenoxy Acetic Acid (4-CPA) is one of the Plant Growth Regulator Hormones (PGRH) that is commonly used in greenhouses, particularly in tomato production, to improve harvest and quality However, remnants of PGRHs on plants may adversely affect human health if their dosing and timing is not strictly followed. They may cause various pathologies, particularly in reproductive system which is highly sensitive to external factors, and lead to testicular atrophy, ectopic testis, and infertility. To our best knowledge, there is no study in the literature that investigated the association of 4-CPA with the oxidant events in testicular tissue. Therefore, we aimed to investigate the effect of 4-CPA on the oxidant and antioxidant systems of the testicular tissue in prepubertal rats. **Material Method:** Study included 40 male Wistar Albino rats that were 20 days old. The rats were randomized into 5 (untreated control, saline control and 25-50-100 mg/kg/day 3 4-CPA) groups. Each rat received intended dose of 4-CPA every day until 50 days of age. Rats

in the 4-CPA groups received 25, 50 and 100 mg/kg/day doses of 4-CPA orally once a day for 30 days (between the 20th and 50th day) between 8-10 a.m. in the morning. Doses of the drug were continuously recalculated according to the body weight. The study was terminated on the 50th day between 8-10 a.m. after administration of the last dose. Levels of malondialdehyde (MDA), nitric oxide (NO) and antioxidant glutathione (GSH) in testicular tissue were measured as indicators of lipid peroxidation using spectrophotometric methods.**Results:** No significant difference was observed between the MDA, GSH and NO levels of the control and saline groups. However, 4-CPA was found to cause dose-dependent increase in MDA and NO levels together with a decrease in GSH levels (0.001).**Discussion and Conclusion:** Endocrine disruptors are known to cause various pathologies in reproductive systems, particularly in humans. Apoptotic death of cells in gonads due to endocrine disruptors has been attributed to inhibition of antioxidant enzymes by various mechanisms and subsequent increase of reactive oxygen radicals in the tissue. Our results confirmed that 4-CPA increase the oxidative stress in a dose-dependent manner and suppress the antioxidant defense mechanisms.

PO3-160 Gonads and Puberty III

Genetic polymorphisms of FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), whose genetic basis is not completely well understood, is the most common endocrine disorder in women and it typically develops during adolescence. The aim of this study is to investigate the possible association between single nucleotide polymorphisms (SNPs) of FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes and PCOS in adolescent girls. DNA samples from forty-four adolescent girls with PCOS and 50 healthy controls were analyzed by PCR-RFLP and direct DNA sequencing to determine the genotypic frequency of 17 different polymorphic loci on the FSHR (A307T, N680S), CYP17 (-34 T/C), CYP1A1 (T6235C), CAPN10 (44, 43, 19, 63), INSR (exon 17 C/T), SERPINE1 (4G/5G) genes. Genotyping of exon 12 (six polymorphisms) and intron 12 (one polymorphism) of INSR gene by direct DNA sequencing was performed for the first time in this study. No significant differences were observed in the genotype and allele distributions of abovementioned polymorphisms between cases and control groups. Our data does not support an association between SNPs of FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes and susceptibility to PCOS or related traits in Turkish adolescent girls.

PO3-161 Gonads and Puberty III

Isolated familial hypogonadotropic hypogonadism caused by a GNRH1 mutation

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In a cohort of non syndromic and normosmic idiopathic hypogonadotropic hypogonadism (IHH), using a candidate gene approach, we investigated whether mutations in the gonadotropin-releasing hormone-1 (GNRH1) gene might be responsible for IHH in humans. We identified a homozygous GNRH1 frameshift mutation (c.18-19insA) in the sequence encoding the N terminus extremity of the pre-pro-GnRH in a young brother and his sister with normosmic IHH. Both unaffected parents were heterozygous for the mutation, as was the unaffected sister. The frameshift mutation was not found in 200 chromosomes from eugonadic controls or in 290 chromosomes from unrelated patients with sporadic normosmic IHH. In 200 ancestrally matched controls we found that one subject was heterozygous for the c.18-19insA GnRH1 mutation. Because the family denied any consanguinity, we hypothesize and confirmed by haplotype analysis that this homozygous mutation results from a founding event in a more distant common ancestor. The frameshift mutation identified here leads, if translated, to a putative aberrant peptide of 42 amino acids, completely lacking the GnRH-1 peptide sequence, including the highly conserved amino acid residues crucial for binding to and activating pituitary GnRH receptor-1. The impact of the c.18-19insA GnRH1 mutation was examined by expressing this mutant in AtT20 pituitary cells. GnRH1 (104 ± 31 pg/ml) was detected in conditioned medium by wild-type GnRH1 transfected cells, indicating that AtT20 cells, expressing convertases, are capable of processing and secreting the GnRH peptide. In contrast, GnRH was undetectable in medium conditioned by the c.18-19insA GnRH1 mutant transfected cells. In conclusion, the GNRH1 mutation identified here is a novel genetic cause of hypothalamic IHH and so far the only known example of a genetic aberration causing complete inactivation of a human hypothalamic hypophysiotropic neurohormone gene. This isolated autosomal recessive GnRH deficiency, reversed by pulsatile GnRH administration, validates the pivotal role of GnRH in human reproduction.

PO3-162 Gonads and Puberty III

First-voided urine LH is a better diagnostic method than GnRH stimulation test in differentiation between precocious puberty and premature thelarche

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Introduction: Unlike precocious puberty (PP), premature thelarche (PT) is considered a benign variant of premature puberty which is not associated with accelerated growth or bone-age (BA) advancement. Early differentiation between these entities is clinically important as therapy is not indicated in girls with PT.
Aims: To compare the diagnostic value of GnRH stimulation test and first-voided urine LH (FVU-LH) levels in differentiation between PP and PT.
Methods: 62 girls with breast budding before 8 years of age (mean age 6.6 ± 1.7 y) simultaneously underwent GnRH stimulation test and FVU-LH measurement. PP was diagnosed based on early breast budding accompanied by BA advancement (>2 SDS, Greulich & Pyle) and/or growth acceleration (>2SDS, US Tanner-Davies). PT was suspected in girls without additional findings or in obese girls (BMI >2 SDS) with BA advancement only. The diagnosis of PT was confirmed after 6 to 12 months of follow-up.
Results: 42 girls were initially diagnosed with PP and 20 girls with PT. 4 PT girls were eventually assigned to the PP group after a follow-up period. No significant differences in basal and stimulated peak LH levels were found between the groups. Compared with PT however, PP was characterized by higher levels of peak LH/ peak FSH ratio (0.76 ± 0.72 *vs.* 0.22 ± 0.12, p=0.002) and FVU-LH (2.2 ± 1.5 *vs.* 1.1 ± 0.3 mIU/L, p<0.001). These two variables were highly correlated: R = 0.735, p<0.001. Cutoff points for these variables were selected By ROC curve, and their sensitivity, specificity, positive- and negative predictive values (PV) for PT are summarized in the Table.

	Sensitivity (%)	Specificity (%)	Positive PV (%)	Negative PV (%)
Peak LH/FSH ratio <0.25	75	74	50	89
FVU-LH < 1.2 mIU/L	75	72	48	89

Conclusions: FVU-LH test is a physiologic, non-invasive method with mildly better diagnostic value than GnRH stimulation test in differentiation between PT and PP. Yet, due to insufficient sensitivity and specificity, both tests are of limited value in the workup of PP, and an observational follow-up is still needed to distinguish between PP and PT.

PO3-163 HPG Axis

Role of leptin on KiSS-1 system and hypothalmus-pituitary axis in mouse

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Purpose : This study was aimed to investigate the role of leptin on hypothalmus-pituitary axis. We observed the effects of leptin on hypothalamic KiSS-1 gene encoding kisspeptin and GnRH gene expression and pituitary LH gene expression at the prepubertal stage and pubertal stage individually. And the effects of GnRH antagonist on these genes were observed to know the dependency on GnRH in the effects of leptin.
Methods : We used 3 weeks-old female ICR mice as a prepubertal group and 4 weeks-old female mice as a pubertal group. The puberty was determined as the presence of vaginal opening. Leptin was directly injected to the lateral ventricle by Laursen and Belknap method. The changes of the expression of GnRH and KiSS-1 mRNA in hypothalamic tissue and LH mRNA in pituitary tissue were observed at 2 hours and 4 hours after the injection. And we observed the changes of these genes expression after injecting cetrotide, a GnRH antagonist, to know the relation of GnRH in the effects of leptin.
Results : The expression of KiSS-1, GnRH and LH gene of pubertal mice was higher than those of prepubertal mice in control group($P<0.01$). In 4 weeks-old pubertal group, leptin injection decreased the expression of KiSS-1 and GnRH mRNA in hypothalmus($P<0.01$) and LH mRNA in pituitary gland(2hr, $P>0.05$; 4hr, $P<0.01$). In 3 week-old prepubertal group, leptin decreased the expression of GnRH mRNA($P<0.01$), but the change of KiSS-1 and LH mRNA expression was not consistent. When GnRH antagonist was treated alone in 4 weeks-old mice, LH mRNA expression was decreased significantly($P<0.01$) and but KiSS-1 and GnRH gene expression was increased(2hr, $P>0.05$; 4hr, $P<0.01$). Additional leptin injection made KiSS-1 and GnRH mRNA expression decreased($P<0.01$) and LH mRNA decreased more(2hr, $P<0.01$; 4hr $P>0.05$).
Conclusion : The findings in this study shows that leptin injection to normal female mice via i.c.v. route make higher ventricular leptin level than physiologic condition, and seems to induce the suppressive effect on the genes expression in hypothalamus-pituitary axis. The observations that GnRH and KiSS-1 expression changed with the same pattern after the injection of leptin or GnRH antagonist treatment suggest that leptin controls GnRH and KiSS system in higher level, then the leptin-kisspeptin-GnRH pathway may exist. The increased response of GnRH and KiSS-1 gene expression to GnRH antagonist means that GnRH and KiSS-1 gene have the same feedback control.

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Incomplete progress through puberty and a large cystic lesion in the hypothalamo-pituitary area in a patient with a heterozygous *SOX2* deletion

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Background: *SOX2* is a member of the SOX family of transcription factors [SRY-related high-mobility group (HMG) box]. Heterozygous, *de novo*, loss-of-function mutations were initially reported in patients with bilateral anophthalmia/microphthalmia, developmental delay, male genital tract abnormalities, oesophageal atresia and sensorineural hearing loss. We have recently reported a number of *SOX2* mutations in patients with anterior pituitary hypoplasia and hypogonadotrophic hypogonadism. Additional features included the association with hypothalamic hamartoma and variable defects affecting the corpus callosum and mesial temporal structures. We herein report a patient with a heterozygous *SOX2* gene deletion associated with an unusual MRI appearance.
Case report: The proband is a female patient of non consanguineous parents from Argentina, who presented at the age of 18 years with pubertal delay. She was born at term, with a birth weight of -1.0SDS, and was noted to have extreme bilateral microphthalmia. She had delayed motor milestones and severely impaired language development. At the age of 18 years, she had a height of -3.12 SDS, with normal concentration of IGF-1 (270.7ng/ml) and basal GH concentration of 6.84ng/ml. Cortisol profile, thyroid function tests and prolactin were normal. Hypogonadotrophic hypogonadism was diagnosed with a flat LH and FSH response to GnRH stimulation. However, at the age of 24 years, she progressed to develop spontaneous but incomplete pubertal development, with Tanner breast stage 2-3. Brain MRI demonstrated a sellar cystic mass of 2cm in diameter, which extended to the suprasellar region. Sequential MR imaging did not reveal any changes over time. The mass was initially felt to be a Rathke's pouch cyst. The patient was referred for genetic screening for identification of *SOX2* mutations. Multiple ligation probe analysis (MLPA) revealed that she was heterozygous for a whole gene *SOX2* deletion. Although described *SOX2* mutations are *de novo*, analysis of parental DNA is necessary in order to confirm this.
Conclusion: Heterozygous *SOX2* mutations are associated with hypogonadotrophic hypogonadism and anterior pituitary hypoplasia. We now describe a case with a large cystic lesion in the intra- and suprasellar area, which extends the spectrum of MR abnormalities associated with this condition. The underlying molecular mechanism that leads to this appearance remains to be established.

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Molecular analysis of PIT1, PROP1, LHX3, and HESX1 in patients with combined pituitary hormone deficiency: multicenter study

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Background: Mutations in the genes of pituitary transcription factors including PROP1, PIT1, HESX1, LHX3 which play a role in the development and differentiation of pituitary gland result in combined pituitary hormone deficiency (CPHD).
Aim: The aim of this study was to investigate the specific mutations in the transcription factors in Turkish patients with CPHD, either sporadic or familial.
Patients and Methods: The study included 39 patients from 28 families (12 sporadic, 16 familial) with CPHD, followed in different centers. CPHD was defined as GH deficiency, associated with at least deficiency of one other pituitary hormone including ACTH, LH, FSH, TSH or prolactin. Based on clinical, hormonal and neuroradiological data, phenotype and relevant transcription factor genes were evaluated. All coding exons of PIT1, PROP1, LHX3, and HESX1 genes were amplified by PCR and sequenced.
Results: In 8 cases from 3 families mutation in PROP1 gene (frame shift mutation in exon 2– p.Glu100GlufsX9) was found. In 2 siblings of a non consanguineous family a novel mutation (IVS1+2T>6) in intronic region was recorded. The parents were carriers for this mutation. The frequency of PROP1 gene mutation was 25.6 % (10/39) in all cases. In the PROP1 genes 10 different polymorphisms, including 2 novel ones, were determined. No mutation was

detected on PIT1 gene however 5 different polymorphisms were identified. In LHX3 gene, there were no mutations but 12 different polymorphisms were noted. A mutation in HESX1 gene was detected in a patient with septo-optic dysplasia on exon 4 which was a missense mutation (p.R160H).
Conclusions: This study is the first to investigate specific mutations in our country in CPHD patients. Novel mutations have been found in PROP1 gene. Knowing the mutations will aid the physician in phenotype-genotype correlation and thus early diagnosis of hormonal deficiencies.

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Anosmia may predict hypogonadotropic hypogonadism in patients with CHARGE syndrome

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Introduction
CHARGE syndrome is characterized by **c**oloboma of the eyes, congenital **heart defects, choanal atresia, r**etarded growth and development, **genital hypoplasia, e**ar anomalies and deafness. Mutations in the CHD7 gene, located on chromosome 8, have been identified causing CHARGE syndrome. Recent studies show that the main features of Kallmann syndrome, i.e. hypogonadotropic hypogonadism, anosmia and abnormal olfactory bulb development, can also be present in patients with CHARGE syndrome. Therefore, we studied whether anosmia could predict the presence of hypogonadotropic hypogonadism in patients with CHARGE syndrome.
Patients and methods
Patients with CHARGE syndrome (CHD7 mutation positive) were included. Pubertal development was assessed by physical examination and scored according to Tanner stages. From patients aged 10 years and older, blood samples were taken to measure LH, FSH, testosterone (males) and estradiol (females). The University of Pennsylvania Smell Identification Test (UPSIT) was used to assess olfactory function.
Results
In total, 20 patients, 10 male and 10 female, were included in the study. Mean (SD) age (yr;mo) for male patients was 15;3 (3;6) and 15;11 (2;8) for female patients. In male patients aged 14 years and older, only one patient did have spontaneous pubertal development. Unfortunately, olfactory function could not be assessed in this patient. In all male patients with anosmia, pubertal development was absent and gonadotropin levels were low. However, 3 of these 5 patients were aged < 14 years, so hypogonadotropic hypogonadism could not be definitely diagnosed yet.
In female patients aged 13 years and older, 3 of 9 patients did have spontaneous pubertal development. In these female patients with spontaneous puberty, two had hyposmia and in one patient olfactory function could not be tested. All female patients with anosmia were prepubertal and had low gonadotropin levels, suggesting hypogonadotropic hypogonadism. However, one of these patients was aged 11 years, so hypogonadotropic hypogonadism could not be definitely diagnosed yet.
Conclusion
Anosmia may predict the presence of hypogonadotropic hypogonadism in patients with CHARGE syndrome. On the contrary, intact olfactory function is associated with normal pubertal development. Early detection of hypogonadotropic hypogonadism in patients with CHARGE syndrome can prevent the unnecessary delay of hormonal pubertal induction, resulting in age-appropriate puberty.

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Diabetes insipidus management in children during nephrotoxic chemotherapy for germinoma

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Introduction: Central Diabetes Insipidus (CDI) is a disorder characterized by impaired ability of the kidney to concentrate urine, leading to polyuria, high serum osmolarity, decreased urinary osmolarity and hypernatremia due to decreased or absent secretion of the antidiuretic hormone (ADH). In children, CDI can be associated with a variety of causes including neoplasms, with germinomas and pinealomas being some of the most common. Usually the management requires adequate fluid intake and use of vasopressine analogs.
Objectives: Case report of the management of a 14 year old boy diagnosed with brain germinoma, panhypopituitarism and DI who underwent nephrotoxic chemotherapy (CT) with high osmolarity, requiring hyperhidration and adjustments of dDAVP dosage.
Material and methods: Review of the medical files and charts of the patient and related literature.
Discussion: A 14 year old boy was diagnosed with a brain germinoma along with panhypopituitarism and DI and was referred to our tertiary care hospital for oncologic evaluation. He was taking levothiroxine, hydrocortisone and dDAVP (10 mcg twice daily). The proposed chemotherapeutic protocol (TCG 99) included 4 inicial cycles of chemotherapy with Etoposide, Ifosfamide and Cysplatin, requiring intravenous (IV) fluid therapy with high volume and hyperosmolarity. During the first cycle he received regular protocol and presented with shock and severe hypernatremia (seric sodium 185mEq/L).
From the second cycle on, to avoid nephrotoxicity, the morning dose of DDAVP was suppressed. During chemotherapy infusion he was managed with hourly replacement of urinary output above 6ml/kg/h with a 2,5% dextrose solution, sodium evaluation every 2 hours, reduction of CT osmolarity (100 vs 150 mOsm/L on the first cicle) and avoidance of manitol in the solution. In this period he presented with severe polyuria (urinary output up to 30ml/kg/h) and sodium concentrations ranged from 137-159,130-158 and 137-166mEq/L on the second, third and fourth cicles, respectively. DDAVP was administered only 4 hours after the end of Cysplatin infusion with subsequent reduction of urinary output.
Conclusion: Diabetes insipidus can cause significant morbidity and mortality especially when associated with other conditions such as neoplasms requiring treatment. In this case strict management of fluid balance and adequation of the chemotherapy protocol successfully prevented electrolyte imbalance and dehydration.

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Sellar osteosarcoma in a 17 years old boy

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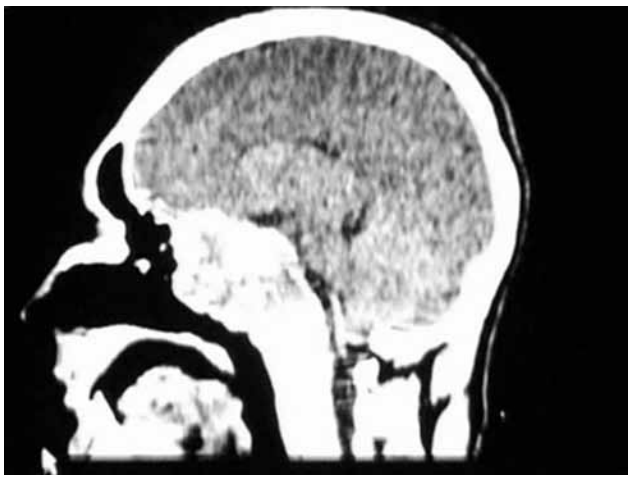
Introduction: Primary osteosarcoma is very rare in the skull bones, even more in the central skull base. Most cases described on the literature are secondary to CNS irradiation, Paget disease, fibrous dysplasia, retinoblastoma or chronic osteomyelitis. The peak incidence of skull osteosarcoma is in the third decade. The clinical presentation of the sellar osteosarcomas is similar to other sellar masses, with visual disturbances and headaches with or without pituitary disfunction with relatively rapid growth.
Clinical Case: We described the case of a 17 years old boy, who was refered because of headaches, diplopia and visual acuity reduction since 16 years old. His pituitary function was nornal and a CT showed a mass that was infiltrating sphenoidal sinus, sellar and parasellar region (71 x 55 x 45 mm), with compression of the optic chiasm and both optic nerves. After contrast some hyposignal areas were seen, suggesting necrosis. At physical examination he was euthyroid, with Tanner stage 4 and testes of 20cc. There were no signs or symptoms of pituitary disfunction. His height was 176,8 cm and weight 65 kg. Surgery was performed with a presumptive diagnosis of clivus chordoma. The post surgery recovery was uneventfull, with full manteinance of pituitary func-

tion 4 months after surgery (Table). He also presented a notable improvement of visual acuity and headaches resolved.

Lab workup		
	Pre-surgery	Post-surgery
TSH (μIU/ml)	1,83	1,12
T4 (μg/dl)	9,76	6
ACTH (pg/ml)	28,5	19,8
CORTISOL (μg/dl)	19	12
PROLACTIN (ng/ml)	16,8	27
IGF1 (ng/ml)	431	378
IGFBP3 (μg/ml)	6,3	5,72
GH (ng/ml)	046	
FSH (mIU/ml)	6,9	6,48
LH (mIU/ml)	5,6	6,06
TESTOSTERONE (ng/dl)	769	558



The histopathology diagnosis was osteosarcoma, osteoblastic type, which has a poor prognosis. The search for methastasis was negative, and he started with chemotherapy (Cisplatine, Doxorrubicine), following protocol EOI.
Conclusion: Osteosarcoma of the sella is rare, but it must be a differential diagnosis of any sellar or parasellar lesion. The presence of neurological symptoms, rapid evolution, and the images on the CT with important compromise of the bones aids to the diagnosis.

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Cognitive effects of aromatase inhibitor therapy in peripubertal boys

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Objective
Aromatase inhibitors, blockers of estrogen biosynthesis, have emerged as a new potential treatment modality for boys with short stature. The cognitive effects of such therapy are unknown. In this study, we explored the effects of aromatase inhibition on cognitive performance in peripubertal boys.
Design
Prospective, double-blind, randomised, placebo-controlled clinical study.
Methods
Twenty-eight boys, aged 9.0–14.5 years, with idiopathic short stature were treated with the aromatase inhibitor letrozole (2.5 mg/d), or placebo, for 2 years. During the treatment, the progression of physical signs of puberty and the concentrations of sex hormones were followed up. A selection of cognitive tests, focusing on memory function, was administered to the participants at entry, at 12 months, and at 24 months after the start of treatment.
Results
Letrozole effectively inhibited the conversion of androgen to estrogen, as indicated by high serum testosterone and low serum estradiol concentrations in letrozole treated boys who progressed in puberty. In both groups there was a gain in performance during the follow-up in tests of verbal performance (Similarities and Comprehension tests), in most of the tests of visuospatial performance (Block Design and Rey-Osterrieth Complex Figure Copying task), and in some tests of verbal memory (List Learning, Digit Span Forwards, and Digit Span total points). No significant differences in cognitive performance in any of the tests were found between the letrozole and placebo treated boys.
Conclusions
Blockade of estrogen biosynthesis with an aromatase inhibitor does not appear to influence cognitive performance in peripubertal males.

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Pharmacokinetics and – dynamics of clonidine during the oral clonidine test

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Background: The oral clonidine test is often performed in children suspected of a growth hormone deficiency. Although widely accepted, the test is associated with adverse reactions, mainly hypotension and sedation. It is not known whether these are related to serum clonidine concentrations, age, or associated hormone deficiencies. A pharmacokinetic study of clonidine during the oral clonidine test has not previously been performed.
Objectives: To describe the serum levels of clonidine in children undergoing an oral clonidine test, and document the pharmacodynamic response, with special attention for cardiovascular parameters, sedation and hormonal response.
Patients and Methods: In 40 children undergoing the oral clonidine test, informed consent was sought from the parents to participate in an observational study. In recruited participants, additional blood was drawn for analysis of clonidine serum levels, and cardiovascular parameters (blood pressure, heart rate) and sedation scores (modified Ramsay sedation score) were noted at 15 minute intervals. Measurements were performed until 210' post-dose.
Results: Maximum serum levels of clonidine were 0.84 ± 0.31 ng/mL 60' after clonidine administration. Clearance of clonidine was slow, with serum concentrations at 210' post-dose of 0.72 ± 0.22 ng/mL. Blood pressure and heart rate declined: at 108' post-dose, systolic and diastolic blood pressure were 85.5 ± 7.0 and 74 ± 11.7% of baseline; heart rate was 88.4 ± 15 % of baseline. This effect persisted until 213' post-dose: systolic and diastolic blood pressure were 84.9 ± 6.3% and 80.6 ± 13.4% of baseline, heart rate was 92 ± 14.1% of baseline. Maximal sedation was reached 80' after clonidine administration (80% of children appeared asleep; Ramsay score of ≥3). At 185' post-dose, this proportion was 48.6%. Growth hormone levels peaked at 60-90' post-dose (in 33 children with a response > 20 mU/L), and fell to baseline by 150'. All children with a normal GH response had reached their peak level by 90'.
Conclusions: This is the first study investigating pharmacokinetics and – dynamics of clonidine during the oral clonidine test. The elimination rate of clonidine is low. In case of a growth hormone response ≥ 20 mU/L, peak levels were always attained by 90' post-dose, however the effect on blood pressure, heart rate and sedation persisted until 210' after clonidine administration.

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Longitudinal assessment of urinary gonadotropin and testosterone levels and their relation to postnatal penile and testicular growth in full term and preterm boys

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Postnatal gonadotropin and testosterone (T) surge is suggested to have a role in the maturation of the gonads. Premature birth may alter the function of the HPG axis, and subsequently have an impact on gonadal development.
We measured gonadotropins and T in serial urine samples from birth to 6 months of age (d2, d7, d30, d60, d90, d120, d150, and d180) in 20 full term (FT) and 20 preterm (PT, gestational age 24,7 – 35,4 wks) boys. Gonadotropins were measured by time-resolved immunofluorometric assays and total testosterone by HPLC-MS/MS. Activity of the HPG axis was assessed by calculating area under the curve (AUC) over the time period. Penile length and testicular volumes were recorded at sampling both manually and by ultrasound.
In FT and PT boys peak median levels of LH were observed at d30. In PT boys, the peak levels were 2.3-fold higher than in FT boys. For FSH, peak was observed at d7 in FT and much later, at d60 in PT boys. T levels increased to maximum at d30 in both groups. In PT boys, the integrated T levels were 2.1-fold higher than in the FT boys (Table). Penile and testicular growth was more marked in PT compared to FT boys, and penile and testicular sizes were similar in both groups from d120 onwards. LH AUC correlated with penile growth, and FSH AUC with testicular growth in the whole group (p=0.001 and 0.003, respectively). Testosterone AUC correlated with penile growth in FT (p=0.015) but not in PT boys.
Postnatal testicular activation can be identified noninvasively in urinary samples. Different pattern of postnatal HPG axis activation is seen in FT and PT boys, PT boys having higher urinary gonadotropin and T levels during the first months than FT boys. High gonadotropin and T levels are associated with rapid postnatal penile and testicular growth in preterm infants. This phenomenon may be important for the functional development of reproductive capacity later in life.

	Full term		Preterm		p
AUC (d2-d180)	Median	Range[1]	Median	Range[1]	
LH (IU/mmol creatinine)	70,4	25,8 - 174,0	150,2	58,8 - 448,6	<0.000
FSH (IU/mmol creatinine)	72,8	40,4 - 127,5	89,5	38,0 - 379,0	(0.081)
Testosterone (nmol/mmol creatinine)	1270	75,6 - 2722	2661	86,3 - 8126	0.023
Δ penile length (%)	17,7	-2,0 - 34,1	31,9	11,0 - 67,1	<0.000
Δ testicular volume (%)	115	39 - 313	436	128 - 2018	<0.000

[1]Note the high inter-individual variation.

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Hypoglycemia during childhood followed by insulin-deficient diabetes mellitus, hypogonadotrophic hypogonadism, hypothyroidism, demyelinating neuropathy and alopecia: a Woodhouse-Sakati-like syndrome or a new neuroendocrine disease?

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Progressive neuroendocrine disorders with multisystemic manifestations are rare and difficult to diagnose. In this study, we characterized an unusual progressive disorder with dysregulation of glucose metabolism, partial anterior pituitary deficiency, demyelinating neuropathy, cognitive impairment and alopecia in three brothers born from first cousins of Senegal origin. Disease onset began in early childhood with growth retardation, hypotrophy and profound asymptomatic hypoglycemia diagnosed between the ages of two and five years. Extensive laboratory analyses revealed normal metabolic and hormonal parameters but spontaneous hypoglycaemia (as low as 1.6 mmol/l), with corresponding incomplete suppression of insulin levels (2 to 4 mIU/l). Imaging studies did not detect any liver or pancreatic lesions. Molecular tests of Kir6 and Sur1 genes, glucose transporters and of congenital disorders of glycosylation were negative. Between the ages of 14 and 16 years, subjects developed slowly progressing non auto-immune insulin-deficient diabetes mellitus (HB1Ac 6.3% progressing to 8.9% and insulin levels in an IV Insulin Tolerance Test at T1+T3 = 33 decreasing to 7.3 mIU/l - normal values >30 mIU/l), central hypothyroidism and hypogonadotrophic hypogonadism (delayed and incomplete puberty). Through adolescence they exhibited movement disorders with ataxia and dystonia due to progressive peripheral sensitive-motor demyelinating polyneuropathy, and pyramidal manifestations mainly affecting the lower limbs. Brain MRI showed moderate sub-cortical temporal white matter disease and small pituitary gland mainly in the older patient (19 years old). Visual and auditory evoked potentials were mildly decreased. All three subjects presented a moderately low intelligence quotient (IQ 70) and varying degrees of dysarthria, difficulty swallowing and frontal alopecia. This progressive multisystem disorder may thus represent a rare inherited single gene disorder. This phenotype is compatible with Woodhouse-Sakati syndrome, but may alternatively correspond to a new neuroendocrine disease (insulin and anterior-pituitary deficiency, neuropathy, dystonia, dysarthria and alopecia-IPiNEDDyA). This family was sufficiently informative to perform a genome mapping. Our current work on characterizing the gene defect could help elucidate the molecular mechanisms involved in the pathogenesis of this neuroendocrine disease and enhance our understanding of neuroendocrine disorders.

PO3-173 HPG Axis

Klebsiella pneumoniae sepsis complicating rotavirus gastroenteritis in two infants with glucocorticoid deficiency

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Klebsiella pneumoniae sepsis complicated rotavirus gastroenteritis in two infants being treated with stress-dose hydrocortisone during their illness (2mg/kg of intravenous hydrocortisone six hourly from admission). One infant had congenital adrenal hyperplasia and developed *K. pneumoniae* sepsis on day 15 of illness. The other infant had bilateral optic nerve hypoplasia with panhypopituitarism. He developed *K.pneumoniae* sepsis on day 4 of illness.
Enteric gram-negative (EGN) bacteraemia is an infrequently recognised complication of rotavirus infection. Rotavirus has not been shown to cause significant gastrointestinal mucosal changes, however rotavirus has been shown to increase epithelial permeability and enhance the ability of bacteria to invade enterocytes (Di Biase et al., 2000, Lundgren and Svensson, 2001, Ramig, 2004). Glucocorticoids are thought to cause delay in gastroduodenal healing most likely due to inhibition of prostaglandin synthesis(Carpani de Kaski et al., 1995). There have been no reports of secondary EGN bacteraemia in infants or children with rotavirus infection on corticosteroids. It is hypothesised that high dose corticosteroids may increase the risk of enteric gram-negative bacteraemia in rotavirus gastroenteritis.

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PO3-174 HPG Axis

Panhypopituitarism in children with hypoxic encephalopathy: report of 2 cases

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Hypoxic encephalopathy is rarely mentioned as a cause of panhypopituitarism in children. Here we report two cases of panhypopituitarism which occurred following severe hypoxic/ischemic brain damage.
Case1
The patient was a male born to nonconsanguineous Japanese parents by cesarean section at 34 weeks of gestation, with a birth weight of 1820g. A ventriculoperitoneal (VP) shunt was performed one month after birth due to idiopathic hydrocephalus. He was diagnosed as Kleinfelter syndrome at 8 months. The diagnosis was confirmed by karyotype analysis that showed 47, XXY. At age 7 years, he presented with headache and vomiting. Computed tomography of the brain revealed no remarkable findings at that time. The following day, he presented with seizure due to VP shunt malfunction and experienced cardiopulmonary arrest. Afterward, he required prolonged mechanical ventilation. At 19 days after the episode, laboratory data indicated central hypothyroidism: TSH 0.03μIU/ml, FT4 0.34ng/dl. Oral administration of thyroid hormone was initiated. One year after the episode, plasma levels of ACTH and cortisol were 4pg/ml and <0.6μg/dl, respectively. Plasma ACTH and cortisol did not respond to CRH test. Maximal cortisol concentration during ACTH test was 0.9μg/dl. Oral administration of glucocorticoid was also initiated.
Case2
The patient was a female born to nonconsanguineous Japanese parents by normal delivery at 39 weeks of gestation, with a birth weight of 2874g. She experienced an acute life-threatening event (ALTE) during kangaroo care in the neonatal period. Laboratory data indicated hypothyroidism and low cortisol at 19 and 23 days following birth, respectively. Oral administration of thyroid hormone and glucocorticoid was initiated.
Panhypopituitarism can develop several weeks after an episode of hypoxic/ischemic damage. Monitoring for endocrine dysfunction during follow-up of these patients is important.

PO3-175 HPG Axis

Successful treatment with GnRH analogue for a boy with traumatic brain injury-induced precocious puberty

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INTRODUCTION: The importance of pituitary dysfunction as a consequence of traumatic brain injury (TBI) has recently been highlighted. Hypopituitarism after TBI is well known, however, precocious puberty (PP) is rare sequelae of TBI. To date, 18 cases of TBI-induced PP have been reported. Out of 18 cases, only 4 patients were boys but none of them were treated with GnRH analogue. CASE: At the age of six years old, the boy fell down from the second floor and had severe head injury (left parietal and occipital lesion). Sequelae of the head injury included seizure, mental retardation, and right hemiparesis. Excessive growth in body height was found soon after the head injury. His growth velocity was 11.5 cm/yr between 7 years and 9 years old. His pubic hair growth was noticed at the age of 9 years and 11 months old. He had become more interested in females and aggressive personality and masturbation had been observed. At age of 9 years and 7 months old, his endocrinological was evaluated. His height and weight were 147.5 cm (+2.6 S.D) and 34.9 kg (+0.54 S.D), respectively. Bone age was advanced to 13 years (+35 %). His pubic hair development was Tanner stage II and testes volume was 20/15 ml (right/left), and his phallus was 11.5 cm in length. LH-RH test revealed marked increase in LH levels (basal and peaked LH levels were 2.4 and 18.3 IU/L, respectively). FSH level increased from 2.7 to 4.5 IU/L. These data indicated that he had pubertal stage due to activation of hypothalamic-pituitary axis. Serum testosterone level was elevated to the pubertal range, 2.69ng/ml. No other endocrine abnormalities were found. Brain magnetic resonance imaging (MRI) showed left cerebral atrophy but no tumor. He was diagnosed PP due to TBI. Although his adult height was predicted to be good, GnRH analogue (50μg/kg) was initiated for his sexual behavioral problems. Two months later, his sexually compulsive behavior was improved. LH and FSH basal levels were 0.3 IU/L and 0.2 IU/L respectively, and testosterone level was 0.27ng/ml.
CONCLUSION: TBI causes both loss-of-function and gain-of-function in hypothalamic-pituitary axis. Our case suggests that it is essential to evaluate the growth of children who had serious head injury. Earlier therapeutic intervention should be recommended not only to improve predicted adult height but also to alleviate psychological problems resulting from PP.

PO3-176 HPG Axis

Prevalence and risk factors for disrupted circadian rhythmicity in children with optic nerve hypoplasia

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OBJECTIVE: Children with optic nerve hypoplasia (ONH) have visual impairment and may have hypopituitarism and developmental delay. Children with ONH have also been reported to have abnormal sleep-wake cycles. We assessed the incidence and nature of sleep-wake abnormalities in children with ONH. METHODS: Rest-activity patterns were assessed in 23 children with ONH using actigraphy. The children also had formal assessment of pituitary function; MRI scans of the head, and assessment of neurocognitive function. RESULTS. Sufficient actigraphy data were obtained on 19 of the children. Analysis of expressed rhythmicity revealed normal patterns in 13 children (68%). Of the six children with abnormal rhythmicity, 3 had fragmented sleep, one had free-running rest-activity cycles, and 2 children were arrhythmic. Of the children with normal rhythmicity: corpus callosum hypoplasia was present in 30%, growth hormone deficiency in 53%, hypothyroidism in 23%, adrenal insufficiency in 30%, diabetes insipidus in 0%, and cognitive impairment in 8%. Of the children with abnormal rhythmicity: corpus callosum hypoplasia was present in 66%, growth hormone deficiency in 66%, hypothyroidism in 50% ($p<0.05$), adrenal insufficiency in 66% ($p<0.05$), diabetes insipidus in 33% ($p<0.01$), and cognitive impairment in 100% ($p<0.01$). CONCLUSIONS: Abnormal rhythmicity is present in 30% of children with ONH. The best predictors of abnormal rhythmicity are the presence of cognitive developmental delay and diabetes Insipidus.

PO3-177 HPG Axis

Leuprolide stimulation tests for the diagnosis of precocious puberty: relative importance of 1 and 2 hr stimulated gonadotropin values

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Background: The two hour Leuprolide stimulation test (L-stim) is currently used to assess the activation of Hypothalamic Pituitary Gonadal axis (HPGA) in patients (pts) with suspected precocious puberty (PP). Previous reports have utilized peak LH values at 30, 40, 60 or 120 min to confirm HPGA activation, although it is unclear which time is most relevant for establishing the diagnosis. In our study protocol, we measure LH and FSH at 60 and 120 min post L-stim.
Purpose: To compare the diagnostic value of 1 hr versus 2 hr post L-stim levels of LH, FSH and LH/FSH ratio in the assessment of PP.
Methods: A Retrospective chart review was done in 92 pts (34 males (M)), who underwent L-stim to confirm HPGA activation. Fifteen pts (11 M) with known disorders of HPGA and 6 M with delayed puberty were excluded. Serum LH and FSH (ICMA- Esoterix Lab; CA) were measured at 0, 60, and 120 after the subcutaneous administration of 20mcg/kg of Leuprolide. We considered pts with stimulated LH values ≥ 5.0 IU/L at 60 and/or 120 min as pubertal (PUB), and those with LH values <5.0 IU/L prepubertal (PRE).

Results: The differences between 1 and 2 hr LH, FSH and LH/FSH ratio between PRE (n=46, M=10) and PUB (n=25, M=8) are indicated in Table.

Difference in 1-hr and 2-hr LH, FSH & LH/FSH ratio in prepubertal & pubertal groups			
	T=60 min	T=120 min	P value (t-test)
PRE LH	2.02 ± 1.13	2.60 ± 1.26	P=0.02
PUB LH	8.98 ± 6.15	11.2 ± 7.08	P=0.24
PRE FSH	9.95 ± 6.04	15.55 ± 8.91	P=0.0006
PUB FSH	13.55 ± 10.06	18.13 ± 11.67	P=0.14
PRE LH/FSH	0.29 ± 0.36	0.25 ± 0.30	P=0.56
PUB LH/FSH	0.94 ± 0.80	0.98 ± 0.92	P=0.86

Mean LH and FSH levels were higher at 2 hrs then at 1 hr in both groups, but reached significance only in PRE. All pts who had LH ≥ 5.0 IU/L at T=60 min (n=17) also had LH ≥ 5.0 IU/L at 2 hrs. The 2-hr LH were ≥ 1-hr levels in 83% pts (59/71). The 17% of pts whose 1-hr LH exceeded 2-hr levels were also in the same pubertal category based on 2 hr values: 7 with PUB and 5 with PRE. In 98.6 % pts (70/71) 2-hr FSH levels were ≥ 1-hr levels. Mean LH/FSH ratio was not significantly different at either time point, within PRE and PUB groups. There was a wide overlap in stimulated FSH levels and LH/FSH ratios between PRE and PUB pts.
Conclusion: A single 2-hr stimulated LH value is sufficient to differentiate between PRE and PUB groups.

PO3-178 HPG Axis

Pituitary adenomas presenting in children and young people: a single centre experience

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Background: Pituitary adenomas are uncommon in childhood and adolescence. We describe one of the largest cohorts of these patients seen in a single centre.
Patients and methods: Retrospective review of patients with pituitary tumors attending University Hospital Aintree in Liverpool, United Kingdom. There were 34 patients aged 21 or younger at diagnosis.
Results: 34 patients (28 female), mean age at diagnosis 17.8 (range 11-21) years, mean current age 31.9 (17-47) years. There were 22 prolactinomas (9 macroprolactinomas), 5 non-functioning adenomas (1 macroadenoma), 4 cases of Cushing's disease, 1 acromegaly, 1 suprasellar cyst and 1 pituitary cyst. Of the 22 prolactinomas (21 female, mean age at diagnosis 18.4 years, mean prolactin at diagnosis 14,731 (1,207-70,000) mU/l), all received dopamine agonists (DA) and only 2 underwent surgery. Two patients had MEN 1 syndrome. Mean current age of this group is 31.4 (17-45) years and mean prolactin on DA is 1,014 (2-5,527) mU/l. For 20 patients with prolactinomas details of their presenting complaints were available. Only 1 patient presented with short stature and she also had delayed puberty. Two were obese at diagnosis and 7 had galactorrhoea. 15 had menstrual abnormalities (2 oligomenorrhoea, 4 primary and 9 secondary amenorrhoea). 2 patients presented with headaches; both had additional symptoms present and only 1 had documented visual field deficits at diagnosis. Currently 3 patients are receiving hydrocortisone, 1 thyroxine, 6 sex steroid hormone replacement and none are receiving growth hormone [although 3 have growth hormone deficiency (GHD)]. Four patients with Cushing's disease were treated with transsphenoidal surgery, and two of these relapsed and required further surgery. Of the 34 patients, 6 had panhypopituitarism, 18 weight gain/obesity, 3 infertility and 9 had documented GHD. Eight patients had treated dyslipidemia and one had hypertension.
Conclusions: This is one of the largest reviews of patients aged 21 or younger when diagnosed with a pituitary tumor, who have been followed up at a single centre. Two thirds of cases were prolactinomas and almost all cases occurred in females. The majority were treated with DA and only two required surgery. Growth problems in these patients are very unusual. Patients with Cushing's disease may relapse after TSS. Non-functioning pituitary adenomas and acromegaly are uncommon. Weight gain and obesity are frequent sequelae of these tumors and their treatment.

PO3-179 HPG Axis

Hypothalamic-pituitary lesions: clinical and endocrine presentation of 198 cases

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By their localization, hypothalamic-pituitary (HP) lesions can disturb endocrine function. Whereas neurological and visual complaints are usually the initial symptoms, abnormal evolution of body weight, statural growth rate, puberty, and diabetes insipidus (DI) may also be revealing of an HP lesion.
Objective: We analyzed the presentation of HP lesions in a large single center population in order to 1) determine if initial symptoms could predict the type of HP lesion; 2) shorten the interval between initial symptoms and diagnosis to avoid emergency surgery and severe neurological or visual sequelae.
Patients: Data of 198 (89 boys) consecutive patients monitored for an HP lesion were analyzed.
Results:

Revealing Symptoms							
HP lesion	Age Dg	N	Neuro	Visual	Endocrine	Other	Dg delay
	yrs [range]						yrs [range]
Cranio	7 [1-15.6]	56	47 (84%)	27 (48%)	13 (23%)	7 (12%)	0.5 [0-11]
OP Glioma	3.7 [0.2-13]	54	12 (22%)	31 (57%)	16 (29%)	0	0.1 [0-2.3]
SSAC	1.9 [0.5-18.2]	25	18 (72%)	9 (36%)	3 (12%)	17 (68%)	ND
Hamar	2.5 [0.3-13.3]	22	4 (18%)	0	17 (77%)	1 (4%)	0.1 [0-3.3]
GCT	10.5 [5.1-15.3]	12	0	0	12 (100%)	0	0.7 [0.2-3.2]
Astro	4.5 [1.5-13.7]	7	6 (83%)	1 (14%)	0	0	0.2 [0.1-2]
Rathk	8.5 [2.5-13.8]	7	0	0	7 (100%)	0	1.5 [0.2-4]
Other	7.7 [0.1-15.7]	15	8 (53%)	2 (13%)	5 (33%)	2 (13%)	0.4 [0.1-5]
TOTAL		198	95 (48%)	70 (35%)	73 (37%)	27 (13.5%)	

Astro: astrocytoma; Cranio: craniopharyngioma; Dg: diagnosis; GCT: germ cell tumor; Hamar: hamartoma; OP Glioma: optic pathway glioma; Rathk: Rathke cyst; SSAC: suprasellar arachnoid cyst.

Neurological or visual complaints were the most frequent revealing symptoms. While abnormal BMI or growth rates were revealing symptoms in 3.5% and 7.5% of cases, these were present at diagnosis in 60% and 72.5% of patients respectively.
Median interval between abnormal weight or height progression and diagnosis was 1.5 [0.5-5.5] yrs and 2 [0.3-6] yrs respectively, and was longer than the median diagnostic delay for other revealing symptoms: 0.4 [0-11] yrs ($p<0.001$). At diagnosis, 44% of patients lacked GH, 19% TSH, 17% ACTH and 14% had DI.
Conclusion: Revealing symptoms were suggestive of the type of HP lesion. Also, abnormal weight or height evolution and endocrine disorders were frequent at diagnosis though rarely the presenting symptom: their characterization may help to suggest an HP lesion.

PO3-180 HPG Axis

A novel missense mutation in the first extracellular loop of the neurokinin B receptor causes hypogonadotropic hypogonadism

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The neurokinin B receptor, encoded by *TACR3*, is widely expressed within the CNS, including hypothalamic nuclei involved in regulating GnRH release. We have recently reported two mutations in transmembrane segments of the receptor, and a missense mutation in Neurokinin B, in patients with normosmic isolated hypogonadotropic hypogonadism (nIHH).

Patients and Methods: We sequenced the *TACR3* gene in a family of Kurdish origin in which three siblings had nIHH. The novel mutant receptor thus identified was studied in a heterologous expression system using calcium flux as the functional readout.

Results: All affected siblings were homozygous for the His148Leu mutation, in the first extracellular loop of the neurokinin B receptor. The His148Leu mutant receptor exhibited profoundly impaired signaling in response to NKB (EC50 = 3±0.1 nM and >5 mM for wild type and His148Leu, respectively). The location of the mutation in an extracellular part of the receptor led us also to test whether senktide, a synthetic NKB analogue, may retain ability to stimulate the mutant receptor. However the signaling activity of the His148Leu receptor in response to senktide was also severely impaired (EC50 = 1±1 nM for wild type and no significant response of His148Leu to 10 mM)

Conclusions: Homozygosity for the *TACR3* His148Leu mutation leads to failure of sexual maturation in humans, while signaling by the mutant receptor *in vitro* in response to either NKB or senktide is severely impaired. These observations further strengthen the link between NKB, the NKB receptor, and regulation of human reproductive function.

PO3-181 HPG Axis

Evidence for postnatal increase of Dlk1 expression within the hypothalamus, a gene involved in cell differentiation

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Molecular mechanisms of the pubertal onset remain largely unknown. The couple GPR54/Kisspeptins was recently described as the integrative hypothalamic centre of all factors playing a major role in pubertal reactivation of the gonadotropic axis. Rodent as well as human models have suggested that GPR54 activation at the end of the juvenile period results from a complex maturation of hypothalamic networks. The increase in expression of KiSS1 at this period is considered as an indicator of the hypothalamic maturation. In this study, we aimed to describe genes showing large variations of hypothalamic expression between post natal day 6 (P6), 20 (P20) or 60 (P60) and therefore associated with the post natal maturation of the hypothalamus. We selected genes located within chromosomal regions associated with disorders of the pubertal timing.

The gene encoding for Delta-like protein 1 (Dlk1) is one of these genes as it is located on human chromosome 14 (14q32). Dlk1 is involved in adipogenesis and hematopoietic cells differentiation. A neuroendocrine function has also been proposed but its postnatal hypothalamic expression has never been studied in detail. In order to gain further insight into neuroendocrine function of Dlk1, we first analysed Dlk1 expression in the hypothalamus of adult mice (P103) and then compared its expression by quantitative PCR at P6, P20 and P60 (four mice in each group). Dlk1 is expressed in adult hypothalamus as two isoforms. Immunohistochemistry with an antibody against Dlk1 showed neuronal expression within arcuate nucleus and ventromedial hypothalamus. Quantitative PCR showed two fold significant hypothalamic expression increase of Dlk1 between P6 and 20 without further change between P20 and P60. Dlk1 is not expressed in KiSS1 neurons and is not regulated by sexual hormones. As Dlk1 was also considered as a protein playing a role in obesity, we performed a dual IHC with a NPY antibody. Dlk1 is not expressed in NPY neurons but confocal analysis showed appositions between both neuron types.

This analysis suggests a possible role of Dlk1 in the postnatal neuroendocrine differentiation of some kinds of neurons which may be involved in the hypothalamic network leading to pubertal onset. The biochemical function of Dlk1 needs to be defined to characterize this hypothalamic function.

PO3-182 HPG Axis

Evaluating the value of a single urinary sample for determining gonadotropin by immunochemiluminometric assays after gonadotropin releasing hormone analogue stimulating test

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Objective To investigate the value of a single urinary sample for determining gonadotropin (UGn) by immunochemiluminometric assays (ICMA) after gonadotropin releasing hormone analogue (GnRHa) stimulating test.

Methods Fifty children suffered from disorders of growth or pubertal development were hospitalized for GnRHa stimulating test. Nocturnal spontaneous 12 h (19:00-07:00) urine before the test, diurnal post-stimulating 5 h (-1.5 h before the test to 3.5 h after the test) single urine in all cases, and diurnal spontaneous 5 h (07:00-12:00) urine before the test in 33/50 cases were collected respctively. Urinary luteinizing hormone (ULH) and urinary follicle-stimulating hormone (UFSH) were assayed.

Results (1) Nocturnal spontaneous urine: The correlation between serum peak luteinizing hormone (PLH) and spontaneous ULH (SULH) contents was 0.631, for serum PLH and the ratio of SULH/*S* 0.609, and for serum PLH and the ratio of SULH/Cr 0.589, respectively, ($P = 0.000$ in three). The correlation between the ratio of SULH/spontaneous UFSH (SUFSH) and the ratio of PLH/the serum peak follicle-stimulating hormone (PFSH) was 0.636, ($P = 0.000$). (2) Diurnal spontaneous urine: The correlation between serum PLH and SULH contents was 0.712, for serum PLH and SULH/*S* 0.699, and for serum PLH and SULH/Cr 0.663, respectively, ($P = 0.000$ in three). The correlation between SULH/SUFSH and serum PLH/PFSH was 0.281, ($P = 0.113$). (3) Diurnal post-stimulating single urine: The correlation between serum PLH and ULH contents was 0.822, for serum PLH and the ratio of ULH/*S* 0.815, and for serum PLH and the ratio of ULH/Cr 0.828, respectively, ($P = 0.000$ in three). The correlation between the ratio of ULH/UFSH and serum PLH/PFSH was 0.892, ($P = 0.000$). When ULH contents, ULH/*S*, ULH/Cr and ULH/UFSH were respectively no less than 0.286 IU, 0.266 IU/m^2, 310.158 IU/mol and 0.062, and the sensitivities were 89.3%, 89.3%, 82.1% and 89.3% respectively, and the specificities were 95.5%, 95.5%, 95.5% and 86.4%, respectively.

Conclusions The ULH contents or ULH/*S* of a single urinary sample after GnRHa stimulating test by ICMA may well reflect the status of the onset of HPGA in children; and their values are better than nocturnal or diurnal SUGn, and nocturnal SULH is better than diurnal SUGn.

PO3-183 HPG Axis

Childhood combined pituitary hormone deficiency: a retrospective study for 5 years

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Objective: To emphasis the characteristics and the management combined pituitary hormone deficiency (CPHD) in childhood.

Methods: A total of 51 CPHD cases were enrolled. They were 45 males and 6 females with a mean age of 14.73 ± 4.47 years. Their birth history, clinical features, laboratory data and therapy were analyzed.

Results: All 51 cases showed short stature with a median of high SDS of -4.72. Among these patients, 16 cases had history of perinatal or birth abnormalities (13 cases of asphyxia, 3 of premature, 1 of small for gestational age and 1 of intracranial hemorrhage). The most common (35 cases) is GN deficiency except the GH deficiency, following by TSH deficiency in 31, ACTH deficiency in 17 and PRL in 2. Combined GH and GN deficiency were noted in 17 cases, followed by GH, ACTH, TSH and GN deficiency in 9, GH, TSH and GN in 7 cases. Most patients (37 cases had GN and/or ACTH deficiency, only 4 without GN and ACTH deficiency at the diagnosis time, as showed in Table 1. Small pituitary were found in 16 cases (31.37%), empty sella in 4 (5.6%), pituitary stalk interruption syndrome in 1, low-density shadow in MIR in 1, tumors in 2, full pituitary body in 3.

Table 1. The pituitary hormones in combined pituitary hormone deficiency patients

GH	TSH	GN	ACTH	PRL	Number (%)
+		+		(1)*	17 (33.33) †
+	+	+	+		9 (17.65)
+	+	?	+		4 (7.84)
+	+	+			7 (13.73)
+		+	+		3 (5.88)‡
+	+		+		1 (1,.96)
+	+	?		(1)*	6 (11.76)
+	+				4 (7.84)
51	31	35	17	2	total

GH, growth hormone; THS, thyroid stimulating hormone; GN, gonadotrophin; ACTH, adrenocorticotropic hormone; PRL, prolactin. *One case with PRL deficiency respectively. † and ‡ with a tumor case, respectively.

Conclusion: CPHD is a common endocrinological disease in child. In children with one pituitary hormone deficiency, other pituitary hormone should be investigated. Genetic disorder, especially Prop1 mutation, may be responsible for the main cause of CPHD.

PO3-184 Obesity, Fat III

Trace elements in obese children

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Diet quality in obese children is poor. Hyperalimentation and feeding habits may alter micronutrient status in obese patient. In this study we investigated 18 trace elements; selenium (Se), zinc (Zn), vanadium (V), molybdenum (Mo), iron (Fe), Copper (Cu), beryllium (Be), boron (B), chromium (Cr), manganese (Mn), Cobalt (Co), silver (Ag), barium (Ba), aluminum (Al), nickel (Ni), cadmium (Cd), mercury (Hg), lead (Pb). status in obese children.

Trace elements levels in obese and control groups

	Obese	Control	p		Obese	Control	p
Hg (µg/L)	0,09 ±0,55	0,12 ±0,66	0,299	Mn (µg/L)	0,72 ±1,51	0,55 ±0,41	0,530
Pb(µg/dl)	4,44 ±7,87	4,19 ±7,37	0,898	Fe(µg/dl)	41,50 ±16,74	49,41 ±24,75	0,131
Ba(µg/L)	3,32 ±5,18	2,21 ±0,85	0,242	Ni(µg/L)	0,78 ±0,68	1,17 ±0,65	0,051
Al(µg/L)	10,08 ±12,48	9,72 ±11,16	0,904	Co (µg/L)	0,14 ±0,13	0,24 ±0,15	0,011
Ag (µg/L)	0,77 ±0,09	0,85± 0,08	0,718	Cu (µg/L)	987,11± 183,83	962,01 ±248,59	0,641
Be(µg/L)	0,067 ±0,09	0,086 ±0,12	0,487	Zn(µg/dL)	67,45 ±18,42	74,45 ±18,42	0,186
B(µg/L)	1,14 ±2,09	1,36 ±1,72	0,640	Se(µg/L)	111,01± 38,24	122,10 ±36,67	0,234
V(µg/L)	0,244± 0,0179	0,261 ±0,012	<0,001	Mo (µg/L)	0,68± 0,38	0,73 ±0,23	0,549
Cr(µg/L)	13,59 ±2,47	13,84 ±1,61	0,624	Cd (µg/L)	0,041± 0,050	0,039 ±0,023	0,831

Values are given as mean±SD

Thirty four obese and thirty three healthy control subjects were enrolled to the study. Serum V, Ni, Co levels were lower than those of the control group. In conclusion this study has demonstrated that there are alterations in some trace elements status in obese children.

PO3-185 Obesity, Fat III

LDL particle size in obese children

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Low density lipoprotein (LDL) has different subtypes. Small dense LDL (sdLDL) (LDL diameter<25,5 nm) is one of these subtypes. After adolescence, prevalence of sdLDL increases with some genetic and environmental effects. In adults it was shown that obesity and insulin resistance affect LDL particle size and sdLDL is associated with cardiovascular diseases in adults. In this study we investigated the effect of obesity and insulin resistance on LDL particle size in Turkish children. Twenty seven obese children (14 girls, 13 boys) mean age 10,31±2,76 and 26 healthy control subjects (16 girls, 10 boys) mean age 10,59±3,03 were enrolled to the study. Ratio of insulin resistance in obese group was 55% (15/27). In control group there was not any insulin resistant subject. Serum triglycerides, VLDL levels were higher and serum HDL level was lower in obese patients than controls. The size of LDL particle was not a statistically different between two groups (26,50±0,62 vs 26,63±0,79 nm (p=0,575)). The size of LDL particle was not correlated with BMI sds, HOMA-IR and serum lipids. In conclusion obese children should be evaluated for dyslipdemia to prevent cardiovascular diseases. But LDL particle size measurement seems not to be necessary in childhood obesity.

PO3-186 Obesity, Fat III

Reversibility analysis of cardiovascular risk factors after a nutritional-hygienic intervention in obese adolescents

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Introduction

The relationship between obesity and cardiovascular risk is already evident in adolescence with metabolic and vascular changes. Some studies have tried to prove that the treatment of obesity in adolescence may modify cardiovascular risk factors and the intima-media thickness (IMT) of the carotid artery.

Objective

To determine whether a nutritional-hygienic intervention can modify cardiovascular risk factors and carotid IMT of obese adolescents.

Material and Methods

Design: Prospective cohort study that examines changes in anthropometry, cardiovascular resistance, lipid profile, insulin and IMT carotid resistance, after a nutritional-hygienic intervention, based on targets set over one year, structured in monthly visits.

Subjects: 146 adolescents aged 9 to 14 years (111 obese and 35 with normal weight)

Results

The two groups were similar in age, sex and maturation, but there were differences in adiposity and related variables. Following the intervention, the obese group showed a good response in respect to BMI in 75% of subjects compared with the control group of non-obese.

Both sexes significantly decreased adiposity using the skin fold evaluation, and showed an improvement in cardiovascular resistance (Astrand test).

Males significantly decreased cholesterol, LDL, platelets and IMT in the internal carotid artery and showed a lower increase in systolic blood pressure, CRP and the number of granulocytes compared with the controls.

Females showed no significant changes in the carotid IMT but a lower increase

in triglycerides and CRP compared with the controls.

Conclusions

75% of obese patients responded well to intervention.

In addition to the adiposity and BMI measures, the intervention achieved changes in cardiovascular resistance, some indicators of inflammation, lipid profile, blood pressure and carotid intima-media thickness.

PO3-187 Obesity, Fat III

Serum levels of FGF21 are reduced and negatively correlated with adiponectin in children with Prader-Willi syndrome

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FGF21 is a novel metabolic regulator that has beneficial effects on glucose homeostasis and insulin sensitivity. FA1 (fetal antigen 1) is another molecule to be involved in fat mass regulation and obesity. In human obesity, serum FGF21 and FA1 levels were increased.

Objective: To compare fasting serum levels of FGF21 and FA1 in Prader-Willi syndrome (PWS) and obese control children, and to correlate these levels with insulin sensitivity and obesity-related parameters.

Patients: Sixteen children with PWS were matched with 16 control subjects for age, sex and BMI.

Methods: Oral glucose tolerance tests (OGTTs) were performed and serum insulin and glucose levels measured in both groups. FGF21, adiponectin, and FA1 levels were measured in OGTT samples at 0 min.

Results: Waist to hip ratio and HOMA-IR were lower in PWS individuals relative to control subjects. Remarkably, serum levels of FGF21 were lower and adiponectin were higher in PWS subjects than in control subjects. FGF21 levels were positively correlated with HOMA-IR and negatively correlated with adiponectin. There was no difference in FA1 levels in both groups and no correlation between FA1 levels and other metabolic parameters.

Conclusion: Previously, FGF21 and FA1 levels were reported to increase with obesity. However, compared with obese controls, our results show PWS individuals have lower FGF21 levels and similar FA1 levels. Our data suggest that insulin-sensitivity, lower waist to hip ratio, lower FGF21 levels and higher adiponectin levels characterize the metabolic profiles of PWS children.

PO3-188 Obesity, Fat III

Precocious abnormal glucose profile in obesity among children and adolescents detected by continous glucose monitoring

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INTRODUCTION: Type 2 diabetes (DM2) in children, associated to obesity, is increasing. Precocious detection is necessary from prediabetic state to establish therapeutic corrections.

OBJECTIVE:

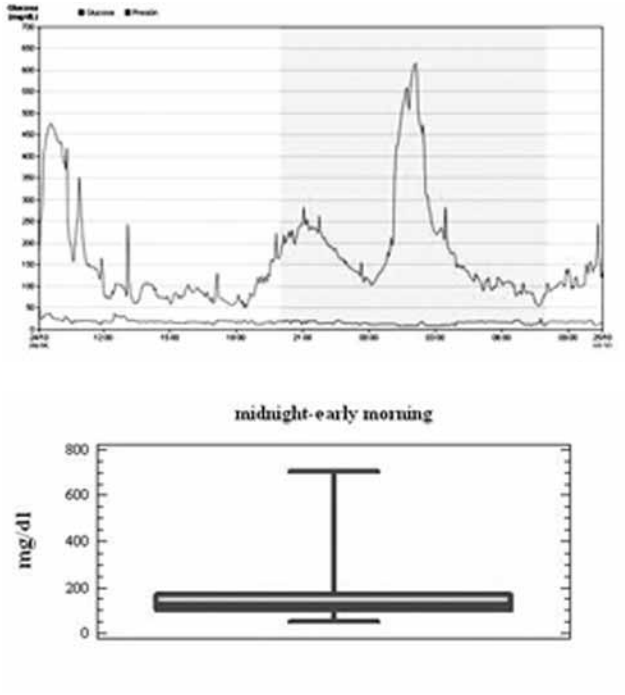
Compare the use of the oral glucose loading test (GLT) with the continuous glucose monitoring (CGM) in the detection of hyperglycaemia.

METHODOLOGY:

A group of 15 adolescents, aged between 9 - 16.6 yrs with a BMI above +4 sd for chronological age, were submitted to a GLT. After that, a Glucoday^{R} sensor (Menarini Diagnostics) for 48 hours was installed by means of a catheter in the abdominal wall. Considered time periods were: 07.00 h (prebreakfast); 00.00 h-06.30 h (midnight-early morning) and 07.30-00.00 h.

RESULTS:

The BMI was 23.5-42.3 kg/m^2 whereas HOMA-IR ranged 3.0-12.4. After GLT, one case was diagnosed by DM2 and for the others no intolerance to carbohydrates was detected. CGM profiles show a postprandial hyperglycaemia during the day but it's statistically more important between midnight-early in the morning (p=0,001).



CONCLUSION:

In our patients, GLT is useful to detect the insulin-resistant situation but not the hyperglycaemia. However, CGM with Glucoday^{R} allows to detect the hyperglycaemia, before being altered in fasting, during the midnight-early morning period, and so to personalize the treatment.

PO3-189 Obesity, Fat III

Microalbuminuria in obese adolescents

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The high prevalence of the obesity in adolescent's population increases the risk of renal injury in this age. The aim of this research was to know the frequency of microalbuminuria (MA) in our obese adolescents patients and its relation with some clinical and biochemical studies that could be a risk factors for renal damage. We studied 57 obese adolescents, of both gender, aging 10 to 15 years. Two samples for MA were collected and drawn blood fasting sample for glycaemia, insulin, total cholesterol and tryglicerides. The MA frequency in our patients was more than 50%., mainly in the group of 13 to 15 years with no gender differences. Although 11 (61, 1%) of 18 patients with high blood pressure had positive MA positive, there were no statistics significant.The risk factors for MA were the presence of dyslipemia and insulin resistance. The intensity of the obesity and lasting over 5 years were no significative for the MA. We also observed that more than 50% of the patient with metabolic syndrome had MA. We point out the importance of the MA study in obese adolescents.

PO3-190 Obesity, Fat III

Biological effects of ghrelin in human preadipocytes and adipocytes

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Objectives:
The hormone ghrelin is the endogenous agonist of the growth hormone secretagogue receptor GHS-R1(a). Ghrelin stimulates growth hormone release and appetite via the hypothalamus. However, little is known about the action of ghrelin in peripheral tissues. Here, we sought to identify possible effects of ghrelin on human preadipocytes and adipocyte biology.
Methods:
Human SGBS preadipocytes and adipocytes were treated with octanoyl-ghrelin. Proliferation, adipogenic differentiation, de novo lipogenesis, and secretory functions were studied.
Results:
GHS-R1a and GHS-R1b as well as their endogenous ligand, ghrelin, mRNA were expressed in human SGBS preadipocytes and adipocytes. Treatment with ghrelin induced preadipocyte proliferation (EC50 ~ 1.2x10-7 M) but did not alter adipogenic differentiation of these cells. Insulin-stimulated lipogenesis was inhibited by ghrelin (IC50 ~ 1.2x10-8 M), basal lipogenesis however was stimulated (EC50 ~1.7x10-8 M). Moreover, exposure of SGBS preadipocytes to ghrelin induced IL-6 and IL-8 mRNA expression suggesting proinflammatory effects of ghrelin in adipose tissue.
Conclusions:
These findings suggest that the direct effects of ghrelin on human adipocytes may play a role in regulating fat cell number and function, and contribute to the pro-inflammatory milieu in adipose tissue of obese patients.

PO3-191 Obesity, Fat III

Study of inflammations' markers and insulin resistance in obese children and adolescents with and without non-alcoholic fatty liver disease

Barbara Predieri[1]; Francesca Lami[1]; Patrizia Bruzzi[1]; Giulia Vellani[1]; Lucia Miglioli[2]; Stefano Bellentani[2]; Fiorella Balli[1]; Amalia Gastaldelli[3]; Lorenzo Iughetti[1]

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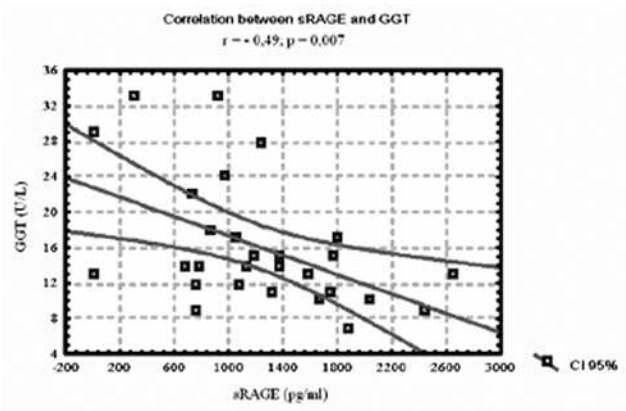
Non-alcoholic fatty liver disease (NAFLD) is a serious problem in childhood obesity; hyperglycemia and oxidant stress lead to the accumulation of advanced glycation end products (AGEs) that with their soluble forms of receptor (sRAGE) could play a role in the pathogenesis of insulin resistance (IR) and the development of NAFLD.
This study was planned to investigate the relationship between the presence of steatosis with anthropometric measurements, abnormal ALT and γ-glutamyl-transferase (GGT), hyperlipidemia, IR (HOMA-IR), and sRAGE in childhood obesity with and without NAFLD.
28 obese children and adolescents (20 M and 8 F; 11.9±2.77 yr.; height SDS 0.55±1.52; BMI SDS 3.11±0.55) were included in the study and were divided in Group 1 (14 with NAFLD) and Group 2 (14 no-NAFLD). sRAGE levels were determined using an ELISA kit.
Group 1 had significantly higher levels of tryglicerides and GGT and significantly lower concentrations of sRAGE than Group 2.

Results			
Parameters/Patients	Group 1	Group 2	p
Age (yr.)	11,9±3,05	11,9±2,59	0,998
BMI (SDS)	3,09±0,72	3,14±0,36	0,833
Height (SDS)	0,42±1,60	0,69±1,49	0,650
AST (U/L)	28,2±12,1	23,9±5,23	0,235
ALT (U/L)	40,4±43,3	26,1±17,4	0,264
AST/ALT ratio	0,91±0,30	1,07±0,32	0,173
GGT (U/L)	19,0±8,38	13,2±4,25	0,031
HOMA-IR	4,63±2,20	3,34±1,31	0,071
TC (mg/dl)	156,3±25,2	144,4±26,5	0,487
HDL-C (mg/dl)	50,4±20,8	49,7±10,7	0,910
LDL-C (mg/dl)	89,1±23,4	82,7±24,7	0,235
Tryglicerides (mg/dl)	101,0±42,5	69,8±30,2	0,034
sRAGE (pg/ml)	883,7±388,4	1553,9±685,1	0,004

No other differences were found. A significant negative correlation was found between sRAGE and GGT (r=-0.49; p=0.00) but not with ALT and HOMA-IR.



Our data demonstate that obese children with NAFLD had higher GGT and lower sRAGE levels than their lean obese counterpart. GGT may be considered as primary marker of liver injury and as a surrogate to suspect fatty liver. The AGEs-RAGE system could play a role in the pathogenesis of NAFLD in childhood obesity.

PO3-192 Obesity, Fat III

High γ-glutamyl-transferase fractions as new markers to identify non-alcoholic fatty liver disease in childhood obesity

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Advanced glycation end products (AGEs) and soluble receptor (sRAGE) could play a role in the development of non-alcoholic fatty liver disease (NAFLD). Normal γ-glutamyl-transferase (GGT) values has been associated with atherosclerosis and predicts the onset of related diseases.
Aim of this study was to determine GGT fractions in obese subjects with and without NAFLD and high-normal range GGT. We try to indentify relationships between NAFLD and ALT, GGT, GGT fractions, hyperlipidemia, and sRAGE.
28 obese children and adolescents (20 M and 8 F; 11.9±2.77 years; height SDS 0.55±1.52; BMI SDS 3.11±0.55) were recruited and divided in Group 1 (14 NAFLD) and Group 2 (14 no-NAFLD). Separation of GGT fractions was performed using liquid chromatography. sRAGE levels were determined using ELISA kit.
Tryglicerides (TG), GGT, big-GGT (b-GGT), and free-GGT (f-GGT) values were significantly higher in Group 1 than in Group 2, while sRAGE levels were lower in Group 1.

Results			
Parameters/Patients	Group 1	Group 2	p
Age (yr.)	11,9±3,05	11,9±2,59	0,998
BMI (SDS)	3,09±0,72	3,14±0,36	0,833
Height (SDS)	0,42±1,60	0,69±1,49	0,650
AST (U/L)	28,2±12,1	23,9±5,23	0,235
ALT (U/L)	40,4±43,3	26,1±17,4	0,264
GGT (U/L)	19,0±8,38	13,2±4,25	0,031
b-GGT (U/L)	1,61±0,85	0,89±0,35	0,007
m-GGT (U/L)	0,36±0,42	0,16±0,23	0,146
s-GGT (U/L)	6,12±4,24	4,02±2,89	0,137
f-GGT (U/L)	10,9±3,77	8,19±1,89	0,023
TC (mg/dl)	156,3±25,2	144,4±26,5	0,487
HDL-C (mg/dl)	50,4±20,8	49,7±10,7	0,910
LDL-C (mg/dl)	89,1±23,4	82,7±24,7	0,235
TG (mg/dl)	101,0±42,5	69,8±30,2	0,034
sRAGE (pg/ml)	883,7±388,4	1553,9±685,1	0,004

A significant negative correlation was demonstrated between sRAGE and GGT (r=-0.49), b-GGT (r=-0.39), small-GGT (s-GGT) (r=-0.47), f-GGT (r=-0.44). GGT and s-GGT were positively associated with total cholesterol (r=0.44) and TG (r=0.42). In Group 1 we found a relative increase in s-GGT and b-GGT fractions; sRAGE significantly correlates only with s-GGT (r=-0.62).
We demonstate that specific GGT fraction profile could be used to identify obese children with NAFLD. It is conceivable that s-GGT may be considered as primary marker of liver injury and a surrogate for suspected fatty liver. AGEs-RAGE system could play a role in the pathogenesis of NAFLD in childhood obesity.

PO3-193 Obesity, Fat III

Does weight affect pulmonary function in healthy adolescents?

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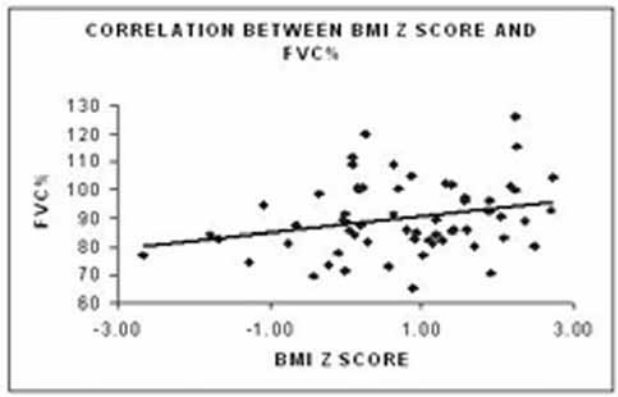
[1]Pediatrics, Infants and Childrens Hospital at Maimonides, Brooklyn, NY, United States; [2]Kids Weight Down Program, Maimonides Medical Center, Brooklyn, NY, United States; [3]Pediatrics, New York Presbyterian Medical Center, New York, NY, United States

INTRODUCTION: Studies have shown that pulmonary function is adversely affected in extremes of BMI. Pulmonary function and airway hyper-responsiveness has been shown to worsen with increasing BMI.
AIM: To measure pulmonary function in healthy early adolescent school children and explore its relation to obesity.
METHODS: A cross-sectional study involving 6th to 8th graders in an urban public school in Brooklyn New York. Anthropometric measurements and lung function studies were done in January 2007. Best of three attempts was registered for lung function.
RESULTS: A total of 98 children (58 girls; 40 boys) participated in the study. Mean age was 12.3 ± 0.9 years, mean BMI was 22.1 ± 5.1 kg/m2. FEV% and FVC % were significantly higher in boys than in girls. Interestingly, FVC% positively correlated with BMI Z score. When analyzing girls and boys separately, similar correlations were found among girls but not among boys. When comparing normal, overweight and obese children (divided by BMI Z score), the FVC% showed tendency to increase with increasing adiposity. This reached significance among girls. No correlation was observed between any of the pulmonary function parameters and waist circumference or body fat percentage,
DISCUSSION: As mentioned in the literature, pulmonary function parameters were higher in boys than girls. However, contrary to other reports, our cohort shows a positive correlation between BMI Z score and FVC%, especially in girls. Further studies are required to explore this in depth.



PO3-194 Obesity, Fat III

Prevalence of vitamin D deficiency in lean as compared to obese children

Radhika Purushothaman[1]; Amrit Bhangoo[1]; Sunil Sinha[1]; Debbie Perez[2]; Kate Pavlovich[3]; Michael Rosenbaum[3]; Deborah DeSantis[2]; Lisa Altshuler[2]; Steven Shelov[1]; Svetlana Ten[1]

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Serum 25-hydroxy vitamin D levels have been found to be inversely correlated to BMI, body fat content and directly with hypertension and degree of insulin resistance.
Objective: Cross-sectional study looking at prevalence of vitamin D deficiency in lean and obese children.
Design/Methods: This was a cross-sectional study involving 3 groups:
1. Group 1: Obese children referred for obesity to our pediatric endocrine clinic.
2. Group 2: Lean children who participated in a school-based intervention study.
3. Group 3: Obese children who participated in a school-based intervention study.

Obesity was defined as BMI above 95th % for age.
Group 1 had baseline biochemical evaluation including vitamin D (25OHD) levels and received diet and lifestyle education as per routine in the clinic. Groups 2 and 3 were middle school children who were part of a multi-center school based intervention study. They had baseline blood work including IVGTTs done after which they underwent 12 weeks of intensive diet and exercise regimen. Blood-work was repeated at the end of 12 weeks. Vitamin D level was measured at this instance. Vitamin D insufficiency was characterized by 25 OHD levels <=20ng/ml and severe insufficiency: <=10 ng/ml.
Results:
Group 1 showed that 55.2% were vitamin D insufficient with 21.6% showing severe insufficiency. The vitamin D insufficient group had significantly higher BMI and systolic pressure and significantly lower HDL and QUICKI when compared to the vitamin D sufficient group.

Comparison of Baseline Characteristics between the 3 groups			
	Group 1 (Obese Children from clinic)	Group 2 (Lean children from school)	Group 3 (Obese children from school)
Number	217 (F:118)	55 (F: 31)	20 (F: 12)
Age	12.9 (5.5)	13.2 (0.8)	13.5 (1)
25-hydroxy Vitamin D <10ng/ml	21.6%	0%	0%
25-hydroxy Vitamin D 10-20 ng/ml	33.6 %	29%	50%
25-hydroxy Vitamin D >20ng/ml	44.8%	70%	50%

Groups 2 and 3 combined showed 22% prevalence of vitamin D insufficiency but no child had severe vitamin D insufficiency.

Conclusion:

Prevalence of vitamin D insufficiency is 26% in lean children which is in sharp contrast to 55.2 % in obese children. Severe vitamin D deficiency was not present in any lean child. This level correlates with other metabolic parameters in obese children but not in lean children. Vitamin D can thus be considered a marker of poor metabolic function.

PO3-195 Obesity, Fat III

Correlation of hyperinsulinemia and waist circumference in childhood metabolic syndrome

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BACK GROUND:

Metabolic syndrome (MS) also known as insulin resistance syndrome is characterized by clustering of central obesity, hypertension, impaired glucose metabolism and dyslipidemia. Central obesity and hyperinsulinemia are amongst the major components of MS.

The objective of our study is to assess the serum insulin levels in obese children and to see the correlation of insulin levels and waist circumference with other metabolic indicators .We also assessed the prevalence of metabolic syndrome in these obese children using two Pediatric definitions.

Patients and Methods:

A total of 262 obese children aged 4 to 16 years, with a BMI greater than 95th percentile attending the endocrine clinic of National Institute of Child Health, Karachi, from November 2005 till May 2008 were included. Children having obesity due to syndromes, medications causing weight gain, chronic illness and developmental disability were excluded. Blood pressure, waist circumference, fasting triglycerides, HDL, insulin and glucose levels were obtained. Obesity was defined as BMI >95th percentile for age and sex according to the U.K. growth reference charts.The prevalence of metabolic syndrome was estimated using two pediatric definitions.

Results:

In our study group of 262 children there was a male preponderance with 158 male and 104 female children. The prevalence of MS varied significantly between 14% and 52% depending on whether insulin levels were included in the definition. There was a significant positive correlation(r) when the metabolic parameters were correlated with waist circumference and insulin levels, except HDL which was negatively correlated. All the metabolic parameters like waist circumference, triglycerides, high density lipoprotein cholesterol and systolic blood pressure increased considerably across the insulin quartile ($p<0.05$). The most noteworthy anthropometric and metabolic abnormality were the waist circumference (46.5%) and insulin levels (58%) respectively.

Conclusion:

This study has emphasized the central key role of waist circumference and hyperinsulinemia in MS in children. There is definitely a need for establishing normal insulin ranges according to age, sex and pubertal status.

PO3-196 Obesity, Fat III

Evaluation of homeostasis model assessment indices in children and adolescents

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Insulin resistance increases pancreatic beta cell function, that later in life, may be followed by type 2 diabetes mellitus. The aim of study was evaluation of homeostasis model assessment index for insulin resistance (HOMA-IR) [fasting insulin (μU/mL) X fasting glucose (mmol/L)/22.5] in normal weight healthy children and its prediction value for increasing beta cell function in overweight children. For this purpose 528 cases (213 males, 315 females; age 4.5 – 18, (mean ± SD) (10.6±2.9) yr) were enrolled into the study. Their BMI was compared to BMI tables and curves of CDC 2000 according to age and gender and BMI-SDS was calculated. Normal weight was considered if BMI was > 5% & < 85%; at risk for overweight, ≥ 85% & <95%; overweight, ≥ 95%. After overnight fast, serum insulin (IRMA) and glucose (glucose oxidase) were measured. Biochemical profile and hormonal evaluation were done for ruling out any disease. HOMA index for beta cell function (HOMA-B) [20 X fasting insulin(μU/mL)/fasting glucose(mmol/mL)-3.5] was determined in normal children with BMI < 85%, and was changed to nominal variable by giving score "1" to the values more than mean + 2SD. It was used for evaluation of HOMA-IR by ROC curve in children with BMI>2SDS. Data were analyzed by SPSS 14. The distribution of these indices was not normal, so Mann-Whitney U test was used for comparing the mean values in different groups. **Results:** HOMA-B and HOMA-IR values in study subjects with different weight are illustrated in table 1.

Table 1, HOMA indices in different weight groups			
weight group	N	HOMA-B	HOMA-IR
normal weight	152	86.8 ± 50.6	1.8 ± 1.2
at risk for overweight	108	146.4 ± 91	2.7 ± 1.7
overweight	268	193.6 ± 140	3.8 ± 2.6
BMI > 2SDS	209	208.25 ± 152	4.1 ± 2.7

These indices were significantly higher in the groups with higher weight ($P\geq0.001$). Area under the curve for HOMA-IR is 0.901, and if this index is 3.6, its sensitivity and specificity for increasing beta cell function will be 79% and 80% respectively, and if it is 4.2, sensitivity and specificity are 74% and 91% respectively.

Conclusion: Weight gain increases insulin resistance and HOMA-IR nearly predict the increased function of beta cells.

PO3-197 Obesity, Fat III

Onset of puberty and deterioration of cardiovascular risk factors: a one-year follow-up study of untreated obese children

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Objective: The course of obesity associated non-alcoholic fatty liver disease (NAFLD) and the cardiovascular risk factors hypertension, dyslipidemia, and disturbed glucose metabolism is largely unknown in untreated obese children.

Methods: We examined prospectively 287 untreated obese children (53% female, mean age 11.4y, mean BMI 28.2 kg/m²) at baseline and 1y later in respect to insulin resistance index HOMA and prevalence of hypertension, dyslipidemia, impaired fasting glucose, and NAFLD defined by ultrasound.

Results: At baseline, 21% of the obese children suffered from hypertension, 22% from dyslipidemia, 5% from impaired fasting glucose, and 29% from NAFLD. These prevalences and weight status remained stable at the 1y follow-up. Stratification by pubertal stage and its change after 1y yielded a significant ($p<0.05$) increase of hypertension (+15%), dyslipidemia (+11%), impaired fasting glucose (+8%), and HOMA values (+0.42) in 62 children entering puberty, while their weight status as SDS-BMI remained stable. A significant ($p<0.05$) decrease of HOMA values (-0.99) and hypertension (-18%), dyslipidemia (-10%), impaired fasting glucose (-6%) as well as NAFLD prevalence (-17%) was observed in 50 children entering late puberty, while their weight status as

SDS-BMI remained stable. Changes in HOMA were only weakly related to changes of cardiovascular risk factors or transaminases (all r<0.2).
Conclusions: Cardiovascular risk factors deteriorated at onset of puberty and improved in late puberty in obese children without change of their weight status. The weak correlation between HOMA and cardiovascular risk factors suggests other factors affecting cardiovascular risk factors.

PO3-198 Obesity, Fat III

The leptin receptor gene polymorphism rs1137101 is associated with worst lipid profile in morbidly obese Brazilian children

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BACKGROUND: Leptin, an important signal for the regulation of body weight, exerts its effects on many tissues, including the hypothalamus, binding to the leptin receptor, a member of the gp130 family of cytokine receptors. The *LEPR* has mapped to 1p31.3 and many variations in the DNA sequence were identified, including the SNP rs1137101 (Gln to Arg in codon 223). A relationship of this variation was observed in obese subjects, where Arg223 homozygote showed lower blood pressure than Gln homozygote. In a Brazilian adult sample this variation was related to increased weight. The same is true for a high risk of lower high-density lipoprotein cholesterol (HDL-c) in the case of familial combined hyperlipidemia.
OBJECTIVES: To investigate the association between the rs1137101 variants in the LEPR gene with BMI, blood pressure, serum leptin and lipid levels in a cohort of morbidly obese Brazilian children screened for *LEPR* mutations.
METHODS: We analyzed a sample of seventeen obese children (5M, 12F) aged 10.1 ± 2.7 years (range 2.5-15.1) with a body mass index (BMI) z-score >2.5 (3.0 ± 0.6, range 2.7-5.5). All exons of *LEPR* were amplified by PCR and submitted to automatic sequencing. The variation rs1137101 found was correlated with BMI z-score, serum leptin levels, lipid levels and blood pressure (BP). Data was submitted to statistical analysis using chi-square test and ANOVA to determine associations between categorical and continuous variables, respectively.
RESULTS: Mean triglyceride levels were 100.1 ± 42.6 mg/dl, mean HDL cholesterol levels were 39.0 ± 8.3 mg/dl, mean leptin levels were 95 ± 85.8 ng/ml, and mean systolic and diastolic BP percentile were 75 ± 25.9 and 77 ± 9.6 respectively. Children carrying the Arg223 in homozygous state (n=4) had higher levels of triglycerides (p=0.039) and a higher ratio of triglycerides to HDL-c (Tg/HDL-c) (p=0.004) than children carrying the Gln223 either in homozygous (n=4) or heterozygous state (n=9). There were no significant differences between them concerning BMI z-score, serum leptin, total cholesterol, LDL cholesterol levels and blood pressure (p>0.05).
CONCLUSION: The presence of rs1137101 polymorphism within *LEPR* encountered in our sample of morbidly obese Brazilian children is associated with a worst lipid profile verified by the higher levels of triglycerides and Tg/HDL-c ratio.

PO3-199 Obesity, Fat III

Leptin and adiponectin are independent predictors of bone mineral density in prepubertal girls

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Aims: Body weight is known to be positively associated with bone mineral density, but as it has been debated whether lean mass or fat mass has a greater stimulatory effect on bone, the relationship between obesity and bone mineral density (BMD) is unclear. Some adipokines, including leptin and adiponectin, appear to participate in bone metabolism and are potential independent contributors to bone mineral density. **Methods:** Study participants included 48 prepubertal girls who were classified into obese (n=23, BMI ≥ 85th percentiles) and control groups (n=25, 25th percentiles ≤ BMI < 75th percentiles) by body mass index (BMI). Serum leptin and adiponectin levels were determined by enzyme immunoassay. Bone mineral density was measured using dual energy X-ray absorptiometry and body composition was measured using bioelectrical impedance analysis.
Results:

Table 1. Correlation of metabolic parameters and body composition with bone mineral density

	BMD_{femur}		$BMD_{L-spine}$	
	Unadjusted	Body weight adjusted	Unadjusted	Body weight adjusted
Body weight	0.751*		0.761*	
Leptin	0.659*	0.128	0.481*	0.409*
Adiponectin	-0.181	0.025	-0.006	0.292
HOMA-IR	0.455*	0.080	0.353	-0.084
Fat mass	0.750*	-0.356	0.681*	-0.578*
Lean mass	0.818*	0.319	0.830*	0.490*

Table 2. Serum leptin, adiponectin levels, HOMA-IR, and body composition as independent predictors of bone mineral density

	BMD_{femur}		$BMD_{L-spine}$	
	β	P	β	P
Leptin	0.555	<0.05	-0.024	0.918
Adiponectin	-0.040	0.743	0.042	0.768
HOMA-IR	0.090	0.495	0.034	0.820
Fat mass	-0.593	<0.05	-0.109	0.740
Lean mass	0.979	<0.05	0.907	<0.05

Conclusions: In prepubertal girls, lean mass has a favorable effect on BMD and is a positive independent predictor of femoral and L-spine BMD. Fat mass is a negative independent predictor of femoral BMD and seems not to protect the bone structure against osteoporosis, despite increased mechanical loading. Serum leptin levels can predict femoral BMD independently and may play a biological role in regulating bone metabolism.

PO3-200 Obesity, Fat III

Adipocytokines and insulin resistance in obese children and adolescents

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Background: Insulin resistance (IR) is the main metabolic complication of obesity. Classic cardiovascular risk factors (CRF) are related to IR. Adipose tissue produces adipocytokines that are implicated in glucose metabolism and inflammation.
Objective: To assess the association among classic CRF, adipocytokines and IR in a sample of obese children and adolescents.
Patients and Methods: Cross-sectional study. Forty-two obese (14 female; age: 7-17) and 40 control (16 female; age: 7-17) children and adolescent were studied. Weight, height, body mass index (BMI), blood pressure (BP) and abdominal circumference (AC) were obtained. After a 12 hour fast glucose, lipoproteins, high sensitivity CRP, adiponectin, leptin and insulin were determined. HOMA index was calculated. Statistical analysis: Chi-square and T-tests, Spearman and Pearson correlation coefficients.
Results: Obese subjects showed significantly higher systolic BP, CRP, leptin and insulin values. HOMA was positively correlated with leptin (0.43 p=0.001), BMI (0.54, p<0.001), AC (0.42 p=0.001), triglycerides (0.47 p=0.002) and CRP (0.39 p=0.007) and negatively with adiponectin (-0.28 p<0.05) and HDL cholesterol values (-0.34 p<0.05). Leptin values correlated positively with BMI (0.72 p<0.001), AC (0.57 p<0.001), triglycerides (0.61 p<0.001) and CRP (0.44 p=0.001) and inversely with HDL (-0.40 p=0.001). Sixty percent of obese patients showed HOMA values ≥ 3, and 79% of the patients with HOMA values ≥ 3 were obese (p =0.004). No significant differences were found in adiponectin, leptin, CRP, BP and lipoproteins between obese

participants with HOMA values ≥ 3compared to those with HOMA < 3. **Conclusion**: IR according to HOMA ratio was common in obese children and adolescents. There is a strong relationship between IR and adipocytokines in children and adolescents.

PO3-201 Obesity, Fat III

RAB5 and GLUT-4 expression in primary cultures of subcutaneous abdominal adipose tissue from obese and lean children and adults in association with insulin levels

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Introduction: Obesity is associated with glucose intolerance and Diabetes Mellitus Type II (DM2). Rab5, a GTPase, regulates Glut-4 trafficking. **Aim:** To study the cellular expression and localization of Rab5 and Glut4 in obese and lean children and adults in association with insulin resistance and diabetes. **Methods:** Primary cultures of pre-adipocytes (PA) and mature adipocytes (MA) were developed from routine surgical biopsies of subcutaneous abdominal adipose tissue from 12 obese (OC) (BMI ≥ 95%) and 6 Tanner-matched lean children (CC) (BMI <85%) from 7-16 yrs of age, and 24 morbid obese (BMI >50) adults: 7 with DM2 (MOD), 4 with high insulin levels (HI) (MOH), 6 without DMII or HI (MOW), and 7 lean controls (BMI < 25) (C). The protein expression (P) and localization of Glut4 and Rab5 were studied by immunofluorescence (IF) and western immunoblotting (WI). Serum Insulin was measured by ELISA. **Results: 1) Rab5 P by IF** and perinuclear (Pn) and cytoplasmic (Cy) localization is increased in MOD and MOH vs, MOW, C, and OC reaching similar but slightly lower levels than pubertal CC; P by IF in pubertal OC is similar to MOW. **2) Rab5 P by WI** is higher in CC and OC with normal insulin vs OC with high insulin levels and MOD **3) Rab5 P by WI** in CC vs. C is 2-fold higher in preadipocytes and 10-fold higher in mature adipocytes. **4) Rab5 P by WI** in children is negatively correlated to insulin (r=-0.728, p=0.026) but positively correlated in adults (r=1, p<0.0001). **5) Glut4** Pn and Cy expression in MOD vs. C is lower. **6) Glut4** Pn expression in CC is increased in early puberty but decreases quickly to prepubertal levels whereas Cy expression in CC decreases as puberty progresses**. 7) Glut4** Pn expression in OC vs. CC is higher but decreases as puberty progresses whereas Cy increases with pubertal progression**. Conclusions: 1)** The increased Cy localization of Glut4 in OC as puberty progresses together with the normal Rab5 protein expression in the OC with normal insulin levels may reflect an attempt to maintain Glut4 trafficking as in the pubertal CC which is not achieved by the OC with high insulin because of the decreased RAB5 P expression. **2)** The increased RAB5 in MOD may cause greater endocytosis of GLUT4 possibly causing problems in GLUT4 trafficking reflected by the decreased GLUT4 expression in MOD which may be a pathological factor playing a role in MOD.

PO3-202 Obesity, Fat III

Studies of different rat models of hypothalamic obesity

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Extreme obesity is a major and unsolved problem in patients with craniopharyngioma (CP). The phenotypic spectrum of disturbed weight regulation is broad and depends on the hypothalamic lesion. Decreased physical activity, reduced sympathetic tone, as well as insulin and leptin resistance have been demonstrated in CP patients. The aim of this study was to create a rodent model that best mimics metabolic changes in CP which is essential for the rational design for use of pharmacologic agents. To accomplish this, two different medial hypothalamic lesions in male Sprague-Dawley rats were performed: Arcuate nucleus (ARC) lesions were performed in neonatal rats by sc injection of either a low or high dose monosodium glutamate (MSG), or vehicle; while paraventricular nucleus (PVN) lesions were performed in adult rats by stereotaxic placement of an electrode and passing anodal direct current (no current for sham group). Body compositions were determined by quantitative magnetic resonance imaging, and serum hormone levels by immunoassays.
Ten weeks after MSG treatment, high dose treated animals exhibited lower body weights (low dose -14%, high dose -23%), body lengths (low dose -5%, high dose -9%), and daily food intakes than did controls. Despite this, animals exhibited higher % body fat (high dose 36% body fat vs 18% in controls, p<0.01). In contrast, PVN lesioned animals exhibited a significant increase in daily food intake compared to controls, accompanied by an increased weight (weight lesion group 555±5 vs 514±4 g in ctrl, p=0.01) due to an increase in body fat 5 weeks after treatment. ARC lesioned rats had higher insulin and leptin levels and lower testosterone levels (all p<0.01) but levels of these hormones did not differ significantly in PVN lesioned rats compared to controls. In summary, both models result in adiposity but by different mechanisms. Adiposity in ARC-lesioned animals was associated with decreased food intake, stunted growth likely due to growth hormone deficiency, hypogonadism, hyperleptinemia and hyperinsulinemia, whereas adiposity in the PVN lesion model was associated with increased food intake and weight gain. Both distinct phenotypes are seen among CP patients. Future studies will examine the effect of ventromedial nucleus lesions or combined lesions of different hypothalamic nuclei, creating suitable models for testing pharmaceutical strategies for a spectrum of different hypothalamic obesity disorders.

PO3-203 Obesity, Fat III

Age and treatment duration are important factors in determining success within paediatric tertiary-hospital weight management services

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Introduction
Many paediatric hospitals are now establishing multidisciplinary obesity services with expertise in general paediatrics, endocrinology and nutrition. Significant improvements in age- and gender-adjusted Body Mass Index (BMI-Z) are possible within such services (in the region of 0.3-0.4)[1,2], in contrast to many community-based interventions (generally ≤0.1). We should continue to strive for reductions ≥0.5 in the knowledge that this level of change is required to improve cardiovascular risk[3] and insulin sensitivity[4].
Aims
To evaluate our tertiary hospital-based Weight Management Service (WMS), and examine factors associated with better outcomes.
Methods
WMS audit: Feb 2005–Aug 2008. Patients were seen 3-4 monthly by a general paediatrician or paediatric endocrinologist, and a dietician. Routine management centred upon standard evaluation, lifestyle recommendations, and education regarding potential complications[1]. Overall aims were weight stabilisation in young children and gradual weight loss in adolescents.
Results
458 overweight/obese youth (median [range] age 11.3 [0.3-21.1] years; mean BMI-Z [SD] +2.47 [0.5]; 48% male) attended during the study period. Of 1529 appointments, attendance was 80% (cancellations 7%; failure to attend 13%). In those receiving follow-up for ≥1year (n=106; median [range] duration 20 [12-40] months), mean [SD] change in BMI-Z was -0.26 [0.3] with 81% reducing their BMI-Z and 17% experiencing reductions ≥0.5.
Multivariate linear regression demonstrated greater reductions in BMI-Z with longer treatment (-0.01 BMI-Z for every month of treatment >12 months; p<0.01) and younger age at recruitment (+0.04 BMI-Z for every year of age at recruitment; p<0.001). Boys did better than girls but this was not significant (p=0.1). In children aged <10 years at recruitment and seen for ≥1 year (n=27),

mean [SD] change in BMI-Z was -0.48 [0.5] with 89% reducing their BMI-Z and 30% experiencing reductions ≥0.5.

Discussion

These data indicate that clinically significant improvements in BMI-Z are achievable within hospital-based services, with best results seen in young children who receive a long duration of follow-up. These data support the efficacy of paediatric hospital-based WMS and help in decision-making around patient selection & service provision.

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PO3-204 Obesity, Fat III

Fasting glucose in at risk obese youth: lowering the lower limit?

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In a preliminary report, we found that obese youth with a fasting preserved glucose (FG) of 90-99 mg/dL on oral glucose tolerance test (OGTT) had similar metabolic parameters to those with impaired fasting glucose (FG>100 mg/dL). Other studies have reported that differences in glucose homeostasis vary among ethnic groups. Therefore, the aim of our present study was to expand our initial observation in more patients (pts) and to analyze for potential differences between at risk obese youth, primarily Hispanic (Hisp) and African Americans (AA).

Retrospective chart review was performed analyzing results of OGTTs done in the last 5 years in obese youth with BMI >95% for age and sex. Pts with FG >126 mg/dl, with psychotropic medications, and incomplete data were excluded. FG and total fasting insulin (FI) were obtained after an overnight fast. Glucose and Insulin were obtained 2 hours (hrs) after the ingestion of dextrose 1.75 g/kg (max 75g). Assays were done in one laboratory. Pts (n=132) were grouped by FG (<80, 80-89, 90-99, 100-125 mg/dl). Since there were no significant difference (NSD) between pts with FG <80 mg/dl vs 80-89 mg/dl, these were combined as group (G)1 (<90mg/dL); (G2 =90-99 mg/dL and G3=100-125 mg/dL). An identical analysis was done for 2 subgroups: AA (n=55) and Hisp (n=62). Statistics used: Mann-Whitney U Test and Kruskal-Wallis Test.

In the entire study population, there was NSD in FI, HOMA, and 2 hr glucose (gluc) between G2 and G3, while in G2, there were higher levels of FI, HOMA, and 2 hr gluc compared to G1 ($p<0.05$). In subgroup analysis, for both Hisp and AA pts, there was NSD in FI levels, HOMA score and 2 hr gluc levels between G2 and G3. Furthermore, Hisp pts in G2 had higher FI levels, HOMA and 2 hr gluc levels compared to G1 ($p<0.05$), while AA pts in G2 had only higher 2 hr gluc levels compared to G1 ($p<0.05$). Two hr insulin levels were not statistically significant between any of the above groups.

Our findings with an expanded pt population substantiate our previous findings that youth with a FG of 90-99 mg/dl may be metabolically similar to those with impaired fasting glucose especially for Hisp and AA pts.

PO3-205 Obesity, Fat III

Obesity-induced liver inflammation is associated with impaired IGF-I-production

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Background: serum IGF-I is mainly produced by hepatocytes in response to GH. Obesity is associated with a state of chronic low-grade inflammation. We aimed to study the relationship between increased serum levels of ALT as a parameter of liver inflammation and IGF-I serum levels in obese children.

Methods: the study-group consisted of 155 obese children, 35 (13 female) with ALT levels above the normal range (>39 U/L) **(= group 1)** and 120 (59 female) with normal values **(= group 2).** All patients were investigated by a standardized program. Fat mass was assessed using DXA (LUNAR-DPX NT, GE-medical Corp.). Fasting IGF-I serum levels were measured by RIA, fasting-insulin by a chemiluminescence assay. The values for fat mass (FM%) and IGF-I serum levels are given as height-adjusted SD scores (SDS_{HA}).

Results:

Characteristics of the two patient groups are shown in the table.

	Group 1 (N=35)		Group 2 (N=120)		
Parameter	Mean	SD	Mean	SD	p=
ALT [U/L]	65	28	24	7	<0.001
Age [yrs]	12.8	3.1	11.8	3.0	n.s.
Height [SDS]	1.20	1.26	1.04	1.34	n.s.
BMI [kg/m^2]	32.7	6.0	29.0	5.2	n.s.
FM% $[SDS]_{HA}$	2.42	0.42	2.24	0.59	n.s.
IGF-I $[SDS]_{HA}$	-0.77	1.63	-0.41	1.42	n.s.
Insulin [pmol/L]	209	96	128	90	<0.001

In group 1 Insulin serum levels were increased, IGF-I-SDS_{HA} was decreased and showed a negative correlation with ALT ($R = 0.43$, $p<0.01$).

In group 2, we found normal IGF-I-SDS_{HA} and significantly lower Insulin levels. There was no correlation of IGF-I-SDS_{HA} and ALT.

Conclusion: in obese children with elevated ALT serum levels, liver inflammation is associated with impaired IGF-I-production. It has to be discussed whether beside insulin resistance liver inflammation plays a role in diminished IGF-I production.

PO3-206 Obesity, Fat III

Components of the metabolic syndrome in obese versus normal weight children and adolescents

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Even if a universally accepted definition of the metabolic syndrome (MS) in children does not exist, its main components such as insulin resistance, dyslipidemia, and hypertension appear to be more common in obese children than in those of normal weight. Moreover, other risk factors, such as a proinflammatory and prothrombotic state, seem to play an important role in the MS and its detrimental sequelae.

The aim of this study was to compare the major components of the MS, as well as other specific markers considered to be associated with the syndrome, between obese and normal weight children and adolescents (controls).

A group of 103 obese children and adolescents (49 boys and 54 girls), aged 5 -16 years (11.27 ± 2.78 years), and 69 age- and sex-matched controls (11.26 ± 2.88 years), underwent the following assessment: body mass index (BMI) and waist to hip circumference ratio (WHR) were calculated and blood pressure (BP) was measured. Fasting glucose and insulin levels were measured and the HOMA index was calculated. In addition, the levels of the following were measured: fasting lipids, apo-A1 and apo-B, uric acid, leptin, visfatin, CRP, transaminases, fibrinogen and spot urine microalbumin.

As expected, BMI was greater in the obese group compared to controls (28.64 and 19.24 respectively), as was the WHR ($P=0.005$). Obese children had a higher systolic ($P<0.0001$) and diastolic ($P=0.01$) BP. Fasting insulin levels and the HOMA index were higher in the obese group ($P=0.0003$ and $P=0.0008$, respectively) without any difference in glucose levels. HDL-Chol and Apo-A1 levels were lower in the obese group ($P=0.0003$ and $P=0.002$, respectively) whereas triglycerides were higher ($P=0.01$). Total cholesterol and LDL-Chol showed no statistically significant difference between the two groups. Leptin was higher in the obese group ($P<0.0001$), while visfatin showed no statistically significant difference. CRP, fibrinogen and uric acid were higher in the obese group ($P<0.0001$ for all three). Regarding the transaminases, AST showed no difference, while ALT was higher in the obese group ($P=0.001$). Finally, there was no statistically significant difference regarding the urine microalbumin excretion.

The results of this study suggest that obese children, compared to their lean counterparts, are at increased risk of developing the main components of the MS. In addition, a proinflammatory and prothrombotic state seems to be present, early in the life of obese children and adolescents.

PO3-207 Obesity, Fat III

Contribution of clinic, metabolic, and genetic factors on hypertension in obese children and adolescents

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The childhood obesity is being recognized as a major health problem, and associated with an increase in hypertension rates. ACE gene has an important role within the renin angiotensin aldosteron system, which plays a critical role in blood pressure homeostasis. The role of ACE gene insertion (I) or deletion (D) polymorphism on blood pressure phenotype is not clear in children. In this study, it is aimed to examine if the association between hypertension and ACE I/D polymorphism, and to examine the contribution of clinic and metabolic parameters on high blood pressure in obese children and adolescents.
The study participants were 199 consecutive obese subjects, mean aged 10.62±3,52. Forty four of them were hypertensive. Hypertensive subjects were older than normotensive subjects (12,59 vs 9,93 years), and most of them were pubertal (77,2% vs. 48,4%). The prevalence of hypertension in obese subjects with II, ID, and DD genotype was similar (22.2, 24, and 20.8% respectively). There was no difference between the hypertensive and the normotensive group according to ACE I/D genotype (table 1), BMIsds, sex, blood glucose level, total colesterol levels, thyroid function tests (sT3, sT4, TSH), and liver function tests (AST,ALT).

The prevalence of ACE genotype in hypertensive obese, normotensive obese and control group

ACE genotype	Hypertensive obese group	Normotensive obese group	Control group
II	6 (13,63%)	21 (13,63%)	28 (15,8%)
ID	20 (45,45%)	76 (49,3%)	82 (46,32%)
DD	18 (40,9%)	57 (37,01%)	67 (37,8%)

IR-HOMA, fasting insulin, triglycerid levels were higher in hypertensive obese patients. In obese children, high IR-HOMA values (odds ratio [OR]: 4,91), puberty (OR: 3,65), presence of family history for hypertension (OR: 1.66), hypertriglyceridemia (OR: 1,5), and low HDL-cholesterol (OR: 1.3), high triglyceride/HDL-cholesterol ratio (>3.5) (OR: 2,69) found as increased risk factors of hypertension.
In obese children and adolescents, blood pressure did not differ by ACE I/D genotype. Hyperinsulinism was significantly related to hypertension. The presence of family history for hypertension, entering puberty, insulin resistance and hypertriglyceridemia consist of important risk factors for developing hypertension. In addition, evaluation of triglyceride/ HDL-cholesterol ratio seems to be more reliable than evaluation of triglyceride and HDL-cholesterol levels alone for predicting hypertension risk.

PO3-208 Obesity, Fat III

Nutritional profile of children and adolescents with solid tumors assisted at a reference service in Fortaleza, Ceara State – Brazil

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Introduction: Infantile cancer is one of the diseases that most affects children and adolescents in the world. Both the tumor and the therapy against it may contribute for the progressive deterioration of nutritional status, due to the effects they cause on the child. The protein-energy malnutrition (PEM), which reaches up to 50% of cases and is associated to the type, the location and the malignancy of the tumor, the stage and the therapy applied.
Aim: To assess the impairment of the nutritional status of children and adolescents with solid malignant tumors
Methods: It was a cross-sectional and documental study held with children and adolescents aged 0 to 19 years, with non-hematologic solid tumors, assisted from February 2007 to January 2009 at the Pediatric Oncology Service of the Albert Sabin Infantile Hospital, independent of the sex and the type of therapy. Data collection included: nutritional assessment, gender, age and anthropometric data (weight variability, height and skin folds: triceps and subscapular). Anthropometric data analysis followed the characterization of nutritional status recommended by the WHO.
Results: Fifty-five patients with solid tumors were assessed, of whom 31 (56%) were children and 24 (44%) adolescents. The children were 55% boys and 45% girls; the adolescents were 46% and 54%. From the 31 children studied, 14 (45%) loss weight after the diagnosis and from the 24 adolescents of the study, 16 (67%) also did so. According to the criteria weight for age, 3% of the children were well underweight for their age, 16% were underweight for the age, followed by 13% of the children who were overweight and the eutrophic ones, comprising 68%. Concerning the criteria weight for height the boys were 100% eutrophic, yet the girls were 14% low weight and 86% eutrophic. Regarding the criteria height for age, 11 (54%) children and 6 (25%) adolescents had low height for age. As for Body mass index (BMI) for age, 3 (14%) children and 6 (25%) adolescents presented themselves under, 1 (3%) child e 1 (4%) adolescent were overweight and 3 (10%) children and 1 (4%) adolescent presented obesity.
Conclusion: The results indicates that the patients with malignant tumors presented impairment of their nutritional status, which reinforces the importance of applying nutritional measures of support immediately after the diagnosis, as essential for the improvement of the adherence and the response to the treatment.

PO3-209 Obesity, Fat III

Nutritional status, self-perception and bulimic behaviors in female nutrition students: an exploratory study

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Background:
Eating disorders are generally characterized by abnormal eating patterns and cognitive distortions related to food and weight, which in turn result in adverse effects on nutrition status, medical complications and impaired health status and function. The body dissatisfaction, associated with eating disorders has been investigated in a possible new risk group: female nutrition students.
Objective:
The aim of the research was to evaluate the nutritional status, the body image perception and the presence of bulimic behaviors among female nutrition students in a Private University of Fortaleza, Brazil.
Methods:
From a total of 452 students derived the sample consisting of 130 females. To evaluate the nutritional status, the classification of body mass index (BMI) was considered; to investigate bulimic behaviors, the BITE (Bulimic Investigatory of Edimburg) was used; and in the investigation of the body image dissatisfaction, both the Body Shape Questionaire (BSQ) and the Figure Ratings Scale were performed.
Results:
The group comprised 130 female students with a mean age of 22.7 (± 4.25) years and BMI of 22.2 (± 2.73) kg/m². From these, 81.6% (n=106) were classified as eutrophic, 13.8% (n= 18) as overweight and 4.6% (n=6) as underweight. According to BSQ, 34.7% had light distortion of body image. The EFS showed that 57% was unsatisfied because of overweight and the BITE indicated that 17% had abnormal eating behavior; four cases of these, with moderate severity. Most part of the students who presented altered results in the referred tests was considered eutrophic: 23.8%, 41.5% and 13%, respectively.
Conclusion:
The study pointed out body dissatisfaction and risk behaviors for bulimia in students with adequate nutritional status. As this study deals with future nutritionists, it is of much relevance, because they deal directly with the treatment of eating disorders.
Keywords: nutritional status, body image, eating disorders, body mass index.

PO3-210 Obesity, Fat III

EAT-26 in the screening of alimentary disorders in adolescent ballerinas

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Eating disorders such as anorexia nervosa and bulimia, by the increase in their number of cases, should be considered a serious threat to public health. Among the various vulnerable groups for such disorders, there are those of physical activity practitioners and particularly, teenager ballet dancers.
Aim: The objective of this study was to apply the Eating Attitudes Test-26 (EAT-26) as a method of screening in a specific group of high-risk for eating disorder, comprised by ballet students, associating this test to body mass index (BMI), body fat percentage (% F) and age.
Method: Ninety adolescent ballet students (aged between 10 to 19 years) were assessed. The research was conducted in an academy of ballet in Fortaleza, Brazil. The anthropometric data such as age (years), weight (kg), height (m), BMI (kg/m^2) and fat percentage (%F) were investigated. The EAT-26 was applied as a method of screening for feeding disorders.
Results: From the studied group, 17 (11%) presented BMI below 18.5 kg/m^2, 26 (25.5%) had BMI over 22 kg/m^2, and 47 (59%) found themselves with BMI in normal range for adolescents. The application of the EAT-26 questionnaire showed positive values (> 20 points) in 15 (16.6%) adolescents and values within the normal range (<20 points) in 75 (83.4%). The percentage of fat found was 20.9 ± 4.7% and was higher the age group of 10 to 13 years.
Conclusion: In this study, by means of the EAT-26, we found a high prevalence of positive results for food disorders in young ballet students, which can be taken as an important indicator of risk in this group. The BMI and %F can be used as methods of body assessment in this population, but are not so representative for the detection of such disorders. Thus, the use of sensitive and easy- to- apply screening methods is indicated in populations at risk, with the purpose of providing early diagnosis and to prevent far more serious eating disorders in adulthood.
Descriptors: Alimentary Disorders; ballerinas, EAT-26.

PO3-211 Obesity, Fat III

Childhood obesity-related cardiovascular risk factors and carotid intima-media thickness

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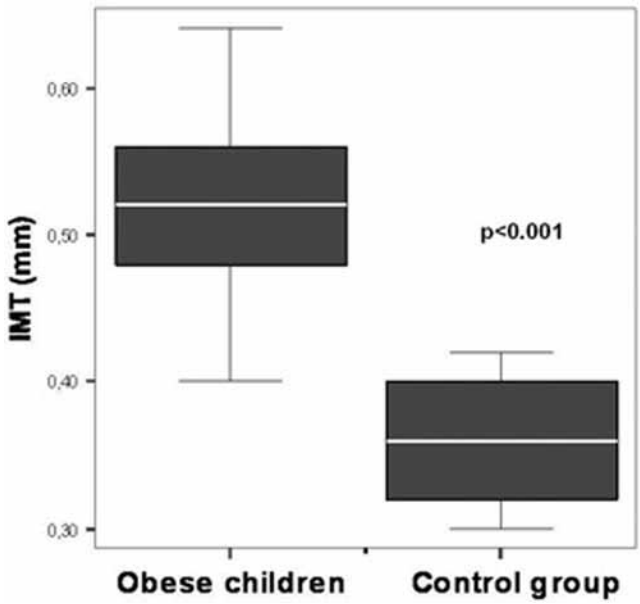
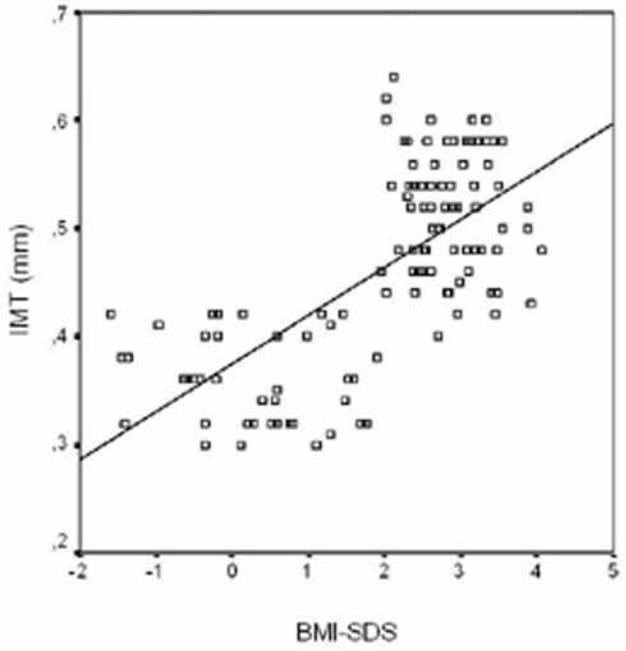
Objective: To investigate the relationship between childhood obesity and carotid intima-media thickness (IMT).
Design: This study included 75 obese subjects (mean age 10.8 ± 2.03 years, mean body mass index–standard deviation score [BMI-SDS] 2.84 ± 0.49) and 40 non-obese control. Systolic and diastolic blood pressure (SBP, DBP) values and waist and hip circumferences were measured. Fasting blood glucose, insulin concentrations, and lipid profiles were assayed. The carotid IMT (cIMT) was measured by ultrasonography.
Results: Waist/hip ratios, SBP, and DBP were significantly increased in the obese group compared to the control group (all p<0.001). LDL-C, HDL-C, and TG in the obese children were significantly different from the control subjects (all p< 0.001). The obese children demonstrated significant differences in a number of clinical risk factors including body weight, BMI, BMI-SDS, SBP/DBP, waist circumference, hip circumference, and waist/hip ratio (all p<0.001). The obese children showed increased mean carotid IMT values [0.52 mm (95% CI, 0.40 - 0.64 mm) *vs.* 0.35 mm (95% CI, 0.24 - 0.38 mm), p<0.001].



The cIMT was closely related to the BMI-SDS, SBP/DBP, waist and hip circumferences, serum TG, cholesterol, LDL-C, HDL-C, fasting serum insulin level, and insulin resistance indices including the HOMA-IR, FGIR, and QUICKI.



The BMI-SDS, TG, and QUICKI were independent predictive risk factors for increased cIMT.
Conclusion: Measurements of BMI-SDS, blood pressure, waist and hip circumferences, serum TG levels, QUICKI, and cIMT can be suitable for pediatric patients and may be used for screening or monitoring therapeutic success in obese children.

PO3-212 Obesity, Fat III

Maternal characteristics associated with offspring cardiovascular disease risk factors

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BACKGROUND:
The mechanisms underlying observed associations between birth weight and risk factors for cardiovascular disease later in life are a source of controversy.
AIM:
This population-based study seeks to better elucidate the relationship between maternal conditions and offspring obesity, hypertension (HTN) and diabetes mellitus (DM), controlling for gestational age, gender and birth weight.
METHODS:
All singleton, term (≥38 weeks), low birth weight (<6.5 lbs) infants born 1/1/76-12/31/82 to mothers residing in Olmsted County, MN were followed in medical records from age 2 to a maximum of 31 years. There were 54 mothers (cases) whose offspring were diagnosed with obesity, HTN or DM. They were matched on offspring gender, date of birth, low birth weight and length of follow-up with control mothers. Maternal medical records were abstracted for pre- and intra-pregnancy data, including socio-demographic factors; smoking, body mass index, weight gain; blood pressure, serum glucose and hemoglobin, urine glucose and protein values; complications of pregnancy, labor, or delivery; and clinical diagnoses. Cases and controls were compared using the chi-square test or Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuously scaled variables.
RESULTS:
Prior to pregnancy, cases were significantly more likely to exhibit pre-HTN or worse, defined as systolic >120 mmHg or diastolic >80 mmHg (55% vs. 34%, p<0.05); cases also exhibited a non-significant trend toward increased anemia, defined as hemoglobin ≤12 g/dl (20% vs. 5%, p=0.09). The difference in pre-pregnancy HTN disappeared during pregnancy (pre-HTN or worse during the second trimester, 55% vs. 52%, p=0.78), as did the trend toward increased anemia (42% vs. 46%, p=0.74). There were no significant differences between groups for other pre-pregnancy variables or any intra-pregnancy variables, including weight gain, HTN, DM, anemia, smoking or diagnoses reflective of fetal hypoxia (pre-eclampsia, eclampsia, placenta previa).
CONCLUSION:
The significant difference in pre-pregnancy HTN disappeared during pregnancy, as did the trend toward increased anemia. Although further research is needed, our data suggest a role for early hypoxia (at conception and in the initial weeks of gestation before seeking obstetric care) in adversely affecting the endocrine development of the fetus, which may then predispose low birth weight infants to increased risk of obesity, HTN and DM later in life.

PO3-213 Obesity, Fat III

Cardiac autonomic balance alters in obese children

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Background: Chronic sympathetic overstimulation and increased cathecolamine levels have been incriminated in obesity. Also higher sympathetic and parasympathetic activity have been found in obese adults. In this cross-sectional, case-control study we aimed to evaluate the cardiac autonomic functions in obese children by using both time- and frequency-domain parameters of heart rate variability (HRV).
Materials and methods: A total of 32 consecutive obese children (11.6 ± 2 years of age) and 30 age and gender matched healthy lean children (11.9±2.9 years of age) were included in this study. Obesity was defined as the body mass index (BMI) being over 97 percentile of the same gender and age. Twenty-four-hour ambulatory electrocardiographic recordings were obtained from each subject with digital holter recorder. The heart rate variability was determined by the software of the same device.
Results: There was no difference in regard of age, gender, total cholesterol, LDL-cholesterol between study group and control group. Subjects in the obese group had significantly higher BMI-SDS, HOMA-IR, VLDL and triglycerides levels than control subjects (p<0.001 for all).
The obese children had significantly lower plasma HDL-cholesterol levels when compared to healthy controls (p=0.004). All parameters of HRV except SDNN were found to be statistically significant between study group and control group. HRV indices for both time- and frequency-domain and their statistical comparison between groups are illustrated in table.

HRV indices for both time and frequency-domain and their statistical comparison between groups

	Obese group	Control group	p value
SDNN	137.8±35.1	142±29	NS
SDANN	101.8±25.1	122±22	<0.001
RMSSD	73.2±37.4	89±45	<0.05
PNN50	26.8±16.4	34±13	<0.05
LF	2.2±1	2.6±0.9	<0.05
HF	2.5±2.1	1.9±1.5	<0.05
LF/HF	1.12±0.56	1.6±0.7	<0.05

Conclusion: These results suggest that there is impaired cardiac autonomic balance in obese children; significantly decreased parasympathetic tone, resulting sympathetic dominance.

PO3-214 Obesity, Fat III

Decreased plasma apelin levels in obese children

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Background: Adipose tissue secretes a variety of biologically active molecules such as apelin which interact with metabolic and endocrine functions and may contribute to the development of insulin resistance.
Methods: A total of 32 obese subjects (5-16 year, 16 girl), and 40 lean subjects (5-15 year, 20 girl) were included in this preliminary case control study. Plasma apelin, adiponectin, high sensitivity C reactive protein (hs-CRP) and lipid profile were investigated. Obesity was defined as the body mass index (BMI) being over 97% percentile of the same gender and age. Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR).
Results: Obese and healthy lean subjects showed no significant difference in terms of age, total cholesterol, LDL-cholesterol and glucose. The obese children had significantly lower plasma apelin and adiponectin levels when compared to healthy controls (p< 0.004 and p= 0.001 respectively). Also, the obese children had higher hs-CRP and insulin levels and insulin resistance by Homeostasis Model Assessment (HOMA) indexes (p< 0.001 for all). High density lipoprotein cholesterol (HDL-C) levels were significantly lower and triglyceride (TG)
levels were higher in obese group (p= 0.001 and p= 0.001, respectively). Both apelin and adiponectin levels were negatively correlated with body mass index (BMI) [(r=-0.32, p=0.007) and (r=-0.55, p< 0.001) respectively], insulin [(r=-0.40, p=0.01) and (r=-0.32, p=0.006) respectively] and HOMA-IR [(r=-0.34, p=0.003) and (r=-0.32, p=0.005) respectively].
Conclusions: The results of the present study show that insulin resistance and inflammation in obese children are associated with low plasma apelin and adiponectin levels.

PO3-215 Obesity, Fat III

How well does body mass index predict differences in body fat during childhood? Findings from a nationally representative sample

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Background: Body mass index is a tool used for assessing body fat in the clinical setting. The purpose of our study was to examine the association between BMI z-score and the total percent body fat for a nationally representative sample of US white, black and Mexican-American children.
Data Source: National Health and Nutrition Examination Surveys (1999-2004).
Study population: 5,320 children aged 8-17 years who underwent total body dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500A).
Main Outcome Measure: Percent body fat as measured by DXA
Analysis: Subjects were stratified by sex, age (8-12 and 13-17 years), and race (white, black, Mexican-American). BMI z-scores were calculated based on the 2000 CDC growth curves. We conducted Pearson correlations between BMI z-score and percent body fat for each of the groups. We fit a multivariable linear regression model to predict percent body fat, using BMI z-score, race and age as covariates. Interactions between race and BMI z-score were also examined.
Results: Correlations were lower for normal weight (range 0.39 to 0.57) compared with overweight children (range 0.70 to 0.78), and did not appear to differ based on age or sex. The table shows the multivariable model, which demonstrated a nonlinear association between BMI z-score and percent body fat and statistically significant differences by race. For a given BMI z-score, black children had 2.5% less body fat and Mexican-American children had almost 1% more body fat compared to white children. We found no significant interactions between race and BMI z-score.
Discussion: Our finding that BMI z-score correlates better with percent body fat for overweight children would support the use of BMI as a measure of body fat for overweight, but not normal weight children. Use of BMI in the clinical setting likely leads to an overestimation of body fat among black children and an underestimation of body fat among Mexican-American children.

Linear regression models predicting percent body fat as measured by DXA

	β coefficient (SE)	
	Males	Females
Black vs. white	-2.61 (0.28)*	-2.59 (0.33)*
Mexican vs. white	0.73 (0.26)*	0.71 (0.19)*
Age (years)	0.65 (0.05)*	0.34 (0.05)*
BMI z²	1.53 (0.10)*	1.13 (0.05)*
BMI z	4.13 (0.11)*	4.44 (0.15)*
Intercept	29.61 (0.62)*	24.63 (0.68)*

* P< 0.01

PO3-216 Obesity, Fat III

Retinol-binding protein 4 (RBP4) in obese children and adolescents. Relationships with insulin resistance indexes, plasma lipids and inflammatory parameters

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BACKGROUND: RBP4 has been reported to be involved in obesity-associated insulin reistance.
AIMS: 1. To determine the relationships between RBP4 and the degree of adiposity, insulin resisitance indexes, plasma lipids and inflammatory parameters in obese chidren and adolescents. 2. To ascertain whether plasma RBP4 levels can help to identify glucose intolerance in these patients.
PATIENTS AND METHODS: Prospective study of 199 obese patients (95 boys), aged between 8 and 16 years (11.8±1.9) with BMI 3.5±0.9 and 53 age-matched controls (23 boys) (11.3±2.1) with BMI 0.5±0.8. Waist perimeter was measured, and plasma lipid fractions were determined: total cholesterol, LDL-C, HDL-C, VLDL-C, lipoprotein A and apolipoprotein B and inflammatory parameters: PCR, homocystein, uric acid and fibrinogen. OGTT was evaluated according to WHO criteria and insulin resistance parameters were calculated. Plasma RBP4 levels were determined by nephelometry.
RESULTS: Plasma RBP4 concentrations (pg/ml) were significantly higher in obese patients with glucose intolerance (45.0±14.1; n=15) compared to those without glucose intolerance (35.9±11.7; n=184) and controls (31.5±12.3); p<0.001. A positive and statistically-significant correlation was found between RBP4 and BMI and BMI z-score (r=0.204 and r=0.213, respectively, p<0.001), waist perimeter (r=0.135, p<0.05), plasma triglycerides (r=0.187,p=0.005) and ApoB (0.187,p=0.007). A negative and statistically-significant correlation was observed between PCR and fibrinogen (r=-0.203;p=0.003 and r=-0.216,p=0.002, respectively). No correlation was found with insulin resístance indexes.
Conclusions: Our results show plasma RBP4 concentrations to be a sensitive marker of metabolic imbalance in obese children and adolescents, particularly those with glucose intolerance. This protein does not appear to contribute directly to the development of insulin resistance in these patients.

PO3-217 Obesity, Fat III

Effect of a high dairy diet on serum antibody titres to heat shock protein 27 in overweight and obese children

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Background: An immune response to heat shock proteins appears to be involved in atherogenesis. To date, there has been no report on the impact of dairy or calcium consumption on serum antibody titres to heat shock protein 27 (anti-Hsp27). We have investigated whether an increase in dairy food consumption is capable of affecting serum antibody titres to heat shock protein 27 (anti-Hsp27) level in children.
Methods: overweight and obese children (n=99, age: 12-18 y, BMI: 27-40 kg/m^2) were randomized to receive a calorie restricted diet providing a 500 kcal/d deficit from total energy expenditure and two (*n= 38), three (n= 26) or four (n*=35) servings of dairy products/day. Serum anti-Hsp27 level in addition to the serum hs-CRP and lipid profile were measured at baseline and after 12 weeks.
Results: Serum anti-Hsp27 concentrations did not change significantly in any of the mentioned groups. Serum hs-CRP and lipid profile did not change significantly either, apart from a significant increase in HDL-cholesterol in the low dairy group.
Conclusion: An increased intake of dairy products does not lead to a significant change in serum anti-Hsp27 level in overweight and obese children.

PO3-218 Obesity, Fat III

The significant improvement on physical fitness and (central) obesity after multidisciplinary treatment in children with obesity is maintained at 1 year follow-up

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The most appropriate treatment for children with obesity(OB) is lifestyle intervention, but reports on its short and long-term effect are contradictory. We

evaluated the effect of a 3 months' multidisciplinary treatment on (central)OB (BMI-SDS, waist circumference(WC) and waist-to-height ratio(WHtR)) and physical fitness(VO_{2peak}-SDS), adjusted for gender, age and weight, immediately after discontinuation of the treatment and after 1 year follow-up(1yr FU). After stratification for gender and ethnicity 79 children were randomly assigned to an intervention(I) (n=40; mean(± SD) age 12.9±1.9yr; 18M/22F; BMI-SDS 3.1±0.7; WC 90.9±9.3cm; WHtR 0.56±0.04; VO_{2peak}-SDS –3.1±0.9) or control group(C) (n=39, age 12.8±1.9yr; 19M/20F; BMI-SDS 3.1±0.7; WC 90.5±7.7cm; WHtR 0.56±0.04; VO_{2peak}-SDS –2.8±0.9). The I group was offered 2 individual and 7 group-meetings, and parents 4 separate and 1 meeting together with the children. Main topics were education on nutrition, physical activity and improvement of self-esteem. The C group was given standard treatment (physical activity and nutrition advice). They were offered the treatment after the 1yr FU measures.

At baseline there were no significant differences between groups for age, BMI-SDS, WC, WHtR and VO_{2peak}-SDS. Furthermore, VO_{2peak}-SDS was significantly inversely correlated with BMI-SDS (r=-0.579;p<0.01), WC (r=-0.347;p<0.01) and WHtR (r=-0.402;p<0.01).

After 3 months' treatment there was a significant time*group effect. BMI-SDS in I group decreased to 2.8±0.9 (significantly different from baseline (p=0.01)) and WHtR was 0.55± 0.06(p=0.05). The other parameters remained stable. In C group a slight but statistically significant decrease in VO_{2peak}-SDS (-2.9±1.2;p=0.04) had occurred and no significant change for the other parameters.

At 1yr FU, BMI-SDS(2.8±1.0) had stabilized in I group, still significantly lower than at start (p=0.01). WHtR showed a slight further decrease (0.53±0.06; p<0.01 in comparison to start). Also WC(88.5±10.6cm;p=0.02) and VO_{2peak}-SDS(-2.1±1.2;p=0.01) were significantly better than at start. In contrast, a further decrease in physical fitness(-3.2±1.3;p=0.00) was shown in C group.

Our 3 months' intervention showed a significant improvement on (central)OB and physical fitness in OB children, which was maintained at 1ye FU. However, the wide majority of the children were still OB. In contrast, a decrease in physical fitness and no improvement in measures for (central)OB were observed in C group.

PO3-219 Obesity, Fat III

Childhood overweight and obesity in "Real life" – severity and morbidity in a multiethnic European cohort

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Objective:

In a multiethnic cohort of overweight to obese children and adolescents differences in the prevalence of elevated metabolic parameters and the metabolic syndrome regarding immigration background were analyzed.

Study design:

In the Berlin childhood obesity cohort 1059 children and adolescents had a recorded immigration background. BMI-SDS was calculated by German reference values and blood pressure was recorded. Metabolic parameters like fasting blood glucose, fasting insulin, R-HOMA and lipids were measured by a fasting venous blood sample. Metabolic syndrome was defined by WHO-criteria.

Results:

In this European obesity cohort the proportion of children and adolescents with German background is 48.0%, 25.1% have a Turkish and 26.9 % have another immigration background, overall 22.7% are overweight, 30.7% are obese and 46.6% are extremely obese. In multivariate models there are significant associations between elevated metabolic parameters and older age and higher BMI-SDS values. A logistic regression analysis shows a greater chance to suffer from a metabolic syndrome with older age and Turkish immigration background.

Conclusion:

Higher BMI-SDS-values and older age are important risk factors for elevated metabolic parameters. In Turkish patients the mean BMI-SDS is significantly higher, although there is no difference in the mean age of the three immigration groups. The results of logistic regression analysis show a higher risk for insulin resistance and metabolic syndrome in older and Turkish children and adolescents. Effective therapy and prevention efforts must be targeted to this special high risk group. More immigration specific research regarding insulin resistance, metabolic syndrome and Type 2 DM is needed in Europe.

PO3-220 Obesity, Fat III

Waist circumference and waist-to-height ratio in Han Chinese children

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Objective: To derive age and gender specific reference values for waist circumference (WC) and waist to height ratio (WHtR) for Han Chinese children and adolescents and to establish the prevalence of excess central adiposity in our study population

Subjects and methods We describe a school based, cross-sectional study of 7326 (3603 boys) Han Chinese students aged 5 to17 years old, living in southwest China in October 2003 and April 2004. Anthropometry was measured using standard procedures. The LMS method was used to establish smoothed percentile curves for WC. Overweight and obesity were defined by both the International Obesity TaskForce criteria (IOTF) and the Working Group on Obesity in Children (WGOC). Excess central adiposity fat was defined by previously published WC cut-points and a WHtR ≥ 0.5.

Results: WC percentile and WHtR curves for Han Chinese children and adolescents were constructed. Based on the IOTF criteria, 26.4% of boys were overweight or obese compared to 16.4% of girls (P<0.001). WC and WHtR cut points identified 31% and 14.8% of boys and 28% and 5.6% of girls, respectively as having excess central adiposity. Young boys (5 to 12 years) had a significant (P<0.001) higher WHtR compared to girls.

Conclusion: We have constructed WC percentile and WHtR curves for Han Chinese children and adolescents. However, the measurements were based on a student population that has a high rate of overweight and obesity. The data will provide a point of reference for future studies measuring the prevalence of overweight and obesity.

PO3-221 Obesity, Fat III

Effects of pregnant cow's milk on blood lipid metabolism in juvenile female SD rat

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Objective: To investigate the effects of estradiol (E_2) and progesterone (P_4) in cow's milk on blood lipid metabolism in juvenile female SD (Sprague-Dawley) rat. M**ethods:** Sixty four female SD rats aged 21 days old were randomly assigned to four groups and fed with quantitative milk from pregnant cows, milk from postpartum cows, commercial whole milk and artificial milk respectively. The body weight and tail length were measured regularly. Selecting randomly 8 rats of each group before puberty (during 28~29 days old), 24h urine were collected to determine total estroil (TE_3), P_4 and creatinine (Cr); and serum P_4 and the activity of uterus peroxidase were determined after these rats were killed (29~30 days old). After the priming of puberty, estrous cycles were observed three times a day by vaginal smear; fasting blood glucose was assayed when the rest of rats were sacrificed at 53~54 days old. When all rats were killed, serum total cholesterol (TC), triglyeride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), body length and reproductive organs weight were determined. **Results:** At prepuberty or puberty, there were no statistically significant differences in body weight, tail length, body length and organ indices among pregnant cows milk, postpartum cows milk, commercial whole milk and artificial milk groups. There were no statistically significant differences in serum P_4, TC, TG, HDL-C and LDL-C, urinary TE_3/Cr, urinary P_4/Cr and the activity of uterus peroxidase in all rats

at prepuberty among 4 groups. Serum HDL-C (mmol/L) of all rats at puberty among the aforesaid ordinal groups were respectively 0.66 ± 0.07, 0.60 ± 0.04, 0.69 ± 0.07 and 0.56 ± 0.12. And serum HDL-C in pregnant cows milk group was higher than that in artificial milk group (P<0.05). Serum HDL-C in commercial whole milk group was higher than that in postpartum cow milk and artificial milk groups (P<0.05 in both), there were no statistically significant differences among other groups. There were no statistically significant differences in serum TC, TG and LDL-C, the time of vaginal opening, estrous cycles and fasting blood glucose in all rats at puberty among 4 groups. **Conclusions:** A certain levels of E_2 and P_4 in milk which come from either pregnant cows or commercial whole milk mainly from pregnant cows may perhaps increase serum HDL-C of juvenile female SD rat.

PO3-222 Obesity, Fat III

The relationship of the level of proinsulin with insulin resistance and factors determining metabolic syndrome in prepubertal children with overweight or obesity

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Purpose: In prepubertal children, we evaluated the change of proinsulin secretion according to body mass index (BMI) z-score, insulin resistance and the existence of metabolic disorders including prediabetes.
Methods: Fasting levels of glucose(G), insulin(I), proinsulin(PI) and adiponectin were measured in total 119 children (80 M, 39 F), who were divided into lean (n=52), overweight (n=14) and obese (n=53) group. Total 67 overweight or obese children underwent a OGTT. We investigated the number of disorders determining metabolic syndrome such as prediabetes, high triglyceride(TG), low HDL-cholesterol (HDLC) and hypertension and classified subjects into two groups with and without disorders.
Results : None were found to have DM. None of overweight children had prediabetes, low HDLC or hypertension and 4 of them had high TG. Nine (17%, n=9), 5 (9.4%), 19 (35.8%) of 53 obese children had prediabetes, low HDLC and high TG levels. Obese, not overweight group had significantly higher fasting G, TG and blood pressure(BP) and lower HDLC than lean group. However, obese as well as overweight group had significantly higher fasting I and HOMA and lower adiponectin levels than lean group. In addition, fasting PI levels (7.22±3.01 vs 12.31±2.91 vs 16.65±6.82, P<0.01) significantly increased in a stepwise fashion, but fasting PI/I ratio was not different between three groups. Fasting PI levels had a significant positive correlation with BMI z-score (r=0.65, P<0.001) and HOMA (r=0.62, P<0.001) but negative correlation with adiponectin (r=-0.49, P<0.001). Fasting PI levels had a significant positive correlation with fasting G, TG, systolic BP and waist circumference. However, PI/I ratio was not correlated with them. The levels of PI, I and PI/I ratio were similar irrespective of whether their glucose metabolism was normal or impaired. However, patients with one or greater metabolic disorders (n=39) showed higher fasing PI (13.16±4.88 vs 17.36±6.87, P=0.008) and HOMA, but lower levels of adiponectin than those without disorders. However, PI/I ratio was not different between them.
Conclusion : PI levels appears to be used as a marker of insulin resistance and early predictor of metabolic syndrome. Secretion of proinsulin increases proportionally to the increased insulin secretion, showing increased ß-cell compensation without failure of proinsulin processing to insulin until certain degree of insulin resistance or metabolic disorders is present.

PO3-223 Obesity, Fat III

Effect of obesity on airway inflammation among non-atopic children

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Purpose: Increased number of macrophages that produce TNF-α, IL-6 and inducible nitric oxide synthetase has been demonstrated in adipose tissue of obese individuals. Thus obesity is accused to induce airway inflammation. Present study was planned to investigate the effect of obesity on airway inflammation among non-atopic children.
Method: Obese and non-obese children aged between 6 to 17 years that were confirmed to have no atopy (i.e. non-atopic) by epidermal prick test were recruited for the study. Nitric oxide level in expired air (eNO) measured by online method using Sievers 280i NOA was used as the indicator of airway inflammation.
Results: Study included 127 children. All cases were classified as normal (n=52), obese (n=30) and morbid obese (n=45) according to body weights and their eNO levels were compared. Median eNO values did not show significant difference between the groups (normal: 18.5 ppb, obese: 18.05 ppb, morbid obese: 20.8 ppb, p=0.374). No difference was observed between median eNO values of the groups even after exclusion of the cases diagnosed as asthma (n=24; normal:2, obese:6, morbid obese:16, p<0.001) (normal: 18.3 ppb, obese: 17.75 ppb, morbid obese: 19.3 ppb, p=0.723)
Conclusion: Although obesity is a risk factor for asthma among non-atopic children, its effect on eNO which is an indirect indicator of airway inflammation could not be demonstrated.

PO3-224 Obesity, Fat III

Total adiponectin and its high-molecular-weight hexamer form are markers of the metabolic syndrome in obese children and adolescents

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Background: Adiponectin is a specific adipocyte cytokine implicated in lipid and carbohydrate metabolism and appears to be the link between obesity, insulin resistance, cardiovascular disease and the metabolic syndrome. Adiponectin does not circulate monomerically in blood since it undergoes a post-translational process which determines the presence of low molecular weight trimers (LMW), medium molecular weight hexamers (MMW) and a high molecular-weight-oligomer (HMW); however, the functions of these peptides in obese patients remain to be established.
Aims: To ascertain whether plasma levels of total adiponectin (total Adp) and its multimeric forms (LMW, MMW, HMW) differ in the presence or absence of the metabolic syndrome in obese children and adolescents.
Patients and Methods: Prospective study of 94 obese patients (33 males) aged between 10 and 16 yrs (12.4±1.6) with BMI 3.5±1.0 and 32 age-matched controls (13 males) (12.0±1.9 años) with BMI 0.3±0.8. IDF criteria (2007) for children and adolescents were used to establish the presence of the metabolic syndrome. Total adiponectin (total Ad) and its multimeric forms (LMW, MMW, HMW) were determined by ELISA (Bülmann; Switzerland).
Results: Thirteen obese patients (13.8%) met the metabolic syndrome criteria. Values of total Adp and its multimeric forms in controls and obese patients with and without the metabolic syndrome are shown in the table.

Total adiponectin and its multimeric forms distribution.

	Control n=32	Obese without Metabolic S. n = 81	Obese with Metabolic S. n = 13	p
Total Adp (µ/ml)	8.41 (2.8)	7.47 (2.6)	5.72 (2.2)	0.01
HMW Adp (µ/ml)	3.69 (2.0)	2.99 (1.6)	1.73 (0.9)	0.003
MMW Adp (µ/ml)	1.95 (0.7)	1.77 (0.8)	1.5 (0.9)	ns
LMW Adp (µg/ml)	2.85 (1.4)	2.69 (1.3)	1.9 (0.8)	ns
HMW Adp/ total Adp	0.41 (0.1)	0.38 (0.12)	0.30 (0.08)	0.02

Conclusions: Plasma values of total adiponectin and high multimeric forms (HMW) are significanty decreased in obese children and adolescents with the metabolic syndrome criteria. These cytokines may constitute a useful parameter for identifying obese children and adolescents with the metabolic syndrome.

PO3-225 Obesity, Fat III

Plasma uric acid: a robust metabolic marker of the metabolic syndrome and insulin resistance in obese children and adolescents

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INTRODUCTION: Hyperuricemia is a characteristic disorder of the metabolic syndrome (MS) in obese adults, although it is not included as a diagnostic criterion and is closely related to insulin resistance. Studies analyzing this association in obese children and adolescents are scarce.
AIMS: 1. To ascertain whether obese children and adolescents with the MS present significantly higher serum uric acid values than those without MS. 2. To establish the relationship between plasma uric acid concentrations and the different components of the MS and insulin resistance indexes. 3. To determine the optimum cut-off point of plasma uric acid to identify the MS in obese children and adolescents.
PATIENTS AND METHODS: Prospective study of 326 obese patients (171 boys) aged between 6 and 20 years (11.7±2.7) and BMI 3.6±1.3 and 36 aged-matched controls (11 boys) (11.4±2.4) and BMI 1.0±0.7. IDF (2007) criteria for children and adolescents were used to determine the presence of MS and the Youden index to estimate the optimum cut-off point of plasma uric acid to identify obese patients with the MS.
RESULTS: Plasma uric acid levels (mg/dl) were significantly higher in obese patients with the MS (5.6.0±1.5; n=36) compared with obese patients without the MS (4.6±1.2; n=331) and controls (4.0±0.8); p<0.0001. A positive and statistically-significant correlation was found between uric acid and BMI and BMI z-score (r=0.515 and r=0.305, respectively, p<0.0001), waist circumference (r=0.535, p<0.0001), plasma triglycerides (r=0.140, p=0.008), systolic blood pressure (r=0.337, p<0.0001), diastolic blood pressure (r=0.213, p<0.0001) and a negative correlation with HDL-C (r=-0.250, p<0.0001). Although no correlation was found with fasting glycemia, the correlation was positive and statistically-significant with insulinemia, (r=0.267, p<0.0001) and HOMA index (r=0.242, p<0.0001) and inverse with QUICKI index (r=-0.243, p<0.0001). Plasma uric acid concentrations over 5.39 mg/dl had 57% sensitivity and 75% specificity (Youden index: 0.32) for identifying obese children with the MS.
CONCLUSIONS: The present study showed that obese children and adolescents with the MS had significantly higher uric acid values compared with those without the MS, and that these values were closely related to the presence of insulin resistance and MS components except fasting glycemia. The optimum cut-off point of serum uric acid to identify obese children with the MS was 5.39 mg/dl.

PO3-226 Obesity, Fat III

The adipocyte role in insulin resistance catch-up growth IUGR rat

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Background: Since Barker introduced the concept of Fetal origins of adult disease(FOAD), many studies had showed the relationship between adult diseases such as diabetes and the abnormality in fetal. At the same time, much more studies show that adipose tissue make a very important role in insulin resistance
Objective:We aimed to investigate whether the differentiation progress and function of adipose tissue has changed in catch-up growth intrauterine growth retardation(IUGR) rat, which lead to obesity and insulin resistance.
Methods: Health female SD rat went to 2 groups. Normal group was fed normal throughout the pregnancy; the IUGR group was fed 30% of the normal group. 8 newborn from the normal fed established the control group; 8 newborn with weight<5.1g of the nutrition restricted established the non-catch up growth IUGR group; 5 newborn with weight<5.1g of the nutrition restricted established the catch-up growth IUGR group. Measure the nose- tail length and weight at 0w, 1w, 2w, 3w, 4w; fast serum glucose, insulin, TG, acylation stimulated protein(ASP) level and OGTT was examined in the 3rd w, then adipose cell from the model adipose tissue was cultured to find out the changes of the differentiation progress in catch-up IUGR rat.
Result: The weight of catch-up group grow fast than the control and non-catch-up IUGR group, there is significant difference between the catch-up group and the control group at 4thw (p<0.01), however, the body length of the three groups have no difference.(p>0.05). Fast and OGTT serum glucose and insulin lever in catch up and non-catch up group is higher than the control group (p<0.01), the glucose has no difference between the two IUGR group (p>0.05), however, the insulin level of catch up is higher than the non-catch up group (p<0.05). TG level in catch-up group is higher than the control group (p<0.05), but has no difference with the non-catch up group.
Conclusion: Catch-up growth IUGR rat grow fast, especially the weight, this may be due to the heavily increased adipose tissue, which lead to high serum TG. The functional changes of the adipocyte results in insulin resistance, and thus make the IUGR rat has high fast glucose level.

PO3-227 Perinatal Endocrinology

Testosterone and estradiol in cord vein serum in boys – correlations to gestational age, size at birth and maternal preeclampsia

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Background
Testosterone is an important regulator of growth and differentiation during fetal development. Estradiol levels in cord venous blood are high compared to later in life, are known to be equal in both genders at birth, and are mainly derived from the placental unit. Preeclampsia (PE) may lead to fetal intrauterine growth restriction (IUGR). Elevated maternal serum levels of testosterone during pregnancy are known to be associated with IUGR. Earlier studies on sex hormones in mothers and their children born with IUGR or after PE are few and inconclusive. Here we present data on a larger male population-based cohort.
Objective and hypothesis
The objective of the study was to measure testosterone and estradiol in cord vein serum in boys born after healthy or preeclamptic or IUGR pregnancies. The hypothesis was that boys born after preeclamptic pregnancies had impaired levels of testosterone.
Population and/or methods
113 newborn singleton boys were studied, 22 born after pregnancies complicated by IUGR and/or PE and 91 born after healthy pregnancies. Gestational age (GA) was mean (SD) 35.2 (1.3) weeks. Birth weight was 2515 (515) g and birth length was 46.4 (2.7) cm. Cord venous blood was collected after birth. Serum testosterone was measured by Spectria testosterone; Orion Diagnostica.

Serum estradiol was measured by Spectria estradiol; Orion Diagnostica. Correlation analyses were made with Spearman non-parametric rank correlation.

Results

Testosterone correlated to estradiol (r=0.36, p<0.001), GA (r=0.42, p<0.001), birth weight (r=0.22, p<0.05) and birth length (r=0.23, p<0.05), but not to birth weight SDS or birth length SDS. Boys born IUGR or after PE had lower estradiol levels 13.9 (14.6) nmol/L than controls 20.0 (14.7) nmol/L, (p<0.05). They had similar testosterone levels 4.5 (2.6) nmol/L as controls 4.0 (1.8) nmol/L, (NS).

Precise conclusions

Boys born with IUGR or after PE were found to have low estradiol but normal testosterone levels, which may be due to low aromatase activity in the fetus, or rather pointing out a low placental estradiol secretion with normal fetal sex steroid production.

PO3-228 Perinatal Endocrinology

Prediction of severe systemic hypotension by serum 17-OH progesterone / cortisol ratio in extremely premature infants

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Objective:

It should be of interest to screen preterm neonates for relative adrenal insufficiency in order to prevent morbidity. Our objective was to evaluate if 17-OHP/cortisol ratio as a marker of immature 11 beta-hydroxylase activity could predict severe systemic hypotension in preterm neonates.

Patients and Methods:

We conducted a prospective, prognostic cohort study in infants less than 32 weeks of postmenstrual age (PMA). From capillary blood deposit on blotter paper, serum cortisol and 17-OHP concentrations were measured at 3 days of age. Death and short term morbidity were monitored prospectively. Logistic regression models were performed to analyze the association between 17-OHP/cortisol ratio and the occurrence of a first severe episode of hypotension.

Results:

105 patients were included in the analysis. 14 patients presented a severe episode of hypotension after 3 days of age and 15 patients presented with bronchodysplasia. Neither 17-OHP/cortisol ratio, nor 17-OHP or cortisol concentrations were associated with the occurrence of a first severe episode of hypotension or with bronchodysplasia. In the univariate analysis, 17-OHP and cortisol were negatively correlated with PMA (respectively -0.36 and -0.40) and birth weight (respectively -0.28 and -0.30). Cortisol, but not 17-OHP, was associated with the type of hospitalization unit, respiratory support and patent ductus arteriosus. 17-OHP/ Cortisol ratio was associated with the type of hospitalization unit.

Conclusions: 17-OHP/cortisol ratio was not predictided for severe systemic hypotension. Serum cortisol concentration was inversely correlated to gestational age and appeared adapted to environmental stress.

PO3-229 Perinatal Endocrinology

Hypercalcemia and hyperphosphatemia secondary to subcutaneous fat necrosis of the newborn – case report

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Introduction: Subcutaneous fat necrosis of the newborn (SFN) is a rare panniculitis, of self-limited course, which can affect full-term newborns who presented perinatal stress. Firm, erythematous nodules and plaques appear up to the 8th week of life, chiefly in face, trunk, tights and upper limbs. Although generally benign, it can be complicated by mild to severe metabolic disturbances, mainly hypercalcemia.

Case report: Female, 29 days, born full-term, Apgar scores 5 (1st min) and 7 (5th min), presented respiratory distress and required orotracheal intubation and transferred to neonatal intensive care unit for 7 days. She recovered well and was discharged from hospital. On the 12nd day of life, it was noted erythematous nodular lesions in malar region, bilaterally, which increased gradually in size. No other complaints. Physical on 29th day of life: healthy infant, afebrile, presenting hard and indurated nodules in both malar regions, measuring the one on the left 2.3x2.2cm and the one on the right 2.6x1.8cm, being the overlying skin reddish, warm and painful on touch. Ultrasonography revealed inflammatory process in subcutaneous tissue and computed tomography image showed obliteration of subcutaneous fat and skin thickening. Lesions reverted spontaneously during the 2nd month. At this time, the infant developed asymptomatic hypercalcemia (highest: 11.6mg/dL; reference 8.1-10.4) and hyperphosphatemia (highest: 8.1mg/dL; reference: 4.8-7.4), which persisted until the 5th month, without complications or need for intervention. Both ion disturbances went through spontaneous resolution. No other metabolic disturbances were detected.

Comments: Although SFN is an uncommon and generally benign condition, it is important to be aware of it, since it can be severely complicated by hypercalcemia, of non-defined etiology. In this case, hypercalcemia was moderate and asymptomatic, not requiring treatment. The observed hyperphosphatemia is not a common disturbance described in literature, being a different aspect in this report. Clinical and laboratorial follow-up of infants with SFN is important until metabolic normalization.

PO3-230 Perinatal Endocrinology

Adipsia or hypodipsia and plasmatic hypernatremia are common manifestations of holoprosencephaly

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Holoprosencephaly (HPE) is the most common structural malformation of the human forebrain that occurs 1 in 8.000 live births. A range of increasing severity is described: lobar (separate ventricles but incomplete frontal cortical separation), semi-lobar (partial cortical separation and absent or hypoplastic olfactory structures and corpus callosum) and alobar HPE (single ventricle and no interhemispheric fissure). Clinical manifestations can include craniofacial anomalies, severe mental retardation, pituitary dysfunction including diabetes insipidus (DI) and seizures. Plasmatic hypernatremia was described in some sporadic cases, but considered as an unusual complication. We follow 8 patients with HPE.

Patients: clinical and radiological features

N	Sex	Age (y)	causes of ad- mittance	Heigth (SDS)	Weight (SDS)	Adipsia/ hypo- dipsia	MRI
1	m	0,25	hyper- natremia	-3,0	-3,8	+	semi- lobar
2	f	0,3	seizure	-0,5	-1,5	+ (DI)	lobar
3	f	0,75	hyper- natremia	-0,8	-0,5	+	hypo- plastic- corpus callosum
4	f	4,02	vomiting	0,08	-0,71	+	semi- lobar
5	f	0,75	growth retard- ation	-2,9	-3,5	+	semi- lobar
6	m	5,5	growth retard- ation	-1,03	-2,57	- (DI)	lobar
7	f	0,45	hyper- natremia	-1,5	-1,3	+	semi- lobar
8	f	6,02	growth retard- ation	-2,16	-1,64	-	semi- lobar

HPE was diagnosed in cases 1-3 after their admission to our Hospital. The other patients were already known to have HPE. Cases 1, 3 and 7 were admitted to our hospital because of the evidence of hypernatremia. Three of the other patients showed plasmatic hypernatremia during the admission. Patients 2 and 6 had DI successfully treated with low doses of DDAVP. Six patients demonstrated adipsia/hypodipsia in spite of severe hypernatremia. Their plasmatic natremia levels returned to normal after forced hydration (oral or i.v.); in case 1 DDAVP was administered at very low dose to obtain persistent normalization of plasmatic natremia. After discharge 6 patients showed many other episodes of hypernatremia due to acute illness or not. None of our patients showed other pituitary disfunctions. In our experience plasmatic hypernatremia with adipsia/hypodipsia are common manifestations of HPE, more frequent than DI. Adipsia/hypodipsia are probably related to a defect in the hypothalamic osmoreceptors that control thirst. This condition had to be treated with forced hydratation and, sometimes, with very low doses of DDAVP.

PO3-231 Perinatal Endocrinology

Higher TSH levels are related to decreased cerebro-cortical bioelectric activity in preterm newborns: a study with spectral EEG

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Objective TSH levels at birth are associated with brain cells receptor saturation for thyroid hormones since intrauterine life. High TSH levels in newborns correlate with adverse neurological and cognitive long-term outcome, but there is no clear evidence of early changes in Central Nervous System development. The aim of this study was to evaluate during the neonatal period the relationship between TSH levels and CNS function, assessed by EEG spectral analysis.
Methods We studied 14 twin pairs, 3 monozygotic and 11 dizygotic, (mean GA 31,6±0,9 weeks), admitted to the Neonatology and Neonatal Intensive Care Unit of S. Chiara University-Hospital in Pisa. We collected blood spot TSH from a heel prick after 72 hours of age, used in neonatal screening for congenital hypothyroidism; all TSH values were < 5mU/L. We also recorded in the first 2 weeks of life a conventional EEG (8 channel), from which 80 sec of artifact-free activity during active sleep were extracted. Absolute and relative spectral power was calculated from delta, alpha, theta and beta activities. We performed a group analysis (Paired samples t test; Group A twin with higher TSH- Group B twin with lower TSH) and a correlation analysis (Spearman correlation - all EEG parameters related to TSH levels)on the data. P values < 0,05 were assumed as significant.
Results: Group analysis did not show significant differences in TSH levels nor in EEG indexes. Significant negative correlations between TSH levels and absolute spectral power of beta2-band ($p<0,01$) and alpha-band ($p<0,05$) emerged from correlation analysis.
Conclusion: Our results suggest that TSH levels at birth are strongly influenced by intrauterine environment, with no significant differences within each twin couple. When the infants are assessed independently from twin birth, higher TSH levels are associated to lower spectral power of rapid activity, which represent a good index of maturation of cerebro-cortical bioelectric activity in preterm newborns below 35 weeks.

PO3-232 Perinatal Endocrinology

Intracellular response to IGF-I in fibroblast cultures from children with idiopathic short stature

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IGF-I promotes cellular division and differentiation by activating several intracellular signaling factors such as IRS-1, AKT and ERK. Some children with idiopathic short stature exhibit high normal GH responses to provocative tests, and high normal IGF-I serum concentrations, suggesting a possible insensitivity to IGF-I (I-IGF-I). Aim: to determine the effects of IGF-I on IGF-IR, IRS-1, AKT and ERK in fibroblast cell cultures from short children with I-IGF-I. We developed primary skin fibroblast cell cultures from 31 prepubertal children: 13 I-IGF-I and 18 controls. We determined the total and phosphorylated contents of IGF-IR, IRS-1, AKT and ERK by Western Blot. The intracellular sensitivity to IGF-I was determined by stimulating the fibroblast cell cultures with recombinant IGF-I 10^{-8} M for 3, 5 and 10 minutes. The phosphorylated IGF-IR, IRS-1, AKT and ERK were determined by Western Blot. Results are shown in the Table as area under the curve (AUC).

table1

		Control (n=18)	I - IGF-I (n=13)
Age at study	years	6.9 ± 0.5	7.8 ± 0.7
Height at study	SDS	0.12 ± 0.20	-2.44 ± 0.50*
BMI at study	SDS	0.15 ± 0.21	-0.52 ± 0.49
IGF-I	SDS	-0.11 ± 0.20	1.35 ± 0.31*
IGF-IR/tyr AUC	AU x min	6.34 ± 1.31	3.40 ± 0.59
IRS-1/ser AUC	AU x min	2.41 ± 0.44	5.51 ± 1.17*
IRS-1/tyr AUC	AU x min	7.94 ± 2.10	3.70 ± 1.10
AKT/thr AUC	AU x min	14.26 ± 3.50	7.58 ± 1.78*
AKT/ser AUC	AU x min	4.38 ± 0.97	3.85 ± 0.81
ERK 42/44 AUC	AU x min	17.40 ± 2.50	13.10 ± 1.60

* $p< 0.05$ I-IGF-I vs control; AU= arbitrary units

As shown in the Table, the study groups had similar ages and BMIs, but as expected, the I-IGF-I group was considerably shorter and had higher serum IGF-I levels. After stimulating with IGF-I, we observed a reduced response for IGF-IR/Tyr AUC, IRS-1/Tyr AUC, AKT/Ser AUC and ERK 42/44 AUC, which reached statistical significance for AKT/Thr AUC in the I-IGF-I group compared to the control group. We also observed higher IRS-1/Ser AUC in the I-IGF-I children compared to controls, suggesting an inhibitory mechanism for IGF-I signal transduction, as has been suggested for insulin.
We conclude that some children with idiopathic short stature associated with serum GH and IGF-I levels in the high normal range, may show evidence of a derangement in intracellular signaling for IGF-I, which may be related to their growth retardation.(FONDECYT 1060784)

PO3-233 Perinatal Endocrinology

Adiponectin and resistin in human cord blood: relation to complicated pregnancies and anthropometric parameters at birth

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Introduction: Adiponectin and resistin is potent regulator of glucose homeostasis and energy metabolism. Previous studies on adiponectin and resistin in complicated pregnancies are still limited and contradictory. In this study we aimed to determine 1) the role of gestational diabetes (GDM) and pregnancy induced hypertension (PIH) on adiponectin and resistin in cord blood, 2) the association of the hormones with anthropometric parameters at birth.
Subjects and Methods: The study included 80 pregnant women (46 healthy pregnant women as control, 14 GDM, 20 PIH at 26-41 weeks of gestation; 36 male, and 44 female). Anthropometric measurements, including maternal weight, length, BMI, and neonatal birth weight, length and Ponderal index were performed. Cord blood samples were obtained from 80 neonates at the time of delivery. Plasma adiponectin levels (RIA) and resistin levels (ELISA) were measured.
Results: There was no significant difference in adiponectin and resistin levels between both sexes. Adiponectin levels were significantly lower in preterm and fullterm groups with GDM and fullterm group with PIH than control group. Resistin levels were significantly lower in preterm and fullterm group with PIH, and significantly higher in fullterm with GDM. Similarly adiponection was significantly lower in LGA than AGA and SGA, and resistin was significantly higher in LGA than SGA. Adiponectin levels were negative correlation with Ponderal index, maternal HbA1c, and BMI. Resistin levels were positively correlation with birth weight and maternal BMI.
Conclusion: Alteration of cord blood adiponectin and resistin in GDM, PIH, and LGA may influence to develop insulin resistance and increasing adiposity through neonate to adult life.

PO3-234 Perinatal Endocrinology

The role of IGF-I and ghrelin in the compensation of intrauterine growth retardation

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Objective: Intrauterine growth retardation (IUGR) is associated with a reprogramming of endocrine pathways that may influence growth and metabolism during later life. The aim of this study was to investigate the role and interplay of IGF-I and ghrelin in IUGR fetuses and neonates in comparison to appropriate for gestational age (AGA) ones, as well as in their mothers.

Design: Levels of IGF-I/IGFBP3, ghrelin, insulin and cortisol were measured in 20 singleton, full-term IUGR and 20 respective AGA infants at birth (UC), on days 1 (d1) and 4 (d4) of extrauterine life and in their mothers.

Methods: Serum IGF-I and IGFBP-3 were determined using two-site chemiluminescence immunoassays and plasma ghrelin levels using the commercial Ghrelin (Total) RIA kit. Insulin and cortisol were measured by the automated chemiluminescence system and electrochemiluminescence immunoassay, respectively.

Results: The ratios of IGF-I to birth-weight were higher in IUGR than in AGA group, at UC (18.2±1.2 vs.14.4±0.9, p=0.05) and d1 (9.6±0.5 vs.6.8±0.3, p=0.05). A significant positive correlation was found between IGF-I and ghrelin levels (r=0.639 p=0.002) and a negative one between IGFBP3 and ghrelin (r=-0.55 p=0.011) only in the IUGR group at d1. In both groups, fetal IGF-I levels negatively correlated with fetal cortisol levels (r=-0.57 p=0.012 in IUGR and r=-0.54 p=0.017 in AGA).

Conclusions: IUGR neonates demonstrate, in respect to their reduced body weight, a relative IGF-I increase, reminiscent of IGF-I resistance, in an attempt to drive energy towards survival on the expense of growth. The observed positive and negative correlations between ghrelin and IGF-I/IGFBP3, respectively, in early postnatal life, indicate that ghrelin might play a role in the compensation of intrauterine undernutrition promoting postnatal growth. On the other hand, cortisol, a stress index, is negatively correlated to IGF-I levels and seems to exert a more pronounced inhibitory effect on IGF-I secretion in IUGR fetuses.

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Gender role behavior in children treated prenatally with dexamethasone

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Prenatal dexamethasone (DEX) treatment has shown adverse influence on brain architecture and behavior in several species. In Sweden, during 1985-1995, 40 fetuses at risk for CAH were treated with DEX to prevent virilization of affected females. We have previously reported that the children exposed to DEX during the first trimester have an impaired verbal working memory indicating that long-term follow up of this group of patients is of extreme importance.

In the present study, we report gender role related behavior, in the same cohort of children, compared to untreated healthy controls.

A short structured interview, The Gender Role Behavior Interview (GRBI), was designed and the psychometric properties of the interview were evaluated. The validity and reliability of the novel screening instrument were investigated in 180 school-age children who also completed the previously validated questionnaire Children´s Sex Role Inventory (CSRI).

A blinded scoring of behavior related to gender role, assessed with GRBI, was performed on results from 26 children that had been exposed to DEX prenatally and on results from 35 age- and sex matched controls. In addition, the two groups were compared on selected items measuring gender role related behavior and gender identity from the Child Behavior Check List.

The results indicate that the interview GRBI is a simple method to assess behavior related to gender role and the instrument can be used in follow-up studies of children that have been exposed to antenatal DEX therapy.

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Analysis of CDKN1C (p57Kip2) in Beckwith-Wiedemann syndrome (BWS) patients: new mutations and novel genotype-phenotype correlations

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Background: Beckwith Wiedemann syndrome (BWS) is a genetic overgrowth disorder characterized by macrosomia, macroglossia, visceromegaly, hemihypertrophy, abdominal wall defects, ear creases/pits, neonatal hypoglycaemia, polyhydramnios, placentomegaly, placental mesenchymal dysplasia, cardiac defects, nevus flammeus, and an increased frequency of embryonic tumours. This condition is caused by a variety of genetic or epigenetic alterations within two imprinting domains on human chromosome 11p15. Several genes in this imprinted cluster encode proteins involved in growth regulation, the paternally expressed (maternally imprinted) *IGF2* and the maternally expressed (paternally imprinted) cell-cycle regulator cyclin dependent kinase inhibitor (*CDKN1C*; also known as $p57^{KIP2}$) among others.

Patients and Methods: all patients with a diagnosis of BWS included in the Spanish Overgrowth Syndrome Registry were evaluated according to a laboratory screening protocol that included karyotype, FISH, MS-MLPA or Southern blot and microsatellite analysis of the chromosome 11p region as well as mutation screening of CDKN1C. Clinical data of the participating BWS children including personal and family history, clinical, laboratory and X-rays, MRI, and CT scan findings, pedigree, and follow-up evolution, were registered in a database.

Results: From a total of 96 BWS patients analysed, we identified seven children with mutations in *CDKN1C*. Six out of seven children inherited the mutation from their apparently asymptomatic mothers, and one mutation was de novo. Three out of the seven mothers developed preeclampsia/HELLP; remarkably all three preeclamptic mothers gave birth to children with BWS due to *CDKN1C* mutations predicted to generate truncated proteins forms. All patients had omphalocele, and most of them also displayed unusual findings in BWS such as polydactyly, cleft palate and hypospadias.

Comments: *CDKN1C* mutations should be firstly suspected in patients with BWS and unusual findings as well as in those BWS neonates born to preeclamptic mothers.

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Lower postnatal serum IGF-I levels in very preterm boys than in girls – association with morbidity

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Background: Reports on preterm children have shown a strong association between poor weight gain and low serum IGF-1 levels. This is consistent with our previous work showing that systemic levels of IGF-1 correlate with the

degree of retinopathy of prematurity (ROP). The time window postmenstrual age (PMA) 30-33 weeks seems important regardless of gestational age (GA) at birth, where male infants are more likely to have neonatal morbidity.
Objective: To investigate the relationship between gender, morbidity and mean IGF-1 level at PMA 30-33 weeks in infants born very preterm.
Methods: We investigated postnatal changes of IGF-1 in 98 infants (45 boys) in relationship to gender and morbidity. Infants were divided into three groups GA< 25 weeks, GA 25-27 weeks and GA 28-29 weeks.
Results: The mean serum IGF-1 level at PMA 30-33 weeks was significantly lower in boys ($p<0.001$) compared to girls born at GA <30 weeks.
In girls born before 28 weeks who developed bronchopulmonary dysplasia (BPD) we found no significant differences in mean IGF-1 levels with those who did not develop BPD. No girl born after GA 28 weeks developed BPD. All boys born before GA 25 weeks developed BPD. Mean IGF-1 levels in boys with BPD born at GA<30 weeks was significantly lower than in boys who did not developed BPD mean 20 ug/L and 28 ug/L, respectively ($p<0.001$).
In girls with proliferative ROP we found that mean IGF-1 levels were significantly lower in all GA (23-29 weeks) studied, mean 36 ug/L and 27 ug/L, respectively ($p<0.001$). All boys born before a GA of 25 weeks developed proliferative ROP. In boys with proliferative ROP with a GA of 25-29 weeks at birth we found that mean IGF-1 levels were significantly lower compared to those who did not develop the proliferative disease, mean 19 ug/L and 28 ug/L, respectively ($p<0.05$).
The mean IGF-1 levels at PMA 30 -33 weeks was significantly lower in boys with proliferative ROP compared to girls with proliferative ROP (20 ug/L and 27 ug/L, respectively, $p<0.05$) and in boys with BPD compared to with girls with BPD (19 ug/L and 30 ug/L, respectively $p<0.001$).
Conclusions: These findings suggest that in very preterm infants boys born preterm have lower postnatal IGF-1 levels than girls. The IGF-1 levels are even lower in infants developing morbidity associated with preterm birth. Thus we found a male disadvantage as opposed to a female advantage and that this may be associated with postnatal systemic IGF-1 levels.

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Cord blood levels of adipocytokines in AGA infants and SGA infants

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Introduction: Adipose tissue secretes a number of hormones called adipocytokines. Adiponectin (Ad) is a major member of adipocytokines, and is involved in glucose and lipid metabolism. Recent report shows high molecular weight form of Ad (HMW-Ad) is the most potent for this action. However, cord blood levels of Ad, particularly HMW-Ad, remain unrevealed.
Aim & Material: To clarify the roles of adipocytokines in neonates, we investigated the cord vein blood levels of HMW-Ad, total Ad (T-Ad), the ratio of HMW-Ad per T-Ad (HMW-Ad/T-Ad) and leptin in 86 neonates (AGA infants:67, SGA infants:19). They were classified into three consecutive age groups [extra preterm (gestational age: 25-27 weeks), preterm (28-36 weeks) and term infants (37-41 weeks)].
Results: The cord vein HMW-Ad levels in term AGA infants (8.6 ± 4.7 μg/ml) were significantly higher than those in preterm AGA infants (3.7 ± 3.5 μg/ml). The cord vein HMW-Ad, T-Ad, HMW-Ad/T-Ad and leptin levels were positively correlated with birth weight and gestational age. However, the cord vein levels of these peptides did not show any significant differences between AGA and SGA infants whose birth weight were same. There are not any significant differences in these peptide levels between in males and in females.
Conclusions: Our study showed that the T-Ad, HMW-Ad, HMW-Ad/T–Ad ratio and leptin levels correlated with not gestational age but birth weight, because cord vein levels of these adipocytokines did not show any difference between AGA and SGA infants who had same birth weight.

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First trimester glycaemia influences birth weight

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Gestational diabetes (GDM) occurs in 2-3% of pregnancies and conveys an increased mortality and morbidity for the fetus. The recent HAPO study demonstrated a continuous risk for macrosomia through the normal range of fasting blood glucose (BG) concentrations at 24 weeks of gestation. We have also demonstrated that fasting BG at 10 weeks of gestation predicts the development of pregnancy induced hypertension and GDM. To determine the impact of early gestation hyperglycaemic status on size at birth we related fasting BG concentration at 10 weeks of gestation to birth weight in 1650 Caucasian singleton pregnancies delivered at term.
Fasting BG was measured at 10 weeks of gestation. Birth weight was recorded as a standard deviation score (SDS). Macrosomia was defined as birth weight SDS > 2. Factors known to influence birth weight such as cigarette smoking and already diagnosed diabetes mellitus were excluded. Results were expressed as Odds Ratios (OR) at different quintiles of BG with and without adjustment for parity. Analysis was based on 1328 complete data sets from a total of 1484 live births.

Odds Ratios for Macrosomia related to Blood Glucose and Parity

Blood Glucose (mmol/l)	N	All mothers		Para ≥ 1	
		OR (95% CI)	p	OR (95% CI)	p
< 4.7	788	1		1	
4.7-5.3	268	2.1 (1.1-4.3)		2.1 (0.9-5.0)	
5.4-6.0	142	2.3 (1.0-5.3)		2.5 (0.9-6.5)	
6.1-6.8	50	1.0 (0.9-1.1)		1.0 (0.9-1.2)	
>6.8	80	3.1 (1.2-8.0)	0.02	4.0 (1.4-11.1)	0.01

These data demonstrate that a fasting BG greater than 6.8 mmol/l at 10 weeks of gestation is associated with a three fold likelihood of a macrosomic baby and accounts for 27% of all the macrosomic babies born (14 of 52). This effect was observed predominantly in multiparous women

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Parity is associated with an increased maternal body mass index, higher blood glucose and insulin at 10 weeks of gestation

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Gestational diabetes (GDM) occurs in 2-3% of pregnancies and is associated with fetal macrosomia. In assessing the impact of maternal fasting blood glucose (BG) concentration on the risk of macrosomia we have demonstrated that multiparity was associated with an increased risk of macrosomia when maternal fasting BG at 10 weeks of gestation was greater than 6.8 mmol/l. To explore this further we have determined the effect of parity on a number of measures of body composition and glucose homeostasis in 1650 Caucasian mothers carrying a singleton pregnancy at 10 weeks of gestation.
Fasting BG and serum insulin concentrations were measured at 10 weeks of gestation and height and weight recorded. Body mass index (BMI) and HOMA measures of beta cell function (B) and insulin sensitivity (R) derived. Parity was rated as nil, one, two and three or greater.
Mothers who carried 3 or more pregnancies were heavier and shorter than those having their first pregnancy. Fasting BG and serum insulin concentrations were highest in the multiparous group as were measures of HOMA-B and HOMA-R.

Anthropometric and Glucose Homeostasis Measures Related to Parity					
Parity	BMI (kg/m2)	Blood Glucose (mmol/l)	Serum Insulin (mU/l)	HOMA-B (%)	HOMA-R
0	23.3 ± 0.1	4.7 ± 0.03	13.2 ± 0.5	408 ± 28	2.9 ± 0.1
1	23.3 ± 0.2	4.8 ± 0.9	13.2 ± 0.7	439 ± 40	3.0 ± 0.2
2	25.3 ± 0.5	4.7 ± 0.7	14.5 ± 1.4	457 ± 67	3.2 ± 0.4
3+	26.2 ± 0.5	5.0 ± 0.9	17.5 ± 1.7	692 ± 116	4.2 ± 0.5
p value	< 0.001	< 0.001	0.03	0.01	0.01

Increasing parity is associated with a higher fasting BG and serum insulin. It is unclear whether this results from an increase in BMI and insulin resistance in these mothers a question that can only be resolved by longitudinal studies

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Growth patterns in children born after ICSI in the first five years of life – results of one controlled and one uncontrolled prospective survey in Germany

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It is well known that children born after assissted reproduction (ART) have a worse neonatal outcome than spontaneously conceived children, with a higher risk of a small for gestational age (SGA) birth or premature birth. The risk of imprinting disorders that affect growth is also higher in ART children. However, there is little longitudinal data on the physical development of ART children.

Objective: Our objective was to assess the growth patterns of children born after intracytoplasmatic sperm injection (ICSI) in comparision to spontaneously conceived (SC) children longitudinal from birth up to age 5.5 years. In addtion we wanted to analyse the influence of a low birth weight and multiple pregnancies in a cohort of ICSI children.

Design and Setting: Prospecive controlled follow-up study and additionally assessement of an uncontrolled cohort of ICSI children

Participants: Participants included 276 term-born singletons conceived by ICSI and 273 matched SC singletons at the age of 5.5 years. An uncontrolled cohort of 1142 ICSI children including 480 children from multiple gestations and 122 SGA children was analysed separately.

Main outcome meassures: Height and weight were assessed longitudinal at 8 different time points from birth up to age 5.5 years.

Results: Overall, the ICSI conception did not influence the children's height. However, at age 5.5 years ICSI boys were significantly taller than their controls. The development of the BMI was significantly influenced by the mode of conception with a lower BMI-SDS in ICSI children up to age 5.5 years. In the second cohort of 1142 ICSI children being born from a multiple gestation and SGA birth significantly (independently) influenced the child's growth up to age 4 years. The BMI-SDS was significantly influenced by SGA birth.

Conclusions: These are the largest cohorts of ART children which have been studied continuously up to the age of 5.5 years with regard to the physical development. Overall, ICSI children show a normal physical development. However, there are differences in the growth pattern and BMI that have to be followed closely. Possible mechanisms that might be responsible for differences in the physical development of ART children include programmed endocrine changes due to the IVF process, epigenetic changes and the parents' subfertility.

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Severe neonatal insulin-resistance caused by compound heterozygous mutations in the insulin receptor gene

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Mutations in the insulin receptor gene lead to a variety of insulin-resistance syndromes. Their clinical expression includes severe neonatal disease, known as leprechaunism, as well as milder forms such as Rabson-Mendenhall or insulin-resistance type A syndromes.

Case report: The patient was the first son of young, non-related parents, born after a 41 weeks pregnancy with intrauterine growth retardation. Birth weight was 2440g (-2.1SD) and birth length 47cm (-2SD). His mother had hypothyroidism and sensorineural deafness.

At physical examination he showed depressed nasal bridge, hirsutism, acanthosis nigricans around the neck, armpits, navel and scrotum. He had a relatively short thoracic box and abdominal distension with hepatomegaly of 4 cms.

Since birth he presented frequent hypoglycaemias, requiring intravenous glucose infusion. From his 5th day of life, postprandial hyperglycemias were detected and insulin therapy was started at maximal dose 3.3 UI/kg/h. At 52 days, he restarted having fasting hypoglycaemias, and insulin was removed. Since then, the patient achieved adequate glycaemic control with regular feeding every two hours.

Hormonal determinations				
Age	Insulin (µUI/ml)	c-peptide (ng/ml)	Hb A1C (%)	IGF-1 (ng/ml)
4 days	850	-	2,4	<25
2,5 months	1749	28,4	5,5	36,2
4,5 months	2267	86,7	6,1	<25
12 months	1036	59,2	5,6	<25

Parents had normal glucose, insulin and c-peptide responses at the oral glucose tolerance test. During follow-up he maintained excellent glycaemic control with only scarce and moderate (non <50 mg/dl) hypoglycemias. His growth was adequate (-1,8SD) to mid-parental height (-1,5SD). Developmental milestones were properly achieved excepting for a mild motoric delay. At the age of 17 months, while dealing with an airway infection, he passed away from respiratory failure.

Genetic study: The 22 exons and introexonic boundaries of the *INSR* gene were PCR-amplified and directly sequenced. Two compound heterozygous mutations within the tirosin-kinase domain of the insulin-receptor were identified [R1026X and D1176GfsX8].

Conclusions: Neonatal onset and habitus of the patient and severity of *INSR* mutations identified lead to diagnosis leprechaunism. Neonatal forms of insulin-resistance need individualized therapy and close glycaemic monitoring. Hypoglycemias may appear as the only symptom of insulin-resistance during neonatal and infancy stages. These patients usually die in infancy as a result of malnutrition and recurrent infections.

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C/EBPβ and 11β-HSD1 mediate surfactant protein production in lung epithelial cells by dexamethasone

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Introduction: Pulmonary surfactant is a complex mixture of phospholipids and proteins produced and secreted by type II alveolar epithelial cells. Surfactant is secreted into the lumen of the pulmonary alveolus, where surfactant phospholipids and proteins act to reduce surface tension and prevent alveolar collapse. In prematurely born infants, inadequate surfactant synthesis can result in respiratory distress syndrome, a major cause of neonatal morbidity and mortality. The production of the protein and phospholipid components of pulmonary surfactant is regulated by natural and synthetic glucocorticoids

(GCs). Previously the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors and glucocorticoid amplifying enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD1) have been postulated to serve a role in surfactant production. Therefore the aim of this study was to investigate how surfactant protein production is achieved by glucocorticoid with C/EBP and 11β-HSD1.
Material and Methods: A549 cells (human lung epithelial cells) were seeded at 1.5 x 10^5 cells per well of six-well plate and cells were treated by dexamethasone (10^{-6}M) for 24 h. RNA was extracted and RNA levels were quantified by real-time PCR.
Results: Dexamethasone increased surfactant protein B (SP-B), 11β-HSD1 and C/EBPβ mRNA in A549 cells. The increase of C/EBPβ mRNA preceded the induction of SP-B and 11β-HSD1 mRNA. Cycloheximide (CHX) abolished the induction of SP-B and 11β-HSD1 mRNA by dexamethasone, whereas the induction of C/EBPβ mRNA was not affected by CHX. C/EBPβ siRNA abolished the induction of 11β-HSD1 mRNA by dexamethasone.
Conclusions: Increased 11β-HSD1 mRNA with C/EBPβ activation could amplify glucocorticoid action within cells, which would facilitate the local generation of SP-B mRNA in lung cells. Present data suggest that activation of both C/EBP and 11β-HSD1 accelerate the transcription of SP-B mRNA, consistent with previous studies showing that knock out mice of C/EBP and 11β-HSD1 resulted in failure of lung maturation. Further studies and understanding of the mechanism between C/EBP and 11β-HSD1 in surfactant proteins production may contribute to treatment of GC for neonatal respiratory syndrome.

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Partial paternal uniparental disomy of chromosome 6 in monozygotic twins with transient neonatal diabetes mellitus

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Transient neonatal diabetes mellitus (TNDM) is a rare disorder, in which insulin-requiring hyperglycemia usually develops within first months of age and diabetes recovers at a median age of 3 months with possible relapse to permanent diabetes in later childhood. Intrauterine growth retardation and macroglossia are other characteristic features. About 70% of TNDM patients are caused by imprinting defects with paternal uniparental disomy of chromosome 6 (pUPD(6)), paternal duplications in 6q24, or loss of maternal methylation at the *PLAGL1* locus in 6q24.
The subjects of the study are a pair of female monozygotic twins. No one in the family was reported to develop diabetes mellitus. The twins were born at 29 weeks of gestation as appropriate for gestational age. They had macroglossia. They received mechanical ventilation for a few days at birth because of respiratory distress syndrome. Their serum glucose levels were around 400mg/dl on 2 days after birth. A continuous insulin infusion with about 0.3U/kg/day began since then. At 19 days of age, serum glucose level decreased gradually, and the older sister and the younger sister remained euglycemia without insulin therapy from 33 and 21 days of age, respectively.
Genomic DNA was extracted from peripheral blood of the twins and both parents. DNA profiling using PCR amplification was performed for polymorphic microsatellite markers from the ABI Prism Linkage Mapping Set version 2. PCR products were electrophoresed by the ABI Prism 310 Genetic Analyzer and analyzed using the GeneScan.
Microsatellite analysis confirmed they were monozygotic. Six informative markers (D6S1574, D6S309, D6S470, D6S422, D6S1610, D6S462), which map to from 6p24 to 6q22.1, showed normal biparental inheritance. However, the five distal informative markers (D6S1569, D6S1577, D6S264, D6S1697, D6S281), which map to from 6q22.3 to the 6q telomeric region, showed absence of a maternal allele and presence of a single paternal allele, indicating paternal isodisomy for this regions.
This is the first description of monozygotic twins with TNDM resulting from partial pUPD(6) involving the distal portion of 6q, from 6q22.3-qter. This suggests somatic recombination occurring at a very early stage of life. This also supports a hypothesis for a meiotic/mitotic mechanism because the monozygotic twins manifested same phenotype. This is an important finding for understanding the mechanisms and time of formation of partial uniparental disomy.

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Results of a collaborative study on generally usable 17OHP reference values for CAH-screening initiated by the International Society for Neonatal Screening (ISNS)

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Assay methods for the determination of 17OHP from filter paper specimens collected in newborn screening programs, have for some time been marred by relatively poor specificity and sensitivity, causing an excessive number of recalls, particularly in premature babies. Beginning of 2008 a new version of the most used 17OHP assay was made available, whose sensitivity and specificity characteristics were markedly improved.
The ISNS took the opportunity to organize a collaborative study with the aim of generating widely useable reference values for the new method and to make these available to all users of this method.
The data were collected from 16 newborn screening laboratories based in Belgium, France, Germany, Israel, Luxembourg, Netherlands, Spain, Switzerland and USA. Inclusion criteria were: Results from routine testing only; sampling age <28 days. Exclusion criteria: proven CAH samples. During the study period January to October 2008 more than 100'000 data sets were collected consisting of: age at sampling (Age), birth weight (BW), gestational age (GA), gender and the value of 17OHP in nmol/L Blood.
Results: Reference value tables were generated for GA from 24 to 42 weeks and for Age from 1 to 21 days. The cut-offs were tested in several screening laboratories and were able to significantly reduce the number of false positive results. Factors influencing the 17OHP values in neonatal screening samples: 1) a highly significant effect of GA, Age, BW and gender on 17OHP; the strongest effect has GA, followed by Age and gender; the contribution of BW, when GA is known, is small. 2) in healthy term babies (≥37 GA) the only significant factor influencing is Age while for preterm babies GA, together with Age, has the strongest influence.
Our study show that it is possible to obtain generally usable reference values for a CAH screening assay; this will help many screening programs in the transition to the new method and avoid the need to run lengthy and costly local studies.
Once more the stronger influence of GA and Age on 17OHP values is demonstrated. It is important to note that the knowledge of GA is only required for interpreting the results in premature babies.

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Protracted hypoglycemia in an infant of a diabetic mother

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Transient hypoglycemia occurs in up to 40 percent of infants of a diabetic mother (IDM). Hypoglycemia typically begins in the first few hours of life and resolves quickly; but it may rarely persist up to seven days. In mild cases, early feeding is usually sufficient to maintain euglycemia, but management with i.v. glucose is sometimes necessary when hypoglycemia is severe. We report protracted hypoglycemia in a full term infant born at 4.4 kilograms to a mother with gestational diabetes. There was no perinatal asphyxia. Hypoglycemia occurred at 2 hours of life, but persisted. Hyperinsulinemia was established by determination of abnormally elevated plasma insulin (117 mIu/ml) and C-peptide (11 ng/ml), but normal serum ammonia (20 umol/L) when plasma glucose (40 mg/dl) was markedly subnormal. Maintenance of plasma glucose required both oral feeding and infusion of i.v. glucose up to 16 mg/kg/min. Attempts to lower the rate of glucose infusion resulted in recurrent hypoglycemia. Persistence of hypoglycemia into the seventh day of life raised the possibility of nesidioblastosis, and diazoxide (7.5 mg/kg/day orally) was initiated. Serum

glucose normalized; i.v. glucose infusion was lowered slowly; and diazoxide discontinued at the twelfth day of life. The resolution of hypoglycemia by 12 days indicated that the hypoglycemia was infact related to gestational diabetes rather than to nesidioblastosis. This case underscores several important considerations in the management of hypoglycemia in an IDM. In typical affected infants, hypoglycemia results from a transient elevation of serum insulin. However,in some cases, such as the one in this report, hypoglycemia may be persistent and severe. Hyperinsulinemic hypoglycemia carries a significant risk for brain damage, particularly when it occurs in the newborn period. Hence, careful monitoring and management of hypoglycemia in an IDM is critical even though the hypoglycemia is expected to be mild and short lasting. Finally other causes of hypoglycemia must be considered if the course is not typical for the hypoglycemia in an IDM, even though there is a clear history of gestational diabetes.

PO3-247 Perinatal Endocrinology

Correlations between pre- and postnatal measurements of penile and clitoral size – a pilot study

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Ultrasound examination, usually at mid-gestation, is routinely used in standard clinical obstetric practice for the prenatal detection of various syndromes, major malformations and fetal growth, as well as the determination of fetal sex and detection of external genitalia anomalies. A reference range for prenatal penile length in relation to gestation age has recently been established and it led to frequent incorporation of the prenatal diagnosis of micropenis or clitoromegaly. Findings outside the normal range often cause parental anxiety, lead to further evaluation and sometimes to pregnancy termination. Comparisons of pre- and postnatal penile and clitoral size are lacking.

Objective: To correlate pre- and postnatal measurement of penile and clitoral width and length.

Methods: Fetal penile and clitoral width and length were measured by high-resolution ultrasonography. Postnatal measurements were carried out during the first postnatal week. Correlation between pre- and postnatal measurements were calculated by the Pearson correlation test.

Results: Paired pre- and postnatal measurements were performed in 31 males and 24 females. The correlations between measurements of fetal penile and clitoral length and width and week of gestation were highly significant ($p \leq 0.001$). Correlation between gestation age at delivery and penile length was marginally significant ($p=0.051$). Correlations between fetal and postnatal penile and clitoral length and width were not significant.

Conclusions: The lack of correlations between pre- and postnatal measurements suggests that prenatal findings of micropenis or clitoromegaly are not reliable indicators of postnatal measurements. This uncertainty mandates exercising caution in parent counseling.

PO3-248 Perinatal Programming

Impact of maternal malaria and blood pressure on aortic pulse wave velocity at birth in Nigerian infants: 'The Ibadan childhood growth and vascular health study'

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Hypertensive heart disease (HD) is an increasingly serious health issue in sub-Saharan Africa. Malaria in those countries is endemic and commoner among pregnant than non-pregnant women with significant consequences to maternal and infant health. We have established a birth cohort in Nigeria to evaluate whether maternal and child malaria may be early risk factors for hypertensive HD. Blood pressure (BP) are being assessed from birth through childhood. However in order to obtain indices of blood vessel status, we are measuring aortic pulse wave velocity (aPWV), as a measure of vascular structure, function and distensibility.

Objectives: To examine the impact of maternal malaria and BP on aPWV at birth in Nigerian infants.

Methods: Healthy women with singleton pregnancies were recruited and followed to delivery at Adeoyo Maternity Hospital, Ibadan, Nigeria. Measures of anthropometry, resting BP and blood film for malaria parasite density were obtained in the women in addition to anthropometry, BP and aPWV on their babies at birth. Data were analysed using Student t test. To assess the determinants of neonatal PWV both univariate and multivariate linear regression analysis were performed.

Results: A total of 178 mother-baby pairs were included. Mean birth weight and length were 2.9 (0.4) kg and 48.5 (2.3) cm and mean BP was 71(12.6) / 36(8.3) mmHg. The mean aPWV was (6.1 (2.9) m/s. There were no significant differences in the mean aPWV of babies of women with malaria and those without malaria (t=1.162, p=0.247).

We have previously shown that maternal SBP is inversely related to neonatal aPWV (Koudsi et al Hypertension 2007). This relationship was not found in the whole cohort nor in infants of mothers with or without malaria. However in babies whose BP fell in the same range as that reported by Koudsi et al, i.e. (65≤SBP<75mmHg), maternal height (r=+0.36, p=0.009), SBP (r=+0.37, p=0.007) and DBP (r=+0.39, p=0.004) were significant negative predictors of neonatal aPWV as well as neonatal DBP (r=+0.33, p=0.015), pulse pressure (r=+0.41, p=0.002) and heart rate (r=+0.35, p=0.011).

In a multivariate regression model, maternal height, SBP and neonatal pulse pressure accounted for 37% of the total aPWV variation.

Conclusions: In this cohort, maternal malaria in pregnancy had no effect on aPWV at birth. We did however confirm the inverse relationship between maternal BP and neonatal aPWV in a subset of babies. We are continuing to monitor aPWV postnatally.

PO3-249 Perinatal Programming

Impact of malaria in pregnancy on birth size and blood pressure in Nigerian infants: 'The Ibadan childhood growth and vascular health study'

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Hypertensive heart disease (HD) is an increasingly serious health issue in sub-Saharan Africa. Malaria in those countries is endemic and commoner among pregnant than non-pregnant women with significant consequences to maternal and infant health. We have established a birth cohort in Nigeria to evaluate whether maternal malaria may be an early risk factor for hypertensive HD.

Objective: To assess the effects of maternal malaria on birth size and blood pressure (BP) in Nigerian infants.

Methods: Healthy women with singleton pregnancies were followed to delivery at Adeoyo Maternity Hospital, Ibadan. Measures of anthropometry, resting BP, blood film for malaria parasites and haematocrit were obtained in 436 women and their babies at birth. Malaria was classified into mild (<1000/µl), moderate (1000-10000/µl) and severe (>10000/µl) based on parasite density through pregnancy and at delivery. Data were analyzed using t-test and analysis

of variance.
Results: Prevalence of malaria parasitaemia among the women was 48.4%. Maternal malaria was significantly associated with younger maternal age (27.7 vs 29.4 years, p<0.001) and being primigravid (p=0.02). Mean haematocrit of women with malaria was significantly lower than those without (31.5 vs 32.2%, p=0. 003).
The birth weight (p=0.005), length (p=0.001), head circumference (p<0.001) and skinfolds (p=0.03) of infants born to women with moderate to severe malaria were significantly smaller than those without malaria.
There were no differences in the mean systolic BP (SBP) (p=0.08) and diastolic BP (DBP) (p=0.44) of babies of women with moderate to severe malaria and those without malaria. However BP is size-dependent. Both SBP and DBP adjusted for weight were significantly higher in babies whose mothers had moderate to severe malaria compared to those with mild malaria.

Table: Infant Blood pressure adjusted by birth weight by Malarial Status

Parameter	Malaria	Mean	Difference	p-value
SBP/weight	Moderate/severe	25.7		
	Mild	24.0	1.7	0.024*
	None	24.8	0.9	0.185
DBP/weight	Moderate/severe	13.4		
	Mild	12.0	1.4	0.006*
	None	12.6	0.8	0.105

Conclusions: There is a high incidence of malaria and anaemia in this apparently healthy cohort of pregnant women associated with shorter, smaller and thinner babies at birth. The weight-adjusted SBP and DBP were highest in those babies whose mothers had a high malarial load. This observation is now being followed into infancy.

PO3-250 Perinatal Programming

Mitochondria: a placental target for leptin?

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Background- In-utero malnutrition is associated with later metabolic and cardiovascular complications but underlying mechanisms are not clear. In pregnant rats, malnutrition induces fetal metabolic programming in offspring, which was shown to be reversed by maternal leptin treatment. During pregnancy, leptin is secreted by the adipose tissue (of the mother and the fetus), and also by the placenta. In addition, leptin receptors were found in the human placenta on its maternal side. Thus, we hypothesized that leptin could act on the placenta to protect the fetus from malnutrition and metabolic programming. As leptin is known to play a role in regulating the efficiency of oxidative phosphorylation in mitochondria in rat liver, we studied the effect of leptin on mitochondria function in human choriocarcinoma cell lines.
Methods- Jar cells of human choriocarcinoma were cultured. The cells were treated with recombinant leptin (0, 10, 50 and 100 ng/mL) during 24, 48 or 72 hours. Respiration rates were measured in intact Jar cells with the Clarke-type oxygen electrode (routine respiration, non-phosphorylating respiration, uncoupling respiration, non-mitochondrial respiration). Determination of enzymatic activities was performed on cell lysates with the Beckman spectrophotometer (respiratory complexes I, II, III and IV and citrate synthase). Mitochondrial DNA (mtDNA) was quantified in each case by long-PCR. Real-time PCR quantified the rates of GLUT1 and GLUT3 mRNA in these cells with or without leptin.
Results- In the culture media, leptin was undetectable. The addition of recombinant leptin induced a significant decrease in enzymatic activities of respiratory complexes I and IV at 48 and 72 hours (p<0.05) while citrate synthase and respiration rates were comparable. MtDNA quantification was unchanged by leptin treatment. GLUT1 and GLUT3 mRNA rates were similar.
Conclusion- Leptin on choriocarcinoma cell lines decreases enzymatic activities of complexes I and IV in electron transport system in mitochondria. These results suggest that leptin may reduce the placental own energy expenditure in favour to the fetus.

PO3-251 Perinatal Programming

Are lipoprotein profiles in cord serum predictive of those in adulthood?

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Introduction:
Serum lipoprotein profiles in children are predictive of those in adulthood, and there is evidence that this association could begin at birth. Very few studies have used neonatal lipoprotein values as baseline values in follow up studies.
Objectives:
Search for predictive factors of adulthood serum lipoprotein profiles in cord serum.
Patients and methods:
Lipoprotein profiles in cord serum of 702 newborns were analyzed. Data for gestational age, birth weight and birth length were collected. Seventy of these patients were recruited for blood sampling and serum lipid profile determination. Spearman´s rank correlations were used to examine tracking of serum lipid. Stepwise multiple regression was performed to identify the best explicative variables of pathologic adulthood profiles (high total cholesterol (TC) and high low density lipoprotein cholesterol (LDLc)).
Results:
Correlations were significant for TC (r=0,28; p<0,05) and LDLc (r=0,47; p<0,001). The best explicative factors in cord serum were Apolipoprotein A and B100, and TC/high density lipoprotein cholesterol ratio, considering birth length and weight, and gestational age.
Conclusion:
There is a correlation between lipid profiles in cord serum, and those in adulthood.
More studies are needed to better define the predictive value of cord serum lipoprotein profiles.

PO3-252 Perinatal Programming

Acylated and desacyl ghrelin concentrations in infants breast- or formula-fed with different protein levels: a randomized controlled trial from 0.5 to 12 months

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Dietary protein intake during infancy may impact long-term growth and body composition. Ghrelin circulates as acyl (AG) and desacyl (DG) forms. Administration of AG stimulates appetite. Both AG and DG decrease energy expenditure. Perinatal ghrelin regulation is unclear. Hypothesis: ghrelin concentrations are modulated by protein content and are positively associated with neonatal weight gain. Objective: to prospectively investigate the effect of breastfeeding (BF), low (1.8g protein/100 kcal; F1.8) or high protein (2.7g; F2.7) formula (FF) on ghrelin and insulin levels and on body mass. Methods: Healthy, full term newborns (53% male) were randomized at 1 wk of age to BF (n=84), F1.8 (n=74) or F2.7 (n=80). BF was diversified with F1.8 after 4 mo if needed. Weight and fat mass were measured at 0.5, 4, 6 (Pea-Pod) and 12 mo (DXA). Insulin and full-length AG and DG (double sandwich ab, Elisa, SPI-Bio) were determined at 0.5, 4 (30-45 min post meal) and 9 mo (1-2 hrs post meal). Statistics: Mixed model analysis; mean (SD); P<0.05. Results: Overall, AG and the % ghrelin that is acylated (%AG) increased in BF, F1.8 and F2.7 from 0.5 to 9 mo (P<0.01). %AG was higher in F2.7 vs F1.8 and BF (P<0.01). In contrast, DG increased with BF [+66 (158) pg/ml] but decreased in F1.8 [-64 (135)] and F2.7 [-55 (178)] between 0.5-4 mo. As a result, FF infants had lower DG levels at 4 mo compared to BF infants.

AG and DG ontogeny and nutrition in human neonates

	AG BF	AG F1.8	AG F2.7	DG BF	DG F1.8	DG F2.7
0.5 mo (n=206)	52 (25)	52 (29)	60 (25)	444 (133)	470 (141)	492 (159)
4 mo (n=202)	82 (37)	72 (36)	85 (46)	516 (172)	411 (136)*	437 (138)*
9 mo (n=175)	138 (79)	139 (83)	155 (89)	696 (335)	707 (321)	652 (245)

pg/ml; Mean (SD); *P<0.01 vs corresponding BF

At 4 mo, weight, fat mass and insulin were lower in BF vs F1.8 and F2.7 (P<0.03) but were similar at 9-12 mo in all 3 groups. There was a negative correlation between AG/DG and insulin at 9 mo (r<-0.24, P<0.01) and between AG/DG change and weight gain between 4-9 mo (r<-0.31, P<0.01) but not in younger infants. Conclusions: 1. The inverse correlation between changes in ghrelin and in weight suggests that ghrelin does not stimulate infant growth. 2. Feeding practice (FF vs BF) but not protein intake (F1.8 vs F2.7) influences DG levels at 4 mo while higher protein affects %AG in infants. We provide novel evidence that early nutrition may affect hormonal modulation during critical windows of development.

PO3-253 Perinatal Programming

Adverse body composition in adult life after perinatal glucocorticoid therapy in prematurely born individuals carrying the 363S variant of the N363S polymorphism in the glucocorticoid receptor gene

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Preterm birth is associated with abdominal fat accumulation, insulin resistance and hypertension, resembling increased glucocorticoid bioactivity. Antenatal or neonatal glucocorticoid therapy does not substantially contribute to these associations. It is unknown whether genetic variants in the glucocorticoid receptor gene could modulate the effect of perinatal glucocorticoid therapy on the above phenotype.
We tested the effects of perinatal glucocorticoid therapy in combination with the R23K and N363S variants, associated with decreased and increased sensitivity to cortisol respectively, on the adult metabolic profile in a birth cohort of men and women born <32 gestational weeks and followed prospectively from birth until 19 years of age. 249 Survivors of the Dutch Project On Preterm and Small-for-gestational-age infants (POPS) cohort underwent anthropometric assessment and blood pressure measurement, and venous blood was drawn for genotyping and determination of fasting glucose, insulin and cholesterol concentrations.
Waist circumference was 1.67 (95% CI: 0.79 to 2.55) SDS in 363S carriers who had been treated antenatally with glucocorticoids (363S/GC+), which was much higher than in the 363S/GC- (0.48 (-0.05 to 1.01) SDS), N363/GC+ (0.27 (-0.01 to 0.55) SDS) and N363/GC- (0.55 (0.41 to 0.69) SDS) groups (p for interaction =0.01). Similar associations were found for waist-to-hip ratio SDS (p =0.02) and fat mass (p =0.08). 363S Carriers who had been treated with glucocorticoids as neonates had a higher absolute (p =0.05) and relative fat mass (p =0.07) compared to the other 3 groups. No such associations were found with the R23K polymorphism.
We conclude that in prematurely born individuals carrying the 363S variant perinatal glucocorticoid therapy strongly predisposes to persistent metabolic adversities.

PO3-254 Perinatal Programming

Auxological and endocrine birth parameters in monozygotic twins with discordant birth weight and association with catch-up growth

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Objective: To investigate the value of birth weight, birth length, and endocrine cord blood parameters as predictors of subsequent catch-up growth in monozygotic twins with discordant birth weight.
Patients and Methods: 27 sets of monozygotic twins were investigated at birth and at the age of 4 years. All twin-pairs had discordant auxological birth parameters, in 13 twin-pairs inter-twin birth weight and/or birth length difference was of more than 1 SD. Cord blood and blood samples at the age of 4 years were analysed for IGF-I, IGF-II, IGFBP-2, ghrelin, leptin, and adiponectin. Correlation coefficients were analysed to detect associations between inter-twin differences (Δ) of birth weight and/or length as well as Δcord blood for endocrine parameters and auxological data (Δheight and ΔBMI) and endocrine parameters at the age of 4 years.
Results: At the age of 4 years 18 twin-pairs had a Δheight < 0.5 SD, 4 had an inter-twin difference for height between 0.5 and 1 SD, and five twin-pairs were still significantly discordant with a Δheight of more than 1 SD. Δbirth length but not Δbirth weight (r=0.01; NS) correlated positively with height at the age of 4 years (r=0.48; p<0.01). ΔIGF-I in cord blood correlated significantly to Δbirth length (r=0.7; p<0.0001) and to Δheight at 4 years (r=0.61;p<0.001). No other endocrine paramter measured in cord blood showed an association to height at the age of 4 years. There was a positive inter-twin correlation for IGF-I in cord blood (r=0.51; p<0.001) which was getting even stronger at the age of 4 years (r=0.87; p<0.0001).
Conclusion: Our data underline the importance of measuring birth length as a prognostic parameter for expected catch-up growth. A larger inter-twin birth length difference increased the risk for insufficient catch-up growth. In contrast, birth weight difference between genetically identical twins did not bear a prognostic value in view of attained height at 4 years. Furthermore, inter-twin difference in cord blood IGF-I was positively correlated to height at the age of 4 years and could be of prognostic value.

PO3-255 Perinatal Programming

Embryonic caffeine exposure induces adverse effects in adulthood on body composition and cardiac function

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PURPOSE: To determine both the short-term effects on cardiac development and embryo growth; and long-term effects on cardiac function and body composition of in utero caffeine exposure.
PROCEDURES: Pregnant mice (C57BL/6) were exposed to hypoxia (10% O2) or room air from embryonic days (E) 8.5-10.5, and treated with caffeine (20 mg/kg, i.p.) or vehicle (normal saline, 0.9% NaCl).
FINDINGS: Caffeine dose results in a circulating level that is equivalent to 2 cups of coffee in humans. Hypoxic exposure acutely reduced embryonic growth by 30%. Exposure to a single dose of caffeine inhibited cardiac ventricular development by 53% in hypoxia and 37% in room air. Caffeine exposure resulted in inhibition of hypoxia-induced HIF1-alpha protein expression in embryos by 40%. When offspring from dams treated with a single dose of caffeine were studied in adulthood, we observed that caffeine treatment alone resulted in a decrease in cardiac function of 38% as assessed by echocardiography. We also observed a 20% increase in body fat with male mice exposed to caffeine.
CONCLUSIONS: Exposure to a single dose of caffeine during embryogenesis results in both short-term effects on cardiac development and long-term effects on cardiac function and body composition.

PO3-256 Perinatal Programming

Newborn screening for congenital adrenal hyperplasia: cost-minimization study in public health program

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Objective: The main goal of newborn screening for Congenital Adrenal Hyperplasia (CAH) is to prevent infant death doe to adrenal insufficiency. All girls with the severe form had genital malformation and CAH should be suspected; boys, however, do not show physical hallmarks. This study determined the economic impact of newborn screening (NBS) for CAH in the state of Goiás-Brazil and proposed ethically sound alternatives methods for the entire country.
Design: The cost of universal NBS-CAH in the state Goiás-Brazil in 2005 was calculated. Modeling of these findings to the entire country (Brazil) was done and three hypothetical decision models were constructed.
Methods The Children was classified in low and high risk to be affected by CAH. Four models were constructed based on two choices: universal (both sex) or selective (only boys) and complete (low and high risk) or reduced (only high risk). The software TreeAge (Williamstown, Mass) was used and sensitivity analysis, calculated.

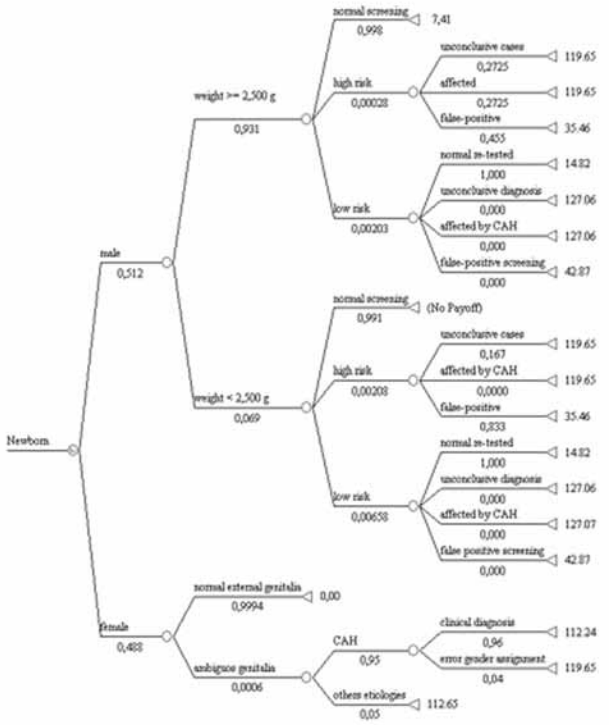

Figure 1:Decision tree for detecting CAH based on clinical signs for girl and NBS for boys. The probability for a particular outcome is indicated below the appropriate branch line and they was based in NBS-Goias-2005 for males and in expert opinion for females. The payoffs are assigned at every terminal node. Population Data bank of 82,603 newborns in the year 2005 in the state of Goiás-Brazil. Results The annual cost of universal complete NBS-CAH screening in Goiás amounted US$609,281, and the estimated annual cost of adding CAH to the newborn screening panel in Brazil (universal complete model) ranges from US$ 22,542,257 to US$ 22,997,121. Using the selective models allows 50% savings; the reduced models only add 0.5 % in savings.
Conclusion: The selective reduced model is the best choice because it detects the patients with risk of dying unrecognized and it represents expressive savings.

PO3-257 Perinatal Programming

The balance between estimated birth brain weight and birth body weight as a predictor of the ratio between circulating levels of insulin–like growth factor binding protein–2 and –3 in newborns free of life–threatening disease: role of gestational age

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The percentage of birth body weight (BW; unit:g) represented by estimated birth brain weight (BRW; unit:g)(BBR; unit:%) was evaluated as predictor of relations between blood serum levels of Insulin–Like Growth Factor (IGF) Binding Protein–2 and –3 (resp. IB2 and IB3) in a human newborn (NWB) sample with availability of the following data: gender (SEX), birth gestational age (GA; unit:completed week), BW, birth head circumference (HC; unit:cm), presence-absence of BW<10th centile for GA (SGA), postnatal age (PNA; unit:day), and IB2 and IB3 measured by radioimmunoassay (unit: microM 100 mL) in each NWB at one of the first 5 postnatal days (x), 5 days after x (y) and 10 days after x (z). 78 NWBs (male SEX, n=43; GA range=28-42; SGA, n=20; GA≤36 complete weeks, n=46; life–theratening disease and/or diabetes mellitus, n=0; mother with diabetes mellitus, n=0) were included in the study. The natural logarithm of IB2 (lnIB2) and IB3 (lnIB3) was calculated. Ratios of lnIB2 to chronologically corresponding lnIB3 (lnIB2dIB3) resulted near-normally distributes. BBR was calculated as follows: " $BBR = 100 \times [(0.037 \times HC^{2.57})\ BW]$ " according to Lindley AA, et al., American Journal of Epidemiology. 2000;152(3):219-225 (BBR range=7.805-16.831). BBR relations to lnIB2dIB3 at x, y and z were evaluated after adjustment by Multiple Linear Regression (MLR) 1) for SEX, SGA and PNA at x (PNAx)(Table 1A), or 2) for SEX, SGA, PNAx and GA (Table 1B)(computations; male SEX, SGA, condition present=1, condition absent=0). Table 1 shows t and r regarding each partial correlation of BBR with lnIB2dIB3 at x, y and z, R2 and F of each MLR model, and corresponding significances (a, p<.05; b, p<.0001; ns, not significant).

Table 1	A)			B)		
vs	lnIB2dIB3x	lnIB2dIB3y	lnIB2dIB3z	lnIB2dIB3x	lnIB2dIB3y	lnIB2dIB3z
BBR, t	2.256a	5.395b	5.748b	1.196ns	1.734ns	1.422ns
BBR, r	.255	.534	.558	.140	.200	.165
R2	.351	.361	.354	.354	.449	.499
F	9.889b	10.311b	10.004b	7.906b	11.744b	14.333b

GA differences may be involved in positive BBR relations to lnIB2dIB3 observed in not life–threatened NWBs after control for SEX, SGA and PNA.

PO3-258 Perinatal Programming

The percentage of birth body weight represented by estimated birth brain weight: predictor effects on circulating insulin–like growth factor (IGF)–I and IGF–II, and on the balance between circulating IGF–I and IGF–II in newborns free of life–threatening disease

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The percentage of birth body weight (BW; unit:g) represented by estimated birth brain weight (BRW; unit:g)(BBR; unit:%) was evaluated as predictor of blood serum Insulin–Like Growth Factor (IGF)–I (IG1) and of IG1 relations to blood serum IGF–II (IG2) in a human newborn (NWB) sample (n=78) with availability of the following data: gender (SEX), birth gestational age (GA; unit:complete week), BW, birth head circumference (HC; unit:cm), presence–absence of BW<10th centile for GA (SGA), postnatal age (PNA; unit:day), and IG1 and IG2 measured by radioimmunoassay in microM 100 mL in each NWB at one of the first 5 postnatal days (x), 5 days after x (y) and 10 days after x (z). 78 NWBs (male SEX, n=43; GA range=28–42; SGA, n=20; GA≤36 complete weeks, n=46; life–theratening disease and/or diabetes mellitus, n=0; mother with diabetes mellitus, n=0) were included in the study. The natural logarithm of IG1 (lnIG1) and of IG2 (lnIG2) was calculated. lnIG1, as well as the lnIG1 ratio to chronologically corresponding lnIG2 (lnIG1dIG2), resulted near-normally distributed. BBR was calculated as follows: " BR= 100 × [(0.037 × $HC^{2.57}$) BW] " according to Lindley AA, et al., American Journal of Epidemiology. 2000;152(3):219-225 (BBR range=7.805-16.831). BBR relations to lnIG1 and lnIG1dIG2 were evaluated with adjustment by Multiple Linear Regression (MLR) 1) for SEX, SGA and PNA at x (PNAx)(Table 1A), or 2) for SEX, SGA, PNAx and GA (Table 1B)(computations; male SEX, SGA, condition present=1, condition absent=0). Table 1 shows t and r of each partial correlation of BBR with lnIG1 and lnIG1dIG2 at x, y and z, R2 and F of each MLR model, and corresponding significances (a, p<.05; b, p<.005; c, p<.001; ns, not significant).

Table 1	A)			B)		
vs	lnIG1x	lnIG1y	lnIG1z	lnIG1x	lnIG1y	lnIG1z
BBR, t	-5.425c	-5.449c	-4.944c	-2.336a	-0.930ns	-0.942ns
BBR, r	-.536	-.538	-.501	-.265	-.109	-.110
R2	.351	.391	.286	.397	.552	.428
F	9.871c	11.732c	7.306c	9.494c	17.765c	10.792c
vs	lnIG1dIG2x	lnIG1dIG2y	lnIG1dIG2z	lnIG1dIG2x	lnIG1dIG2y	lnIG1dIG2z
BBR, t	-4.246c	-4.004c	-3.770c	-1.880ns	-0.824ns	-0.977ns
BBR, r	-.445	-.424	-.404	-.216	-.097	-.144
R2	.301	.261	.193	.328	.349	.262
F	7.855c	6.430c	4.362b	7.015c	7.732c	5.099b

BBR predictor effects on lnIG1 and lnIG1dIG2 observed after control for SEX, SGA and PNA in not life–threatened NWBs were apparently related to GA.

PO3-259 Perinatal Programming

Adiponectin in cord blood is associated with weight gain until the age of 4 years

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Objective: To investigate the impact of birth weight, and endocrine paramters in cord blood on subsequent weight development in monozygotic twins.
Patients and Methods: 27 sets of monozygotic twins were investigated at birth and at the age of 4 years. All had discordant birth weight, 13 had an inter-twin birth weight difference of more than 1 SD. Cord blood and blood samples obtained at the age of 4 years were analysed for endocrine parameters including adiponectin. Correlation coefficients were analysed to detect associations between inter-twin differences (Δ) of birth weight with the of the Δendocrine parameters and with ΔBMI after 6, 12, 24, and 48 months.
Results: Mean of Δ birth weight SDS between twin-pairs was 0.95 (range, 0.2 to 3.7), decreased to a mean of 0.6 at the age of 4 months (range, 0.5 to 4.3), but did not change significantly thereafter (mean ΔBMI at 6 months: 0.48; range, 0.58 to 3.36; mean ΔBMI at 12 months: 0.45; range, 0.86 to 2.23; mean ΔBMI at 4 years: 0.63; range, 0 to 3.11). In parallel, we observed catch-up in length in most of the twins during the first years of life. Mean for inter-twin difference of birth length SDS was 1.17 (range, 0.5 to 2.8), mean inter-twin difference for height SDS at 1 year: 0.49 (range, 0 to 2.61).
ΔBMI at the age of 4 years correlated positively with cord blood adiponectin (r=0.4; p<0.05)
Conclusion: In genetically identical twins, catch-up length was mainly observed during the first year of life. This was proceeded by a catch-up weight with the major changes of BMI occuring during the first four months of life. Twins with a greater inter-twin difference concerning weight and height at the age of 1 year showed no significant approximation thereafter. Only cord blood adiponectin, but none of the other endocrine cord blood parameters was of predictive value for subsequent weight development in monozygotic twins.

PO3-260 Perinatal Programming

The influence of the IGF system and ethnicity on growth, fat distribution and blood pressure: results for the first 4 years of life in the Manchester growth and vascular health study

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Small size at birth followed by rapid post-natal growth is a risk factor in the aetiological pathway to adult cardiovascular disease (CVD). However there are ethnicity related differences in the risk for developing later morbidities: in particular South Asians (SA) have high rates of diabetes and CVD worldwide when compared with Europeans (E).
We have established a birth cohort of British-born E and SA babies to identify predictive markers of vascular health in early life. In this report, the relationship between the IGF system and ethnicity on growth and blood pressure (BP) from birth to 4 years has been assessed.
210 children (142 E, 68 SA) were evaluated at one or more time points: birth, 3, 6, 12, 24, 36 and 48 months. At each visit growth measurements, BP and a blood sample were taken in a standardised manner with consent. Weight (Wt), Height (Ht) and Head circumference (Hc) measurements were converted to SDS. Skin-fold thickness (SFT) was used as the raw value in mms. IGF-I and IGFBP-3 were assayed by Immulite 2000. A mixed effects regression model using data at all time points was used to assess the overall association of time, ethnicity, sex, IGF-I and IGFBP-3 on the dependent variables Wt, Ht, Hc, SFT and BP.
Mean levels of IGF-I & IGFBP-3 in cord blood were 76µg/L & 1.9mg/L in E and 67µg/L & 1.7mg/L in SA respectively. Levels of IGF-I & IGFBP-3 had risen to 122µg/L & 4.4mg/L respectively in both E and SA by 3 years. Wt, Ht and Hc SDS were all influenced by ethnicity (smaller in SA, p<0.001, p=0.03, p<0.001 respectively) and Wt and Ht SDS were positively associated with IGF-I and IGFBP-3 (for Wt, p<0.001 for both, for Ht p=0.015 for both); however only Wt SDS was associated with IGF-I (p<0.001) and IGFBP-3 (p=0.006) when both were entered into the model. Subscapular but not triceps SFT was associated with sex (lower in males, p=0.004) and with IGF-I and IGFBP-3 (higher levels, greater SFT) when entered separately into the model but only by IGFBP-3 (p=0.001) when both were entered. Systolic BP was associated with ethnicity (lower in SA, p=0.02) and IGFBP-3 (higher levels, greater BP, p=0.05). No variables impacted on diastolic BP.The IGF system was most significantly associated with weight, with IGFBP-3 being associated with central but not peripheral fat. IGFBP-3 but not IGF-I was associated with systolic BP. Thus IGFBP-3 appears to be an important marker of body composition and BP in early life independent of ethnicity and IGF-I.

PO3-261 Perinatal Programming

Long-term effects of prenatal pesticide exposure on several endocrine functions in children

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Background
Maternal pesticide exposure may cause adverse effects in male genital development. Obesity and early onset of puberty have recently been proposed as yet

other adverse health effects of exposure to endocrine disrupting chemicals during critical stages of development.

Following the observation of a high prevalence of cryptorchidism, decreased penile length, testicular volumes, and reproductive hormone levels in three months old sons of greenhouse workers, a follow up study was undertaken to examine long-term effects on endocrine function in children prenatally exposed to modern pesticides.

Methods

Pregnant women working in greenhouses were categorized as exposed or unexposed to pesticides. At follow up at age 6 to 10 years 177 children (94 boys and 83 girls, 112 exposed, 65 unexposed) underwent a standardized examination including weight, height, calliper measurements of skinfolds, puberty staging (Tanner), testis volume by sonography, and blood sampling. Exposure status was unknown to the examiner.

Linear regression analysis was made on log-transformed variables adjusting for age and puberty. Fisher's exact test and binary logistic regression were used in analysis of breast development.

Results

Testicular volumes in prepubertal boys were significantly lower in the exposed than in the unexposed group (geometric means: 517 vs. 451 mm^3, p= 0.027). Accumulated prevalence of cryptorchidism (congenital + acquired) in the exposed group was 13.2 %. No cryptorchidism was found among unexposed boys. Exposed boys had higher BMI-z-scores, body fat percentage and larger skinfolds (beta= 0.241, p=0.016, and beta= 0.247, p= 0.015, beta=0.240, p= 0.017) as well as higher IGF-I z-scores and androstendione concentrations (beta=0.258 p=0.021, and beta= 0.243, p= 0.026) than the unexposed. Exposed girls had significantly higher serum concentrations of androstendione (beta=0.283, p= 0.021) and lower IGF-I z-scores (beta = -0.362, p= 0.005) than the unexposed. BMI z-scores were not significantly different between exposed and unexposed girls. Significantly more exposed girls had breast development (B2) than the unexposed (p= 0.039, age adjusted p=0.054). Among exposed girls younger than 8.5 years, 6/22 had B2, compared to 2/18 unexposed girls.

Conclusion

Our data support the hypothesis that early developmental exposure to currently used pesticides has gender dimorphic effects on reproductive development and body composition that may have implications for future health.

PO3-262 Perinatal Programming

Fatty acid metabolism is programmed toward lipogenesis in intrauterine growth restricted rat offspring

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Background: Intrauterine growth restriction (IUGR) leads to increased risk of adult obesity. Nutritional programming by maternal food restriction during rat pregnancy results in IUGR pups. By 3 weeks of age, young IUGR offspring develop hypertrophic adipocytes before development of hypertriglyceridemia and obesity, implicating adipose tissue as the primary source of these abnormalities. Therefore, fatty acid synthesis pathways are known targets for prevention and treatment of obesity.

Objective: To study the effects of nutritional programming on fatty acid metabolism in primary rat adipocyte cell cultures from IUGR pups compared to Controls at 3 weeks of age using stable isotope tracers.

Methods: Primary rat subcutaneous adipocytes were cultured from either IUGR pups or Controls at age 3 weeks. Undifferentiated cell cultures were exposed to medium with either 1) $U^{13}C_6$-glucose alone or 2) unlabeled glucose with $U^{13}C_{18}$-stearate for 24 hours. A second set of cultures underwent differentiation induction with MDI and then were exposed to medium with 1) $U^{13}C_6$-glucose alone or 2) unlabeled glucose with $U^{13}C_{18}$-stearate for 24 hours. Fatty acids were extracted and analyzed by gas chromatography/mass spectroscopy. The M4/M2 ratio was determined to measure ^{13}C enrichment in acetyl precursors and the fraction of new synthesis (FNS) determined from isotope incorporation.

Results: In undifferentiated cells cultured with $U^{13}C_6$-glucose, there was an increase in the M4/M2 ratio for palmitate, stearate, and oleate in adipocytes from IUGR versus Control. There was a trend toward an increase in the FNS of palmitate, the main product of de novo lipogenesis, in IUGR. In differentiated IUGR cell cultures with $U^{13}C_6$-glucose, there was an increase in the M4/M2 ratio for palmitate, and an increase in the FNS for palmitate and stearate. There was no difference between IUGR vs Controls in uptake of $U^{13}C_{18}$-stearate in either undifferentiated or differentiated cells.

Conclusions: The increased M4/M2 ratios in the IUGR cells indicate increased glucose utilization toward fatty acid synthesis in adipose tissue. The increased FNS indicate increased de novo synthesis of fatty acids in IUGR pups. Effects of nutritional programming toward lipogenesis in the fatty acid metabolic pathways are therefore already present in the subcutaneous adipose tissue of young IUGR pups prior to the onset obesity suggesting that the critical window for intervention in young pups is less than 3 weeks of age.

PO3-263 Reproductive Endocrinology

Alteration of the menstrual cycle in adolescence: early clinical manifestation of the metabolic syndrome?

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The relationship between menstrual cycle and Metabolic Syndrome (MS) is insulin resistance/hyperinsulinemia. The mitogenic effects of hyperinsulinemia may affect menstrual cycle through their direct action on ovaries, adrenal glands, hepatic production of SHBG, and on regulation of the feedback of the gonadotropins. These alterations may appear early, preceding metabolic alterations of MS. **Objective:** Evaluate menstrual cycle in teens who present risk factors for e MS. **Method:** Observational, comparative, and transversal-cut study with 37female adolescents between 12 and 19 years, and the presence of at least one of the following risk factors for MS; Overweight, Obesity, "*Acanthosis Nigricans"*, Polycystic Ovary Syndrome (POS).All the adolescents underwent a clinical evaluation, with gathering of anthropometric data, blood pressure, presence of "*Acanthosis Nigricans"*, and/or hyperandrogenism skin manifestations, evaluation of pubertal development, and laboratory evaluation composed of Lipidogram, evaluation of glycose and hormonal metabolism. Two groups were created according to the menstrual cycle pattern. G-1 adolescents with irregular cycles since menarche, G-2 with regular menstrual cycles since menarche. **Results:** 37 adolescents evaluated, 27 formed G-1 and 10 formed G-2. In the statistical analysis of the differences in clinical and laboratory variables between the groups, it was observed that G-1 presented insulin after TOTG120 (p =0,001), HOMA (p = 0,035) and Triglyceride (p = 0,028) higher and HDL (p = 0,039) lower than G-2. Analysis of the clinical/laboratory categorical variables : G1 with family history for Diabetes Mellitus (*p = 0,041), POS (p = 0,008)and MS (p* = 0,024) higher thanG2.**Conclusions:** The results demonstrate that IR/Hyperinsulinemia is the bridge between alterations in Menstrual cycle, MS and POS.In MS, due to its evolutionary character as well as environmental and genetic influences, metabolic alterations might not yet be laboratorially detectable in adolescence, but alterations of menstrual cycle certainly can. The clinical evaluation of adolescents should include an assessment of menstrual cycle, in which alterations in pattern might represent first sign of a systemic and evolutionary disease such as MS, whose clinical manifestation may occur only at adult age.Appreciationof alterations in the menstrual cycle in adolescence can be an opportunity for an early diagnosis of MS and the beginning of effective preventive actions.

PO3-264 Reproductive Endocrinology

Transabdominal ultrasound (TAUS) in perimenarchal adolescents with clinical features of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is increasingly appreciated in adolescents. Yet, diagnostic criteria for this group remain controversial; in particular, the utility of transabdominal ultrasound (TAUS). We examined TAUS as it relates to other markers of PCOS, like hyperandrogenism, in girls with clinical complaints of menstrual irregularity and/or acne.

Methods: Cross-sectional analysis of 47 treatment naïve adolescents enrolled into an ongoing cohort study from our multi-specialty adolescent PCOS program. TAUS was read by a single radiologist, blinded to clinical information. Readings were divided in three groups: NAO (normal appearing ovaries), CPO (classic polycystic ovaries: bilateral ovarian volumes >10 cm^3 and multiple peripheral follicles with no dominant follicle >1.6 cm or large cysts) and VPO (variant polycystic ovaries: unilateral polycystic-appearing ovary, other ovary normal or with cysts > 1.6 cm). Biochemical measures included testosterone (T), androstendione (A), 17-Hydroxyprogesterone (17-OHP), LH /FSH ratio. Metabolic parameters included HOMA-IR and lipid profile. Student t-test was performed and data is presented as mean ± SD.

Results: The mostly (85%) Caucasian cohort (age 15.5 ±1.9 years) with clinical concern of PCOS had a BMI of 31.0 ± 7.8 kg/m^2. TAUS analysis revealed CPO in 45% (21/47), NAO in 38% (18/47) and VPO in 17% (8/47). Those with CPO had significantly higher T (67 ± 7 ng/dl) than those with NAO (35 ± 4 ng/dl; p = 0.0002) or VPO (33 ± 12 ng/dl, p = 0.003). CPO subjects also had higher serum A (p= 0.006), 17-OHP (p < 0.0001) and LH/FSH (p = 0.045) compared with NAO but no significant differences to VPO. Subjects with NAO (BMI 32.0 ± 1.8 $kg/m^{2)}$ and CPO (32.2 ± 1.6 kg/m^2) were more overweight than those with VPO (25.9 ± 2.7 kg/m^2, p=0.045). Metabolic parameters of insulin resistance such as HOMA and lipid profile were more strongly associated with degree of obesity (p = 0.0008) than TAUS category.

Conclusions**:** Symptoms of puberty are often indistinguishable from PCOS. In our study TAUS delineated adolescents with biochemical features of PCOS from those with a normal biochemical profile. This suggests that in obese adolescents of similar clinical presentation, TAUS may aid in diagnosing PCOS vs "simply" obese, insulin resistant girls who present with symptoms of puberty. Furthermore adolescents with TAUS-VPO may represent a population of adolescents in whom weight gain leads to CPO, despite presently normal androgen levels.

PO3-265 Reproductive Endocrinology

The role of environmental endocrine disruptors in the onset of precocious puberty

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Objective: To explore the role of environmental endocrine disruptors(EEDs) in the onset of precocious puberty.

Methods: The blood samples were collected from 110 cases of precocious puberty and 100 cases of normal children. The concentrations of octylphenol(OP), bisphenol A(BPA) and di-n-butyl phthalate(DBP) in the serum were measured by using reversed-phase high performance liquid chromatography(HPLC) and gas chromatography. The volume of uterus and ovary, and the content of estradiol(E_2) in the serum of precocious puberty were determined at the same time. The contents of EEDs in the serums and the indices of the target organs were analyzed by the methods of correlation and regression.

Results: In normal control group, OP,BPA,DBP[2.72μg/l, 2.11μg/l, 0.52μg/l(median, the same below)] were detected in 5%, 2%, 4% of serum samples, respectively. In precocious puberty group, OP,BPA,DBP(7.38μg/l,9.15μg/l, 2.76μg/l)were detected in 33.6%, 40.9%, 27.3% of serum samples, respectively. The levels of EEDs in the serum of the precocious puberty group were notablely higher than that of the control group($P < 0.001$). In precocious puberty group, positive correlations were found between the contents of OP,BPA,DBP and the volume of uterus ($P < 0.05$ or 0.01), the contents of OP,DBP and the volume of ovary also had a positive correlation($P < 0.05$ or 0.01).

Conclusion: A part of the present normal children has contaminated by EEDs, the children of precocious puberty have been much more heavily exposed to EEDs than the normal children. There is a close statistical relationship between EEDs and the onset of precocious puberty, and EEDs are important factors inducing the disease.

PO3-266 Reproductive Endocrinology

Uterus and ovaries in girls and young women with Turner syndrome evaluated by ultrasound and magnetic resonance imaging

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Background: Conventional pubertal induction regimes in Turner syndrome (TS) may be insufficient in terms of uterine growth. Ovarian size has not been thoroughly evaluated in TS. The objective was to determine uterine and ovarian size in relation to circulating hormones and history of hormonal replacement therapy (HRT) in Turner syndrome (TS).

Methods: A cross-sectional study of 41 girls and young women with TS (age 17.0±3.3, range 11-25 years) examined by magnetic resonance imaging (MRI) and transabdominal ultrasound (US). Two groups of controls were used for comparison. One group consisted of 50 healthy age-matched controls examined by MRI. The other group consisted of 107 historic, healthy, and Tanner-stage-matched controls examined by ultrasound (US). Uterine and ovarian volumes were main outcome measures.

Results: In TS, 73% received HRT (n=30). Breast development was delayed by 3-4 years at each Tanner stage and uterine volume reduced in TS at Tanner stage B5 compared to controls by US (TS vs. C: 33.6±18.2 vs. 50.2±18.0 ml, P=0.007). Uterine volume in TS was smaller than in age-matched controls by MRI (29.5±25.1 vs. 54.3±23.3 ml, P<0.0005). Uterine volume measured by MRI correlated significantly to age, BMI, BSA and weight in both TS (R=0.40-0.61, all P<0.01) and age-matched controls (R=0.32-0.47, all P<0.003). Ongoing HRT and age at initiation of HRT was not associated to uterine size. Current dose of 17β-estradiol correlated strongly (R=0.68, P<0005). Age and s-estradiol was significant explanatory contributors of uterine size in TS. A relatively poor agreement was found between uterine volume measured by MRI and US in TS (p=0.2). Ovaries were detected in 27% (n=11) of TS patients by US and in 44% (n=17) of TS patients by MRI. Ovarian volume was lower in TS patients compared to both groups of controls (TS vs. C-US: 7.32±12.93 vs. 14.14±11.00 ml, P=0.06, and TS vs. C-MRI: 5.41±8.77 vs. 13.61±5.39 ml, P<0.0005). In TS, follicles were detected in one or both ovaries in 24% (n=10) by US in 33% (n=13) by MRI.

Conclusions: Pubertal development was delayed, and uterine and ovarian volumes were reduced in TS compared to age- and Tanner-matched controls. Determinants of uterine size are manifold with influence from many co-variables, but age and serum concentration of estradiol were significant in our study population. MRI was superior to US in evaluating presence of ovaries and follicles.

PO3-267 Reproductive Endocrinology

Metformin for the treatment of hyperandrogenism in adolescents with type 1 diabetes mellitus: a double blind randomized study

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Aim To study the effect of metformin on hyperandrogenism and ovulation in adolescents with type 1 diabetes mellitus (DM1).
Methods DM1 girls (N=24) who had clinical (Ferriman-Gallwey (F-G) ≥5) and/or biochemical hyperanandrogenism (testosterone level >0.6 ng/ml or free androgen index (FAI) >6), who required high daily insulin dose (≥0.7 UI/kg/day) and had suboptimal metabolic control were recruited. Patients were randomized in a double-blind placebo-controlled trial to metformin (850 mg bid) or placebo. Treatment was given for 9 months. Steroid and gonadotropin levels were measured at the beginning and at the end of the trial. Ovulation was determined by progesterone levels measured in a spot sample during 18-23-28 days of each menstrual cycle (RIA). Rate of ovulation is expressed as number of ovulatory cycles in 100 days. An intention to treat analysis was performed. Statistics: Paired T test or Wilcoxon paired test for parametric and non-parametric variables, respectively.
Results No differences in the F-G score, rate of ovulation, HbA1c levels or daily insulin dose was observed in girls treated with metformin compared to those treated with placebo. Girls treated with metformin showed a greater decrease in basal testosterone, FAI, androstenedione, 17OH Progesterone and estradiol levels.

Table

	Basal		Delta Final-Initial	
	Metformin	**Placebo**	**Metformin**	**Placebo**
N	13	11	13	11
Ferriman-Gallwey Score	5 (4-24)	11 (8-15)	0.1 ± 1.0	0.6 ± 0.7
Ovulatory rate (N/100 days)	1.0 ± 1.4	0.7 ± 1.4	0.1 ± 1.0	0.4 ± 1.0
17OH Progesterone (ng/ml)	1.5 ± 0.8	1.3 ± 0.6	-0.5 ± 0.7+	-0.2 ± 19.6
Testosterone (ng/dl)	59.7 ± 22.2	49.9 ± 21.2	-13.0 ± 16.4*+	2.8 ± 19.6
Free androgen index	11.0 ± 10.9	8.8 ± 9.3	-7.3 ± 10.1+	-3.3 ± 10.2
DHEAS (ng/ml)	1867 ± 1019	1440 ± 473	-138 ± 485	228 ± 504
Andro-stenedione (ng/ml)	2.1 ± 0.7	1.8 ± 0.7	-0.4 ± 0.5+	-0.1 ± 0.8

* Metformin vs placebo P<0.05 +: Final vs initial
Conclusions Treatment with metformin of hyperandrogenic adolescents with DM1 produces a significant decrease in serum androgens compared to placebo, but it did not have any significant effect on clinical parameters such as hirsutism score, ovulatory rate and metabolic control.
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PO3-268 Reproductive Endocrinology

Clinical and metabolic profile of adolescent girls with polycystic ovary syndrome from India

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Methods: Adolescent girls with polycystic ovary syndrome (PCOS) attending our endocrine clinic were prospectively followed to evaluate their clinical and metabolic profile. PCOS was diagnosed on the basis of revised Rotterdam criteria of 2004. Secondary causes of PCOS were ruled out. Metabolic work up included an oral glucose tolerance test and lipid profile, done after an overnight fast.
Results: During 2003 to 2009, one hundred and four girls with PCOS, age range 13.9 – 20 years, median 17 years were seen in our clinic. Of these, 79 girls were prospectively followed. Oligomenorrhea (84%) was the commonest presenting symptom followed by hirsutism. The mean body mass index (BMI) was 24.98 ± 5.98 kg/m^2, 66% of them were obese or overweight (BMI ≥ 23 kg/m^2, according to criteria for Asians). Eight (10.2%) had hypertension.Thirty percent girls had hyperlidemia, predominantly raised LDL cholesterol and hypertriglyceridemia. Family history of diabetes was present in 37.5% girls. As per the International Diabetes Federation criteria, 14% girls had metabolic syndrome, which is higher than the 0.8% prevalence reported in the general population in this age group from New Delhi, India. Sixteen of these 79 girls had abnormal glucose tolerance in the form of impaired glucose tolerance (12) and impaired fasting glucose (4). None had diabetes. In a population based study from Chennai, India, prevalence of <1% of diabetes has been reported in women aged 15-24 years. Comparing the girls with abnormal glucose tolerance with normal glucose tolerance, there was no statistical difference in their mean age, BMI or waist circumference. Prevalence of hypertension, metabolic syndrome or family history of diabetes also did not differ in the two groups. Prevalence of abnormal glucose tolerance was similar in both lean and overweight or obese girls with PCOS (OR 0.77, p=0.68).
Conclusion: Indian patients with PCOS have a high risk for abnormal glucose tolerance and metabolic syndrome during adolescence. Lean girls have the same prevalence of abnormal glucose tolerance as overweight or obese girls.

PO3-269 Reproductive Endocrinology

Bilateral undescended testes – the main reason for impaired male fertility in Noonan syndrome?

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Aim: To study in a prospective design the genital tract function in males with Noonan syndrome, by means of genital development and regularly hormonal levels during pubertal years.
Background: A couple of reports have examined the genital tract function in males with Noonan syndrome and found that undescended testes (UT) could be the main course of impaired fertility. Whether surgical treatment and thereafter normal testes development normalizes the gonadal status is not examined.
Methods and population: Twelve short males diagnosed with Noonan syndrome were followed throughout puberty (start median age 10.7, range 10 to 12 years) until adulthood (median age 21 years, range 18-26). We found UT - bilateral in four males and unilateral in one male. All were surgical treated at a young age. One other male was found to have central hypogonadism and one had small testes after treatment with Metotrexate due to juvenile arthritis. Gonadotrophins (LH and FSH), testosterone and inhibin B were followed yearly and testicular volume determined by orchidometer.
Results: Half of the males who underwent bilateral surgical treatment of UT had normal testicular volumes (> 15 ml bilateral) and half of them subnormal testicular volumes (largest testes 15 ml or less). Despite the surgical treatment and despite normal testicular volume achieved in half of them, all those males with bilateral UT had at adult age significantly elevated LH and FSH levels compared with the spontaneous descended group (10.9 and 25.8 U/L versus 4.4 and 5.1 U/L, p<0.01) and compared with a reference population of age-matched healthy men (4.0 and 2.5 U/L). Testosterone levels were surprisingly elevated in the UT-group compared with the spontaneous descended (20.1 versus 15.8 nmol/L, p<0.05). Inhibin B concentrations reached a peak value during early to mid-puberty in all patients, but to a lower level in the bilateral UT-group (median 116 pg/mL compared to 227 pg/mL, p<0.01).
Conclusion: As reported by one previous published paper, bilateral UT was found to be the main factor contributing to impairment of testicular function in a substantial minority (30%) of men with Noonan syndrome. We found that this was unrelated to if surgery was performed at young ages and despite achieved normal testicular volume. Whether the gonadal status per se or perinatal programming of the hypothalamus-pituitary-gonadal axis is the background for this impaired testicular function remains to be elucidated.

PO3-270 Reproductive Endocrinology

Does the balanced X-autosome translocation affect the normal ovarian development?

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Premature ovarian failure (POF) is characterized by hypergonadotropic hypogonadism and amenorrhea for more than 6 months before the age of 40. Chromosomal rearrangements of the Xq chromosome as deletions or X-autosome translocation are associated with POF. A critical region for normal ovarian function has been proposed for Xq13-q26. Within this zone the most frequent breakpoints involve two specific regions associated with POF: POF1 located in Xq26-qter and POF2 in Xq13.3-q21.1.

We identified two balanced X-autosome translocations by high resolution cytogenetics methods in a female affected with POF and in a girl with growth retardation and in her carrier mother with the same karyotype and POF.

Patient 1: 19 years old with primary amenorrhea. Karyotype: 46,X,t(X;11)(q21;q21). After 3 years of estrogen and progesterone replacement therapy she had spontaneous pregnancy and delivered normal girl.

Patient 2: 9 years old. Height: below Pc3. Without hormone therapy she started breast development at 10.6 years old. Menarche: 11.5 years old with regular menses until now. Karyotype:46,X,t(X;10)(q26.1;q26.2) inherited from her carrier mother. She had two normal brothers, one of them carried the balanced translocation. Mother: Height: Pc3-10. Menarche at the age of 12 with normal menses. At the age 35 she began with irregular cycles and entered in menopause at 40 years. She had an infertile sister. FISH using the whole X chromosome painting and tel Xq/Yq probes analysis confirmed the X translocated. The triplet (CGG)n of FMR1 gene (Xq27.3) and the STRs of DXS1205 (Xq27.2), DXS1091 (Xq28) and DXS8069 (Xq28) linked to POF1 region were performed in the mother and her daughter. The results were normal amplification of CGG triplet and revealed two alleles. The STR of DXS1205 evidenced only one allele while the remains STRs showed two alleles.

We conclude that different breakpoints on Xq critical region not always affect the genes involved in ovarian development. POF could be caused by haploinsufficiency of some genes, a position effect on the autosome translocated or by meiotic disturbances which lead to apoptosis of germ cells in the ovary essential for the development of ovarian follicles. If the balanced translocations Xq-autosome lead to disruption of the genes involved in the ovarian development one could expect that the carriers would have streak gonads. Instead, the apoptosis of the asynapsed oocytes may preserve the ovarian reserve allowing some period of fertility.

PO3-271 Reproductive Endocrinology

Familial partial androgen insensitivity syndrome presenting at adolescence with gynaecomastia: a case report

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Androgen insensitivity syndrome (AIS) is an X-linked disorder caused by mutations in the AR gene, impairing the androgen-dependent male sex differentiation to various degrees. Partial androgen insensitivity syndrome (PAIS) can occur in isolated form or in familial form. Usually this disorder presents at birth with the ambiguity of genitalia. Here we describe a 15 years male (youngest sibling among – 5 siblings) patient referred to our clinic for complaints of bilateral gynecomastia. On examination, he was found to have undescended inguinal testis on left side and 8 ml right testis with perineoscrotal hypospadias and gynaecomastia. Hormonal analysis is given in table-1. Molecular analysis revealed that a missense mutation in exon 7, encoding substitution of histidine for arginine at codon 840 of the androgen receptor (AR) gene.

His elder 31 year old sibling was also diagnosed to have similar problem at age 17 years when he presented with breast enlargement. Both these siblings could not discuss their problem with other members in the family or friends due to social stigma. The older sibling was unmarried, did not undergo corrective surgery and had accepted the situation. He had a deep concern for the younger sibling who also had similar disorder. He insisted for early surgery so that he could lead a normal life. We present here in these two siblings, socio-cultural factors that could affect the presentation and interfere with management of intersex patients.

Hormonal analysis (table 1)

Hormonal Profile	Patient-1	Patient-2
LHRH Stimulation Test	LH(IU/L) FSH(IU/L)	LH(IU/L) FSH(IU/L)
(Time - Hrs)	T(ng/ml)	T(ng/ml)
0	4 6 445	7.2 4.3 600
2	26 12 490	29 6.5 580
4	25 13 445	66 12.2 600
8	30 16 540	54 11.1 540
24	35 21 690	21 5.8 800

PO3-272 Reproductive Endocrinology

Polycystic ovary morphology (PCOM) does not affect ovulatory function in healthy postmenarcheal adolescents

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Background:Polycystic ovaries are a diagnostic criteria for polycystic ovarian syndrome (PCOS), however a high rate of this appearance is relatively prevalent in non-hyperandrogenic adolescents. Aims:To evaluate the frequency of PCOM in adolescents at two, three and four years after menarche (PM), its association with ovarian function and body composition and its evolutionary pattern over time.Methods:We performed a prospective study of 20 healthy adolescents (13.8±0.8 years) from 2-4 years PM.All the girls were followed during 6 consecutive months 2-2.9 and 3-4 years PM with salivary samples for measurement of progesterone (PG) obtained during the 13,18,23 and 28th days of each cycle.Annual hormonal profile (leuprolide test),ultrasound (US),body composition (DEXA) was performed. PCOM was diagnosed according to the Rotterdam criteria.Statistical analysis:Cohen's Kappa.Comparison of hormonal findings in girls with and without PCOM (PCOM+ and PCOM-, respectively), was performed with Mann-Whitney's U test. Results:PCOM was observed in 40,40 and 33.3% of these girls at 2,3 and 4yr PM,respectively. Concordance between the PCOM diagnosis at 2 and 4 yr PM was 52.6%;p=0.6, between the 2 and 3 yr PM was 71.4%;p= 0.03 and 3 and 4 yr PM was 79 %;p=0.01. After the first US,5 and 3 girls showed disappearance and development of PCOM,respectively.Linear regression showed no influence of PCOM over frequency of ovulatory cycles or cycle duration.

Cycle duration and ovulation rate in adolescents with and without PCOM

	PCOM+	PCOM-	p
Cycle duration (days) 2-3yr PM	32.1±4.0	33.4±6.1	NS
Cycle duration (days) 3-4yr PM	32.1±1.8	32.2±6.1	NS
Ovulation rate (n/100 days) 2-3yr PM	1.1±0.9	1.2±0.6	NS
Ovulation rate (n/100 days) 3-4yr PM	1.2±1.1	1.6±1.1	NS

At entry lower basal FSH (4.3±0.9 vs.5.4±0.9mUI/ml;p=0.04) and lower stimulated estradiol levels (35.0±11.7 vs.40.4±19.2pg/ml;p=0.01),were evidenced in PCOM+ and PCOM- girls,respectively. PCOM + and PCOM- girls had similar hormonal profile 3 and 4 yr PM.The stimulated 17OHPG,testosterone levels and body composition were similar in both groups.Conclusions:Presence of PCOM is a frequent and inconstant finding in healthy adolescents and does not appear to affect ovulatory function or body composition. PCOM diagnosed during the first few years PM is not associated with a higher anovulatory rate or hyperandrogenism. PCOM should not be included as diagnostic features of PCOS at this age. (FONDECYT 1050452)

PO3-273 Reproductive Endocrinology

Could ovarian volume alone help in the diagnosis of adolescent polycystic ovary syndrome?

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Transvaginal ultrasound (TVUS) criteria for polycystic ovaries (PCO) in adult women are based on visualization of ≥10 peripheral follicles measuring <10 mm in diameter, rounded ovaries, and increased ovarian volume (>10ml). In adolescents TVUS is often replaced by trans-abdominal ultrasound (TAUS); however, the ability to count the number and size of follicles is limited. Ovarian volume is an easier, less user-dependent measure for describing ovarian morphology that may have clinical significance in PCO. Elevation of serum testosterone is a well-validated diagnostic criterion for PCOS and has been shown to correlate with typical PCO using TVUS. However little is known about how accurately is relates to increased ovarian volume in TAUS.

38 adolescents (age 15.0 ± 0.5 years) attending the MAPP (multi-specialty adolescent PCOS program) enrolled in this ongoing cohort study. TAUS was read by a single radiologist, blinded to all clinical information. Ovarian volume was calculated with three diameter measurements using the prolate ellipse formula (longitudinal D1 x anteroposterior D2 x transverse D3 x 0.523). Total ovarian volume was obtained by adding the volume of both ovaries. Largest single ovarian volume included only the larger of the two ovaries. Biochemical measures of hyperandrogenism included total testosterone (T) and sex hormone binding globulin (SHBG); free T was calculated. Correlation analysis was performed using GRAPH-PAD PRISM.

This obese group (BMI 31.2 ± 1.3 kg/m^2) with clinical concern for PCOS had a mean total ovarian volume of 21.1 ± 2.1 ml (range 2.5-50.2 ml), whereas the mean single largest ovary volume was 13.2 ± 1.3 ml (range 1.3-30 ml), indicating that asymmetrical ovarian enlargement was not uncommon. It was found that T levels correlated with both single largest ovary volume (r= 0.4, p = 0.01) and total ovarian volume (r=0.44, p= 0.006). The same relationship existed with free T. When ovarian volume was analyzed on a continuum there was an 82% likelihood that an adolescent whose ovaries were both <10 ml had normal T (< 50 ng/dl) levels.

CONCLUSIONS: Our study demonstrated a statistically robust association between TAUS ovarian volume measures and serum testosterone levels. While magnetic resonance imaging (MR) and trans-rectal or TVUS may better visualize ovarian follicles, TAUS has been shown to be equal to MR in measuring ovarian volume. TAUS is a safer, cheaper, and more readily available imaging modality to diagnose PCO.

PO3-274 Reproductive Endocrinology

Menstrual irregularities and their relationship with HbA1c and insulin dose in adolescents with type 1 diabetes mellitus

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Objective: To evaluate the prevalence and risk factors of menstrual cycle (MC) irregularities in adolescents with type 1 diabetes mellitus (T1D) treated with multiple daily insulin injections

Design: Prospective controlled study.We studied 56 adolescents with T1D treated with ≥ three daily doses, and 56 healthy adolescents (C). Both groups were paired according to gynecological age and body mass index (BMI).

A prospective record of MC was obtained for six consecutive months. MC duration and variability assessed by variation coefficients and the presence of oligomenorrhea and amenorrhea were determined.

Results: MC duration was 48±39 and 32±7 days in T1D and C girls, respectively (P<0.01). Oligomenorrhea (58.9% vs. 19.6%, P<0.001) and amenorrhea (10.7 vs. 1.7%, P=0.05) were more prevalent in T1D than in C. Oligomenorrhea was observed in 53.3 and 21.1% of the T1D girls with optimal metabolic control and the C girls, respectively (OR= 4.7; CI= 1.4-15.7; P<0.05). Girls with an HbA1c level of 7.6-8.9% exhibited increased cycle duration, MC variability, and a prevalence of oligomenorrhea.(Table 1) Regression analysis showed that for each point of increase in HbA1c, the MC duration increased by 5.1 days. Cycle variability was associated with a higher daily insulin dose.

Characteristics of menstrual cycles according to metabolic control in T1D girls compared with the control group. Data are presented as the average ± standard deviation.

		T1D		CONTROL
HbA1c (%)	<7.6	7.6-8.9	> 9	
N	15	18	23	56
Menarche (years)	12.2 ±0.9	12.5±1.4	13.0 ±1.3**	12.1±1.0
Menstrual cycle duration (days)	34.9±8.9	48.6±34*	57±52.3***	32.7±8.6
Variation coefficient (%)	27.6±15.6	35.5±17.8*	27.4±21.7	24.3±18.7
Girl had at least one cycle with (%):				
Oligomenorrhea	53.3*	72.2***	54.5**	21.1
Amenorrhea	0	11.1	18.2*	3.5
Polymenorrhea	60.0	50.0	18.2*	43.9

*P<0.05 **P<0.01 ***P<0.001

Conclusions:

Irregular and long MCs are a prevalent problem in adolescents with T1D. Despite optimal metabolic control, a higher prevalence of oligomenorrhea was observed. This is the first report to describe the high variability of MC in T1D. HbA1c and insulin dose are important factors related to menstrual irregularities in T1D.

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PO3-275 Reproductive Endocrinology

A familial translocation t(2;8) with a submicroscopic deletion in breakpoint 2p16.3 and a paternally inherited point mutation of the FSH receptor gene explain primary amenorrhea and delayed puberty in a 17-year-old girl

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Case Report: A 17-year-old girl presented with primary amenorrhea and incomplete pubertal development (B3, PH3). In addition, she had lichen sclerosus et atrophicans of the vulva. Psychomotor development and longitudinal growth had been normal. Other symptoms or syndromal signs were lacking. Endocrine testing revealed hypergonadotropic hypergonadism. At laparoscopy, ovaries of normal size and a rather small uterus were seen. Ovarian histology showed a high number of primordial follicles. Surprisingly, secondary or tertiary follicles were completely lacking. This led us to hypothesize a defective FSH action as the cause of the amenorrhea. **Materials and Methods:** Karyotyping, fluorescence-*in situ*-hybridization with probes of the *FSH-R* locus, a whole genome array analysis, and a mutation analysis of the FSH receptor (*FSH-R*) gene were carried out. **Results:** In the patient, a translocation t(2;8)(p16.3or21;p23.1) was found, that appeared to be cytogenetically balanced. This was seemingly confirmed by the finding of the same translocation in the unaffected mother. Since the precise breakpoints of this translocation could not be delineated cytogenetically, involvement of the *FSH-R* gene could not be ruled out. In addition, analysis of the *FSH-R* gene in the patient revealed a single non-synonymous point mutation in exon 10, with no wildtype sequence present. The point mutation was also found in heterozygous form in the father, but not in the mother. Further work-up with two BAC probes including the

FSH-R gene demonstrated a deletion with a breakpoint within the gene. Array testing (GeneChip®HumanMapping 250 K Sty Array, Affymetrix) showed a 163 kb deletion in 2p16.3, including exons 9 and 10 of the *FSH-R* gene. **Conclusions:** Deletion on the maternally derived allele demasks the heterozygous point mutation on the paternally derived allele, prohibiting *FSH-R* gene activity. Thus, two very rare distinct events occuring jointly in this patient explain the primary amenorrhea to be caused by FSH resistance.

PO3-276 Reproductive Endocrinology

A single nucleotide polymorphism in *STK11* influences insulin sensitivity and metformin efficacy in hyperinsulinemic girls with androgen excess

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Background & **Aim:** Serine-threonine-kinase STK11 catalyzes the AMP-activated protein kinase complex. We tested the hypothesis that STK11 modulates insulin sensitivity and metformin efficacy by studying the effects of a single nucleotide polymorphism (SNP; rs8111699) in *STK11* on endocrine-metabolic features and on the body composition of hyperinsulinemic girls with androgen excess, before and after 1 yr on metformin.
Subjects & **Methods:** The study population consisted of 85 girls, of whom 18 were prepubertal (mean age 8 yr, BMI 19 Kg/m^2) and 67 postmenarcheal (14 yr; 22 Kg/m^2). The prepubertal girls combined a history of low birthweight with the appearance of precocious pubarche, secondary to an exaggerated adrenarche (JCEM, August 2006 and May 2008). Postmenarcheal girls had hyperinsulinemic ovarian androgen excess. Metformin was dosed at 425 and 850 mg/d in younger and older girls, respectively. *STK11* rs8111699 was genotyped; the endocrine-metabolic features were assessed in fasting state and, when applicable, in the follicular phase; body composition was estimated by absorptiometry.
Results: Genotype effects were similar in prepubertal and postmenarcheal girls, and are therefore reported together. At baseline, the mutated G allele in *STK11* rs8111699 was associated with higher insulin and IGF-I levels, and with a lower glucose-to-insulin (G/I) ratio (all p<0.005). The 1-yr response to metformin differed markedly by *STK11* genotype: GG homozygotes (N=24) had robust improvements of endocrine-metabolic features and body composition, GC heterozygotes (N=38) had intermediate responses, and CC homozygotes (N=23) almost no response. Major differences were found for the 1-yr changes in circulating insulin, IGF-I and G/I ratio (all p<0.0001), HDL, triglycerides and SHBG (all p<0.01), and lean mass, total fat and abdominal fat mass (all p<0.0001).

Table 1. One-year response to metformin by STK11 genotype

	CC (n=23)	GC (n=38)	GG (n=24)	P
Insulin (mIU/L)	0.4 ± 0.9	-3.5 ± 0.7	-4.9 ± 0.7	<0.0001
Fat mass (kg)	0.9 ± 0.4	-0.5 ± 0.3	-1.2 ± 0.4	<0.0001

Conclusion: The *STK11* rs8111699 SNP was found to influence insulin sensitivity in hyperinsulinemic girls with androgen excess, and to increase metformin efficacy with a stepwise increment from C/C over G/C to G/G genotype. These observations represent a first leap forward into the pharmacogenomics of pediatric insulin sensitization.

PO3-277 Reproductive Endocrinology

Long-term testicular function and spermatogenesis after childhood ALL

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Infertility is a potential complication of childhood cancer treatment. In males chemotherapy (especially alkylating agents) and radiotherapy may impair spermatogenesis and testosterone production. The aim of this study was to evaluate long-term testicular function after childhood acute lymphoblastic leukemia (ALL) and to identify treatment modalities associated with preserved fertility in adulthood.
The study included 51 male long-term ALL survivors (median age 29 yrs), treated for childhood leukemia at Helsinki University Hospital in 1970-1995, who were examined 10-38 years after the ALL diagnosis. Cumulative doses of irradiation and cytotoxic agents were recorded from hospital charts. Testicular volume, testicular endocrine function and sperm quality were assessed. The findings were compared with 56 age-matched healthy control men (median age 30 yrs) without history of cancer.
Serum levels of FSH and inhibinB, testicular volume and sperm count differed significantly between the groups and correlated strongly with ALL therapy. Testicular volume and testosterone concentration was significantly decreased from control values after therapy with no or low cumulative dose (<10 g/m2) of cyclophosphamide while total semen count and semen quality were similar to controls. CNS irradiation (mean 23 Gy) associated with a further decrease in testicular volume but was not alone associated with impaired spermatogenesis. Patients with high cumulative dose of cyclophosphamide (>20 g/m2) showed markedly decreased sperm concentration, inhibinB and testicular volume. Cumulative dose of methothrexate did not correlate with testicular function. All patients after 24Gy testicular irradiation were azoospermic. Altogether 25% of the ALL survivors and 43% of the controls had fathered a child.
ALL treatment with no or low dose of cyclophosphamide does not impair spermatogenesis but may impair Leydig cell function. High cumulative dose of cyclophosphamide is damaging to spermatogenesis. No recovery in gonadal function occurred after testicular irradiation with 24Gy.

PO3-278 Reproductive Endocrinology

Age at menarche in Chilean adolescents with polycycstic ovary syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age .It is a lifelong disorder, often diagnosed during late adolescence. Literature about age at menarche in PCOS is scarce and report a later age at menarche, 20% older than 14 years. The aim of our study was to evaluate age at menarche in adolescents with the diagnosis of PCOS, the correlation with maternal age at menarche and markers of hyperandrogenism and compare these data with age at menarche in normal Chilean girls of the same socioeconomical level (n=304). Subjects and methods: Retrospective data analysis of adolescents diagnosed with PCOS who were at least two years after menarche, according to NIH criteria. Birth weight, age at menarche, maternal age at menarche, current age (at least 2 yrs post menarche) weight, height, BMI, Ferriman-Gallwey score, acne and androgen levels were recorded. Results: 78 adolescents with PCOS, current median age 16, 8±0, 2 years. In 68 the maternal age at menarche was found. 5,1% were born LGA and 6,2% pre-term. None of them were born SGA. Age at menarche in PCOS girls was 12,3±0,15 yrs. Maternal age at menarche was 13,01±0,2 yrs (delta-0,62±0,2 years p<0,01). Age at menarche in girls from the community was 13,05±0,05 (P<0,01). BMI was 0,81±0,12 sds(4,5±0,2 years after age at menarche). PCOS girls with Ferriman-Gallwey score ≥6 had menarche at 12,2±0,2 vs Ferriman-Gallwey score <6 12,7±0,3years (p=NS). PCOS girls with moderate/severe acne had menarche at 12,2±0,3 vs PCOS with no acne 12,7±0,3years (p=NS). No differences in age at menarche according to Free androgen index (FAI) <4,5 or ≥4,5 was detected. Corrected by maternal age at menarche PCOS girls with FAI≥4,5 had menarche 6 months earlier than their mothers and 3 months earlier than PCOS girls with FAI<4,5. Conclusion: Chilean adolescents with PCOS had menarche earlier than their mothers and normal counterparts. We postulate a potential role for insulin and androgens in pubertal time course.

PO3-279 Reproductive Endocrinology

Androgen receptor gene CAG repeat polymorphisms in girls with hyperandrogenic states

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BACKGROUND/AIMS: Hyperandrogenism is a common condition considered an independent cardiometabolic risk factor for insulin resistance and type 2 diabetes. The Androgen Receptor (AR) CAG repeat length polymorphism has been associated with low or high androgenic activity. Shorter CAG tracts seem to be more transcriptionally active and therefore related to a potent androgenic effect on target tissue and a risk condition for cardiovascular disease. Few data has been published regarding in vivo expression pattern of AR in muscle biopsies. The aim of the present study is to analyze AR expression in muscle and CAG tracts length in DNA of hyperandrogenic pediatric patients and their relation to free androgen index (FAI), SHBG and cardiometabolic risk factors.
METHODS: Twenty two hyperandrogenic girls (mean age 14.4years) clinically and biochemically hyperandrogenic were included. RNA of external vaste was obtained as well as Leukocyte DNA. Girls were classified in two groups: A) PCOS diagnosis according to Rotterdam criteria (n=13) and B) NON-PCOS hyperandrogenic group (n=9).Glucose, insulin, lipid profile, androgens and SHBG were determined after a 12hr. fasting condition and during follicular phase of the menstrual cycle when possible. Other conditions such as non classical congenital adrenal hyperplasia, hyperprolactinemia, adrenal or ovarian tumors and thyroid abnormalities were ruled out. The AR CAG repeat polymorphism was evaluated by PCR and polyacrilamida elecrophoresis gel. The mean biallelic was calculated in each case. The expression profile was performed with QRTPCR analysis.
RESULTS: Biallelic average of CAG length of AR was 23.8±1.26 for group A and 22.8±1.87 for group B ($p<0.05$), No statistically differences were found among groups while comparing FAI and SHBG. Metabolic syndrome prevalence was not statistically different between groups (38.4 Vs 42.8% respectively), nevertheless, a tendency to higher systolic blood pressure and low HDL levels was evident in group B. The AR expression pattern showed consistent differences among the groups compared with normal and metabolic stable controls.
DISCUSSION: A shorter AR CAG repeat length with a higher androgen sensitivity could explain the higher cardiovardiovascular risk observed in hyperandrogenic NON-PCOS girls.

PO3-280 Reproductive Endocrinology

Human steroidogenic factor-1 (hSF-1) induces progesterone biosynthesis, suppresses proliferation, and promotes apoptosis of ovarian surface epithelial cancer cells

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The majority of cancers derived from ovarian surface epithelial (OSE) cells are lethal. Estrogens promote proliferation of OSE cells, whereas progesterone inhibits proliferation and promotes apoptosis of OSE cells. Human steroidogenic factor-1 (hSF-1) induction of the steroidogenic acute regulatory protein (StAR) gene is central to progesterone biosynthesis. We examined tissue sections from six normal ovaries for expression of hSF-1 and StAR by immunohistochemistry. Five out of the six normal ovaries showed hSF-1 expression and all six normal ovaries showed StAR expression in OSE cells. However, a panel of immortalized and malignant OSE cell lines, including SKOV-3, BG1, Caov-3, and OVCAR-3 ovarian cancer cell lines, and human OSE cells immortalized by SV40 large T antigen (IOSE 121) did not express hSF-1 and StAR. Transient expression of hSF-1 in SKOV-3 cells activated expression of StAR, including steroidogenic enzymes, p450scc and 3βHSD-II mRNAs. We also measured progesterone biosynthesis by ELISA in a panel of ovarian cancer cell lines and IOSE 121 cells transiently transfected with hSF-1. Progesterone biosynthesis was induced in picomolar concentration in hSF-1 transfected ovarian cancer cell lines and in micromolar concentration in immortalized IOSE 121 cells. Progetesterone biosynthesis was induced 4-50 fold in hSF-1 transfected ovarian cancer cells compared to vector transfected cells. In immortalized IOSE 121 cells progesterone induction was 240 fold in hSF-1-transfected cells as compared to vector-transfected cells. Furthermore human steroidogenic factor-1 suppressed proliferation and promoted apoptosis of SKOV-3 cells and suppressed SKOV-3 cell growth induced by ERαand estradiol. Our immunohistochemistry studies on normal ovarian tissues and cell culture studies in ovarian cancer cell lines suggest that hSF-1 may decrease OSE cancer cell numbers directly by apoptosis and indirectly by promoting StAR, p450scc and 3βHSD-II activity and progesterone biosynthesis, thereby opposing estradiol-induced proliferation. Thus, down-regulation of hSF-1 expression may contribute to progression of ovarian epithelial cancers and hSF-1 expression may be useful as a prognostic bio-marker in ovarian epithelial cancer.

PO3-281 Reproductive Endocrinology

Clinical, hormonal, genetic and enzymatic characterization of five patients with mutations in P450 oxidoreductase

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P450 oxidoreductase (POR) deficiency is a newly-described disorder of steroidogenesis with variable clinical phenotypes: severe mutations result in genital ambiguity in both sexes and the Antley-Bixler skeletal malformation syndrome (ABS); mild mutations only cause adult infertility (Nat Genet 36: 228, 2004; Am J Hum Genet 76: 729, 2005). POR donates electrons to all microsomal P450 enzymes, including P450c17 (17α-hydroxylase/17,20 lyase), P450c21 (21-hydroxylase) and P450aro (aromatase). We describe five patients with POR deficiency. Patient 1: a term newborn 46,XY male with ABS and micropenis had a newborn screening 17OHP of 60 ng/ml (nl<50) at 2 d. At 2 mo, serum cortisol was 9.3 µg/dL but rose to only 13 µg/dL after ACTH. Patients 2 and 3: two 46,XX adolescent sisters presented with primary amenorrhea. For patient 2, LH=9, FSH=3.9 mIU/ml, E2=25 µg/ml, low serum androgens, elevated 17OHP and cortisol response to ACTH was 9.7 to 8.5 µg/dL. Patients 4 and 5 were 46,XX females with ABS and genital ambiguity. Patient 4, now 21 yo, has severe scoliosis and no breast or pubic hair development: FSH=8.8, LH=1.3, E2 undetectable, and poor cortisol response to ACTH. The coding regions of the *POR* gene were amplified by PCR and sequenced. Patient 1 was compound heterozygote (CH) for A287P and delE217; Patients 2 & 3 were CH for L577R and N185K; Patient 4 & 5 were CH for G539R and a frameshift mutation. L577R, N185K and delE217 are novel mutations; each was re-created in human POR cDNA expression vectors by site-directed mutagenesis. POR and human P450c17 were expressed in bacteria, and assayed in isolated bacterial membranes. The ability of POR to support 17α-hydroxylase activity and 17,20 lyase activities of P450c17 were assayed by conversion of labeled progesterone to 17OHP and 17OH-pregnenolone to DHEA. Steroids were separated by TLC and quantified by phosphorimmaging. Varying substrate concentrations and incubation times permitted calculation of enzymatic parameters. N185K and delE217 had no measureable activity. As assessed by Vmax/Km, L577R supported 51% of 17α-hydroxylase activity but only 25% of 17,20 lyase activity. In previous assays, G539R supported 46% of the 17α-hydroxylase but only 8% of 17,20 lyase activity. These patients illustrate that mildly elevated newborn 17OHP can suggest the Dx, cortisol reserve is poor and adult infertility may be the sole manifestation. POR assays based on P450c17 correlate well with hormonal and clinical phenotypes.

PO3-282 Reproductive Endocrinology

Unilateral polycystic ovary in adolescents: is it a milder variant of bilateral PCOS?

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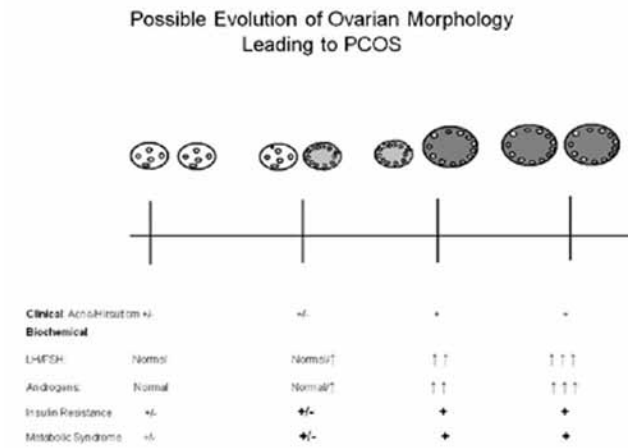
Background: Adult studies have shown that unilateral polycystic ovary (UniPCO) may be milder clinical condition of classic polycystic ovarian syndrome (PCOS) with bilateral polycystic ovary (BiPCO). These characteristics have not been examined in adolescents.
Objectives: To evaluate clinical, biochemical & radiologic features in adolescent females with UniPCO v/s BiPCO in cases of PCOS and to compare the association of insulin resistance (IR) & metabolic syndrome (MS) between 2 groups.
Methods: A retrospective chart review of girls with the diagnosis of PCOS was performed. They were divided into 2 groups: PCOS with UniPCO & BiPCO. Girls with UniPCO (n=10) ranged from 10.1-18.8 yrs (mean 15.3) and those with BiPCO (n=13) ranged from 12.5-17.7 yrs (mean 15.6). Clinical, biochemical & sonography data (Ovarian volume, follicular morphology) were reviewed. Association of IR and MS between 2 groups was also compared.
Results: No difference was seen between PCOS adolescents with UniPCO and BiPCO in regards to clinical parameters (ethnicity, hirsutism, acne, acanthosis nigracans, menses, body mass index, blood pressure). LH/FSH ratio (3.6 +/- 0.5 vs. 2.4 +/- 0.3) were significantly higher ($p<0.05$) in BiPCO subjects. No difference was seen in free testosterone, lipids, MS or IR between groups. Ultrasound showed a mean ovarian volume of 13.2 +/-1.5 cc on the affected side in UniPCO and 16.1 +/- 1.2 cc in BiPCO. Ovarian follicles location was mostly peripheral in both uniPCO (affected: 80% vs. unaffected: 70%) and BiPCO(65%). Multiple follicles were found in majority of cases.
IR and MS was present in 40% girls with UniPCO & 38%, 23% respectively in girls with BiPCO.
Repeat ultrasound in 4 UniPCO girls showed progression to BiPCO in 3 subjects (mean progession time, 18.3 months).
Conclusion: Amongst PCOS groups, UniPCO may be a forerunner of BiPCO. UniPCO may represent an early point along the continuum, and that over time, the unaffected ovary will continue to enlarge, and LH & androgens will subsequently rise.



Metabolic abnormalities of UniPCO highlights that though it may be a precursor of BiPCO, it still imparts considerable metabolic health risks.

PO3-283 Reproductive Endocrinology

The relationship between the levels of estradiol and progesterone in commercial whole milk and seasons alternation

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Objective: Much interest has been focus on relationship between the animal-derived estrogens in human diet and health, especially in milk. There is a correlation between the milk's consumption and the increased risk for hormone-dependent cancers in human. We aim to observe the relationship between the levels of estradiol (E_2) and progesterone (P_4) in commercial whole milk and seasons alternation. **Methods:** Samples of commercial whole milk for three brands (A, B and C) were collected once every half a month in a year, and there were 24 samples (24 lot numbers) for every brand. Total E_2 (TE_2) and P_4 in whey were assayed by immunochemiluminometric assays. **Results:** When a year was divided into the first stage (from March 16 to August 15, 2007, in China) and the second stage (from August 16, 2007 to March 15, 2008 in China), P_4 (ng/mL) at the first stage were respectively 1.70 ± 0.36, 1.81 ± 0.69 and 1.36 ± 0.48 in the order of brand A, B, and C; and at the second stage were 1.47 ± 0.31, 1.16 ± 0.69 and 0.89 ± 0.44 respectively; and P_4 in brands B and C at the first stage were respectively higher than one at the second stage ($P<0.05$ in both), while there was no statistically significant difference between both stages in brand A; and P_4 in brand A at the second stage was higher than one in brand C ($P<0.05$); and there were no differences in P_4 among other brands at the first or second stage; and there were no statistically significant differences in TE_2 among the same brands at different stages or different brands at the first or second stage; at the first stage, the highest of highest/lowest in TE_2 and P_4 among lot numbers of a brand for three brands were respectively 2.61 and 5.33, and among lot numbers of three brands respectively 2.74 and 5.33; and at the second stage, the highest of highest/lowest in TE_2 and P_4 among lot numbers of a brand for three brands were respectively 2.41 and 5.64, and among lot numbers of three brands respectively 2.91 and 6.24; and P_4 (ng/mL) at the first stage (1.62 ± 0.55) was higher than one in the second stage (1.18 ± 0.55), ($P=0.001$); and there was no statistically significant difference in TE_2 between both stages. **Conclusion:** The levels of TE_2 in whey of commercial whole milk fluctuate at a greater degree, especially in P_4; the levels of P_4 are high from March 16 to August 15, 2007 in China, which may be in relation to milk from pregnant cows.

PO3-284 Reproductive Endocrinology

Mutation screening of the insulin-like growth factor (*IGF-1*) and insulin-like growth factor-1 receptor (*IGF1R*) genes in patients with intrauterine growth retardation (IUGR) and postnatal catch-up growth failure: identification of a novel frame-shift mutation in the IGF1R gene

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Purpose: Around 10% of infants born intrauterine growth retardation (IUGR) remain short, and the causes of their growth deficits are often unclear. Insulin-like growth factor-1 (IGF1) and insulin-like growth factor 1 receptor (IGF1R) genes might cause some cases of prenatal and postnatal growth failures. To investigate the cause of growth retardation in IUGR, we analyzed *IGF1R and IGF1* genes in patients with IUGR with postnatal catch-up growth failure. **Methods:** Mutation screening of the *IGF1R and IGF1* genes has been undertaken in 23 patients with IUGR and postnatal catch-up growth failure (height more than 2 SD below the mean for age after 24 months of age). Auxological and endocrinological profiles were examined in all patients. Genomic DNA was extracted from peripheral leukocytes. All coding regions of the *IGF1R and IGF1* genes were amplified by polymerase chain reaction (PCR) and directly sequenced.

Results: Out of 23 patients with IUGR, a 6.8-year-old Korean girl with short stature had a mutation in the *IGF1R* gene. She was born at 41st week gestation as the second child of a nonconsanguineous marriage. Her birth weight was 2,450 g (-1.975 SDS). Her height was 102.8 cm (-2.68 SDS), and her weight was 17.7 kg (-0.839 SDS). Physical examination was unremarkable. Her puberty staging was Tanner B1P1, and her bone age was 5 years and 9 months, as determined by the Greulich-Pyle method. Blood chemistry analysis showed normal serum TSH and free T4 levels. Her serum IGF-1 level was 196 ng/mL (43-373 ng/mL) and IGFBP-3 level was 2,625 ng/mL (2,000-4,210 ng/mL). The height of her father was 150.6 cm (-3.769 SDS) and her mother 156 cm (-0.340 SDS). The patient's elder brother was born at 40 weeks of gestation with a birth weight of 2,550 g (-2.215 SDS) and was reported to have height of 114.2 cm (-2.964 SDS) at 9.5 year old. *IGF1R* gene analysis revealed a heterozygous mutation of c.420del (p.Ala140fsX20) of *IGF1R* in exon 2, resulting in a frame-shift with premature termination of IGF1R protein. Her father and elder brother also carried the same mutation, indicating an autosomal dominant inheritance.
Conclusions: Three members of one family who showed intrauterine and postnatal growth retardation, with normal serum IGF-1 levels, demonstrated a novel frame-shift mutation of the *IGF1R* gene. The study suggests *IGF1R* mutation is an uncommon cause of intrauterine and postnatal growth failure in humans.

PO3-285 SGA

The polymorphism Asp9 Asn of lipoprotein lipase gene in children with low birth weight (below 2500g)

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Children who are born with low birth weight (less 2500g) are known to have an increased risk of heart diseases in adult live. Lipoprotein lipase (LPL) plays a regulatory role in the pathogenesis of hyperlipidemia and a modulatory role in the control of inflammatory response.
The aim of this study was to determine whether the presence of polymorphism Asp9Asn in gene of lipoprotein lipase is associated with susceptibility to lipid disturbances in children with low birth weight.
Methods: The associations between Asp9Asn polymorphism in the gene for lipoprotein lipase, apoptosis activation, lipid peroxidation and lipid profile were examined in 165 children with low birth weight aged 4 -11 years and in 39 children born with normal weight as a control group.
Results: The polymorphism Asp9Asn of the LPL gene is presented only in 3 children with LBW, but not in control group. The frequency is 0.023.
In the group with polymorphism gene 2 children with LBW have the 50 Kb domains on the DNA electrophoretic profiles and susceptibility to apoptosis and infection, but 1 child with LBW does not have it. The effect of the Asp9Asn polymorphism within LPL gene on the serum total HDL levels were not observed. The levels of HDL-cholesterol, LDL-cholesterol, triglycerides and lipid peroxides were on normal levels. Among all the children with the polymorphism, the lowest level of HDL-cholesterol and higher level of triglycerides than normal levels were observed in one girl only. Two children have BMI SDS above 1.6 and they are overweight without the growth disturbances. One child has a normal weight and height.
Conclusions: 1.In children with low birth weight the Asp9Asn polymorphism in LPL gene is rare and is not connected with lipid pattern disturbances.
2.The Asp9Asn polymorphism of LPL gene is not reason for heart diseases in adult live in children with LBW.

PO3-286 SGA

Catch-up growth in late preterms: does it occur beyond the age of two?

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Objective:
Information on growth of *late* preterms (GA 32-35 weeks) is scarce. Earlier we found that late preterms showed a tendency towards catch-up growth up to the age of 2 years. Our aim now was to investigate whether growth patterns of late preterms showed proceeding catch-up growth during the next 2 years, up to the age of 4 years.
Methods:
In a community based cohort study of children born in 2002 and 2003 (n=2460, of whom 1150 *late* preterms; response rate 80%), we retrospectively extracted data on growth since birth. Sources were files of Preventive Child Healthcare Services throughout the Netherlands, hospital charts and national perinatal registration files. Body height and body weight were transformed in standard deviation scores
Results:
- *Late* preterms had significant catch-down growth (mean -1.5 SD-score for body height and -1 SD-score for body weight) from birth until discharge several weeks later.
- After term, till 4 years, catch-up growth was on average + 1 SD-score for height and + 1 SD-score for weight.
- Approximately one third of total catch-up growth (0.3 SD-score) took place between the age of 2 and 4 years.
Conclusion:
Late preterm children show significant catch-up growth. Beyond the age of 2 years, catch-up growth proceeds, but is insufficient in 8% of *late* preterm children compared to children born at term. Close surveillance of growth is required in *late* preterm children as it seems likely that this group follows a different growth pattern.

PO3-287 SGA

Growth of late preterm infants during the first four years of life

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Objective:
Previous studies showed that *early* preterms (gestational age [GA] <32 weeks) have an increased risk for growth retardation. Information on growth of *late* preterms (GA 32-<36 weeks) is scarce. Our aim was to investigate catch-down and catch-up growth following birth until 4y of age in *late* preterms.
Methods:
We performed a community based cohort study on children born in 2002 and 2003, aged 3 years and 9 months at entry (n=2460, of whom 1150 *late* preterms; response 80%). We retrospectively extracted data on growth since birth from files of Preventive Child Healthcare Services throughout the Netherlands. In addition, growth data were extracted from hospital charts and national perinatal registration files.
Results:
- *Late* preterms had significant catch-down growth (mean -1.5 SD-score for body height and -1 SD-score for body weight) from birth until discharge several weeks later.
- After term, till 4 years, catch-up growth was on average + 0.75 SD-score for height and + 0.5 SD-score for weight. However, approximately 13% of *late* preterms remained below -2SD (P2) for height at the age of 4y.
Conclusion:
Late preterm children have catch-down growth following birth and catch-up growth following discharge. Up to 4y of age, catch-up growth is insufficient in approximately one seventh of the *late* preterm children on a normal growth

chart. Our data indicate that close surveillance of growth is required in *late* preterm infants during the first years of life as *late* preterms possibly follow different growth patterns.

PO3-288 SGA

Increased insulin resistance and oxidative stress in obese and non-obese pre-pubertal children born small and large for gestational age

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Birth weight (BW) and obesity are associated with an increased risk of adult diseases. Insulin resistance (IR) seems to play a key role in small (SGA) and large (LGA) for gestational age children, whereas no data on oxidative stress are available. The aim was to evaluate the effect of BW and obesity on oxidative stress and IR in pre-pubertal SGA and LGA than appropriate for gestational age (AGA) children.
We performed a cross-sectional study comparing oxidative stress and IR in 103 children divided into six groups according to BW (26 SGA, 15 AGA, 16 LGA normal-weight children) and obesity (15 SGA, 15 AGA, 16 LGA obese children). Infants born from mothers with gestational diabetes mellitus, hypertension, obesity were excluded. Indexes of IR (homeostasis model assessment, glucose to insulin ratio) and marker of oxidative stress (urinary isoprostanes) were evaluated.
Homeostasis model assessment was higher in both normal-weight SGA and LGA than in normal-weight AGA children (all $P \leq 0.02$). Furthermore, a difference was detected between obese SGA and obese LGA subjects than normal-weight SGA (all $P \leq 0.0007$) and LGA children (all $P \leq 0.01$), respectively. Glucose to insulin ratio was lower in the three obese groups than normal-weight AGA (all $P \leq 0.009$) and normal-weight SGA children (all $P \leq 0.02$). Furhermore, a difference was detected between obese SGA and obese LGA compared to normal-weight LGA subjects (all $P \leq 0.0002$). Isoprostanes levels were higher in both normal-weight SGA and LGA than in normal-weight AGA children (all $P \leq 0.002$). Moreover, both obese SGA and LGA showed higher levels than obese AGA subjects (all $P \leq 0.01$) and in comparison to the three normal-weight groups (all $P \leq 0.04$).
In conclusion, increased IR and oxidative stress are already present in pre-pubertal normal-weight SGA and LGA children with a continuous alteration in relation to obesity, suggesting that BW and adiposity represent two independent risk factors for degenerative diseases.

PO3-289 SGA

The PPARγ Pro12Ala polymorphism and risk factors for cardiovascular disease: a longitudinal study in short children born small for gestational age treated with growth hormone

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The Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPARG) gene has been associated with type 2 diabetes mellitus (DM), BMI, and growth and small for gestational age (SGA) birth is associated with a higher risk for type 2 DM and cardiovascular disease (CVD) in later life. Growth hormone (GH) treatment of SGA children has divergent effects on determinants of the risk for type 2 DM and CVD.
Objective: To analyze the contribution of the *PPARγ* Pro12Ala polymorphism to GH-induced changes in determinants of metabolic and cardiovascular disease in short SGA children.
Methods: *PPARγ* was genotyped in 238 Caucasian short SGA children (mean age 7.5 years) and we sought associations with determinants of metabolic and cardiovascular diseases before and during GH therapy. We evaluated height, weight, blood pressure, serum lipids, body composition by DXA (n = 79), and glucose homeostasis by frequently sampled intravenous glucose tolerance test (FSIGT) (n = 51) and HOMA-IR (n = 148) at start and after 4 years of GH treatment.
Results: At baseline, the Ala12 allele was not associated with any determinant of metabolic and cardiovascular disease. After 4 years of GH treatment, the increase in weight for height SDS and in body mass index (BMI) SDS was significantly greater in carriers of an Ala12 allele than in non-carriers. The change in all other evaluated parameters was not associated with Pro12Ala genotype.
Conclusion: The Ala12 variant of the PPARγ gene promotes weight gain during GH treatment but is not associated with changes in determinants of metabolic and cardiovascular diseases in Caucasian subjects born SGA.

PO3-290 SGA

Low-birthweight children develop low SHBG and high DHEAS levels, and aggravate their visceral adiposity and hypoadiponectinemia between 6 and 8 years of age

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Background
Most children born small-for-gestational-age (SGA) normalize their body size by late infancy. Between 2-6 yr, such SGA children tend to become hyperinsulinemic, hypoadiponectinemic and viscerally adipose, even in the absence of overweight. Here, we report on their further course between 6-8 yr.
Study Design & Population
Longitudinal study over 2 yr comparing data from 32 appropriate-for-gestational-age (AGA) versus 32 SGA children, matched for gender, height, weight and BMI at the age of 6 yr.
Main Outcomes
Fasting insulin, DHEAS, SHBG, high-molecular-weight (HMW) adiponectin, leptin, IGF-I; body composition by absorptiometry; abdominal fat partitioning by magnetic resonance imaging.
Results
Between 6-8 yr, novel AGA-vs-SGA divergences emerge (higher DHEAS and lower SHBG in SGA; $P<0.001$) and some earlier divergences widen further (HMW adiponectin, visceral fat; $p<0.001$) while others stabilize (fasting insulin, IGF-I).
Conclusion
SGA children with spontaneous catch-up growth develop high DHEAS and low SHBG levels, and aggravate their HMW hypoadiponectinemia and their visceral adiposity between 6-8 yr of age.

PO3-291 SGA

Study of the regulation of the *IGFBP3* gene expression in short children born small for gestational age

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Most children born small for gestational age (SGA) show catch-up growth during the first 2 years of life, but approximately 15% of them continue to be short throughout childhood, adolescence and in adulthood. The functional role of the polymorphisms in the genes involved in the pathway of insulin-like growth factors (IGFs) remains to be elucidated in determining SGA phenotype and SGA-related outcomes.
In the *IGFBP3* gene's promoter region we have identified 4 new polymorphisms (-1112 G/A, -1239 G /A, -1510 C /T and -1556 G /A) in SGA children, three of which have turned out to be statistically significant when compared to controls. Moreover, a preliminary analysis of regulatory regions of the *IGFBP3* gene has highlighted the presence of putative p53 binding consensus sequences. It has been already shown that p53 family members (p53, p63,p73) are involved in the *IGFBP3* expression regulation. Indeed it has been reported

that *IGFBP3* is a p53 direct target.
The aim of our study is to analyze in SGA children the functional significance of the polymorphisms we identified in the promoter region of *IGFBP3* gene and to search whether the members of the p53 oncosuppressor gene family, in particular p63 and p73 proteins, are involved in the regulation of *IGFBP3* gene expression.
As to the analysis of the polymorphic sites identified in the *IGFBP3* promoter region, we focused our attention in particular on the 1239 and 1510 polymorphic sites: the first site is part of a consensus sequence for NF-kappaB (Nuclear Factor-kappaB) transcription factor involved in the immune response, in cell proliferation, differentiation, adhesion, angiogenesis and apoptosis; the second one is part of a consensus sequence for binding to the E2F transcription factor which regulates the expression of the genes involved in cell cycle progression, DNA synthesis, repair, recombination and a variety of other cellular processes. The polymorphic regions of *IGFBP3* promoter and the p53 responsive elements have been amplified and cloned upstream from the luciferase reporter gene of pGL3basic and pGL3promoter reporter vectors. The recombinant reporter vectors have been transfected into the H1299 cell line and into the HepG2 (hepatocarcinoma) cell line in the presence or absence of insulin and the effect of the polymorphisms and of the p53 family members on the reporter gene transactivation compared to control has been evaluated at different times after trasfection (i.e. after 24h, 48h, and 72h).

PO3-292 SGA

The metabolic risk status in non-obese prepubertal children born small for gestational age

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Background: Children born small for gestational age (SGA) are at risk for developing insulin resistance (IR), type 2 diabetes, hyperlipidemia, hypertension and cardiovascular disease in adulthood. The antecedents of metabolic disturbance may be present early in childhood before puberty. A key feature of metabolic syndrome is resistance to the action of insulin. Low grade chronic inflammation and adipose tissue function has also been proposed to be an underlying mechanism for IR.
Objective: To evaluate IR and early markers of metabolic disturbances among non-obese prepubertal SGA children.
Method: Study group consisted of 34 prepubertal, term, SGA children aged 6-10 years and was further subgrouped due to catch-up growth (CUG).Control group included 47 prepubertal, term, appropriate for gestational age (AGA) children aged 6-10 years. After an overnight fast, blood samples were obtained for glucose, insulin, triglycerides (TG), total colesterol, LDL-colesterol, leptin, adiponectin, tumour necrosis factor alfa (TNF-α), insulin like growing factor1 (IGF-1), IGF binding protein1 and 3 (IGFBP-1, IGFBP-3). The homeostasis model assessment of IR (HOMA- IR) was calculated. A year later 16 SGA children underwent an oral glucose tolerance test (OGTT); glucose and insulin levels were obtained at basal and 120 minutes after giving 1.75 g/kg of 25% glucose solution (maximum 75 g) orally.
Results: No significant difference in insulin, glucose and HOMA-IR was found between SGA and AGA children. SGA children had higher triglycerides, TNF-α and IGF-1 than AGA children ($p<0.05$; $p<0.0005$; $p<0.01$). SGA children with CUG had higher leptin, IGFBP-3 ($p<0.01$; $p<0.001$) and lower IGFBP-1($p<0.05$) than SGA children without CUG. IGF-1 tended to be higher among SGA children with CUG ($p=0.053$). One subject was diagnosed with impaired glucose tolerance (120' glu: 146 mg/dl, insulin: 134μU/ml) and another with fasting hyperglycemia (0' glu: 108 mg/dl) during OGTT.
Conclusion: IR was not determined with simple surrogates of insulin sensitivity such as basal insulin or HOMA-IR among non-obese prepubertal SGA children. There was also no difference for adiponectin and leptin secreted from the adipose tissue. However higher levels of triglycerides, IGF-1 and TNF-α can be early markers of decreased insulin sensitivity which could have been shown by more sophisticated, sensitive methods such as clamps.Thus SGA children should be followed for metabolic changes starting from early childhood.

PO3-293 SGA

SGA classification: important of use of correct newborn tables with sex differentiations

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Previews: A correct SGA classification in newborn periodic is very import in order to detect in the future the children who no have a correct catch up and value the need or not of use of rhGH.
In Spain, tables of Delgado et al (An Esp Pediatr. 1996;44:55-9.) had been used for this fact and accept from our Local Commite for use of rhGH. These tables have not sex difference. Recently, Carrascosa et al (An Esp Pediatr. 2008) has publish a new tables of newborn growth with sex differentiation named "Spanish study of growth".
Objective: Study the difference between the use of these different growth newborn tables in the classification of SGA in our population.
Methods and Analysis: Study and classication of 4.934 newborn life (♂2.485) from single pregnant of our Hospital (years 2005-2006) about weight, tall in relation of their age of gestation respect Delgado tables (1.996) and Carrascosa (2008). SGA was if weight of tall < 2 SDS. (Stadiometer and precisions bascule. Study type T-Student and Z-score. SPSS vv 14.0).
Results: 345 SGA with Delgado tables and 319 with Carrascosa tables (p:0.085). 4,2/4.8% from the total. About sex: BOYSè 98 with Delgado, and 140 with Carrascosa (p:0.002). Infra-estimation of SGA if only we use Delgado tables of 30% p:0.001 IC 95% [26-33]. GIRLSè 247 with Delgado, and 179 with Carrascosa (p:0.005). Upper-estimation of SGA if only we use Delgado tables of 28% p:0.001 IC 95% [22-31].
Conclusions: The use of newborn tables without sex differentiation could be a sesgo of selection in the SGA classification. In our study, there is a infra-estimation of the boys (because the boys have more weight and tall respect girls); and a upper-estimation of the girls. We recommend the use of tables with sex differentiation like Carrascosa 2008 in the SGA classification.

PO3-294 SGA

Changes in somatotrophic hormone concentrations in cord blood from small (SGA), appropriate (AGA) and large (LGA) for gestational age newborns

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Introduction: Fetal growth is the product of the availability of adequate oxygen and nutrients supplies, combined with the effect of growth factors. The insulin-like growth factor (IGF) system is an important endocrine factor influencing fetal growth. **Objective:** To study placental GH (pGH), IGF-I/II, IGFBP-1/2/3, ghrelin and ALS concentrations in cord blood from SGA, AGA and LGA full term newborns. **Methods:** We studied cord blood from 80 SGA (birth weight (BW) = -1.97 ± 0.08 SDS), 66 AGA (BW= 0.36 ± 0.10 SDS) and 63 LGA (BW= 2.69 ± 0.12 SDS) term gestations (37-41 weeks). We determined the cord blood concentrations by specific immnunoassays. **Results** are shown in the table as mean ± SEM, and statistical differences were determined by ANOVA.

Table

	SGA (n=80)	AGA (n=66)	LGA (n=63)
IGF-I (ng/ml)	68 ± 3.1*,**	78.2 ± 2.9	109.4 ± 4.3***
IGF-II (ng/ml)	577.9 ± 18.9*	655.1 ± 22.1	624.4 ± 34.4
IGFBP-1 (ng/ml)	165.5 ± 21.5 *,**	100.5 ± 20.3	62.4 ± 16.0
IGFBP-2 (mg/L)	2.8 ± 0.1*,**	2.3 ± 0.1	1.9 ± 0.2
IGFBP-3 (mg/L)	0.98 ± 0.08**	1.10 ± 0.07	1.40 ± 0.08
ALS (nmol/L)	42.4 ± 3.8**	46.7 ± 2.7	58.8 ± 3.8***
Ghrelin (pg/ml)	531.9 ± 30.3*,**	607.9 ± 29.2	456.4 ± 31.8***
pGH (pg/ml)	996 ± 190	636 ± 157	722 ± 156

p< 0.05, *SGA vs AGA; **SGA vs LGA, ***LGAvs AGA

We observed a significant direct correlation between birth weight and IGF-I (r=

0.53), IGFBP-3 (r= 0.431) and ALS (r= 0.38) cord levels, and a significant inverse correlation between birth weight and IGFBP-1 (r= - 0.521) and IGFBP-2 (r= - 0.426) cord levels.
Conclusion: The lower cord blood concentrations of IGF-I, IGFBP-3 and ALS and the higher cord blood concentrations of pGH, IGFBP-1 and IGFBP-2 observed in SGA newborns suggest that these growth factors may play a role in the development of fetal growth restriction. In contrast, the higher cord blood concentrations of IGF-I, IGFBP-3 and ALS and the lower IGFBP-1 and IGFBP-2 concentrations observed in LGA newborns suggest that they may facilitate the development of fetal macrosomia. (FONDECYT 1061082)

PO3-295 SGA

Differences in IGF-I/IGF-IR/IRS-1/ERK/AKT protein contents in human term placentas according birth weight

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Introduction: IGF-I and its receptor (IGF-IR) participate in both pre and postnatal growth. The human placenta expresses the mRNA and the protein for IGF-I and IGF-IR and their intracellular signal components (IRS-1, ERK y AKT). **Objective:** To study the protein contents of IGF-IR, IRS-1, ERK and AKT in human full term placentas (37 -41 weeks of gestation) from SGA, AGA and LGA newborns. **Methods:** We collected placentas from 31 SGA (birth weight (BW) = -1.91 ± 0.15 SDS), 27 AGA (BW= 0.25 ± 0.14 SDS) and 30 LGA (BW= 2.55 ± 0.19 SDS) newborns. We determined the protein contents by Western Blot in the chorionic (CP) and basal (BP) plates of the placentas using specific antibodies and the contents were normalized using beta-actin. **Results** are shown in the table as mean ± SEM: The differences were studied by ANOVA.

table1

		SGA	AGA	LGA
IGF-I (ng/g placenta)	CP	89.2 ± 6.1**	75.7 ± 4.4	39.1 ± 2.0***
	BP	81.9 ± 5.8**	68.4 ± 4.6	41.6 ± 2.3***
IGF-IR (AU)	CP	0.78 ± 0.16*,**	0.45 ± 0.07	0.26 ± 0.03
	BP	0.62 ± 0.08**	0.55 ± 0.16	0.24 ± 0.03***
IRS-1 (AU)	CP	0.34 ± 0.12	0.37 ± 0.19	0.27 ± 0.05
	BP	0.32 ± 0.07	0.48 ± 0.24	0.28 ± 0.05
AKT (AU)	CP	0.80 ± 0.19**	0.62 ± 0.17	0.34 ± 0.04
	BP	0.65 ± 0.18	0.62 ± 0.16	0.39 ± 0.06
ERK42 (AU)	CP	0.49 ± 0.11**	0.32 ± 0.07	0.17 ± 0.02
	BP	0.37 ± 0.07**	0.38 ± 0.12	0.18 ± 0.03***
ERK44 (AU)	CP	0.47 ± 0.10**	0.48 ± 0.15	0.17 ± 0.03***
	BP	0.35 ±0.05**	0.39 ± 0.08	0.16 ± 0.02***

p<0.05, *SGA vs AGA; **SGA vs LGA; ***LGA vs AGA. AU=arbitrary units

We observed an inverse correlation between birth weight with IGF-IR contents in CP (r= -0.299, p=0.005) and BP (r= -0.370, p=0.001). These inverse correlations were also observed for AKT, ERK 42 and ERK 44 in both plates of the placenta.
Conclusion: The higher contents of IGF-I, IGF-IR, AKT and ERK 42/44 in SGA placentas and the lower content of IGF-IR, AKT and ERK42/44 in LGA placentas suggest that these placental IGF-IR signal transduction proteins may influence fetal growth. (FONDECYT 1061082).

PO3-296 SGA

Insulin-like growth factor-I, d3-*GHR* gene polymorphism, prematurity and height at start as determinants of one year growth response during high dose GH; preliminary results from the NESGA study (NESGAS)

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Children born small for gestational age (SGA) without catch-up growth are now treated with growth hormone (GH) with good, although variable, outcome. However, the optimal dose and possible long term side effects still remain to be assessed. A common polymorphism in the GH receptor gene (d3-GHR) has previously been described to influence the response to GH treatment.
The North European Small for Gestational Age Study (NESGAS) is a multicenter study involving the UK, Ireland, Sweden and Denmark. Baseline data were collected for 110 patients (69 males), 62 patients (39 males) have completed first year of treatment with a GH dose of 67µg/kg/day. Serum IGF-I was determined by immunoassay, and the GHR-exon-3 locus was determined by simple multiplex PCR.
Mean age at start of treatment was 6.31 years (SD 1.82). After 12 months of GH therapy height and IGF-I significantly increased ΔHSDS = 0.96 SDS (SD 0.41 SDS), and ΔIGF-I SDS = 3.55 SDS (SD 1.73). In the entire cohort, 61% were homozygous for the wild type *GHR* genotype (fl/fl), 32% were heterozygous for the d3 allele (d3/fl) and 7% were homozygous for the d3 allele (d3/d3). There was a trend towards less severe growth retardation at baseline among children with d3/fl or d3/d3 (HSDS -2.98) than among children with the fl/fl genotype (HSDS -3.21), this did not reach significance (P=0.13).
There was a strong association between ΔIGF-I SDS and ΔHSDS (B 0.14, SE 0.03, P<0.0001). First year ΔHSDS was inversely associated with baseline IGF-I SD scores (B -0.103, SE 0.04, P=0.016), HSDS (B -0.203, SE 0.07, P=0.007), and HSDS corrected for midparental height (B -0.172, SE 0.05, P< 0.0001). d3-*GHR* genotypes were not associated with ΔHSDS or ΔIGF-I SDS. In a multivariate analysis of possible predictors of one year growth response only baseline HSDS at baseline remained significant (table).
In conclusion, low IGF-I levels and low height at baseline were associated with a better first year growth response to GH treatment. There was no effect of gestational age at birth or the d3-*GHR* polymorphism on first year growth response.

Table1: Possible predictors of 1 year growth response

Independant variables	B coefficient	SE	P-value
Gender	0.064	0.112	0.570
Gestational age	-0.016	0.014	0.258
IGF-I (SDS)	-0.074	0.043	0.092
Height (SDS)	-0.181	0.079	0.026
d3-GHR	-0.033	0.111	0.766

Dependent variable: ΔHeight (SDS)

PO3-297 SGA

Phenotypic characteristics of short children born SGA. Baseline data from the North European small for gestational age study (NESGAS)

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Children born small for gestational age without catch-up growth at four years of age can be treated with growth hormone (GH) with a good outcome, however, the optimal dose and possible long term side effects still remain to be assessed.
The North European Small for Gestational Age Study (NESGAS) is a multi-

center study including centres from the UK, Ireland, Sweden and Denmark. Patients were recruited over a 5 year period (2003-2008). A total of 110 patients have been enrolled and 62 patients have completed first year of treatment.
The inclusion criteria were: birth weight (BW) and/or birth length (BL) SDS <-2 SD for national references, gestational age (GA) > 28 weeks, height SDS (HSDS) at baseline < -2.5 SD and HSDS more than 1 SD below midparental HSDS. All patients were treated with GH 67 µg/kg/day for the first year and were then randomised into three different groups of treatment. Serum IGF-I was determined by immunoassay. Auxology was recorded at baseline before start of GH treatment. We present baseline characteristics of these subjects.
Baseline data was collected on 110 patients (69 males) prior to starting GH treatment. Mean GA was 35.26 weeks (SD 3.95 weeks) and mean BWSDS was -2.82 SDS (SD 0.92 SDS). Mean age at start of GH treatment was 6.30 years (SD 1.75 years), mean HSDS was -3.15 SDS (SD 0.66 SDS), mean weight SDS was -3.04 SDS (SD 1.71 SDS) and mean IGF-I SDS was -1.11 SDS (SD 1.22 SDS). Patients were divided into 3 GA groups: < 32 weeks, 33-36 weeks, and > 37 weeks. There was a negative association between GA and HSDS at baseline (B -0.15, SE 0.78, P=0.05). Conversely, there was a positive association between GA and WSDS at baseline (B 0.47, SE 0.21, P=0.03). Patients were grouped as IGF-I deficient (IGF-I SDS < -2), IGF-I sufficient (-2 < IGF-I SDS < -1) and IGF-I resistant (IGF-I SDS >-1). IGF-I deficient children had significantly lower HSDS compared to IGF-I-sufficient and -resistant children, but BW, BL, blood pressure and lipids were similar in the 3 groups. There was no association between GA and IGF-I at baseline.
In conclusion, premature SGA children were significantly shorter and heavier than mature SGA subjects, and IGF-I deficient subjects were significantly shorter compared to IGF-I-sufficient and IGF-I resistant children.

PO3-298 SGA

Individual height velocity outcome after one and two years of either individualized or fixed dose growth hormone (GH) treatment in short children born small for gestational age (SGA): results of the OPTIMA trial

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The first year (yr) height velocity (HV) response to GH treatment in short children born SGA is highly variable. However, there is no scientific consensus on how a 'sufficient' response is defined. The EU Regulatory Authority approved indication considers a HV standard deviation score (SDS) of ≥+1 during the 1st yr sufficient to continue treatment. Here we report the HV SDS outcome after Yr 1 and 2 in short children born SGA, treated either with an individually adjusted GH dose (IAD) or a fixed high dose (FHD) from the randomized, open-label OPTIMA trial.
Children born SGA (birth weight <10th percentile and/or birth length <-2 SDS) with height (Ht) SDS ≤-3; age ≥3yr; pre-pubertal; bone age ≤9/10yr for girls/boys) were randomized to receive GH at FHD (0.067mg/kg/d) or IAD (0.035mg/kg/d up to month 3; followed by 0.067mg/kg/d up to month 24 if predicted 1-yr change in Ht SDS (ΔHt SDS) <0.75 (IA high); or continuation on start dose until month 12, then shift to 0.067mg/kg/d if actual 1-yr ΔHt SDS <0.75). Results are presented for pts in the 2-yr extension full analysis population (N=175; FHD 91, IAD 84).
Both groups were comparable at baseline (FHD/IAD: 55%/52% male, (mean±SD) age 6.7±2.4/6.8±2.5 yrs, Ht SDS -3.9±0.6/-3.8±0.6, HV SDS -1.6±1.7/-1.6±1.8). In the IAD group, of the 44 pts remaining on the low GH dose of 0.035mg/kg/d during Yr 1, 33 also received the low dose during Yr 2 (IA low-low); dose was increased to 0.067mg/kg/d in 11 pts at start of Yr 2 (IA low-high). ANCOVA indicated a statistically significant, but clinically not relevant difference in absolute HV after 2 yrs of -0.69 cm between the IAD and FHD group (IAD-FHD, least-square mean -0.69; 95%CI [-1.07;-0.31]).

HV SDS after 1 and 2 yrs of GH

HV SDS	FHD	IA high	IA low-low	IA low-high
1 yr: N	91	40	33	11
Median (Q1;Q3)	5.1 (3.1; 6.4)	3.4 (2.2; 4.7)	3.8 (3.0; 5.0)	2.2 (1.7; 2.2)
n (%) ≥+1 SDS	89 (97.8)	32 (80.0)	33 (100.0)	10 (90.9)
2 yrs: N	88	40	32	10
Median (Q1;Q3)	3.4 (2.4; 4.6)	2.8 (1.6; 3.6)	2.9 (2.3; 3.4)	2.3 (1.7; 2.6)
n (%) ≥+1 SDS	85 (96.6)	34 (85.0)	29 (90.6)	9 (90.0)

After 1yr of GH, the percentage of patients in the IAD groups who achieved a HV SDS ≥ the +1 SDS 'cut-off' for treatment continuation ranged from 80.0-100.0%. Although mean HV SDS declined in Yr 2 of GH treatment as expected, the percentage of patients meeting the 'criterion' in these IAD groups ranged from 85.0-90.6% at the end of Yr 2.

PO3-299 SGA

High frequency of endocrine tumours in mulibrey nanism

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Mulibrey nanism (MUL) is a monogenic growth disorder. Patients are born small for gestational age (SGA) with immature craniofacial features. Other clinical characteristics are progressive postnatal growth failure, mild dysmorphic features, cardiopathy, and tendency for a metabolic syndrome but no major neurological handicap. MUL is caused by recessive mutations in the *TRIM37* gene encoding for the peroxisomal TRIM37 protein with E3 ubiquitin-ligase activity.
We analyzed the frequency and pathology of malignant and benign tumours in the national cohort of 92 Finnish MUL patients with an age-range of 0.7 to 77 years. The subjects had clinical and radiological evaluations. Histological and immunohistocemical analyses were performed on specimens obtained from biopsy, surgery or autopsy.
The results indicate that the MUL patients have disturbed organogenesis and a high frequency of both benign and malignant tumours in several internal organs, many of them of endocrine origin. A total of 237 tumorous lesions were detected in 70 of the 92 patients (76%). Sixteen malignancies occurred in fourteen patients (15%); eight of them in the kidney (six Wilms' tumours), three in the thyroid gland, two gynaecological cancers, one gastrointestinal carcinoid tumour, one neuropituitary Langerhans cell histiocytosis and one case of acute lymphoblastic leukaemia (ALL). Tumours detected by radiology in the liver and other organs mainly comprised strongly dilated blood vessels (peliosis), vascularised cysts, benign adenomas and nodular lesions, as well as fibrous dysplasia of long bones. The majority of the lesions showed strong expression of the endothelial cell markers CD34 and CD31 as well as the myocyte marker α-smooth muscle actin.
Our findings show that MUL is associated with frequent malignant tumours and benign cystic adenomatous and vascular lesions particularly in endocrine tissues, as well as disturbed organ development. This speaks for a central role of TRIM37 protein in the cellular functions regulating proliferation, migration and angiogenesis.

PO3-300 SGA

Influence of preterm birth and birth size on gonadal function in young men

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Background / Objectives: Preterm birth has been associated with reduced reproduction rates and being born small for gestational age with reduced gonadal function. We hypothesized that alterations concerning gonadal function in young men are not due to preterm birth or being born small for gestational age (SGA), but to other (environmental) factors.
Methods: In 207 young men of the PROGRAM/PREMS cohort study, aged 18-24 years, the influence of preterm birth, birth length and birth weight on serum levels of anti mullerian hormone (AMH), inhibin B, Testosterone, SHBG, LH, FSH, FSH/inhibin B-ratio, LH/T-ratio and T/SHBG-ratio were analyzed with multiple regression modeling. In addition, markers of male gonadal function were analyzed in 4 subgroups: men either born SGA with short stature (SGA-S) or with catch-up growth (SGA-CU), or men born Appropriate for Gestational Age (AGA) with Idiopathic Short Stature (ISS) or with normal stature (Controls).
Results: Preterm birth and SGA did not affect gonadal function. After adjustment for age, birth size, adult height, fat mass and socio economic status (SES) preterm birth even showed a positive relation with inhibin B. Higher SES was associated with higher inhibin B levels. Higher fat mass and maternal smoking were associated with decreased T-levels and increased LH-levels, respectively. After adjustment for confounders, there were no significant differences in gonadal function between the subgroups.
Conclusion: Preterm birth and SGA did not affect gonadal function in young men. Factors associated with a reduced gonadal function were: maternal smoking during pregnancy, lower SES and a higher fat mass.

PO3-301 SGA

Insulin, adiponectin, leptin and IGFBP-1 levels in short children born small for gestational age

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Introduction: Intrauterine growth restriction is associated with an increased risk of developing metabolic and cardiovascular diseases later in life. Small for gestational age (SGA) children may develop an impaired insulin sensitivity compared to children born appropriate for gestational age (AGA).
Adiponectin, a peptide derived from fat cells promotes insulin sensitivity and hypoadiponectinemia has been associated with insulin resistance. Elevated plasma leptin levels have been demonstrated to correlate with insulin resistance. Insulin like growth factor binding protein-1(IGFBP-1) has been reported to be a useful marker of impaired insulin sensitivity and predictor of insulin resistance.
Objective: The aim of this study was to determine whether being born SGA has an impact on serum adiponectin, IGFBP-1, leptin and insulin levels.
Patients and methods: The study was carried out in 34 short SGA and 20 short AGA prepubertal children. Children born preterm, with complicated neonatal period, endocrine, metabolic or genetic disorders were excluded from the study. Serum adiponectin, IGFBP-1, leptin, fasting glucose, insulin, the homeostasis model assessment insulin resistance index (HOMA- IR) and anthropometric indices were evaluated in all of them.
Results: There were no significant differences in age (5, 4 ±1, 27 vs. 5, 73±0, 98 yrs) as well as in height (-3,61±0, 78 vs. -3,29±0,75 SDS) between SGA and AGA group. SGA children had higher fasting insulin levels and HOMA-IR than AGA children (insulin, 4,18±0,99 vs. 2,79±0,93 mU/l p<0,001; HOMA-IR, 0,55±0,73 vs.0,25±0,52, p<0,05). Glucose levels were similar in both groups. Adiponectin levels were lower in SGA than in AGA children (10,75±3,07 vs. 13,02±2,37 mg/l, p<0,025). No significant differences in serum IGFBP-1 (SGA group 210,56±118,54 vs. AGA group 243,02±117,48 µg/l, p>0,1) as well as leptin levels (SGA group 2,7±2,2 vs. AGA group 2,15 ±1,5 µg/l, p>0,1) were stated between the groups.
Conclusions: Short SGA prepubertal children show the presence of reduced adiponectin levels and predisposition to develop insulin resistance. IGFBP-1 and leptin levels do not differ between short SGA and AGA children.

PO3-302 SGA

Neurocognitive development in children born small for gestational age (SGA) due to intrauterine growth retardation (IUGR)

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This study analyzes the psychomotor and intellectual development of SGA children due to IUGR. **Patients.** n=163, ages: 3 months-17 yrs; 56 males. **Methods**. Tests: 0-2 yrs: Brunet Lezine; 3-6 yrs: McCarthy; 7-15 yrs: Weschler Intelligence; 16-18 yrs: Weschler Adult Intelligence. Reference values: Longitudinal Study of Normal Spanish Children (3m-18yrs) performed by the same psychologist who studied the patients. Factors analyzed: spontaneous growth, catch up present (+) (n69) or absent (-) (n90), GH therapy in the catch up - cases (n47), gestational age: term (n101) or preterm (n62), socioeconomic level of the family (Graffar Scale), Apgar score, neonatal comorbidity and head circunference (n82). Statistical study: comparison of means (p<0.05). **Results**. The mean IQ was -1.1(0.5) SD at a mean age of 6.0(3.8) yrs; over the yrs IQ remains between -0.6 SD (the best mean score of 99 cases controlled yearly along 3 yrs) and -1.3 SD (the lowest mean score of 11 cases controlled yearly along 7 yrs). Catch up - cases show a lower IQ compared to catch up + with statistical significance at 6 yrs of age (-1.1 vs - 0.4 SD). Being term or preterm have no impact on IQ in the catch up + and in the catch up - group it only show influence at 10 yrs of age (-0.2 SD vs -1.6 SD, p>0.05). In the catch up - group there was no statistical difference between GH treated and untreated. SGA with catch up + show better IQ than catch up - children treated with GH being significant at 9 yrs of age and after 2 yrs of therapy (-0.4 SD vs -1.2 SD). In the catch up - group treated with GH the IQ in the preterm SGA were worse than in the term cases. The parents´socioeconomic level did not show influence. 22% of the SGA children show IQ equal or less than - 2 SD and 10.1% less or equal - 3 SD. The familial socioeconomic level, gestational age, head circunference, Apgar socre and comorbid perinatal factors did not play a significant role, with few exceptions, on IQ development. The most affected areas were: 3m-2yrs: postural control (-1.3 SD); 3-6yrs: motor function (-1.1 SD); after 7yrs: performance IQ (-1.0 SD); in the verbal scale the arithmetic test showed the lowest value. (-0.9 SD). **Conclusions**. SGA children due to IUGR have in many cases an impaired IQ that worsens with age, independently of the child growth although it has less effect on those whose spontaneous growth is better, and up to 22% show an IQ severely affected. Prevention of IUGR is of most importance.

PO3-303 SGA

Similar change in height standard deviation scores during the first 2 years of growth hormone therapy for children with growth hormone deficiency, multiple pituitary hormone deficiencies and short children born small for gestational age: data from the NordiNet® international outcome study (IOS)

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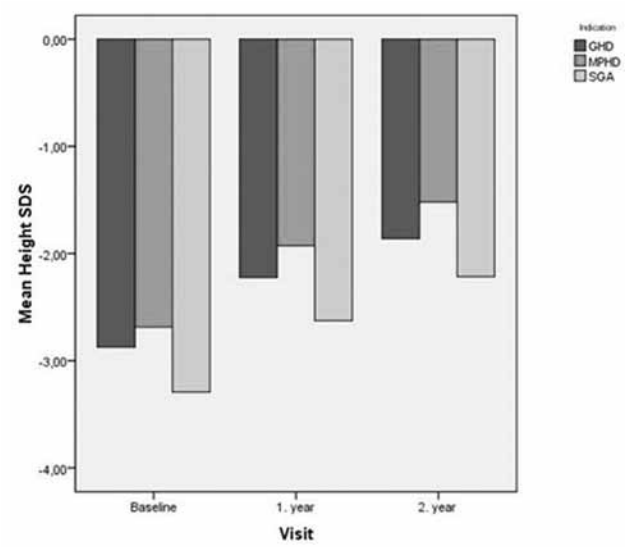
Background: Growth rates after initiation of therapy with growth hormone (GH) are generally expected to be different in different indications. The goal of this study was to assess growth response as indicated by change (Δ) in height standard deviation scores (Ht SDS) during the first 2 years of GH therapy (Rx) for 3 conditions: small for gestational age (SGA), GH deficiency (GHD), and multiple pituitary hormone deficiency (MPHD). The relationship of Δ Ht SDS was determined for other factors including condition/indication, age at onset of GH Rx, Ht SDS at baseline, GH dose, insulin-like growth factor I (IGF-I) and pubertal status based on age.

Methods: Data from NordiNet® IOS have been analyzed for growth rates among 1224 children with SGA (423), GHD (686), and MPHD (115) having Ht SDSs and average dosage (mg/kg/day) at baseline, 1 and 2 years. Descriptive statistics and analysis of variance were done for condition/indication, age at onset of Rx, average GH dosage, and HtSDS at baseline, using Δ Ht SDS as the dependent variable. These data were reanalyzed adding an age variable since the older group would include some pubertal individuals, males< or >12 years and females< or >11 years. The subset with IGF1 levels were also analyzed.

Results: Similar, good growth responses indicated by change in HtSDS were present for all 3 groups (Figure 1). Significant differences were present for dose of GH at onset and during the 2 years of Rx. After 1 and 2 years of treatment the Δ Ht SDS was significantly related to indication, age onset of therapy and HtSDS at baseline. Average GH dosage as a co-variant significantly influenced the Δ Ht SDS after 1 year ($p<0.0001$). When pubertal categories were added to the model, this was also significant with P= 0.038 at 2 years. Δ IGF1 levels were significant at 1 and 2 years comparing to baseline (<.0001).



Conclusions: Δ Ht SDS is similar in patients with SGA and GH deficiency patients. Δ Ht SDS was related to age of onset of therapy, baseline Ht SDS, and dosage of GH therapy, and reflected in similar changes in IGF1 levels. Further data are needed to assess the interactions of pubertal status and dosage of GH.

PO3-304 SGA

Distance to target height as a prerequisite for growth hormone (GH) treatment in short children born small for gestational age (SGA); valuable or not?

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GH treatment is approved as a therapy for short children born SGA. The criteria to receive GH differ between Europe and USA. The role of distance to TH in European criteria has been subject to discussion.

Aim: Our objective was to investigate whether short SGA children with a distance to TH of <1 SD have significantly lower response to GH treatment than those with a distance >1 SD.

Methods: 465 short children born SGA were divided into 3 groups according to the distance to TH (distance ≤1 n=15; distance 1-2 n=127; distance ≥2 n=323). Short and long term growth response on GH were analysed, 150 children had reached adult height (AH).

Results: Multiple regression for dependent variable height gain SDS (AH SDS – height SDS at start) showed a significant influence of distance to TH. This influence remained significant after correcting for multiple variables as sex, age at start, GH dose, bone age delay, birth length SDS, IGF1 and IGFBP3. Looking to father and mother height SDS separately, only mother's height SDS turned out to be positively correlated. Multiple regression showed no significant influence of a distance to TH <1 SD, however this was a small group of children. When growth was investigated individually, a wide range of responses was seen. The three groups showed better results of GH treatment when the distance to TH was larger, regarding first year growth, height gain SDS, AH SDS and percentage of AH > -2SDS. Nevertheless, these differences disappeared after correction for sex and initial age.

Conclusions: Distance to target height in short children born SGA plays, next to other variables, a significant role in the response to GH treatment. Especially the height of mother seems to be relevant. The European prerequisite of a distance to TH of >1 SD is not supported by our study; the cut-off level might be worth reconsidering.

PO3-305 SGA

Distribution of birth weight according to age of mothers from families in risk situation

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Birth weight is a sign of fetal development and it has been associated with diseases later in life. This study aimed to identify the prevalence of low and high birth weight infants from pregnant women with different ages from low social class. Methods: We evaluated 518 women who are part of a population study conducted with low-income families in Curitiba - Parana - Brazil. All babies were born at term (more than 37 weeks of gestation). Birth weight was measured soon after birth. Low birth weight (LBW) was considered when igual or less than 2500g and high birth weight (HBW) over 4000g. A descriptive analysis of data (percentage) was used. Results: Table 1 shows birth weight distributed by mothers' age during pregnancy. About 11% of babies were born with low weight, 83% with appropriate weight and 6% were born with high weight.

Distribution of birth weight by age of the mother during pregnancy.

Age (years)	LBW (n=56)	Normal (n=432)	HBW (n=30)
15-19,9	32,1% (18)	27,5% (119)	13,3% (4)
20-24,9	19,7% (11)	24,6% (106)	26,7% (8)
25-29,9	21,4% (12)	28,2% (122)	33,3% (10)
30-34,9	12,5% (7)	14,1% (61)	16,7% (5)
35-39,9	8,9% (4)	5,1% (22)	10% (3)
40- 44,9	5,4% (3)	0,5% (2)	0% (0)

We observed that pregnancy during adolescence (15 - 20 years) resulted in 32% of babies with LBW. This frequency decreased with increasing maternal age. Conclusion: In a population of risk with low economic income, the prevalence of LBW is high, especially during adolescence. Medical and social politics are necessary to avoid the pregnancy during adolescence and improve the prenatal care.

PO3-306 SGA

Clinical and gonadal hormones characteristic during puberty in unselected girls born ether small for gestational age (SGA) or appropiate for gestational age (AGA)

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Introduction: There are limited and controversial data concerning pubertal characteristics in girls born SGA. The aim of the study was to characterize pubertal course longitudinally in matched healthy girls born either SGA or AGA recruited from the community. Methods: Inclusion criteria were breast Tanner stage II and a normal BMI. Girls were followed during three years with a complete physical exam, bone age, pelvic ultrasound and gonadal hormones. Results: Twenty five AGA and 15 SGA completed three years of follow up with a similar bone age (12,6 vs 13,1 years(y)), delta bone age (3,1 vs 3,2 y), and delta height(19±3 vs 17±4cm) in AGA vs SGA respectively (p=NS). Height SDS (-0,09±0,2 vs -0,98±0,28) was lower in SGA (p<0,05),but BMI SDS was not (p=0,19). Sixty % of the total group achieved breast Tanner 5 and 38% Tanner 4 at the third year. SGA girls advanced a little faster during the first two years. At the third year 52% of SGA vs 50 % of AGA had Tanner 4 of pubic hair and 32% SGA vs 44 % AGA were in Tanner 5 (p=NS). Ferriman score and ovarian volume and number of follicles between AGA and SGA were no different as well as the age of menarche (corrected by maternal age of menarche) 12±0,2y (AGA) vs 12,6±0,2y (SGA). BMI at the third year was inversely correlated to age of menarche in the whole group (r=-0,45 p<0,01). A greater delta between weight at enter of the study and birth, was correlated with lower age of menarche only in the SGAs (r-0,58 p=0,01). At the third year basal and post stimulated (LHRH-a) levels of gonadotropins and androgens (17OH Prog, T, Adione, DHEAS)) were similar in both groups and did not show differences related to weight catch up growth. Estradiol was higher in the AGA group (97,1 ±10,8 vs 67,7±16,6 pg/ml, p=0,016) and it was directly correlated with higher delta BMI (BMI at third year minus entrance) only in the SGAs r=0,89 p<0,01. Conclusion: Unselected SGA girls show a slightly faster pubertal development but no differences in internal genitalia and gonadal hormonal patterns. Girls who experienced higher weight catch up had lower age of menarche and higher levels of basal estradiol.

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PO3-307 SGA

Paternal allele of *IGF2* gene haplotype CTG is associated with fetal and placental growth in Japanese

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Objective: Insulin-like growth factor II (IGF-II) associates with feto-placental growth in rodent and human. Our aim is to evaluate whether common genetic variation of *IGF2* contributes to feto-placental growth.

Subjects and methods: We determined three tag-SNPs to investigate haplotype frequency of *IGF2* relative to size at birth in 134 healthy Japanese infants. In addition, a total of 276 healthy infants, who were born at ≥ 35 weeks of gestational age between October 2004 and September 2007, were investigated to determine if common genetic variation of *IGF2* might contribute to feto-placental growth using haplotype analysis. Quantitative methylation analysis of the *IGF2/H19* was performed using the MassARRAY Compact system. We analyzed the IGF-II levels in cord blood in only 142 of 276 infants in both groups (29 of CTG infants and 113 of non-CTG infants).

Results: In the initial study, the frequency of *IGF2* haplotype CTG from the paternal allele in small for date (SFD) infants was significantly higher than that in non-SFD infants (37.9% vs. 14.3%, $p < 0.05$). In a second study, the CTG haplotype infants exhibited significantly lower birth length, birth weight and placental weight compared to non-CTG infants. The CTG *IGF2* haplotype did not associate with the methylation status of *H19/IGF2*. Although the data was only partial, there was no significant difference in cord serum IGF-II levels in the two haplotypes.

Conclusion: Inheriting the CTG *IGF2* haplotype from a paternal allele results in reduced feto-placental growth, but it is not associated with the methylation status of *IGF2/H19*.

PO3-308 SGA

Is skinfold thickness (SFT) an alternative to DEXA for the assessment of body composition in newborns with or without fetal-growth-restriction?

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BACKGROUND: Dual Energy X-ray Absorptiometry (DEXA) is recognized as a reference method for the assessment of body composition. However it is not easily accessible, especially for newborns.**OBJECTIVE**: To compare body composition assessed by DEXA and SFT in a population of term newborns with or without fetal growth restriction (FGR).**DESIGN, SETTING AND METHOD**: This study is made from a cohort of newborns, at risk of being born small for gestational age, in whom fetal growth was prospectively monitored. Fetal growth restriction (FGR) was defined as the loss of more than 20 percentiles of estimated fetal weight or birth weight between gestational week 22 and birth. 139 newborns with fetal growth restriction (FGR+) and 128 without fetal growth restriction (FGR-) were examined. Fat mass percentage (FM%) was estimated at day 3 postnatal by DEXA and from SFT sum using the Durnin and Womersly equation. SFT sum was calculated from biceps, triceps, suprailiac and subscapular skinfold measurement by a trained pediatrician using Harpeden skinfold caliper. **RESULTS**:

	FGR- (n=128)	FGR+ (n=139)	P
M/F(ratio)	61/67	67/72	
Gestational age (wk)	39.3 +/- 0.1	39.2 +/- 0.1	0.58
Birth weight (g)	3179.69 +/- 41.82	2924.39 +/- 40.13	<.0001
Ponderal Index (kg/m3)	25.7 +/- 0.2	25.2 +/- 0.2	0.037
SFT sum (mm)	18.2 +/- 0.3	15.9 +/- 0.3	<.0001
FM%_SFT	15.81 +/- 0.3	13.80 +/- 0.3	<.0001
FM%_DEXA	20.46 +/- 0.6	17.62 +/- 0.5	0.0008
ΔFM%	4.64 +/- 7.6	3.8 +/- 6.7	0.35 / 0.39*
lean mass (g)**	2588.18 +/- 31.7	2447.51 +/- 34.4	0.001
Fat mass (g)**	689.48 +/- 25.2	541.77 +/- 24.2	<.0001

*ΔFM%=FM%_DEXA-FM%_SFT, **P further adjusted for gender, gestational age and ponderal index, *** Measurements with DEXA.

A significant but modest correlation between FM% estimated by DEXA and by SFT sum was found (r^2= 0.04; p= 0.0005).**CONCLUSION:** These data showed non significant differences in the estimation of FM%, using at day 3 postnatal DEXA or SFT measurements even after correction for gender, gestational age or ponderal index. However whether there was FGR or not, SFT sum tends to underestimate FM% in comparison to DEXA. Given the poor correlation between the two methods, measurement of SFT is not recommended for

the assessment of body composition of newborns in clinical situations where reduced fat mass is anticipated.

PO3-309 SGA

Changes in body composition as assessed by DXA scan after one year of high dose growth hormone treatment in children born SGA. Results from the NESGAS group

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Introduction: Small for gestational age (SGA) (birth weight SDS ≤ -2.0) with persistent short stature (height SDS ≤ -2.5) at age of 4 years is an increasingly recognised indication for treatment with Growth Hormone (GH). Children born SGA have lower lean body mass and higher fat content than their AGA peers, despite their low body mass index. The NorthEuropean Small for Gestational Age Study (NESGAS) Group has been recruiting patients for treatment with GH since 2005. DXA scan is performed at baseline and at one year following commencement of GH treatment (GH dose 67 µg/kg/day), in order to assess bone mineral density (BMD), bone mineral content (BMC) and body composition (percentage tissue fat) along with auxological measurements, insulin sensitivity and IGF values.
Objectives: To assess changes in body composition following one year of GH treatment.
Methods: Patients were recruited in participating centres in Denmark, Ireland, UK and Sweden. Auxology was assessed and body composition was determined by DXA scan. IGF-I analysis was performed centrally in Denmark. Assessments were repeated after one year of GH treatment and changes in baseline and one year variables were analysed.
Results: Data were obtained on 61 patients (37 boys, 24 girls) born SGA and treated with GH. Mean age at start of GH treatment was 6.3 years, with mean height SDS -3.1 (SD 0.54) and mean BMI SDS -1.4 (range 2.3 to -3.9). Following first year of GH treatment significant changes were demonstrated with increments in mean height (SDS -2.1; range -0.8 to -3.6 p<0.001) and mean BMI (SDS -1.0; range +2.0 to -3.6 p<0.001). Minor but significant increments in mean BMD (p=0.02) and mean BMC (p<0.001) were observed. Mean lean body mass increased from 15051g to 18904g, however, this was not significant (p=0.4). A significant reduction in percentage tissue fat from a mean at baseline of 14.9% to a mean of 11.5% after one year of GH was observed (p<0.001). Higher ΔIGF-I SDS was associated with greater decrease in body fat percentage (Pearson correlation =-0.3, p=0.03).
Conclusions: Improvements in body composition of children born SGA with short stature, as assessed by DXA scan, occur as early as one year after commencing high dose GH treatment. These changes may not be reflected by large changes in BMI. The greater lean body mass and the lower tissue fat should be beneficial in future adult health outcomes, however long-term follow up studies are required.

PO3-310 SGA

Factors determining adult height of short children born small for gestational age (SGA) and Silver-Russell syndrome (SRS) treated with growth hormone (GH): analysis of data from KIGS

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Aims Although GH is an approved indication SGA and SRS since 2001 (USA)/2003 (Europe) information about the adult height (AH) is limited. In order to optimize and individualize GH treatment we investigated the factors determining height outcome based on patients documented within KIGS (Pfizer International Growth Database).
Patients and Methods
161children with SGA (SRS; N= 55) [57% male] have reached adult height (aHT) by Jan 2009. aHT was taken at an age >16 yrs (boys) and > 14 yrs (girls) plus a HT velocity < 2 cm/yr or when the growth pattern indicated the end of growth. – Postnatal HT data were transformed into SD scores (SDS) based on Swiss (Prader) references. Multiple regression by the all possible approach was conducted using Mallows's C(p) as described before (Ranke 2003).- Data at/before GH start [median(10./90.p)]: birth weight = -2.8(-4.1/-1.8) SDS; Age = 7.8 (3.8/11.7) yrs; HT = -3.7 (-5.2/-2.4) SDS; HT minus MPH = -2.8 (-5.0/-1.2) SDS; GH dose = 0.25 (0.17/0.46) mg/kg week (>=6 inj./wk). GH dose was constant over time. – DeltaHT during the first year on GH was 0.7 (0.3/1.3) SDS.
Results
Males reached a median adult height of 161.9 cm at a median age of 17.3 years of age and females 148.5 cm at 15.7 years after a median of 7.2 years(5.0-12.1).This corresponds to a ht SDS of -2.2(-3.9 to -1.0) and a parental adjusted ht SDS of 1.1(-3.1 to 0.2).The median change in ht SDS during GH was 1.4(0.2 to 2.7).
Factors significantly contributing in the multiple regression analysis of adult ht SDS were in the order of explanatory importance:Ht SDS at GH start,first year delta htSDS, duration of GH,mothers ht SDS,birth length SDS and a diagnosis of SRS. And in analysis of change in ht SDS from start to adult height: first year delta ht SDS, duration of GH and htSDS at GH start- MPH SDS.
Conclusions a) With the presently applied treatment (low-dose regime) about 50% short children with SGA/SRS reach a normal adult height. b) Adult HT and total HT gain can be predicted with high accuracy and precision after the 1st year of treatment. Thus, in children growth during GH treatment can be estimated with these algorithms. Patients whose height outcome on GH is likely to be poor may be excluded from ineffective long-term therapy.
(1) Ranke, Lindberg, Cowell, Wikland, Reiter, Wilton, Price - JCEM 2003; 88:125-131

PO3-311 SGA

Small for gestational age (SGA) status is associated with metabolic syndrome in overweight children

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Background: Children with former small for gestational age (SGA) status are at risk of both obesity and metabolic syndrome (MetS) later in life. However, it is not studied yet if overweight former SGA children had an increased risk for MetS compared to overweight children with former appropriate for gestational age (AGA) status.
Methods: We analyzed blood pressure, lipids, glucose, and insulin in 803 overweight children (4% SGA, mean age 11 ±0.1 years, BMI 27.3 ±0.2, SDS-BMI 2.32 ±0.02). An oral glucose tolerance test (oGTT) were performed in all 35 former SGA children and 147 randomly chosen former AGA children. We defined the Metabolic Syndrome according to the definition of Weiss (BMI > 97th percentile + 3 of the following criteria: blood pressure > 97th percentile for height, age and gender, triglycerides >95th percentile, HDL <5th percentile,

impaired glucose tolerance).
Results: After adjustment for age, sex, pubertal stage, and BMI-SDS, former SGA status was significantly related to blood pressure, triglyceride, insulin, and 2h glucose levels in oGTT. The MetS prevalence was more than doubled in SGA subjects compared to AGA subjects (adjusted odds ratio was 4.08 (95%CI 1.48 to 11.22) for SGA children).

Prevalence (standard error)				
Variable	SGA (n=35)	AGA (n=726)	LGA (n=42)	Exact adjusted p-value*
Hypertension	43 (8.4)	24 (1.6)	21 (6.3)	0.032
Triglycerides > 95th percentile	26 (7.4)	15 (1.3)	31 (7.1)	0.024
HDL-cholesterol < 5th percentile	14 (5.9)	16 (1.4)	15 (5.5)	0.962
Impaired glucose tolerance**	51 (8.4)	18 (3.2)	25 (6.7)	<0.001
Metabolic syndrome**	40 (8.3)	17 (3.1)	25 (6.7)	0.018

Table: Prevalence of metabolic syndrome and its components among 803 children and adolescents; * adjusted for age, sex, SDS-BMI, and pubertal stage; ** n=190
Conclusions: Overweight children with former SGA status had an increased risk for hypertension, hypertriglyceridaemia, impaired glucose tolerance, and consequently a more than doubled risk for the metabolic syndrome. Therefore, SGA status seems to be a risk factor for the MetS independently of weight status.

PO3-312 SGA

Effect of growth hormone treatment on insulin secretion and sensitivity in relation to growth of children born small for gestational age

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NESGAS is a randomised multi-centre trial to evaluate the safety and efficacy of GH treatment at varying doses in short SGA children without catch-up growth. The aim of this analysis was to explore the relationship between changes in insulin secretion and sensitivity, IGF-I levels and growth over one year of treatment.
We studied 42 (30♀ 12♂ pre-pubertal children born SGA (aged 3.9-9.9 years) who had failed to show catch-up growth and were naïve to GH treatment. Fasting blood samples were taken to assess IGF-I, glucose and insulin levels. Subjects underwent a short intravenous glucose tolerance test to measure acute insulin secretion, and HOMA was used to calculate insulin sensitivity. The disposition index gave an estimate of insulin secretion for the degree of insulin sensitivity. These measurements were repeated after treatment with GH (67μg/kg/day) for 12 months.
GH treatment resulted in increases in SDSs for height (-3.2±0.6 to -2.3±0.7, p<0.001), height velocity (-1.2±1.4 to 3.4±1.8, p<0.001) and IGF-I (-1.0±0.9 to 1.4±1.5, p<0.001). HOMA decreased (206.2±60.7 to 118.6±50.6, p<0.001), but basal insulin levels (17.8±9.0 to 40.2±23.9pmol/l, p<0.001) and acute insulin secretion (area under the curve: 1584.9±954.0 to 2929.4±2599.4pmol/l*min, p<0.001) increased so that the disposition index remained similar (33.8±18.0 vs 31.3±14.2, p=0.8). In a linear regression model, sex and acute insulin secretion were the most important determinants of the improvement in height velocity with GH therapy (R2=0.323, p=0.03). The increase in IGF-I levels showed no direct association with the change in height velocity.
GH therapy for 12 months effectively improved growth in short SGA children. Increased insulin secretion adequately compensated for the fall in insulin sensitivity with treatment, thus subjects appear to have a normal β-cell reserve. Enhanced insulin secretion, but not increased IGF-I levels, was closely related to the improvement in height velocity.

PO3-313 SGA

Low body adiposity and high leptinemia in breast-fed infants born small-for-gestational-age

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Background
Leptinemia reflects body adiposity, not only before birth, but also beyond infancy. We studied whether this is also the case in infants born small-for-gestational-age (SGA), as compared to infants born appropriate-for-gestational-age (AGA). Early infancy is thought to be a time window that is critical for the lifelong settings of weight control.
Study Design & Population
We measured circulating leptin at age 4 mo, and studied the partitioning of weight gain (0-4 mo) in breastfed infants born AGA (N=46; 23 girls, 23 boys) or SGA (N=28; 14 girls, 14 boys).
Main Outcomes
Body composition (absorptiometry); leptin, high-molecular-weight (HMW) adiponectin, insulin, IGF-I.
Results
Compared to AGA infants, the SGA infants were found to amplify their hypo-adiposity across early infancy, and to have two-fold higher (P<0.001) levels of circulating leptin at 4 mo.
Prefeeding HMW adiponectin, insulin and IGF-I were similar in AGA and SGA infants.
Conclusion
Breastfed infants with a low weight at birth and with a low body adiposity at age 4 mo have elevated levels of circulating leptin. It remains to be studied whether such paradoxical hyperleptinemia in early infancy contributes to program the sequence from fetal underweight to adult overweight.

PO3-314 SGA

Final height of short children born small for gestational age: influence of GH treatment

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Objectives: To assess the long term effect of Growth Hormone (rGH) treatment on final height in short children born Small for Gestational Age (SGA) without catch up growth.
Methods: Data of growth follow-up and final height from 74 short children born SGA were collected. 38 children were treated by rGH at a mean dosage of 0.055±0.015 mg/Kg/j during an average of 5.1 ±3 year (rGH-treated group). They were compared to a non randomized control group of 36 untreated short children born SGA (untreated group).
Results: At the age of 6 [3.2 – 14] years and 5 [3.2 – 16] years, mean height was –2.55 ±0.8 SDS and –3.1 ±0.6 SDS respectively in untreated and rGH-treated group. During childhood, height was significantly reduced in the rGH-treated group. In contrast to the untreated group, the rGH-treated group showed a significant increase in height. The mean final height was –1.9 ±0.9 SDS for rGH-treated group and –2.5 ±0.7 SDS for untreated group (p= 0.004), resulting from a mean height gain of 1.2 ±0.9 SDS and 0.05 ±0.9 SDS respectively (p= 0.0001). The potential for spontaneous catch up growth in short children born SGA is limited and they remain 1.6 ±1.2 SDS under their target height. In contrast GH treatment increase final height and the rGH-treated group came closer to their target height (-0.5 ±1 SDS) With rGH treatment, the height gain attained before the onset of puberty is maintained to final height. A multivariate analysis identified 3 independent predictors of final height, namely maternal height, GH cumulative dose and delay bone age at puberty. Height gain is correlated positively with duration treatment.

Conclusion: GH treatment improves the final height of short children born SGA. We suggest to start GH treatment at an early age in order to achieve a normal height before the beginning of the puberty and to carry on through puberty to maintain benefit of GH treatment

PO3-315 SGA

High serum cortisol levels associate with low birth length and reduced insulin sensitivity in young adults born small for gestational age

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Low birth weight (LBW) is linked to increased risk for insulin resistance (IR) in later life. Altered activity of the hypothalamic-pituitary-adrenal (HPA) axis is one of the suggested mechanisms connecting LBW to IR. Our aim was to study the association of insulin sensitivity with birth size and glucocorticoid parameters in young adults born small (SGA) or appropriate (AGA) for gestational age. Seventy 20-year-old subjects (35 SGA, 13 males; 35 sex- and age-matched AGA controls) born full-term were evaluated. The mean (±SD) birth weights and lengths of the SGA and AGA subjects were 2.47 (±0.33) kg (-2.39±0.55 SDS) *vs.* 3.43 (±0.53) kg (-0.25±1.10 SDS) and 46.1 (±2.2) cm (-2.31±0.96 SDS) *vs.* 50.2 (±2.3) cm (-0.18±1.17 SDS), respectively (P<0.001 for all). Venous blood and urine samples were taken after an overnight fast. Serum (S) and urine (U) cortisol (F) and cortisone (E) concentrations were measured by liquid chromatography – tandem mass spectrometry. Insulin sensitivity was assessed with QUICKI {Quantitative Insulin Sensitivity Check Index = 1/[log(glucose) + log(insulin)]}. The data were analysed with SPSS for Windows. The paired and independent samples t tests were used for the normally distributed variables, while the Wilcoxon matched-pairs signed rank test and the Mann-Whitney test were used for the non-normally distributed ones. Either Pearson or Spearman correlation coefficients were computed as appropriate. The means of S-F, S-E, S-F/E, U-F/E or QUICKI did not differ between the SGA and AGA subjects (561 *vs. 550, 76 vs. 80 nmol/L, 7.0 vs. 6.7, 0.75 vs. 0.68, 0.70 vs. 0.65; P*>0.21 for all). S-F/E and U-F/E correlated well (r=0.317; *P*=0.008) suggesting that both S- and U-F/E ratios can be used to estimate 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity. The S-F levels in the SGA group correlated positively with S-insulin (r=0.411; *P*=0.014) and negatively with QUICKI (r=-0.388; *P*=0.021). The SGA subjects in the highest S-F tertile were significantly shorter at birth (-2.76 *vs. -2.07 SDS; P*=0.038) and had higher S-insulin (8.31 *vs. 6.37 mU/L; P*=0.007), lower QUICKI (0.64 *vs. 0.74; P*=0.012) and higher S-triglycerides (1.30 *vs. 0.89 mmol/L; P*=0.005) when compared to the SGA subjects in the lower S-F tertiles. These statistically significant findings were specific for the SGA group only. In conclusion, high serum cortisol levels were associated with smaller length at birth and increased risk for IR among young adults born SGA.

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High fat diet during pregnancy leads to changes in murine gene expression known to have roles in fetal growth and glucose metabolism

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To determine the effects of in utero high fat diet (HF) on genes that regulate fetal growth and glucose metabolism.
Female CD1 mice (10-12 wks), were fed a HF (59% fat) or control breeding chow (C, 22% fat) for 2 wks prior to mating and throughout pregnancy. WT females were bred to glucose transporter 4 heterozygous male mice (G4+/-, a model of insulin resistance), which may be more sensitive to alterations in intrauterine milieu.
Mothers were sacrificed at embryonic day (e) 18.5. Maternal GLUT4, GLUT1, hexokinase (HK), phospho-Akt/total Akt protein expression in gonadal and renal fat and skeletal muscle were assessed by Western blot analysis. Maternal body weight (BW) gain and fetal BW were determined (n=30-51/group). Embryos and placentas were evaluated for gene expression (qRT-PCR). Statistical analysis was performed using ANOVA and two tailed t test.
HF exposure was associated with a 25% decreased in maternal BW gain (p<0.01) that was accompanied by a 50% reduction in expression of GLUT 4 and HK in maternal gonadal and renal fat, with no change GLUT1 or Akt, or GLUT4, HK, GLUT1 and Akt expression in skeletal muscle. WT and G4 HF fetuses weighed 35% less (p<0.01). In placenta, HF exposure decreased by 50% expression of growth-promoting genes; bone morphogenetic protein-4 (p<0.05), epidermal growth factor receptor (p<0.05), and maternally expressed pleckstrin homology-like domain, family A, member 2 (p=0.03) in WT-HF fetuses compared to C, and increased the IGF2 and IGF1R expression in both in WT and G4 embryos as a counter regulatory mechanism in response to the growth retardation. In liver, HF decreased by 50% expression IGF1 in WT and G4 fetuses (p<0.01). A 2-4 fold increase in phosphoenolpyruvate carboxykinase and glucose 6 phosphatase mRNA expression in HF fetuses liver was associated with higher fetal glucose levels (63±6 in C vs. 90±7mg/dl in HF, p<0.01) in both genotypes.
HF diet was associated with decreased maternal BW gain, and tissue specific alteration in GLUT4 and HK expression. HF diet led to a reduction in fetal weight, and this effect was associated with a decreased expression of several growth-promoting genes, and with a marked increase in the gluconeogenic pathway leading to higher glucose levels. Thus, HF feeding during pregnancy may be an important mechanism during development linking poor fetal growth to the inability of properly sense nutrients, leading to an uncontrolled glucose production in prenatal life.

PO3-317 SGA

Russell-Silver syndrome with 11p15 epimutation: analysis of growth, bone maturation, puberty and response to GH treatment on a large series of 101 patients

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Russell-Silver Syndrome (RSS) is characterized by intrauterine and postnatal growth retardation with spared cranial growth, dysmorphic features, frequent body asymmetry and bone age retardation. Our group has identified loss of methylation of the ICR1 domain of 11p15 as a major mechanism leading to RSS. No longitudinal growth parameters are available on a large series of RSS with 11p15 epimutation.
Objective: To assess these parameters in 101 RSS patients with 11p15 epimutation.
Studied population and methods: We analyzed anthropometric data, bone maturation, pubertal growth, in a retrospective cohort of 101 RSS patients with 11p15 epimutation.
Results: At birth, growth retardation is more severe for height than weight, while head circumference (HC) is spared (table1). However from 2 until 10 years, weight is more impaired than height suggesting undernutrition. At 5 years, half of the patients are treated with Growth Hormone (GH). Though, regardless of the GH treatment status, growth does not normalize before puberty. Despite medium heights at -1.6 SDS at age 13, final heights (FH) are low (approaching -3 SDS), even when GH was administrated (table 2) suggesting a poor pubertal growth spurt. Precocious puberty (PP) was noted only for 2 boys. Thirteen over 26 patients caught up their bone age retardation before 10 years.

table1

age (n)		Weight (SDS)	Height (SDS)	HC (SDS)
birth (101)		-3.1 [-4.7;-1.3]	-4.4 [-9;-0.4]	-1 [-3.7;3.6]
2 yrs (64)		-4.4 [-8.5;-0.6]	-3.2 [-7.5;-0.1]	-0.3 [-3;2.5]
5 yrs	**GH- (18)**	-3.5	-2.2	-0.2
	GH+ (24)	-5	-2.8	-0.7
8 yrs (girls)	**GH- (6)**	-1.9	-1.9	-0.8
	GH+ (6)	-3.9	-2.6	-1.2
10 yrs (boys)	**GH- (10)**	-2.8	-1.8	-1.5
	GH+ (4)	-2.4	-1.8	-1.6
13 yrs	**GH- (10)**	-0.9	-1.3	0.5
	GH+ (4)	-2.5	-2.2	-0.7

GH+: GH treated; GH-: no GH treatment; [range]

table 2

	Boys GH+ (n=4)	Boys GH- (n=6)	Girls GH+ (n=5)	Girls GH- (n=2)
FH (cm)	154.6 [138;163.5]	158 [152;156.5]	147.2 [139.5;156]	144 [144;144]
FH (SDS)	-3.3 [-6;-1.8]	-2.8 [-1.9;-1.4]	-2.9 [-4.2;-1.3]	-3.4 [-3.4;-3.4]
PP	2/4	0/3	0/5	0/1

Conclusion: Most of the RSS children show no spontaneous catch up growth at two years and worsen their weight deficit in the first years of life. Final heights are short, even with GH treatment, maybe due to impaired pubertal growth and rapid bone maturation. Undernutrition may also play a role and explain part of a possible GH resistance.

PO3-318 Testis

Abnormal expression of estrogen receptor alfa and androgen receptor in human Leydig cell (LC) hyperplasia of dysgenetic testes and in LC tumors

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High levels of aromatase (ARO) and estrogen receptor alpha (ERα) are associated with LC proliferation and tumorigenesis (Sirianni et al, 2007). LC hyperplasia was described in transgenic mice overexpressing ARO (Li et al, 2001) and in many 46,XY dysgenetic gonads (Wikström et al, 2008, Lourenco et al, 2009). In the absence of androgen receptor (AR), fetal LC function is normal, but adult LC development fails (O´Shaughnessy et al, 2002). We have postulated that, during normal prepuberty (PP), the LC pool might be modulated by IGFs, estrogens and androgens. We have also reported in PPLC a high expression of ERβ and ARO while no expression of ERα was found, suggesting a paracrine action of estrogens on steroidogenesis control (Berensztein et al, 2006). We have hypothesized that lack of androgen action and/or an increment of estrogen action might be involved in the development of LC hyperplasia, in testicular dysgenesis. The aim of this study was to analyze the immunoxpression of ERα, ERβ, ARO and AR in LC of human dysgenetic testes and of PPLC tumors (PPLCT).

Clinical Material: Group (Gr) 1: two 46, XY DSD patients, one PP and the other pubertal (PU) aged 18 and 148 months, with heterozygous SF1 gene mutations (W279X and Y183X); Gr2: two 46, XY patients aged 15 and 53 months, with complete androgen insensitivity, (R831X and c1550-1569del); and Gr3: three 46,XY patients aged 19, 46 and 80 months, with a PPLCT. Testis was removed by orchidectomy or biopsy. PP testis controls (PPC) from necropsies (n=15) and PU varicocele biopsies (PUC) (n=5) were used for comparisons. In GR1, histology revealed gonadal dysgenesis and LC hyperplasia. All LCs were positive for ARO, ERα, and ERβ in the cytoplasm, while AR was absent. For comparison, ERα was absent in PPC LC but present in PUC LC. Nuclear AR expression was moderate in PPC LC (7 to13%) and high in PUC LC (52%). LC hyperplasia, with high expression of cytoplasmic ERα, ERβ and ARO and absence of AR was found in all patients from Gr2 and Gr3. We propose that in dysgenetic testes, the hyperplastic LCs are potential estrogen biosynthesis sources and display a modified ER expression pattern. The high estrogen levels and the exclusive presence of ERα could amplify E2 signaling, contributing to LC growth and proliferation. Estrogens through ERα could also play a role in the neoplastic transformation of LCs. The absence of AR could be involved in the alteration of LC differentiation of dysgenetic testes during PU development.

PO3-319 Testis

Management of undescended testis in Italy: the use of hormonal therapy. The Italian collaborative study group on the undescended testis

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BACKGROUND: Recently a consensus among specialists from the Nordic countries on the state-of-the-art in the treatment of undescended testicles has been published. The guidelines clearly state that "hormonal treatment is not recommended, considering the poor immediate results and the possible long term adverse effects on spermatogenesis. Thus, surgery is to be preferred".

AIM: We conducted a study with the aim of describing the current management of cryptorchidism among Italian family paediatricians (FP), and in particular the use of hormonal therapy.

METHODS: An online questionnaire was filled in by 141 Italian FP belonging to 18/20 Italian regions. The questionnaire requested information on all children with cryptorchidism born between 1/01/2004 and 1/01/2006, their characteristics and management. A preliminary data analysis was performed.

RESULTS: Overall they followed 168 children with undescended testis. Among those, 21% had at diagnosis a retractile testis, while 79% had a true cryptorchidism. Mean age at diagnosis was 0,7 years (range 0-4.1). In 19% of cases cryptorchidism resolved spontaneously at a mean age of 1,8 years (range 0,2-3,8).

Among 136 children with persistent cryptorchidism, 34 children (25%) were treated with hormonal therapy as first line treatment, of whom 56% with HCG (mean age 2.2 years, range 0.5-4.2), 44% with GnRH (mean age 1.5 years, range 0.5-3.5). Success rate was of 29% (n=10/34), and was higher among those treated with HCG (8 patients versus 2 treated with GnRH). Among the remaining 24 children whom did not respond to hormonal therapy, 17 (71%) underwent orchidopexy (mean age at surgery 1.8 years, range 0.7-3.1), 2 were still awaiting surgery, 4 were managed with a "wait and see approach", 1 had a missing data.

Out of 136 children with persistent cryptorchidism, 81 underwent orchidopexy as first line treatment (mean age at surgery 1.8 years, range 0.1-4.7, of which 16% before 1 year of age), while 21 children did not undergone any intervention.

Hormonal therapy was also prescribed after successful orchidopexy as adjunctive therapy in 12% of cases.

CONCLUSIONS: In Italy there is still a high percentage of children with undescended testis treated with hormonal therapy both as a first line and adjunctive therapy, although not recommended by the recent guidelines. Moreover, an important delay in orchidopexy was recorded.

PO3-320 Testis

Immunoexpression of germ cell markers, insulin receptor/IGF system, aromatase (ARO) and estrogen receptor beta (ERβ) in the postnatal human testis

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The mechanisms that are involved in the regulation of the germ cell (GC) pool before puberty are still poorly understood. Previously, we have reported that ARO and estrogen receptor beta but not ERα are expressed in GCs of the prepubertal human testis (HT) (Berensztein, Ped Res 2006). The subject of our current investigation focuses on the identification of different subpopulations of GC in HT, as a function of age. HT preparations (n = 36) were divided in 3 age groups (Gr): Gr1 neonatal (NEO) (<1 month old), Gr2 post natal activation (1-7 month old), Gr3 early and late prepuberty. Gonocytes and spermatogonial cells were identified by histology. Immunoexpression of OCT3/4 (marker of fetal GC), C-kit (marker of immature GC), MAGE-A4 (marker of spermatogonia), ERβ, ARO, IGFI, IGFII, type I IGF receptor (IGFR I) and insulin receptor (IR) was studied. Data were expressed as % of positive GC, (mean±SD).
We found that gonocyte number decreases with age (Gr1, 20.9±3.8%; Gr2, 8.9±4.3%; Gr3, 3.5±2.3%).OCT3/4 expression was not detected in any age group. In the case of C-kit, the highest expression was observed in Gr1, followed by Gr2 and Gr3 (41.7±14.3, 24.4±17.3, 14.5±13.2, $p<0.05$). High expression of MAGE-A4 (only determined in Gr2 and Gr3) was found. In all groups, high ERβ, IGFII and IR and moderate IGFI and IGFR I expression was observed. ARO expression in Gr1 and Gr2 was significantly higher than in Gr3 (45.2±25.5, 45.4±22.3, 19.6±14.9, $p<0.05$). Gonocytes were negative for MAGE-A4, ERβ and IR while in some gonocytes positive C-kit and ARO were found. In all spermatogonia, MAGE-A4, ERβ and IR stained positive, but positive C-kit and ARO were only observed in some cells. Interestingly, colocalization studies in GR1 showed that some spermatogonia were C-kit positive/ERβ negative while others were C-kit negative/ERβ positive.
Taken together these results, it could be suggested that: 1) different expression pattern of receptors, like the decrease in C-kit expression with age, characterize gonocytes and spermatogonia, (2) within spermatogonia, different subpopulations could be identified, (3) local estrogens acting through ERβ might stimulate spermatogonium proliferation, and finally (4), since the percentage of the IGF system, IR and MAGE-A4 expression in spermatogonia does not change as a function of age, it can be proposed that IGFs and insulin might have a role in maintaining an adequate pool of spermatogonia, in the prepubertal HT.

PO3-321 Testis

Chronic stimulation with insulin-like growth factors (IGFS) 1 and 2 modulates Leydig cell response to HCG in cell cultures of human prepubertal testes

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We have previously reported the expression of IGF1, IGF2, IGF receptor type 1 (IGFR1) and insulin receptor (IR) in human prepubertal testes of 3 age groups (GR): GR1, neonates; GR2, postnatal activation; GR3, early prepuberty. We have also demonstrated an effect of IGF1 on cell proliferation and apoptosis, P450scc expression and testosterone (T) secretion in primary culture.
A role for IGFs in the modulation of Leydig cell differentiation was proposed (Berensztein et al, Ped Res 2008). In this study, we analyzed the effect of chronic stimulation with IGF1 and IGF2 on hCG acute response in human prepubertal testicular cells in culture. Human prepubertal testes, were collected at necropsy as previously published (Berensztein et al, Ped Res 1995).Chronic IGF1 (50 ng/ml, 4 days) significantly increased T response (by immunoassay) to acute (4-hours at 100 ng/ml) hCG stimulation (396 ± 133% of basal; mean ± SE; $n = 7$ cultures; $p < 0.05$). Similar responses were observed with chronic IGF2 (50 ng/ml) stimulation in two additional cultures: increased T secretion (232 and 175% of basal, respectively) and T response to acute hCG stimulation (238 % of basal), as well as increased mRNA expression of 3β-hydroxysteroid-dehydrogenase type 2 and 17α-hydroxylase enzymes (5.7 and 8.2 fold, respectively, determined by real time RT-PCR), These IGFs effects were blocked by the presence of 100 nM picropodophyllin, a selective inhibitor of IGFR1. We concluded that IGF2, the main IGF expressed in human prepubertal testicular interstitium, increased T secretion per se, as well as the expression of steroidogenic enzymes. IGF2 also increased T secretion after acute hCG stimulation. These results are in favor of the hypothesis that the IGF system modulates functional differentiation of Leydig cells in human prepubertal testis.

PO3-322 Testis

The protein kinase A pathway is involved in early Leydig cell differentiation

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Leydig cells (LC) are the major source of testosterone (T) in the male, and differentiation of LC in the testis plays a critical role in the prenatal development of the male phenotype. Recent findings indicate that platelet-derived growth factor (PDGF)-α receptor positive testis peritubular cells (PTC) have potential to differentiate into adult LC lineage and are suggested to be putative stem Leydig cells (PSLC). However, the regulation of this maturational process remains to be elucidated. The aim of this study was to investigate the signaling pathways involved in the differentiation of PSLC into the adult LC lineage. PDGF-α receptor (PDGFR) positive PSLC were purified by magnetic cell separation (MCS) of interstitial cells from testes of 8 day-old rats. By immunostaining, 98% of PSLC isolated by MCS were positive for PDGFR and LIF receptor. The PSLC also expressed α-actin and OCT-4, indicating that PSLC belong to the PTC lineage and have potential of pluripotency. Unstimulated PSLC in primary cultures produced high levels of 5α-reduced androgens, 5α-androstane-3α, 17β-diol (3α-diol), but showed no detectable production of T, androstenedione (A) and progesterone (P). This indicates that PSLC display an immature pattern of steroidogenesis and that 3α-diol seems not to be a metabolite of T. To investigate the role of the protein kinase A (PKA) signaling pathway in differentiation of LC, primary cultures of PSLC were exposed to (Bu)2cAMP and cholera toxin for 7 days and steroidogenic products were determined by RIA. We demonstrated that activation of PKA significantly stimulated P and to a lesser extent A production in a time-dependent manner, while T was not detectable and 3α-diol remained the predominant steroid produced by PSLC. In the same experimental paradigm, incubation of PSLC with human chorionic gonadotropin, insulin-like growth factor-I, T3, desert hedgehog and PDGF-α for 7 days did not induce synthesis of any of the above steroids other than 3α-diol.
All together, our findings suggest that PSLC treated with PKA activators expressed 3βHSD and P450c17 activities, which are markers for Leydig cells. Additional signals and extended time may be required to activate 17β-HSD and produce T by these cells.
We conclude that PKA may play an important role in triggering the early differentiation of PSLC into the adult Leydig cell lineage. Moreover, potentials of PSLC for regenerative therapy in subjects with undeveloped Leydig cells may be worth to explore.

PO3-323 Testis

Ultrasound screening study in testes of cryptorchid patients operated on during childhood

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The urgency of this problem depends on high prevalence of cryptorchidism in population, the risk of infertility and malignancy.
To study prevalence, peculiarities of parenchymatous abnormalities in adolescents and young adults, treated for cryptorchidism in childhood.
53 patients (age 15,6 ±2,3) were investigated after 4 to14 year treatment for cryptorchidism:18 (age 16,2±2,8) with bilateral and 35 (age 15,2±2,1, p=0,18) with unilateral. The mean age to the moment of treatment was 7,3 ±3,6 years. Patients underwent through ultrasound scrotal examinations by linear transducer with frequency 10.5 MHz (n=53) and determination of basal level of follicle-stimulated hormone (n=27).
The mean volume of operated testes (8,13 ±3,2 ml) significantly lower than the volume of spontaneously descended testes (14,41±3,9 ml, p=0,000). In 25 (78,1%) of 32 cases with unilateral cryptorchidism the undescended testes were hypotrophic compared with spontaneously descended testes. Inversely correlation between the volume of operated testes and the level of FSH (r=-0.63, p=0,000) was found in this group. The echo pattern of testicular tissue in all spontaneously descended testes was normal, but among operated testes in 50,7% cases was found the lowering of echo pattern of parenchyma. Testicular microlithiasis was found in 6 (11,3%) of 53 cases (4 patients with unilateral and 2 patient with bilateral cryptorchidism). Two patients with unilateral cryptorchidism had bilateral testis's damage. Abnormalities of the appendix were found in 43% cases operated and in 37,4 % (p=0,85) spontaneously descended testes. Significant reduction of volume and echo pattern of testicular tissue of operated testes, negative correlation between the volume of testes and level of FSH, high prevalence of appendix's damage leads to high risk of infertility. High prevalence of testicular microlithiasis confirmed its communication with cryptorchidism, pointed to increased risk of testicular cancer and the conduction of annual ultrasound examination.

PO3-324 Testis

Klinefelter´s syndrome – from diagnosis to the onset of puberty: experience of a paediatric hospital

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BACKGROUND: Klinefelter's syndrome (KS) was initially characterized by gynecomastia, small testes, lack of facial and body hair and azoospermia. In 80 to 90% of cases the karyotype is 47, XXY, but mosaics and additional sex chromosomes may be present.
OBJECTIVE: to review the current knowledge about KS before puberty and examine a paediatric population with KS: age, associated diseases, growth and development and circumstances that led to diagnosis.
MATERIAL AND METHODS: Population: 28 boys with KS followed in consultation of Paediatric Endocrinology. Clinical data were obtained through consultation of clinical process.
Statistical analysis used descriptive methods and the results were presented in absolute and relative frequency, mean and standard deviation.
RESULTS: Karyotype: 22 (79%) were 47, XXY, 3 (11%) were mosaics and 3 (11%) had other variants.
Age of diagnosis: 10 were diagnosed before birth by amniocentesis. Of the remainder, 3 were diagnosed in the neonatal period and 13 during childhood and adolescence.
In 3 boys diagnosed in the neonatal period, the dysmorphic face (n = 2) and sexual ambiguity (n = 1), led to diagnosis. Of the remainder, 10 were diagnosed during the investigation of development delay and learning difficulties, and the other because of: dysmorphic face, scrotal abnormalities, bone marrow compatibility and germinoma study. At the first appointment of Paediatric Endocrinology, the mean age was 7.69 ± 5.46 years.
The mean weigth SDS was -0.52 ± 1.78 and of height -0.79 ± 2.09. Seven children had short stature and only 1 had high stature.
Related diseases: 15 had development delay, 2 attention deficit and hyperactivity, 7 genito-urinary abnormalities, 6 osteoarticular anomalies and 5 cardiac anomalies. The boys with 47,XXY karyotipe had on average, fewer diseases than those with more extra X chromosomes.
DISCUSSION: As described in the literature, in about 80% of cases the karyotype was 47, XXY and almost all boys had medical and / or psychological problems.
Our results showed in this population a high proportion of individuals with short stature. The main problem presented by these pre-pubertal patients was development delay, suggesting that many of the affected individuals has only mild phenotypic changes, contributing to the under diagnosis. Family doctors, paediatricians and speech therapists must pay attention to this common but under diagnosed syndrome, to identify individuals at younger ages, allowing early intervention.

PO3-325 Testis

Human testicular xenografts as a model for testis development and disease

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Disorders of sex development (DSD) occur in early fetal life and in many cases involve an abnormally developed gonad. Much knowledge of fetal gonad development is based on work in rodents, in which fundamental differences exist in comparison to the human. One such distinction is the development of testicular germ cell tumours (TGCT), which occur in humans and have a higher prevalence in patients with many forms of DSD. Such tumours do not develop in rodents. As both DSD and TGCT originate in fetal life our aim was to develop a model of in vivo fetal human testis development. Such a model would be valuable for studies which attempt to disrupt normal testis development and could provide insight into the development of both DSD and TGCT.
We grafted testis tissue from first trimester (n=4) and second trimester (n=5) human fetuses subcutaneously onto the back of immunodeficient nude mice (n=20, total grafts=84). Six of the mice receiving second trimester grafts were treated with hCG (20IU, 3x/week), whilst control animals received vehicle. The grafts were retrieved after 6 weeks and examined for structure and morphology. In addition sections were studied by immunohistochemistry for signs of differentiation and proliferation in germ, Sertoli and Leydig cells.
Our results demonstrate that these grafts will survive and develop within the hosts. First trimester grafts, in which the cords were not fully formed prior to grafting, developed seminiferous cords with normal appearance. The structure and morphology of the seminiferous cords are maintained for the duration of the grafting period. In addition we have shown that germ cells undergo proliferation and differentiation as demonstrated by immunohistochemistry using antibodies to Ki67, OCT4, VASA and MAGE-A4, whilst Sertoli cells also proliferate (Ki67). Leydig cell function is demonstrated by positive immunostaining for 3beta-HSD. Animals treated with hCG had significantly increased seminal vesicle weight and grafts retrieved from these animals were 25% larger.
This is the first demonstration of human testicular cord formation in vivo. We have also demonstrated that Leydig cells within the grafts are responsive to hCG and can produce testosterone. This model can be used as an in vivo system to investigate normal testis development and may be useful to investigate disruption of testis development. Such studies may have relevance to the pathogenesis of DSD and TGCT.

PO3-326 Testis

Developmental expression pattern of fibroblast growth factor receptor 3 (FGFR3) and H-RAS reveals a novel signalling pathway involved in male germ cell maturation and proliferation

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Our recently published observation of a surprisingly good sperm concentration in a patient with the non-mosaic Klinefelter syndrome and achondroplasia caused by a gain-of-function mutation in the *FGFR3* gene suggested the involvement of FGFR3 in testicular function, and prompted this study. To investigate the role in development and proliferation of male germ cells, we examined by immunohistochemistry the expression pattern of FGFR3 and its downstream signalling partner, H-RAS in normal human testes from 16 weeks of gestation (w.g.) until adulthood. In the fetal testis specimens from 16-22 w.g., a moderate FGFR3 staining was present in the cytoplasm of an increasing number of germ cells (5-40%), followed by a transient decline between 22-32 w.g. and a subsequent rapid increase in the number of FGFR3-positive germ cells from 32 w.g. (10%) until birth (85%). A weak cytoplasmic H-RAS staining was detected in a few single germ cells in 16 w.g. Between 19-33 w.g. a nuclear H-RAS staining was demonstrated with varying intensity in an increasing number of germ cells. A prominent expression of both FGFR3 (cytoplasmic) and H-RAS (nuclear) was seen in a subset of spermatogonia in the infantile testes until about 1.5 –2 years of age, after which the expression decreased to 2-5%. At the onset of puberty and spermatogenesis (10-12 years of age), FGFR3 expression increased again and was seen in the majority of spermatogonia (cytoplasm and the cell membrane), whereas H-RAS remained nuclear in a subset of spermatogonia but was abundantly present in the cytoplasm of spermatocytes and spermatids.

These findings indicate that FGFR3 signalling via H-RAS is activated in fetal germ cells after their differentiation from gonocytes into pre-spermatogonia and is downregulated during the transient arrest of germ cell proliferation in mid-gestation and during childhood. The mini-puberty period is a notable exception where a transient activation of the FGFR3/H-RAS pathways occurs, probably due to the increased hormonal activity in the pituitary-testis axis. After the onset of puberty and spermatogenesis, FGFR3 signalling via H-RAS continues only in a subset of spermatogonia, most likely type A, whereas H-RAS is apparently activated as a partner in another, yet unknown signalling pathway in meiotic and post-meiotic germ cells. Taken together, the results indicate the role of FGFR3 and H-RAS signalling pathway in the differentiation and proliferation of spermatogonia.

PO3-327 Thyroid III

The use of combined liothyronine and thyroxine therapy for consumptive hypothyroidism associated with hepatic haemangiomas in infancy

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Background: The rare association between consumptive hypothyroidism and congenital hepatic haemangiomas is believed to be secondary to conversion of thyroxine to biologically inactive reverse triiodothyronine by type 3 iodothyronine deiodinase (D3)

Clinical history: Neonatal hypothyroid screening detected a raised TSH in a premature male infant. Congenital cutaneous haemangiomas over his chest wall, back and limbs were noted by the parents to be increasing in size. Thyroid function tests (TFT) at 4 weeks of age revealed a TSH of 66.2mU/L(N<6) and a free thyroxine (fT4) of 16.9pmol/L(19-39). The child was mildly jaundiced and anaemic with normal liver function tests. A nucleotide thyroid scan demonstrated avid uptake of isotope in the region of a normally located thyroid gland. An abdominal ultrasound scan identified marked hepatomegaly with multiple hepatic lesions. He was commenced on levothyroxine (T4) 25mcg daily. Thyroid autoantibodies were negative.

Subsequently, the infant deteriorated with rapidly increasing hepatomegaly, respiratory compromise and bradycardia requiring ventilatory support. Cardiac dysfunction and neuroblastoma stage 4S were excluded with a normal echocardiogram, bone marrow aspirate and skin biopsy. An abdominal MRI confirmed the diagnosis of neonatal haemangiomatosis.

His TSH had increased to >375mU/L [fT4 10.2pmol/L; fT3 1.5pmol/L; reverse T3 (rT3) 15,950pmol/L]. T4 therapy was discontinued and liothyronine (T3) commenced, leading to improvement of his TSH and fT3 values.

Vincristine and dexamethasone were given to reduce the haemangiomas. After 6 weeks, the rT3 fell to 8500pmol/L with a TSH of 5.48mU/L, fT3 6.6pmol/L and fT4 16.0pmol/l. A gradual improvement of the abdominal distension correlated with a reduction in the hepatic lesions on abdominal USS and a parallel regression of the cutaneous haemangiomas. At six months of age, the fT4 fell below the normal range and T4 was re-introduced. T3 was successfully weaned and at 18 months of age the child is well on 25mcg T4 with TSH 0.9mU/L, fT4 16.3pmol/L and fT3 9.4pmol/L.

Conclusion: Treatment with exogenous T4 may be ineffective if it is rapidly converted to inactive rT3. Over activity of D3 can lead to concentrations of reverse T3 much greater than previously reported. We suggest a combination of T3 and T4 therapy to prevent the sequelae of peripheral and central hypothyroidism and control the over-stimulation of the TSH-thyroid-rT3 axis until the haemangiomas regress.

PO3-328 Thyroid III

Effect of iodinated contrast medium on neonatal thyroid function following in-utero exposure

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Background: Theoretical concerns exist regarding the effect of in utero exposure to iodinated contrast medium on neonatal thyroid function. If demonstrated, the lack of an effect would allow the use of contrast media in pregnancy without significant concern for neonatal thyroid dysfunction.

Objective: To determine if exposure to iodinated contrast in utero has any effect on neonatal thyroid function.

Methods: The study identified consecutive pregnant patients that underwent CT pulmonary angiogram (CTPA) for suspected pulmonary embolism (PE) between 2004 and 2008. An IRB approval was obtained from Women and Infants Hospital and the Rhode Island Department of Health. Maternal medical history, delivery dates, type and dose of contrast were determined from record reviews. Newborns were then matched with mothers' records. The corresponding newborn screening (thyroxine (T4) and thyroid stimulating hormone (TSH)) results were obtained from the department of health.

Results: A total of 350 mothers with suspected pulmonary embolism received iodinated contrast as part of a CT pulmonary angiogram protocol. To date, 239 thyroid function values were reviewed. All the children had T4 levels that were appropriate for gestational age. Only one patient had an abnormal TSH on day of life 2, which normalized on day 6 and remained normal on day 20 without any thyroid replacement therapy.

Conclusion: Despite theoretical concerns about the risk of neonatal thyroid dysfunction following in-utero exposure to iodinated contrast media, the incidence is likely very low. The limitation of this study is the sample size. A prospective is on going to address this concern.

PO3-329 Thyroid III

Relapse of hyperthyroidism after transient radioactive iodine-induced hypothyroidism in a 14 year old girl with Graves' disease

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Background: Recovery of thyroid function in patients following hypothyroidism induced by Radioactive Iodine (RAI) treatment for Graves' disease has been described in adults but reports on children are scant. In a 36-year retrospective analysis of the efficacy and safety of RAI in 116 patients under 20 years, Read et al described a 14 1/2 year old boy who was hypothyroid and treated with L-Thyroxine for 16 years and had relapse of hyperthyroidism.
Objective: To report a case of relapse of hyperthyroidism after transient radioactive iodine hypothyroidism in a teenage girl with Graves' disease, and explain possible mechanism behind transient hypothyroidism and relapse of hyperthyroidism.
Design/Methods: Descriptive report of the clinical presentation, management and long term follow-up of a teenage girl with Graves' disease developing transient RAI-induced hypothyroidism with subsequent relapse of hyperthyroidism.
Case Report: We treated a 14 year old, 60-kg, girl with Radioactive Iodine (15 mCi of I-131) after 3 years of poorly controlled thyrotoxicosis. She developed clinical hypothyroidism (TSH 110 uIU/ml, total T4 4 ug/dl) two months after treatment, and was replaced with 150 mcg Levothyroxine. Two months after starting Levothyroxine, biochemical hyperthyroidism ensued necessitating progressive reduction of Levothyrosine to achieve normal thyroid function tests (TFT). She had complaints of palpitations, tremors and weight loss. Levothyroxine was eventually discontinued 15 months after RAI treatment due to complaints of palpitations, tremors and weight loss. TFT's showed suppressed TSH 0.02 UIU/ml, mildly increased T3 222 ng/dl, normal FT4 1.63 mg/dl, and increased Thyroid stimulating Immunoglobulin 241% (normal <125). Twenty-four hour I-123 thyroid scan showed increased uptake at 57% consistent with recurrence of Graves' disease.
Conclusion: Relapse of hyperthyroidism following transient hypothyroidism induced by Radioactive iodine treatment is uncommon in the pediatric population. Mechanisms explaining this phenomenon include impaired organification of iodide with normal iodide trapping by thyroid cells and alterations of thyroid autoimmunity (TSH blocking antibody and TSI) after RAI treatment. With the increasing use of RAI treatment in the pediatric Graves', this phenomenon needs to be appreciated and further studied.

PO3-330 Thyroid III

Graves' disease in children: possible determinants of remission

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Hyperthyroidism in children is mainly caused by Graves' disease (GD), prevalence 0.1-3/100.000. In GD, the immune system produces thyroid-stimulating hormone receptor antibodies (TRAb), that stimulate the thyroid to produce excess thyroid hormones. The optimal treatment for GD in children is controversial. Anti thyroid drugs (ATD) are often used initially, but many children eventually require alternative therapies. The aim of the study was to evaluate the possible determinants of GD remission in 124 children afferent at 6 Italian Paediatric Endocrinology Departments, with a logistic regression model. Odds Ratio (OR) and 95% Confidence Interval (95% CI) were calculated and reported. Mean age at onset was 11.2 years (±3.0 SD), male to female ratio 1:4.9, mean duration of follow-up 6.5 years (±3.3 SD). Most frequent signs and symptoms at onset were goitre (77.5%), tachycardia (75%), weight loss (50%), ophthalmic abnormalities (35%), hypertension (9.2%). Clinical severe presentation (tachycardia, hypertension, weight loss and/or ophthalmic abnormalities) was present in 67.5% patients. A positive family history for thyroid disorders was present in 65.5% patients. At diagnosis all patients showed positive titre of TRAb, mean fT3 was 17.5 pg/ml (n.v. 1.8-4.2), mean fT4 was 48.3 pg/ml (n.v. 8-19) and mean TSH was 0.13 μU/ml (n.v. 0.5-4.4).
At onset, all patients were treated with ATD (methimazole at mean dose 0.63 mg/kg/die), 30.6% of children achieved remission after only ATD therapy. We analyzed in logistic regression model the following variables, as possible determinants of GD remission: sex, pubertal stage, severity of disease at diagnosis, BMI z-score (≤-1.18/>-1.18), TRAb at diagnosis (≤31/>31), dose of therapy (≤0.44/>0.44), treatment duration (≤3.45/>3.45), time of TRAb normalization (≤1 years/1-2 years/>2 years). The time of TRAb normalization ≤1 years or 1-2 years and the BMI z-score ≤-1.18 were identified as important factors associated with GD remission (table 1). Therefore, the BMI z-score at diagnosis and the time of TRAb normalization may guide long-term GD management decisions in the children.

Best fitted logistic regression model. Outcome variable: remission

Determinants	OR	95% CI	P#
Time of TRAb normalization			
(1-2 yrs vs ≥2 yrs)	6.85	2.0-23.	<0.0001
(<1 yr vs ≥2 yrs)	20.64	5.8-73.14	
BMI-z score (≤-1.18 vs ≥-1.18)	3.99	1.3-12.7	0.016

P value refers to the Likelihood Ratio test

PO3-331 Thyroid III

A case of iodine deficiency, hypothyroidism, goiter and severe food allergies in two caucasian North American brothers

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Iodine deficiency is a rare entity in pediatric population in developed countries. It is still endemic in the areas of the world with low dietary supply of iodine. When iodine intake is abnormally low, adequate secretion of thyroid hormones is achieved by marked modification of thyroid activity: preferential synthesis and secretion of T3 and increased secretion of TSH. Goiter is a mechanism of adaptation to iodine deficiency.
We report on two brothers from the US, both with severe food allergies and soy milk intake, who presented with hypothyroidism and thyromegaly secondary to iodine deficiency.
Brothers presented simultaneously to endocrine clinic at age 5 7/12 yrs (J.I.), and 3 10/12 yrs (N.I.) respectively. They developed thyromegaly few weeks prior to initial evaluation. Laboratory evaluation was consistent with hypothyroidism, low urinary iodine levels and negative thyroid antibodies.

Table 1

Baseline	J.I.	N.I.
Free T4 (0.73-1.77 ng/dL)	0.94	1.12
T4 (7.3-15.0 mcg/dL)	7.9	8.1
TSH (0.7-5.7 mIU/mL)	12.7	36.5
T3 (ng/mL)	2.0	2.9
TPA (<60)	3	3
TGA (<60)	12	12
Urine iodine (42-350 mcg/L)	42	57

Bone ages were concordant to chronological ages. Linear growth was not affected in older brother while younger brother's linear growth was slightly affected. They had normal newborn screening. Thyroid hormone replacement was initiated.
On follow up visit they were hormonally and clinically euthyroid. Three months later thyroid hormone replacement was stopped in preparation for thyroid uptake scan. Scans showed symmetric increased uptake at 4 and 24 hours respectively. Perchlorate discharge test was performed and ruled out peroxidase deficiency. Urinary iodine levels continued to be low or low normal.
Presently brothers remain off thyroid hormone replacement and are clinically and hormonally euthyroid. The diet is being supplemented with iodized salt and milk-free multivitamins with iodine. They continue to have appropriate linear growth.

Table 2		
Off L-thyroxine - on Rx Iodine supplement	J.I.	N.I.
Free T4 (0.73-1.77 ng/dL)	1.42	1.42
T4 (7.3-15.0 mcg/dL)	10.5	10.2
TSH (0.7-5.7 mIU/mL)	2.1	1.4
T3 (127-221 ng/dL)	170	181
Urine iodine (42-350 mcg/L)	92	178

Children with restricted diets due to food allergies might be at the increased risk for hypothyroidism secondary to iodine deficiency. Adequate iodine supplementation would prevent hypothyroidism.

PO3-332 Thyroid III

Longitudinal growth in children with congenital hypothyroidism – a follow up study

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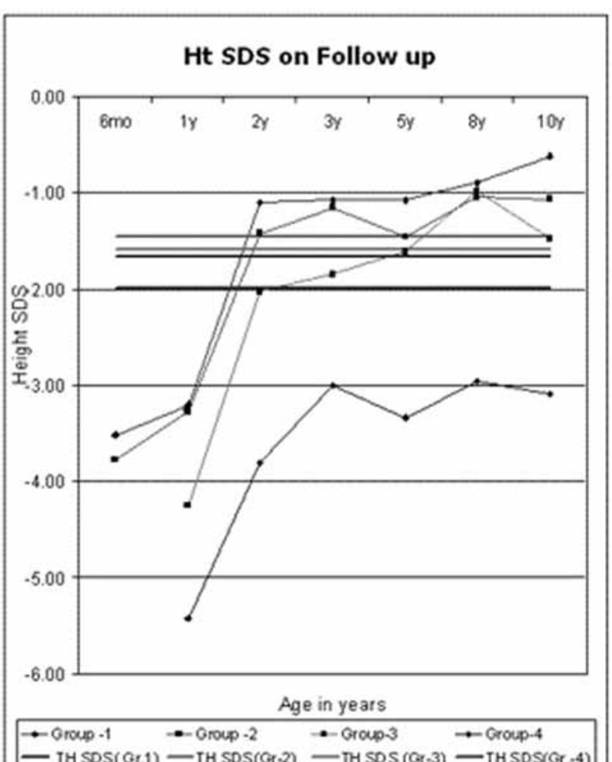
In the absence of neonatal screening for congenital hypothyroidism (CH) delayed diagnosis is not uncommon. To assess the influence of age at onset of treatment on subsequent growth, records of 134 (52M; 82F) children with CH, in a non endemic area, seen over the last decade and followed up regularly from diagnosis up to 10 years of age were studied.
Based on age at onset of treatment these children were divided into 4 groups. Group 1: <3 month (n=45); Group 2: 3-6months (n=23); Group 3: 6mo-1yr (n=35); Group 4: 1yr- 3yr (n=31). Detailed history, anthropometry, hormonal evaluation and thyroid scan/sonography at diagnosis and follow up records of Ht SDS, serum T4, TSH levels and thyroxine supplementation dose at 6mo,1,3,5and 10 years were compared by unpaired t test between the groups. At presentation, the mean Ht SDS in Group1(-2..26±1.7) and Group2(-4.25±1.7) were significantly ($p<0.01$) less affected than Group3 (-5.01±1.59) and Group4(-4.71 ±1.83).The mean level of T4 and TSH in Group 1 & 2 was significantly ($p<0.05$) lower than Group 3 & 4.The mean dose of L-thyroxin supplementation in groups 1& 2 was significantly ($p<0.05$) higher (10.56± 1.95 µgm/kg/d) than group 3 &4 (7.81 ± 3.08 µgm/kg /d) at the start of therapy.
The mean Ht SDS at 6mo,1yr,3yr,5yr and 10 yr in all groups is shown in figure 1.



Children with onset of therapy beyond 1 year of age remained significantly ($p<0.05$) short for their target Ht SDS (TH SDS) at the last follow up as compared to those being treated before 1year of age. The mean level of T4 and TSH did not differ significantly between the groups. The mean dose of L-thyroxine in group 1&2 was significantly ($p<0.05$) higher than group 3 & 4 till 3 years of follow up. Although at presentation, the mean HtSDS in athyrotics was more affected than the ectopic or the dyshormonogenesis cases, no significant ($p>0.05$) difference was found on follow up. The growth outcome is not affected by the etiology of CH but the onset of therapy after 1 year of age has significant negative effect. Replacement dose L-thyroxine is higher for the first 3 years of life in children treated before 6 months of age.

PO3-333 Thyroid III

Congenital hypothyroidism associated with goiter: risk of nodules and thyroid cancer

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Objective: Describe development of goiter and nodules in a subset of patients with congenital hypothyroidism (CH), with resultant risk of thyroid cancer.
Results: Table showing characteristics of four patients with congenital hypothyroidism and nodular goiter.


Table showing characteristics of four patients with congenital hypothyroidism and nodular goiter

Patient	Sex	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Surgery	Pathology
1	F	N/A	[illegible]	[illegible]	[illegible]	[illegible]	Not performed	[illegible]	[illegible]
2	F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Not performed	Total thyroidectomy	[illegible]
3	M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	N/A	N/A
4	F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	N/A	N/A

[illegible]

Discussion: We describe 4 cases of congenital hypothyroidism and goiter that developed thyroid nodules; evaluation of 2 by FNA showed benign thyroid tissue, but 2 developed thyroid cancer. While not proven, most likely these 4 patients had a form of dyshormonogenesis. Overall, we estimate there are ~ 27 patients with CH and goiter between the ages of 1-18 yrs in Oregon. This would mean an incidence of nodules of ~15% and thyroid carcinoma ~7.5%. These patients appear to require higher l-T4 doses to suppress TSH into the normal range, and even then they have intermittently elevated TSH concentrations which may play a role in development of nodules and cancer. Previously similar patients are reported to have primarily follicular carcinoma, an uncommon form of thyroid cancer in children.
Conclusion: We conclude that patients with CH and goiter require more intensive monitoring and treatment to try and reduce nodule formation and perhaps reduce the risk of cancer.

PO3-334 Thyroid III

Ectopic thyroid gland presenting as a midline neck mass

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An ectopic thyroid is a common cause of congenital hypothyroidism, and is typically identified by thyroid scan in the newborn period. By contrast, ectopic thyroid presenting as a midline neck mass in childhood is rare. It can be mistaken for a thyroglossal duct cyst, and incorrect diagnosis results in unnecessary surgery. A two and a half year old girl presented a midline neck mass that had appeared 6 months earlier. The child was asymptomatic. Newborn screening for congenital hypothyroidism had indicated normal thyroid function. The mass was located in the midline of the neck just inferior to the hyoid bone. It was 2.8 cm x 2.0 cm, round, firm, non-tender, and moved with protrusion of the tongue and with swallowing. No thyroid tissue was palpable in the normal location. The serum level of free T4 (1.0 ng/dL) was normal (0.9-1.6 ng/dL), but the serum TSH (8.28 mIU/L) was slightly above the normal range (0.5-4.3 mIU/L). Ultrasonography indicated bilobar, homogeneous tissue in the midline of the sublingual region. No thyroid gland was identified in the normal location. A Tc-99m pertechnetate scan identified a single area of uptake in the midline beneath the tongue. On evaluation 9 weeks after the first visit, the patient continued to be euthyroid. Serum level of free T4 (1.0 ng/dL) was unchanged, but the serum level of TSH (14.57 mIU/L) was higher than previously. Daily levothyroxine 37.5 mcg was initiated; and 8 weeks later, serum free T4 (1.1 ng/dL) and TSH (2.1 mIU/L) were normal. Four months later, the patient was clinically and biochemically euthyroid and the neck mass had decreased in size to 1.5 cm x1.5 cm. After 9 months, the neck mass was no longer present. Ectopic thyroid gland tissue presenting as a midline neck mass is rare, and may be mistaken for thyroglossal duct cyst. Evaluation by ultrasonography, radionuclide scan, and determination of serum free T4 and TSH should be performed. The initial treatment of ectopic thyroid in the absence of thyroglossal duct cyst should be thyroid hormone replacement. The patient we present underscores the importance of evaluation, and the possibility of complete resolution of the neck mass with medical therapy. Surgery should be reserved for children who have dysphonia, dysphagia, and dyspnea that does not respond to treatment with levothyroxine, or for children who present with severe symptoms of obstruction.

PO3-335 Thyroid III

Clinical utility of time-of-day normal ranges for TSH

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TSH concentrations are known to double in a circadian pattern (highest values 2200-0400h; lowest values 1000-1800h). However, most laboratory assays for TSH provide a single normal range, not time-based.

The current project was performed in order to assess the clinical utility of time-of-day-based normal ranges for TSH.

Method: Normal ranges for TSH at 0800 and at 1600h were developed from previously obtained data in 100 normal children (42 girls, ages 5 to 18y). TSH at 0800h, TSH at 1600h, and AM to PM TSH ratio from 22 children (9 girls) with mild primary hypothyroidism and from 23 children (11 girls) with central hypothyroidism were compared to results in normal children. (mean ± SD)

Results:

Diagnosis	N	TSH 0800h (mU/L)	TSH 1600h (mU/L)	AM/PM TSH ratio	FT4 (ng/dL)
Normal (95%CL)	100	2.7±0.9 (1.3-4.5)	1.5±0.7 (0.7-2.9)	1.9±0.5 (1.3-3.0)	1.4±0.2 (1-1.8)
Primary hypothyroid (range)	22	7.6±4 (5.0-12.0)	3.8±2.6 (1.5-7)	2.2±0.8 (1.6-2.8)	1.2±0.2 (0.9-1.5)
Central hypothyroid (range)	23	2.8±2.3 (0.1-4.8)	2.2±1.8 (0.1-3.3)	1.3±0.3 (0.9-1.9)	0.9±0.2 (0.5-1.2)

In normal children, 0800h TSH was significantly higher than 1600h TSH ($P < 0.05$).

In primary hypothyroidism, 0800h TSHs were elevated above normal. However, in all but 2 patients, 1600h TSHs would not have been considered elevated based on the usual laboratory normal range of 0.5-4 mUn/L. In contrast, when compared to 95% CL at 1600h, 70% would be identified as elevated.

In central hypothyroidism, TSHs at 0800 and 1600h were within time-of-day normal ranges, but AM/PM TSH ratio was low in 54%.

Conclusions: Time of day normal ranges for TSH can be used for clinical diagnosis of mild hypothyroidism. Either AM or PM TSH compared to time-of-day normal range can be used to identify TSH elevation in patients where it would otherwise be missed.

When FT4 is in the lowest third of the normal range, low AM/PM TSH ratio can confirm central hypothyroidism. When there remains suspicion of central hypothyroidism with a normal AM/PM TSH ratio, patients should undergo a formal TSH surge test for confirmation of the diagnosis.

PO3-336 Thyroid III

Effects of long-term l-thyroxine treatment on endothelial function and carotid intima media thickness in young adults with congenital hypothyroidism

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Background: Patients with Congenital Hypothyroidism (CH) display subtle abnormalities of the cardiovascular system that are related to non physiological fluctuations of TSH levels and occur despite careful replacement therapy.

Objective: Aim of the present case-control study was to evaluate the effects of long-term levothyroxine (L-T4) replacement therapy on the vascular district in patients with CH by assessing endothelial function with flow-mediated vasodilation (FMD) and carotid intima-media thickness (CIMT).

Patients and methods: Forty young adults with CH aged 18.9 ±0.2 years and 40 age and sex-matched controls underwent brachial artery reactivity evaluation by FMD and CIMT measurement by Doppler ultrasound at the time of the study. Hypothyroidism was diagnosed by neonatal screening and L-T4 treatment was initiated within the first month of life and adjusted to maintain TSH levels in the normal range and free thyroxine in the high-normal range.

Results: Compared to healthy controls CH patients displayed a significantly reduced vasodilatory response with lower FMD values (14.5±0.9% vs. 8.0±0.9%, $p<0.0001$) and increased mean internal CIMT (0.61±0.01mm vs. 0.70±0.04mm, $p<0.0001$). Stepwise regression analysis revealed that pubertal and total mean TSH was an independent determinant of FMD ($p<0.0001$) and CIMT ($p<0.0001$) respectively. In addition, the number of episodes of subclinical hypothyroidism (TSH>5.0 mU/L with normal FT4) during puberty ($r=-0.53$, $p< 0.003$) was an additional risk factor for endothelial dysfunction.

Conclusions: Young adults with CH, treated with long-term L-T4 replacement therapy, display endothelial dysfunction and increased CIMT.

FMD is as s measure of preclinical cardiovascular risk, thus it may be used in adolescents with CH to identify those patients who require careful follow-up with frequent dosage adjustment in order to prevent early atherosclerotic changes of CIMT.

PO3-337 Thyroid III

A new case of congenital hypothyroidism due to a new genetic alteration in the sodium-iodide symporter

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Introduction

Iodide transport defect (ITD) is a disorder characterized by an inability of the thyroid to maintain an iodide gradient across the basolateral membrane of follicular cells which often results in dyshormonogenetic Congenital Hypothyroidism (CH). The defect has been shown to arise from autosomal recessive mutations of Sodium-Iodide Symporter (NIS) gene.

We describe a new NIS mutation in a patient with the complete clinical picture of ITD at birth.

Clinical report

We describe a 14 years old girl with CH. At 4 years of age, following a temporary discontinuation of hormone therapy, a 123-I scan showed no thyroid tissue in the neck. After a period of inadequate compliance to treatment, she presented goiter, and a ultrasonography showed a normally located gland, with a higher volume and three nodules.

Materials And Methods

Thyroid uptake measured as percentage of administered oral 131-I (50 uCi, 1,85 MBq), scans with 99-Tc, 131-I and with MIBG; the saliva to plasma (S/P) iodide ratio; gene sequencing; functional analysis of expression vector.

Results

Thyroid uptake of 131-I was 1.5%. Scans performed with 99-Tc and 131-I failed to show thyroid tissue and no drug accumulated in the salivary glands or stomach, while a scan with MIBG showed a normal uptake in the gland. The S/P ratio was 0.5. Gene sequencing of the proband revealed the presence of a six nucleotide in-frame deletion, from nt 1206 to nt 1211, in exon 7 of the NIS gene. The nucleotide deletion corresponded to the deletion of aminoacids 287 and 288 of the human NIS protein (del 287-288) located at the beginning of the VIII transmembrane segment. The patient was homozygous for this deletion, while both parents and the brother were heterogygotes for the del 287-288. COS-7 cells transfected with the mutant del 287-288 of NIS concentrated 125-I similarly to cells transfected with the empty vector suggesting that the mutation was the direct cause of the phenotype in this patient. Cells co-transfected with both wt NIS and del 287-288 of NIS showed a lower iodide uptake activity as compared to cells transfected with wt NIS alone, but uptake was similar to that in cells co-transfected with wt NIS and the empty vector.

Conclusion

We describe a new case of CH due to a new deletion in the NIS gene. In agreement with the autosomal recessive inheritance pattern of ITD, the patient was homozygous for this deletion, while both parents and the brother showed the same deletion in the heterozygous state.

PO3-338 Thyroid III

A new case of congenital central hypothyroidism due to an intronic defect in the TSHβ gene

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Central Hypothyroidism (CeH) is a rare thyroid hormone production defect due to an insufficient stimulation of a normal thyroid gland. Candidate genes for isolated CeH include TSHβ (several cases reported) and TRHR (two cases reported so far). Here, we report the clinical and genetic studies in a male infant, second child of Italian unrelated parents, delivered at term after an uneventful pregnancy. He was referred to our Clinic at 44 days of age for severe hypothyroidism, not detected at the TSH-based neonatal screening. Physical examination showed weight 3.850 Kg, length 53 cm, cranial circumference 37 cm, feeble cry, weak suction and reflexes, hypotonia, dry skin, jaundice, anterior fontanel enlarged (4x4cm) and posterior still open (4x2cm), large protruding tongue, cold extremities, umbilical hernia. The Beclard's nuclei were absent. Hormone profile (See also table) showed low basal TSH and FT3/FT4. Other pituitary functions, including PRL, GH, LH/FSH and adrenal axis were normal. TRH administration was followed by an impaired TSH but normal PRL response.

Proband hormone profile in basal conditions and after TRH test 200 μg i.v.

	Basal	time 30'	time 60'
TSH μU/ml	0.17	5.94	0.21
PRL ng/ml	27.6	85.9	not detected
fT4 ng/ml	0.20		
GH μU/ml	30.3		
ACTH pg/ml	250		
FSH/LH mUI/ml	8.4 / 8.2		

CeH was diagnosed and L-thyroxin replacement therapy (12 mcg/kg/day) promptly started. Genomic DNA molecular analysis by direct sequencing of the TSHβ gene coding exons (2 and 3) and the surrounding intronic region was performed. While the coding region of the gene was normal, we identified an h omozygous mutation in the intronic splice site region between the two coding TSHβ exons, IVS2+5 GtoA. Parents are heterozygous for the same mutation, while the oldest sister is entirely wild-type. The family history, back to the 4th generation is negative for consanguinity and thyroid diseases. This mutation was already reported in literature in five cases of Turkish origin with consanguineous parents, low but detectable TSH and high glycoprotein α-subunit levels. Surprisingly in our case, unlike all others, no consanguinity between parents was reported. The mild TSH response to the TRH test observed in the present study and in one of the previously reported cases indicates that the mutant gene partially escapes the aberrant splicing, yielding a certain production of normal transcript and protein but with an altered biological activity.

PO3-339 Thyroid III

Genetic background of neonatal transient hyperthyrotropinemia

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Background: About 30-50 % of children with elevated thyroid stimulating hormone (TSH) concentrations at newborn screening have transient forms of the disorder. The causes of transient hyperthyroropinemia or transient hypothyroidism include prematurity, maternal thyroid disease, and the excess or the lack of maternal iodine intake. On the other hand, it has been reported that subclinical hypothyroidism persisted in about 30 % of false positive children at newborn screening in late childhood.

Aim: The aim of this study is to determine whether neonatal transient thyroid dysfunction is influenced by genetic background.

Subjects and Methods: We studied fourteen children with transient thyroid dysfunction at the age of 9.8±2.9 years (range, 6.25 to 15.0). Subjects with known underlying causes of transient thyroid dysfunction were excluded. At neonatal screening, serum TSH concentration was 22.2±10.1μU/ml (range, 14.5 to 43.4). Serum TSH and free T4 concentrations at first visit to the hospital on day 31±17 (range, 11 to 56) of life were 13.0±7.6μU/ml (range, 5.5 to 32.78) and 1.42±0.27 ng/dl (range, 1.09 to 1.85), respectively. Seven children had not received levothyroxine (l-T4) replacement. The remaining seven children started l-T4 replacement during neonatal period and stopped it after the reevaluation of thyroid function at the age of 5.5±2.2 years (range, 4.0 to 9.0). Genomic DNA was isolated from peripheral blood lymphocytes. The complete coding regions of the TSH receptor (TSHR), thyroid peroxidase (TPO), and thyroid oxidase 2 (THOX2) genes were analyzed by direct DNA sequencing.

Results: One untreated child was heterozygous for a TPO gene mutation (P883S), and one treated child was heterozygous for a TSHR gene mutation (R450H). THOX2 gene mutation was not identified.

Conclusions: Two out of 14 children (14 %) with neonatal transient thyroid dysfunction had genetic background. Although the further examination is needed, it is possible that genetic background contributes to the development of neonatal transient thyroid dysfunction.

PO3-340 Thyroid III

Celiac disease in children with autoimmune thyroid disease

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Background:
The prevalence of CD in children with autoimmune thyroid disease (ATD) in the US is unknown. We designed this study to determine the prevalence of antibodies associated with CD and of biopsy proven CD in children with ATD.
Patients and Methods:
300 patients with positive anti-thyroid antibodies were prospectively enrolled. Tissue transglutaminase IgA (tTG-IgA) and total IgA levels were obtained to screen for CD. Those with a positive tTG-IgA were offered biopsy.
Results:
We found 1) a 4.7% correlation between positive tTG-IgA and ATD (fig.1), and 2) the prevalence of biopsy confirmed CD in ATD was 2.3% (fig.2). Our population was enriched with both type 1 diabetics (DM1) (4.3%) and Down Syndrome (DS) (3.4%). 15% of the DM1 and 22% of the DS patients had CD in addition to ATD. Excluding individuals with these co-morbidities and Turner syndrome, the prevalence of CD in ATD is 1.4%. Finally, the likelihood of biopsy proven celiac disease in patients with ATD and positive tTG-IgA is approximately 50%.
Discussion:
The prevalence of CD in the US in children is approximately 0.31% [1]. The prevalence of CD in children in Italy was found to be 0.5% [2]. Two European studies have found a prevalence of CD of 7.8% and 4.9% among children with ATD [3, 4]. We also found an increased prevalence of CD (2.3%) in our patients with ATD and 1.5% in those with no co-morbidities. This is higher than the prevalence of CD in the US population but lower than reports from Europe. However, this increase in prevalence of CD in ATD over the general population may not be enough to justify screening of patients with ATD who don't have co-morbidity.
Fig. 1

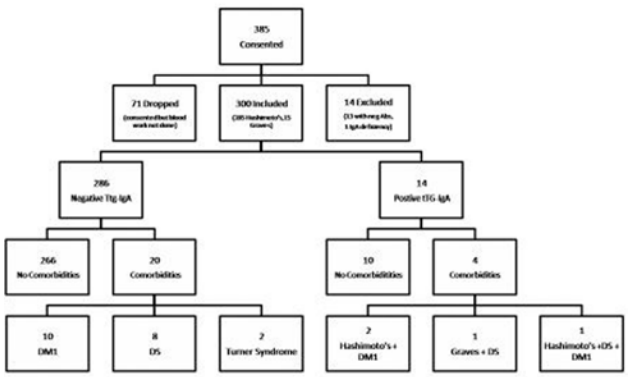
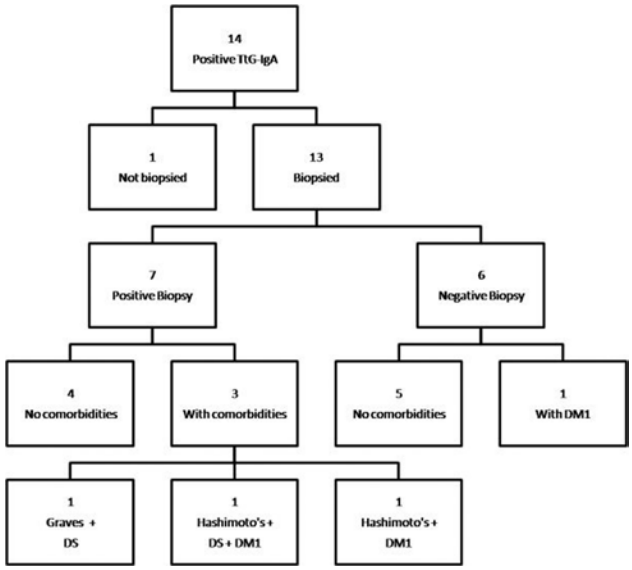


Fig. 2



PO3-341 Thyroid III

Detours on the road to diagnosis of Graves disease

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Background: The clinical presentation of children with hyperthyroidism may mimic other pathologic processes. Prior to the correct diagnosis being made, some children are seen by multiple subspecialists and undergo medical evaluation and treatment for other conditions. However, it is unclear how often such diagnostic detours take place in children with Graves disease (GD).
Objective: To determine the frequency at which spurious diagnoses and unnecessary evaluation and/or treatment occurs prior to the diagnosis of GD in pediatric patients.
Design/Methods: A retrospective chart review of children with GD seen in the pediatric endocrine clinics at Riley Hospital for Children from 1998 to 2007 was performed.
Results: Seventy-six subjects (61 girls) aged 11.9 ±3.8 years diagnosed with GD were identified. The most common presenting symptoms were arrhythmias (48.7%), temperature intolerance (47.4%), weight loss or poor weight gain (39.5%), increased appetite (39.5%), and neck swelling/goiter (34.2%). Seventeen (22.4%) were referred to subspecialty clinics prior to the diagnosis of GD, including cardiology, psychiatry, gastroenterology, and ophthalmology. Five were evaluated by more than one subspecialist. Six patients were hospitalized, and 2 visited emergency rooms. Eighteen (23.7%) underwent non-thyroid related studies including EKG, echocardiogram, Holter monitor, abdominal CT, endoscopy, head CT, and brain MRI. Twelve (15.8%) were diagnosed with ADHD. Eighteen (23.6%) were started on medications prior to the diagnosis of GD including psychostimulants, antidepressants, beta blockers, ACE inhibitors, analgesics, and anti-reflux agents.
Conclusions: In children with GD, medical evaluation and treatment for presumed other conditions is common. Since symptoms of hyperthyroidism overlap with those of many other disorders, thyroid function testing prior to subspecialty referral should be considered. A high index of suspicion for GD on the part of primary care providers may help to avoid unnecessary referrals, extensive testing, and medications that may be costly, delay eventual diagnosis, and needlessly affect quality of life.

PO3-342 Thyroid III

Plasma C-type natriuretic peptide forms in prepubertal children with acquired thyroid disease: correlation with thyroid status and height velocity

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Background: C-type natriuretic peptide (CNP) plays an essential role in endochondral bone growth, but rapid degradation limits its detection in the circulation. In contrast, the amino-terminal propeptide of CNP (NTproCNP), a stable by-product of CNP synthesis, is easily measured in plasma and strongly correlates with skeletal growth and markers of bone formation in normal health. Although thyroid disorders may profoundly affect linear bone growth in children, the relation of CNP synthesis to thyroid status and bone growth is unknown. Accordingly, we have studied changes in plasma CNP forms in prepubertal children with acquired thyroid disease before and during corrective treatment.
Objective: To correlate changes in plasma levels of CNP forms with changes in thyroid hormones (TH) and height velocity (HV) in children with acquired hypo- and hyperthyroidism.
Methods: We measured plasma CNP, NTproCNP, and TH levels before and at multiple time points during the first 6 mo of T4 treatment for hypothyroidism (n=8) and methimazole treatment for hyperthyroidism (n=5). Height was measured by stadiometer and HV was annualized. Associations between variables were investigated by Pearson correlation analysis and regression analysis of HV and CNP forms and other markers of bone growth.
Results: At baseline, CNP and NTproCNP were positively correlated (r=0.83, p=0.0004); CNP correlated with T3 (r=0.64, p=0.02) and T4 (r=0.59, p=0.03); and NTproCNP correlated with T3 (r=0.70, p=0.008), T4 (r=0.67, p=0.01) and bone specific alkaline phosphatase (BsAP) (r=0.81, p=0.01), but not IGF-I. After 2.5-6 mo of treatment, the change in NTproCNP was significantly positively correlated with changes in T3 (r=0.79, p=0.01) and T4 (r=0.72, p=0.03). Regression analysis of HV at individual time points demonstrated a positive correlation with NTproCNP (r=0.51, p=0.0006), but not with CNP. Regression analysis of HV vs IGF-I (r=0.49, p=0.02) and vs BsAP (r=0.65. p=0.0013) also showed significant positive correlations.
Conclusions: Plasma levels of CNP and NTproCNP correlate with TH levels in children with acquired hypo- or hyperthyroidism. NTproCNP levels change in parallel with thyroid hormones and BsAP in response to treatment. HV correlates with NTproCNP, IGF-I, and BsAP in children with thyroid disease. These findings suggest novel mechanisms underlying changes in bone growth in children with acquired thyroid disorders.
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PO3-343 Thyroid III

A 19-week fetus with non-immune hypothyroidism and goiter: treatment or observation?

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A 33-year-old G3P1 woman was referred because of the discovery of a goiter in her fetus at 19 week of gestation: the fetal thyroid was enlarged (1.9 ml) without signs of tracheo-oesophageal compression (i.e. normal volume of amniotic fluid, normal volume of the lungs). On blood obtained by cordocentesis, TSH, free T4 and Tg values were 90.74 mU/L [1-9], 3.68 pmol [2-7.5], 12.39 µg/L [NA], respectively. Maternal thyroid function was normal (TSH 0.85 mU/L; free T4 8.89 pmol/L) and there were no antibodies against Tg or TPO in maternal plasma. We decided to follow the fetus with echographies q 2 weeks and to treat if signs of tracheo-oesophageal compression (i.e. increased volume of amniotic fluid, neck hyper-extension) developed. At 25 weeks, the volume of the fetal thyroid (4.6 ml) and the amount of amniotic fluid (75th centile) had increased so intra-amniotic (i.a.) L-T4 was initiated at a dose of 200 µg q 2 weeks. Two weeks after the first dose, the thyroid volume (3.3 ml) and the amniotic fluid volume (55th centile) had decreased. Altogether, i.a. L-T4 was injected three times (25, 27 and 29 weeks). Planned doses after 29 weeks were cancelled because of frequent contractions and stabilization of the fetal goiter. At 38 weeks, the mother spontaneously delivered a boy without any obstetrical and neonatal complications (Apgar 9 9; BW 3205 g; no signs of tracheal compression). Neonatal cord blood revealed TSH 224.05 mU/L [<30], free T4 11.76 pmol/L [12-19.4] and Tg 3,55 µg/L[31-101]. The goiter was confirmed by echography (7.2 ml [0.83 ml]). L-T4 50 µg die p.o. was started at day 1 of life. Tg gene analysis is in progress. In conclusion: 1) the pituitary feedback system is already operative at 19 weeks; 2) i.a. L-T4 decreased the size of a fetal hypothyroid goiter and the associated hydramnios. The benefit-to-risk ratio of i.a. L-T4 needs to be carefully evaluated.

PO3-344 Thyroid III

Serum TSH confirmation of neonatal screening: need for time adjusted values during the second week of life

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Objective: In our experience, patients requiring retesting due to abnormal values in the neonatal screening for congenital hypothyroidism usually return at 7 to 15 days of life (median: 13). Therefore, and given the changes made by manufacturers in the TSH measurement kits (June 2006), our objectives were: 1) to assess whether those methodological changes would modify reference values during the neonatal period; 2) to evaluate if during the second week, variations in TSH create a need for time-adjusted values. **Materials and methods:** 80 serum samples with TSH values (mIU/L) ranging 0.04-34, processed by Immulite, pre-changes (batch 340) and post-changes (batch 351) were evaluated and, in 252 full-term healthy newborns: Group A (GA), 48-72 hours of life, n= 152 and Group B (GB): 7-14 days of life, n= 100, TSH post-change, T3(nmol/L), T4(nmol/L) and T4L(pmol/L) were measured. **Results:** A high correlation was found between the results obtained with both batches (r=0.995; p<0.0001. Spearman Regression analysis). Consistency in TSH measured with both batches was evaluated (Bland and Altman), obtaining the ratio: batch 351/340, as a measurement of the difference. The ratio was: 1.31 ± 0.03 (mean ± SEM), confidence interval 95%:1.26-1.37. The results obtained in full-term healthy newborns (median and range) were, GA:TSH: 4.70(0.80-15.4);T3: 2.4(1.3-4.9);T4: 237(143-309) and T4L: 29.3 (18.3-43.9) and GB, TSH: 4.06(1.10-10.2); T3: 2.9 (1.7-4.4); T4: 188(136-309) and T4L: 24.4(18.8-37.7). In Group B, we evaluated the correlation between the day of sample collection and the TSH value obtained (Spearman Regression analysis: r= -0.3087, p= 0.0260. Based on the trend observed, GB was subdivided into: 7-10 days of life (early), n= 65 and 11-14 days of life (late), n= 35. TSH values were: early: 4.2(1.5 -10.2) and late 3.8(1.1-7.1) p <0.03 (Mann-Whitney Test). **Conclusion:** -the values obtained in GA and GB for TSH, slightly higher than those published in the literature, would be linked to the mean overestimate of 31% in current kits**.** – The differences found between the early and late groups, and the negative correlation between the sampling day and the TSH value, reinforce the need to obtain reference ranges for early and late stages in the second week of life for a proper interpretation of doubtful measurements in neonatal screening.

PO3-345 Thyroid III

A missense mutation of the thyroid hormone receptor beta (THRB) in a child with Down syndrome (DS)

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Background: Thyroid dysfunction is common in children with DS. However, not all are secondary to autoimmune conditions or thyroid dysgenesis, and

many cases remain unclassified.
Objective: To characterize the etiology of abnormal thyroid laboratory tests in a child with Down syndrome.
Methods: 14-month-old Caucasian boy with trisomy 21 was evaluated for abnormal thyroid labs (table). Past medical history includes microphallus (phallus stretched length 2.6 cm, 10th %ile), chronic otitis media and seasonal allergies. Serum TSH concentrations were persistently slightly increased, as were free T4 and total & free T3 concentrations. There was no goiter and growth in height was normal for a boy with DS. He had no symptoms suggestive of hyper- or hypothyroidism. BP and heart rate have been normal for age. Pituitary MRI was normal. Maternal grandfather has history of thyroid disease. DNA analysis for THRB mutation was sent.

Thyroid test (ref range)	12 month	15 month	21 month	26 month	28 month	28.5 month	32 month
FT4 (0.8-1.8 ng/dL)	2.4	1.9	2.26	3.3		8.9	2.7
T3 Total (0.2-2.0 ng/mL)				2.59		3.17	2.14
FT3 (1.80-4.20 pg/mL)				5.55		7.34	4.69
TSH (0.4-5.5 uU/mL)	6.52	4.30	5.35			4.69	
TSI (<130%)					107		96
TBG (16.6 -29.8 mg/L)				25.7			

Results: THRB mutation analysis revealed a heterozygous P453A mutation, previously reported to cause autosomal dominant resistance to thyroid hormone. The child is asymptomatic and thus no treatment has been initiated.
Conclusions: This is the first description of thyroid hormone resistance syndrome in DS. Although elevated TSH concentrations are common in DS, often not requiring intervention, other specific causes of abnormal thyroid tests should be excluded.

PO3-346 Thyroid III

Hashimoto's thyroiditis: a rare cause of severe growth failure. Report of 6 cases

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Autoimmune thyroiditis, the most frequent cause of acquired hypothyroidism in childhood, leads mostly to mild symptoms : asymptomatic enlargement of the thyroid gland or less often primary mild hypothyroidism. We report 6 children who presented with arrested growth for several years due to Hashimoto's thyroiditis.
Characteristics of the patients are summarized in Table 1.

Characteristics of the patients						
Patient	1	2	3	4	5	6
Sex	F	M	F	F	F	F
Age at diagnosis (yrs)	10.3	8.8	7.5	11.2	9.7	9.7
Height at diagnosis (SDS)	-1.4	-4	-1	-4.7	-2	-3.5
Growth velocity (cm/yr)	2	1	1	0.7	1.3	0.5
slow growth duration (yr)	2	3.8	2	6	2.5	3
Bone age (yr)	8.8	5	4	6	7.8	5.8
Goiter	no	no	no	no	yes	yes
FT4 (pmol/l)	<2	<2	<2	5.2	3.5	5.4
TSH (µU/ml)	895	1154	584	582	540	1844
TPO antibodies (U/ml)	650	20450	+	2760	23750	25310
Thyroid ultrasound	atrophy and heterogeneous	atrophy and heterogeneous	atrophy and heterogeneous	atrophy and heterogeneous		small goiter
Therapy	LT4 2.5 µg/Kg.d	LT4 2.5-3µg/Kg.d	LT4 2.2-2.5 µg/Kg.d	LT4 3.5 µg/Kg.d + GH + GnRHa	LT4 2 µg/Kg.d + GH	LT4 2.5 µg/Kg.d+ GH + GnRHa
Last height (SDS)	-1.65	-2.7	+1.5	-3.6	-1.4	-3
Mid- parental height (SDS)	-0.4	0	+3	-0.6	-1.4	-0.4

We describe 6 children (5 girls, 1 boy) aged 7.5 to 11.2 years old, with a drastic decrease in growth velocity (0.5 to 2 cm/yr), lasting for 2 to 6 years. Four of them had no goiter. Symptoms of hypothyroidism were moderate in spite of very low FT4 serum levels (<2 to 5.4 pmol/l). TPO antibodies were increased in all subjects and ultrasound showed atrophic heterogeneous thyroid in 4 of them. MRI of the brain performed in first line diagnosis in 2 patients showed enlarged homogeneous hypophysis. L-thyroxine therapy (2-3.5 µg/Kg.d) increased growth velocity (8-11 cm/yr) but puberty started during the first year of therapy in 3/6, compromising predicted adult height because of very short stature in 2 (-3.5, -4.7 SDS). Combined therapy with GH and GnRHa was initiated.
In conclusion, Hashimoto's thyroiditis should be evoked as the cause of severe growth deceleration in childhood, even in the absence of clinical symptoms of hypothyroidism and goiter. L-thyroxine therapy induces catch-up growth but may be not sufficient to normalize compromised final height in the severely growth retarded children and use of GH (possibly combined with GnRHa) therapy should be considered.

PO3-347 Thyroid III

A patient presenting with central hypothyroidism, developmental delay and poor head control. Should we be checking T3 levels?

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Central hypothyroidism(CH) is rare and seen 1:100,000 in the newborn(NB) period. It is usually associated with a hypothalamic-pituitary anomaly and can be seen as part of panhypopituitarism, isolated TSH deficiency or thyroid hormone resistance. We present a 5 yr 6 mos old boy who presented to the Endocrine clinic at 10 mos of age with a history of developmental delay and failure to thrive. He started to have constipation at 2-3 mos of age followed by poor weight gain and developmental delay. Birth history and birthweight were normal. He had normal thyroid NB screening results. Family history was positive for a maternal uncle who had hypotonia /cerebral palsy and died at 20 mos of age. Physical exam: significant for central hypotonia with poor head control. Height was in 50th %, weight was <3rd % and head circumference was 10th %. Work-up showed a normal TSH =3.3 mcIU/ml (nl 0.35-6.0), low Total T4 = 3.5 mcg/dL (nl 5.0-13) and low Free T4= 0.4 ng /dl (nl 0.8-1.9) Patient was started on 50 mcg daily L-Thyroxine with the diagnosis of CH. Low dose ACTH stimulation test was normal. MRI of the brain showed slightly decreased myelination pattern with no hypothalamic or pituitary abnormality. Despite L-Thyroxine treatment there was no change with regard to clinical picture especially the cognitive and psychomotor delay. At 2.5 years of age during a Genetics outpatient visit Free T4 and T3 levels while on L-Thyroxine treatment showed low Free T4 0.5 ng/dl and high T3 of 290 ng/dL (normal 50-261 ng/dL). The diagnosis of Allan-Herndon-Dudley syndrome was suspected. Patient and his mother were found to have a 1492G->A mutation in exon 5 of the MCT8 gene resulting in a D498N change in the monocarboxylate transporter 8 protein.
Several cases of X-linked hemizygous mutations in the MCT8 gene have been reported so far. Phenotype is characteristic for truncal hypotonia with especially poor head control, combined with limb spasticity and severe intellectual / motor disability. Serum T3 level and FT3 levels are elevated whereas serum

T4 and FT4 levels are low and TSH levels are within upper range of normal or even elevated. MCT8 plays an essential role in T3 supply to neurons which are the primary targets of T3 action especially during brain development.
Conclusion : Any patient presenting with above phenotype and CH should be checked for T3 levels and if elevated, the possibility of a mutation in the MCT8 gene should be investigated.

PO3-348 Thyroid III

Urinary iodine levels and thyroid function tests of neonates and their mothers in Aydin Province

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Introduction: Iodine deficiency causes some developmental and functional diseases which can be prevented with iodine prophylaxis. According to WHO, TSH >5 mU/ml in more than 3% of the population and average urinary iodine levels <100 µg/L are defined iodine deficiency. We studied thyroid functions, breast milk iodine levels and urinary iodine levels in neonates and in their mothers in Aydin province.
Material and Method: Blood and urine samples were taken simultaneously from 400 newborns between 4-7 days of life and their mothers for determination of TSH, FT4, TT4 and TT3 and urinary iodine levels in provincial and district hospitals.
Results: Mean urinary iodine levels of neonates and mothers were 141.8±13.6, 128.8±12.4 µg/L, respectively. Mean of thyroid function tests was TSH; 7.26±7.87 (0.34-54.8) mU/ml, TT4; 13.9±3.6 (1.85-24) µg/dl, FT4; 1.68±0.33 (0.78-3) ng/dl, TT3; 187±60.4 (55-456) ng/dl in neonates and TSH; 1.71±1.35 (0.02-12) mU/ml, TT4; 12.9±2.37 (6.54-20.3) µg/dl, FT4; 1.29±0.22 (0.52-2.22) ng/dl, TT3; 173.2±39.3 (83-331) ng/dl in mothers. The ratio of neonates who are recalled back with TSH>9.1 mU/ml was 22.5% and with TSH> 5mU/ml was 47% and with congenital hypothyroidism frequency was 1.25%.
Conclusion: We obtained urinary iodine levels of neonates and mothers as normal. This situation shows the emphasis of iodinated salt usage by local health policies. The absence of iodine deficiency in those infants (47 %) with high TSH levels make us think of other causes. Some other etiologic and physiopathologic mechanisms such as umbilical care with iodinated solutions, insufficient iodine intake in pregnancy and natural goitregens taken by the mother are thought to be effective in thyroid metabolism.

PO3-349 Thyroid III

Radioactive iodine^{131} treatment during the second trimester of pregnancy: *a case report and review of literature*

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A woman was treated with radioactive iodine^{131} while she was in the 20th week of an unexpected pregnancy. The boy, born after 35 weeks of gestation, had no signs of hypothyroidism. During follow-up, he developed normally, free thyroxine hormone levels were subnormal and thyroid stimulating hormone levels were normal. Ultrasound examination showed a hypoplastic thyroid gland. At 15 months of follow-up, treatment was still not necessary.
Second trimester accidents with radioactive iodine are exceptional, therefore predicting neonatal outcome is difficult. This case emphasizes the variability of clinical outcome after accidental iodine^{131} exposure, influenced by administered dose, fetal uptake and timing in pregnancy.

PO3-350 Thyroid III

Thyroid and somatotropic axis function in children diagnosed of attention deficit and hyperactivity disorder (ADHD)

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Introduction: Attention deficit and hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorder in childhood. Exact aetiology is unknown although sometimes it has been associated with alterations of thyroid hormones. Also it has been linked both the disease and its treatment with methylphenidate with abnormalities of the somatotropic axis.
Objective: To evaluate thyroid function and somatotropic axis in prepubertal children diagnosed with ADHD and to examine whether variation exists after six months of treatment with methylphenidate.
Patients and methods: Prospective study of prepubertal children diagnosed with ADHD in neuropediatrics out patients from 2007 October to 2008 May. All patients had informed consent and the study was approved by the ethics committee of the Cruces hospital. We studied the following variables: height, BMI and differential height (DH: current size and height SDS according to target local reference charts), differential bone age in years(DBA: chronological age-bone age, using as Greulich and Pyle) Free T4 (ng / dl), TSH mU/ml), IGF-1 (ng/ml) and IGFBP3 (µg/ml), complet blood count and blood chemistry. The data were analyzed using SPSS.15 for windows.
Results: We studied 49 prepubertal patients with an **average age** of 9.3 years (r: 6.4-13.8). Male 86%. *At diagnosis*, the result of variables were: **height(SDS)**mean:-0.25, with a range(r): -0.26 ± 1.22 and **BMI(SDS)**mean: 0,07, (r)from -1.9 to 2.6; **Free T4**: mean:1.3 r: from 1.1 to 1.6; **TSH**: mean 2.1, r from 0.52 to 4.8; **dif EO**: mean: 0.47 (r from -2 to 3.7), **IGF-1**: mean 188, (r) from 61 to 612; **IGFBP3**: mean: 4.3, (r) from 2.1 to 6.2. *After six months of treatment with methylphenidate:* **height (SDS):** mean -0.51,(r)from from -2.3 to 2; **BMI(SDS)** mean -0.45, (r) from -1.99 to 1.37); **FT4**: mean 1.3, (r) from 0,9 to 2); **TSH**: mean 2.4, (r) from 0,79 to 8,5; **IGF-1**: mean: 197, (r)from 44 to 825; **IGFBP3**: mean: 4 5 (r) from 2,4 to 6,7. We found no difference between groups except in the variable BMI (SDS) with significant difference ($p<0.00$).
Conclusions: 1. Thyroid disorders are not consistent with ADHD. 2. Our patients with ADHD are normal in auxological data and analytical study. 3. Treatment with methylphenidate, at least in the short term does not affect the somatotropic axis or thyroid function. 4. Treatment with methylphenidate causes a weight reduction without affecting the rate of growth.

PO3-351 Thyroid III

Association of autoimmune thyroiditis with CTLA-4 +49 A/G gene polymorphisms

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Autoimmune thyroiditis is one of the most common human autoimmune diseases caused by an interaction between susceptibility genes and environmental triggers. Based on functional and experimental data, the gene encoding cytotoxic T lymphocyte-associated antigene 4 (CTLA-4) has been suggested as a candidate gene for conferring susceptibility to autoimmunity.
In our study we attempted to evaluate the association between CTLA-4 A/G gene polymorphisms and autoimmune thyroiditis. To this purpose, polymorphisms of the exon 1 of the CTLA-4 +49 A/G gene were determined by group-specific polymerase chain reaction amplification (PCR) method based on time-resolved fluorometry (TRF) technique, in 58 patients with autoimmune thyroiditis, aged 12.8±1.4 years, as well as in 100 controls from the general population.
Autoimmune thyroiditis was diagnosed at an age of 9.32±2.4 years, through

positive antithyroid antibody testing and ultrasound findings suggestive of the disease (heterogenity and hypertrophy of the gland). The TSH levels at diagnosis were 11.87±3.35 μIU/ml and the exogenous thyroxine requirements at last examination were 1.6μg/kgr/day.
The CTLA-4 +49A/G polymorphisms seem to be related to autoimmune thyroid disease. The AG and GG genotypes are related to a moderate risk for autoimmune thyroiditis (p<0.001, OR= 2,85 and p=0.045, OR=1,43 respectively). Furthermore, the G allele increases the risk for autoimmune thyroiditis(p=0,003, OR= 1,628).
Our results support the hypothesis that CTLA-4 may play a role in regulating self-tolerance by the immune system and in the pathogenesis of autoimmune thyroiditis.

PO3-352 Thyroid III

Association of autoimmune thyroiditis with HLA-DQB1 and DQA1 gene polymorphisms

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Autoimmune thyroiditis is a complex disease, caused by an interaction between susceptibility genes and environmental triggers.
The histocompatibility leucocyte antigen DQB1 (HLA-DQB1) polymorphisms may modulate the susceptibility to the disease. The aim of our study was to assess the association between autoimmune thyroidits and the HLA-DQB1 and HLA-DQA1 polymorphisms.
To this purpose, molecular typing of six HLA-DQB1 alleles (DQB1*0201,*0301,*0302,*0602,*0603,*0604) was carried out, using the group-specific polymerase chain reaction amplification technique, to 58 patients with autoimmune thyroiditis, aged 12.8±1.4 years, as well as to 1784 controls from the general population. Further typing of three DQA1 alleles(DQA1*0201,*0301, *0201) was performed in HLA-DQB1 *0201, x carriers. Autoimmune thyroiditis was diagnosed at an age of 9.32±2.4 years, through positive antithyroid antibody testing and ultrasound findings suggestive of the disease (heterogenity and hypertrophy of the gland). The TSH levels at diagnosis were 11.87±3.35 μIU/ml and the exogenous thyroxine requirements at last examination were 1.6μg/kgr/day.
A higher incidence of the HLA-DQB1*0201,0302 (p=0.022, OR:4.3), the HLA-DQB1* 0302,x (p=0.035, OR:1.78) and the HLA-DQB1* 0201,x (p=0.042, OR:1.53) genotypes and a lower incidence of the HLA-DQB1*0301,x (p=0.012, OR:0.22) genotype was found in the patients with autoimmune thyroiditis compared to the general population. A positive association was found with the HLA-DQB1*0302 allele (p=0.034, OR:2.14) as well as with the HLA-DQB1*0201 allele(p=0.044, OR:1.46) and a negative association with the HLA-DQB1*0301 allele (p<0.001, OR:0.22). Statistically significant associations of autoimmune thyroiditis were found neither with the HLA-DQB1*0602,x, the DQB1*0602,x, the DQB1*0301,0302, the DQB1*0201,0301,the DQB1*0302-0602 and the DQB1*0602,0201 genotypes nor with the HLA DQB1*0602-*0603-*0604 alleles. Patients with autoimmune thyroiditis with the DQB1*0201, x genotype carry the DQA1*0501 allele(p=0,04, OR: 1,67) in a higher incidence compared to controls.
Our results support our hypothesis that the HLA-DQB1 and DQA1 gene polymorphisms play an important role in susceptibility to or protection against autoimmune thyroid disease.

PO3-353 Thyroid III

New TTF1/NKX2.1 mutation in a child with congenital hypothyroidism and neurodevelopment delay

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Thyroid transcription factor 1 (NKX2-1/TITF1) mutations are associated with important neurologic deficits and/or congenital hypothyroidism (CH) and/or neonatal respiratory distress, causing the phenotype described as brain-lung-thyroid syndrome.
We studied three children with congenital hypothyroidism, choreoathetosis/neurodevelopment delay, and perinatally respiratory distress (in two of the three children), with the aim to find the genetic defect that causes this phenotype.
After isolation of genomic DNA from peripheral blood, the three exons of the TTF1 gene were amplified. The PCR products were then purified and sequenced using an automated sequencing system. In two children DNA analysis did not reveal mutations in the entire coding region of the TTF1 gene, but in a child, the only one without pulmonary diseases, we identified a new heterozygous frameshift mutation (p.H90TfsX10 in exon 2), causing the formation of a premature stop codon.
The clinical spectrum of this child was characterised by normal neonatal anamnestic data with delivery at term and adequate weight and length for gestational age; Apgar score was 10/10 and no respiratory distress occured in the first years of life. He resulted positive at neonatal screening for CH with elevated serum TSH (> 200 mU/L) and very low FT4 levels (0.21 ng/dl), the thyroid morphology was characterized by an ectopic gland. At 9 days of life L-Thyroxine replacement therapy was introduced with a dosage of 12.2 mcg/kg/day.
By age of 6 months the child manifested neurological retard (QS 87, locomotion 61) with locomotion disorders characterized by ataxia and spasticity. Brain magnetic resonance imaging showed no defects. Neurologic and metabolic tests didn't reveal a specific syndrome or diagnosis.
Our study is in accordance with data of the literature showing that the majority of patients with TTF1 defect displays neurological and/or thyroidal problems and, less frequently, lung disease. However, the association between CH and ataxia is not always related to mutations of TTF1 gene.

PO3-354 Thyroid III

The polymorphism of CYP27B1 gene is associated with autoimmune thyroid diseases in Chinese Han population

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Objective: To investigate the relationship between CYP27B1 gene polymorphism and autoimmune thyroid diseases in Chinese Han population. **Methods:** A case-control study including 158 patients with GD, 174 patients with HT and 172 matched controls was conducted. Three genotypes of CYP27 B1 gene -1260 site (CC,AA and AC) were determined by restriction fragment length polymorphism (PCR-RFLP) assay and confirmed by sequencing. Another case-control study including 172 patients with GD, 180 patients with HT and 197 matched controls was conducted. Three genotypes of CYP27 B1 gene +2838 site (CC,TT and TC) were determined by polymerase chain reaction-sequence specific primer(PCR-SSP) assay and confirmed by sequencing.**Results:** These genotypic distributions didn't deviate from Hardy-Weinberg equilibrium. There were significant difference in the genotypes and alleles in -1260A/C and +2838 T/C polymorphism between GD or HT and control group(-1260:GD: genotype frequencies $\chi^2=6.80$, $P<0.05$, allele frequencies $\chi^2=6.79$, $P<0.05$; HT: genotype frequencies $\chi^2=8.39$, $P<0.05$; allele frequencies $\chi^2=8.71$, $P<0.05$;+2838: GD: genotype frequencies $\chi^2=13.1$, $P<0.05$, allele frequencies $\chi^2=14.5$, $P<0.05$; HT: genotype frequencies $\chi^2=12.2$, $P<0.05$; allele frequen-

cies χ^2=*8.30, P*<0.05;). **Conclusion:** The polymorphism of CYP27 B1 gene is associated with GD or HT in Chinese Han nationality.

PO3-355 Thyroid III

Male predominance, high prevalence of thyroid autoantibodies and homozygous TPO mutation presenting with three different haplotypes in a single inbred consanguineous kindred with congenital hypothyroidism

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A high incidence of severe hypothyroidism due to an iodide organification defect was identified in the youngest generation of five consanguineous nuclear families of a single inbred Arab kindred from northern Israel. The pedigree was consistent with autosomal recessive heredity. Of 18 males and 13 females in the affected generation, 9 males and a single female were affected by the disease. Of 25 family members tested 5 had positive thyroid autoantibodies. Initial studies suggested that association with the TPO gene was unlikely, as 5 of 9 affected siblings were heterozygous at the TPO locus. All affected individuals shared heterozygosity for a single common haplotype in the TPO locus, but had 3 different variations for the other allele. Nevertheless, all affected individuals and none of the non-affected were homozygous for a single mutation, C to T transition of nucleotide 1708 in exon 10 of the TPO gene. The mutation replaces arginine at position 540 with a premature termination signal, resulting in a 539 amino acid protein in place of the original 933 aa TPO protein, abrogating the peroxidase enzymatic domain of TPO. These findings suggest either that the mutation has occurred some generations ago, or that the TPO locus is a recombination "hotspot". The pitfalls in homozygosity mapping are highlighted, and the male predisposition and high prevalence of thyroid autoimmunity to the disease is discussed.

PO3-356 Thyroid III

Hashimoto encephalopathy – diagnostic and therapeutic challenge

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Hashimoto encephalopathy (HE), neurologic disease associated with Hashimoto thyroiditis, has been recognized in pediatric patients in recent years. We report three girls that were treated and followed up in our institution.
Patient 1 A 11-year-old girl was diagnosed with hypothyreosis due to Hashimoto thyroiditis and started on L thyroxine. A month later, she was admitted because of seizure and coma, without signs of infection. After the introduction of steroid treatment her status improved and she was discharged and remained well in 3 year follow up.
Patient 2 A 13-year-old girl was admitted because of seizures and deterioration of consciousness. Laboratory evaluation revealed Hashimoto thyroiditis and she was started on L-thyroxine. Clinical improvement occurred after the introduction of IV methylprednisolone, followed with prednisolone in next 6 months. She remains without neurologic symptoms in 2 year follow up.
Patient 3 A 15-year-old girl was transferred from psychiatry ward because of anemia. Diagnostic work up revealed hypothyroidism and she was started on L-thyroxine. Her confusion worsened, she developed dysartry and stupor. After the introduction of steroid treatment her neurologic status improved, but her hyperactivity, agressive behaviour and fears remained unchanged.
HE should be considered in children with seizures, coma and behavioural changes that are otherwise unexplained. If antithyroperoxidase antibodies are positive, steroid treatment might improve the outcome.

PO3-357 Turner, Noonan II

Peculiarities of puberty formation in adolescents living in the ecologically adverse Aral Sea area

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Drying of the Aral Sea resulted in pollution of air, water (mineralization of clear water) and soil which in its turn caused deterioration of economic and social conditions of the region.
The ecological factors affect the health of children and adolescents of the Aral Sea area as these adverse factors undoubtedly produce their effects on physical and sexual development of adolescents.
The goal was to study peculiarities of puberty formation in adolescents residing in the ecologically adverse Aral Sea area.
Materials and methods: Using a method of random sampling an epidemiological survey with regards to age and gender was undertaken to evaluate a physical and sexual development of adolescents of the Aral Sea area. We examined 1120 adolescents (600 girls and 520 boys) aged 12 to 17. Anthropometry (SDS), sexual development evaluation according to Tanner, clinical and hormonal studies (FSH, LH, GH, testosterone, estradiol) and definition of bone age (BA) were performed.
Results: the epidemiological researches demonstrated that weight deficit was present in 67% of adolescents and 85% of the examined had anemia. Stunting (-3SDS) prevailed more in boys vs. girls. The difference between BA and a chronological age (CA) made 2.6+0.4 years. Sexual development retardation was revealed in 23.6% of boys, late puberty in 8.6% of boys (aged 15 to16), hypogonadism in 0.42% of adolescents while the onset of puberty in other adolescents was normal.
Girls had stunting (44%), irregular puberty (4.5%), sexual development retardation (14.8%), later menarche (20.5%) and primary amenorrhea (9.6%).
Thus, the results obtained suggest that adolescents of the Aral Sea areas lag behind considerably in their physical and sexual development under the influence of various adverse factors.

PO3-358 Turner, Noonan II

MR-aortic compliance and 3D-MR-angiography in children and adolescents with Ullrich-Turner syndrome (UTS)

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Objective: Increased risks of congenital heart defects, progressive aortic root dilatation and aortic dissection exist in UTS with a prevalence of 40%. Abnormal biophysical wall properties may be responsible for these findings. To avoid major cardiovascular events it is important to recognize the high-risk patients early. There is no clear guideline how to monitor the cardiac disease progression in children and adolescents and which diagnostic item is best to be used. So far there are no data at which age the risk for cardiac disease progression increases.
Methods: In a prospective, cross-sectional study 37 conscious children and adolescents with UTS without a history of cardiac malformations (median age: 13.5 [6.7-20.5] years) and 12 healthy controls (median age: 14 [9-18] years) were examined in a 1.5 Tesla whole-body MR scanner. 2D-CINE MRI of the aortic distensibility was performed. All measurements were positioned perpendicular to the descending aorta at the level of the diaphragm. Aortic compliance (C) was calculated in relation to body surface area and considering blood pressure. To assess aortic morphologic changes and to prove normal-sized aorta, contrast-free 3D-MR angiographies were performed in all patients. Echocardiographies and Electrocardiograms were also performed. Patients were treated with GH for median 2.94 [0.0 to 8.86] years and their median height SDS was -2.13 [-3.44 to -0.39].
Results: Patients with UTS showed similar compliances of the aorta as the healthy controls: C= 7.02 ± 2.82 vs. 5.54 ± 2.41 [10^{-5}*Pa^{-1}*m^{-2}]; *t*-test: p=0.13.

Contrast-free angiography revealed two girls with a mild dilatation of the aorta and one patient with a mild elongation of the aorta. Echocardiographies revealed normal left ventricular functions. In three patients QT intervals were prolonged.
Conclusion: Aortic morphology and distensibility can be assessed by MRI without using contrast agents and without sedation in children and adolescents with UTS. Reduced aortic compliance might be a predictor for hypertension or aortic dissection. The results indicate that alterations of the aortic wall may first occur beyond adolescence and therefore regular examinations might not be necessary in younger children. Further studies are warranted in order to investigate the significance of MRI measurements in aortic root dilatation and aortic dissection of UTS patients.

PO3-359 Turner, Noonan II

Uterine volume in young adult women with Turner syndrome

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Turner Syndrome (TS) is characterized by ovarian digenesis. Hormone replacement therapy (HRT) with estrogen is usually necessary to induce and/or maintain secondary sexual and uterine development.
Objective: To determine the uterine volume in young adult women with TS and to evaluate oral estrogen therapy and other factors which could influence the uterine size.
Methods: Thirty-nine women with TS, aged 16.3 to 29.6 years (mean age 22.8) followed up in Pediatric Endocrinology Unit at Unicamp were selected. Criteria of inclusion were: 1- patients with spontaneous puberty who had undergone pelvic ultrasound at least 1 year after regular menstrual cycles; 2- patients with induced puberty who had US one year after menarche or who received HRT for at least 4 years. Retrospective data were collected for clinical parameters of age, karyotype, pubertal development, menarche, duration of HRT and uterine volume. The uterine volume was evaluated by pelvic transabdominal ultrasonography (US). Subjects were divided into 3 groups: complete spontaneous puberty, incomplete spontaneous puberty and without spontaneous puberty. This study was approved by the local ethics committe.
Results: The mean uterine volume (UV) was 47.1cm^3 (14.1 to 109.8). Analyzing the karyotype, there was no significant difference (p=0,372) in the UV between the groups 45,X (n=16; UV=42.9±25.3cm^3) and others (n=23; UV=50.1±27.1cm^3).
From 39 women, 5 had spontaneous puberty (UV=43.5±12.0cm^3), 4 had incomplete spontaneous puberty (UV=54.3±42.2cm^3) and 30 without spontaneous puberty (UV=46.6±26.2cm^3) and no significant difference was found in the UV between the groups (p=0.978). All the patients with spontaneous puberty, 50% of those with incomplete puberty and 80% of those without spontaneous puberty had normal uterine volume. The mean age of initiating HRT was 15.2 years for the incomplete puberty group and 12.6 years for those without spontaneous puberty. The mean duration of TRH was 9.8 years and the women were divided in quartiles in order to analyze the influence of therapy length in the UV. There was a significant difference (p=0.029) between the group that received HRT for more than 7.1 years (n=9; UV=66±31.9cm^3) and the group that received less than 3.25 years (n=8; UV=28.5±11.7cm^3).
Conclusions: Most of studied group of TS women had a uterine volume similar to that found in the normal population and a small uterine size may be related to insufficient length of hormonal replacement therapy.

PO3-360 Turner, Noonan II

The G allele at *KRAS* IVS4 +92 is associated with higher frequency of congenital heart disease and severity of short stature in Noonan syndrome patients with *PTPN11* mutations

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Introduction: Noonan syndrome (NS) is mainly characterized by dysmorphic facial features, congenital heart disease and postnatal short stature. Heterozygous mutations in *PTPN11* are the most frequent gene alteration observed in NS patients. However, there were no clear genotype-phenotype correlations in NS patients with *PTPN11* mutations. Mutations in *KRAS* gene were also observed in NS patients. **Objectives:** To evaluate if alterations in *KRAS* can modify the phenotype of NS patients with *PTPN11* gene mutations. **Patients and Methods:** 58 patients with NS with heterozygous mutations in *PTPN11* were selected. Exon and intron boundaries of *KRAS* were directly sequenced. Association between three polymorphisms and NS phenotype was investigated. The variables analyzed were congenital heart defect (type and frequency) and height SDS. Differences between genotypes were analyzed by Student t-test and Fisher's exact test. **Results:** Among patients carrying one of the two most frequent *PTPN11* mutations in our cohort (p.N308D – 15 patients and p.Y63C – 5 patients) a wide phenotype variation was observed. No mutations in *KRAS* were found but three polymorphisms were identified: IVS5 -9 C>T (rs 12313763), c.704 T>C (rs 17473423) and IVS4 +92 A>G (described for the first time). All 3 *KRAS* polymorphisms were in Hardy-Weinberg equilibrium with minimum allele frequencies >10%. The IVS4 +92A>G polymorphism was associated with higher frequency of heart disease (52% in A/A patients vs. 100% in patients G/G or G/A, p = 0.004) and the severity of short stature (height SDS = -2.1 ± 0.9 for genotype A/A vs. -2.9 ± 1.3 for the genotype G/G or G/A, p = 0.048). Multiple regression showed that age (p = 0.006), IVS4 +92 A>G polymorphism (p = 0.005) and IVS5 -9 C>T polymorphism (p = 0.005) independently influenced height SDS and together explain 30% of the variability of stature observed in NS patients with *PTPN11* mutations. **Conclusions:** No mutation in *KRAS* was observed in NS patients harboring mutations in *PTPN11*. Severity of NS phenotype is not associated with *PTPN11* mutation. However, our results suggest that a common variant in *KRAS* may modulate the phenotype of NS patients with mutations in *PTPN11*: the G allele at *KRAS* IVS4 +92 is associated with higher frequency of congenital heart disease and severity of short stature in Noonan syndrome patients with *PTPN11* mutations.
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PO3-361 Turner, Noonan II

Morphologic remodelling and dilatation of the aortic root in patients with Turner's syndrome without congenital heart disease (CHD): comparison of echocardiography and MRI

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Turner syndrome (TS) is at risk of aortic dilation (AoDil) and dissection. The prevalence of AoDil in TS with no risk factors and the complex rearrangement of the Ao root associated with AoDil are unknown. Our aim was to evaluate the presence of a morphometric remodeling of the Ao root to see if it contributes to AoDil and to compare the accuracy of echo and MRI to the image of the Ao.
Methods - 71 TS patients without CHD (15-35 yrs) were studied with echo and MRI: 56 were or had received GH and 60 EE-P therapy. 71 age-matched

female controls were evaluated with echo. Ao diameters were measured at annulus, sinuses of Valsalva, sino-tubular junction (STJ) (transition between sinuses of Valsalva and tubular portion of ascending Ao), and ascending Ao. Karyotype distribution was: 45,X - 35%, X-mosaicism - 18%, X-structural abnormalities -40% and Y-mosaicism - 7%. Plotting TS subjects on prediction bands of controls, the values of Ao diameters above 99th percentile for controls were considered as abnormal.

Results - TS had larger diameters at the sinuses of Valsalva, STJ and ascending Ao than controls of equivalent CA and BSA, but taller. AoDil was found at the sinuses of Valsalva (echo – 15.5%; MRI – 12%), ascending Ao (echo – 10%; MRI – 7%), but especially at STJ (echo – 28%; MRI – 14%). TS had an abnormal architecture of the Ao root, affecting the typical shape: relative dilation of STJ versus controls ($p<0.0001$), with slight increase in ascending Ao/Valsalva sinuses ratio ($p=0.377$). 45,X subjects had larger STJ and ascending Ao than other karyotypes ($p=0.047$ and $p=0.012$). At multiple linear regression the karyotype was the strongest predictor of STJ ($p=0.0138$) and ascending Ao ($p=0.0085$) and not GH or EE-P therapy.

Conclusions - Dilatation of the STJ is frequent in TS subjects and is more common than dilation of the ascending Ao, even without Ao defects. STJ is a critical area with important structural and mechanical functions. Remodelling of the Ao root, leading to the loss of the normal shape, seems a characteristic of TS and may be the result of an intrinsic Ao wall anomaly. Reshaping of the Ao root, related to an abnormal Ao wall, may be important in triggering Ao dissection even without other risk factors. Echo and MRI gave similar results, but echo had a slightly higher prevalence of AoDil caused by some distortion, in particular in the individuals with a bad thoracic window, emphasizing the role of MRI in TS .

PO3-362 Turner, Noonan II

Juvenile rheumatoid arthritis (JRA) and inflammatory bowel diseases (IBD) in patients with Turner syndrome (TS): data from the Genentech national cooperative growth study (NCGS) of recombinant human growth hormone (rhGH)

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Background: The rate of autoimmune (AI) thyroid disease is increased in TS with an estimated risk as high as 50% over a lifetime. However, reports of other AI diseases such as JRA and IBD in TS are scant. One study reported the frequency of TS among JRA patients to be 0.12% (Zulian 2000) which corresponds to a 3-6 fold increase in what would be expected compared to the number of TS girls in the general population. TS with IBD has been reported but no large series can provide actual risk data.

Hypothesis: That the presence of JRA and IBD among the 5477 children/adolescents with TS in the NCGS can provide insight into the risk of AI disorders as well as provide data for the growth response to rhGH treatment (Rx) in affected TS patients.

Methods: TS patient records in NCGS were examined for mention of either JRA or IBD. First yr growth rates for TS girls with/without JRA or IBD were compared.

Results: The prevalence of JRA among 5477 TS patients was 0.26% (n=14), greater than prevalence estimates of JRA in the general population (0.03-0.11% Anderson Gare 1999). The prevalence of IBD among the TS cohort was 0.13% (n=7). Published prevalence estimates for one IBD, Crohn's disease, in the pediatric population ranged between 0.01-0.02%. The mean 1st year growth response to rhGH of the TS+JRA with at least one yr of data was a Ht increase of 0.5 SD (n=9) and TS+IBD of 0.4 SD (n=4) similar to the general NCGS TS population, 0.5 SD (n=3860). There were no new rhGH associated safety signals in the small JRA or IBD cohorts.

Discussion and Conclusions: These NCGS data are consistent with literature case reports suggesting a higher risk of JRA and IBD in TS. Although those who were Rx with rhGH for ≥ 1 yr had growth comparable to unaffected TS girls, several in both groups did not complete 1 yr. Given the known effect on growth of the concomitant medications used for Rx (primarily corticosteroids) earlier institution of rhGH in all girls with TS could allow a longer Rx window that might precede the onset of AI disorders requiring corticosteroids, and therefore maximize the window where growth promotion Rx can be of benefit.

References:

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PO3-363 Turner, Noonan II

A questionnaire summary of the follow-up status in adult Turner women

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To investigate follow-up status of medical conditions in adult Turner women, anonymous questionnaires were sent by mail to the members of Turner Societies in Tokyo and Osaka older than 16 years old. Answers were obtained from 83 women (52%) of 152 persons.

Heights of girls with TS was 147.2 ± 4.9 cm (148.4±4.2 cm with GH therapy and estrogen therapy, 143.3±4.9 cm without GH therapy, body weight 47 kg(range 30-64kg), BMI 21.9(range 15.2-30.6), abdominal circumference 68.8±8.7 cm.

Attending physicians for their follow-up were pediatricians in 34 persons, obstetricians in 25 and internists in 9. Comparing to the recommended interval of the examination suitable for each possible complication, the achievement quotient rate was 61% for bone mineral measurement, 41% for cardiac evaluation and 41% for audiological examination. Their menstrual cycle was normal cycle 83%, abnormal menstrual cycle 6% and absence of menstruation 10%. These unsatisfactory results suggest that not only patients but also physicians needed detailed reporting and shared medical knowledge for favorable continuous medical care.

Comparing to the recommended interval of the examination suitable for each complication

Examination	Achievement
Bone mineral measurement	61%
Cardiac examination	48%
Audio logical examination	41%
Blood examination	88%
Urinalysis	78%

PO3-364 Turner, Noonan II

Aortic dimensions in girls and young women with Turner syndrome – a magnetic resonance imaging study

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Objective: An aortopathy is evident in adolescent and early adult Turner syndrome (TS) where a grossly increased risk of aortic dissection is encountered. The extent aortic dilation and aneurism as well as the predictors of aortic dimensions have, however, only been sparingly investigated in the young.

Aim: We set out to measure aortic dimensions and to elucidate the predictors hereof in girls, adolescents and young adults with TS.

Design: Aortic dimensions were determined in a cross-sectional study by magnetic resonance imaging scans conducted on TS ($N = 41$) (age: 17 ± 3 years, range: 11 to 24 years) and age-matched healthy female controls ($N = 50$).

Setting and analysis: Thoracic aortic diameters were measured at 9 positions and adjusted for body surface area (BSA). Student´s t-test, Pearson´s coefficient of correlation, and multivariate regressions analysis were used.

Results: Absolute and BSA-adjusted aortic diameters in TS were smaller

(three positions in the aortic arch, all $P < 0.005$) or comparable (descending and ascending aorta) to healthy contemporaries. Aortic dilation (at one to four positions) presented in four TS patients in the uncorrected diameters and in five patients after BSA-adjustment. Dilation never affected the aortic arch but was found in both the ascending and descending aorta. Aortic diameters correlated to height, weight, body mass index, and BSA at all positions ($R = 0.34\text{-}0.60$, *all* $P < 0.04$). Aortic arch and descending aortic diameters were predicted by the presence of aortic coarctation ($R = 0.35\text{-}0.52$, $P < 0.03$). In TS patients with a bicuspid aortic valve, descending aortic diameters were enlarged ($R = 0.38$, $P < 0.03$).

Conclusion: Mean ascending and descending aortic diameters in girls and young women with TS were comparable to those of age-matched healthy contemporaries, while aortic arch diameters were smaller. The prevalence of aortic dilation and aneurism was lower than previoulsy observed in adult TS. BSA predicted aortic size at all positions and thus holds the promise of an adequate identification of pediatric TS patients at increased risk of dissection in childhood and adulthood. It is pivotal to conduct longitudinal studies of aortic dimensions in TS to understand how and when the dimensions increase and the association to BSA is lost, as encountered in MRI studies of adult TS.

PO3-365 Turner, Noonan II

Congenital anomalies of the major intrathoracic arteries in Turner syndrome: a magnetic resonance imaging study

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Objective: Ectatic aortopathy and congenital arterial anomalies cause excess morbidity and mortality in Turner syndrome (TS), and this vasculopathy seemingly extends into the conduit arteries.

Aim: We investigated the prevalence of congenital arterial anomalies of the major intrathoracic arteries, their interaction with arterial dimensions, and association with karyotype.

Design: A cross-sectional magnetic resonance imaging (MRI) and echocardiographic study of adult TS ($N = 99$) compared with female controls ($N = 33$).

Setting and analysis: The MRIs were evaluated for congenital arterial anomalies as well as for conduit artery and aortic dimensions. Aortic valve morphology was determined by echocardiography. The results were analysed with Students t-test, chi-square test, Fisher´s exact test and Pearson´s coefficient of correlation.

Results: In TS, the relative risk of any congenital anomaly was 7.7 ($P = 0.003$) and 6.7 of ascending aortic dilation ($P = 0.02$). A bovine aortic arch was seen in both TS (27%) and controls (9%). Other anomalies were only encountered in TS; elongated transverse aortic arch (ETA) (47%); bicuspid aortic valve (BAV) (27%); aortic coarctation (CoA) (13%); aberrant right subclavian artery (8%) and aortic arch hypoplasia (2%). The innominat and left common carotid arteries were enlarged in TS ($P < 0.001$). Significant associa tions in TS were as follows: BAV with CoA, ETA, and ascending aortic dilation; CoA with ETA and descending aortic dilation; and 45,X with CoA, ETA, and ascending aortic dilation. In TS, aortic and conduit artery dimensions also correlated. Dilation of the conduit arteries was related to BAV, CoA, ETA, and 45,X.

Conclusion: An increased risk of congenital arterial abnormalities, aortic dilation and enlargement of the conduit arteries was found in TS without distinct patterns of co-segregation. Consequently, cardiovascular follow-up should be offered to all TS patients irrespective of karyotype.

PO3-366 Turner, Noonan II

Thirteen cases of deletion Xp21.2 in the same presumed constitutional short stature family

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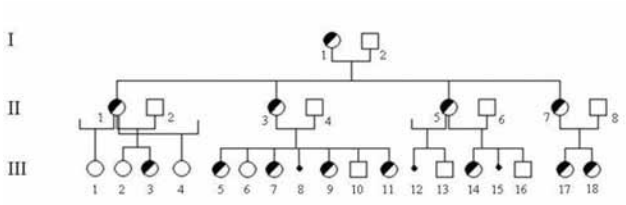
The clinical symptoms of the girls suffering from Turner's syndrome are rarely described the way Mr Turner initially did in 1938. The phenotype is sometimes very moderately evocative but the small size is a constant sign. Moreover, the traditional concept of infertility is not constant. We report the case of a family transmission of distal extremity deletion of the short arm of one of the X chromosome observed on three generations of women, concerning 13 subjects. To our knowledge, it is the biggest family presenting Xp21.2. deletion.



Diagnosis of the girls Turner is not always easy because of frequent pairing of small size women with a small spouse. The diagnosis of constitutional small size is sometimes quickly done without biological exploration. Systematic blood chromosomic study of the girls having a statural defect, makes the diagnosis of this type of patients possible.

Then, they can benefit from a recombining growth hormone treatment but also explorations for the search of particular cardiac and renal complications.

In order to support feminisation and help for procreation an estrogen-progestagen replacement treatment is to be considered on a case-by-case basis depending on the aspect of the ovaries and the hormonal tests results.

PO3-367 Turner, Noonan II

The impact of oestrogen and oxandrolone on pubertal progression and height velocity in Turner syndrome

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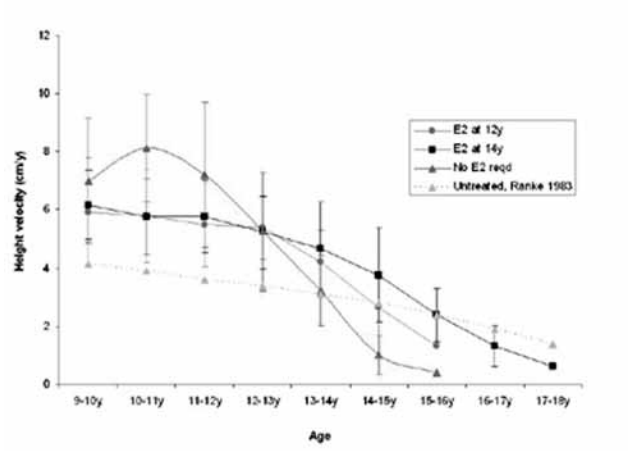
Short stature is almost universal in Turner syndrome (TS). Although not classically GH deficient, supra-physiological doses augment final height (FH). The UK Turner study has examined the influence of Ox/placebo and E2 at 12 or 14y on FH in girls receiving a standard dose of GH. Here we describe their impact on pubertal progression and height velocity (HV). **Subjects:** Recruits were randomised to a) Ox (0.05mg/kg/d max 2.5 mg) or placebo from 9y and b) to oral Ethinyloestradiol (y1: 2µg/d; y2: 4µg/d; y3: 4-mo each of 6/8/10µg/d) or placebo at 12y with E2 at 14y. Girls enrolled >12y were automatically assigned to E2 at 14y ("late group"); girls with spontaneous puberty (SP) did not receive E2. **Results:** 91 girls were analysed: 55 randomised to E2; n = 28 at 12y (11 Ox); n = 27 at 14y (12 Ox); late group n = 19 (9 Ox) and SP n = 17 with (10 Ox).

Baseline characteristics and pubertal progression: mean (SD)				
	E2 at 12y (n=28)	E2 at 14y (n=27)	Late group: E2 at 14y (n=19)	SP: No E2 reqd (n=17)
Age (y)	9.6 (1)	9.6 (1.2)	12.7 (0.4)	9.7 (1.2)
Bone age (y)	9.4 (1.4)	9.3 (1.7)	11.7 (1.3)	10.2 (1.9)
Height (cm)	122.4 (6.7)	123.8 (6.9)	135.9 (5.8)	124.6 (8.1)
Age at Tanner B2	12.9 (1.2)	14 (1.4)	14.5 (1.1)	11.4 (1.2)
Age at Tanner B3	13.8 (1.1)	14.5 (1.3)	15.4 (1.5)	12 (1.5)
Age at Tanner B4	14.7 (1)	15.4 (1.6)	16.5 (1.3)	12.7 (1.1)
Age at Tanner B5	14.8 (0.5)	17.2 (0.8)	16.7 (1.9)	13.4 (0.5)

Ox did not alter age at any pubertal stage achieved (unpaired t-tests p>0.05). Girls with SP had normal timing of puberty reaching menarche at mean age 12.7y and had a pubertal growth spurt. By contrast, girls receiving E2 completed puberty 2 or 4y later than average without growth acceleration. HVs differed in those with SP and E2 therapy at 12 or 14y (GLM repeated measures p<0.05). **Conclusions:** Possible explanations include 1) Girls with SP have mosaic karyotypes and may have less severe skeletal dysplasia 2) SP is more growth-enhancing than artificial induction with oral E2. As oral E2 may not be the optimum mode of pubertal induction a more physiological induction with transdermal natural E2 may improve growth during puberty in girls with TS.



PO3-368 Turner, Noonan II

Growth hormone in Turners syndrome: a comparison of two decades of treatment

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Recombinant growth hormone (GH) has been licensed for use in Turner syndrome since 1988 to promote an increase in height. In 1999 the national KIGS board published an audit of ten years data on GH treatment in 485 girls with Turner syndrome (TS). To assess whether practice and outcomes have changed in the ten years since the original audit, the board has compared data on 333 children treated between 1997 and 2007. Data were obtained from the UK KIGS database.

Standard deviation scores are standardised for growth in untreated girls with TS.

In the current audit 1 year of GH therapy led to an increase in mean height SDS from 0.44(0.89) to 1.00(0.94) and in mean height velocity SDS from -0.75(1.84) to 2.67(2.70).

In the previous audit, mean age of treatment had fallen from 10.4 to 8.5 years (p<0.0001). In the last decade, this trend has ceased with treatment start remaining at 8.8 (3.6) years. Similarly, girls moved from 3 to 7 injections per week during the last audit. TS patients have remained on 7 injections per week over the recent decade.

Mean GH dose was 0.04 mg/kg/day in 1997-07 compared to 0.035mg/kg/day in 1986-96. However, this higher dose did not translate into a benefit in overall mean change (Δ) in height SDS over the first year of treatment +0.53 (1997-07) versus +0.61 (1986-96).

To explore whether earlier treatment has affected near adult height in TS we looked at GH treatment in 96 girls. Mean height SDS at the start of treatment was +0.21(0.75) for the 1997-07 cohort compared with -0.20(0.87) for the 1986-96 cohort, possibly indicating a secular trend in growth. The mean increase (Δ) in height SDS at final height was +0.62(0.57) in the earlier cohort compared with +0.68(0.79) in the later cohort. TS patients reaching near adult height commenced GH at 8.0(2.7) years compared with previous decade 10.7(1.6) years. The Turner adjusted height SDS at near adult height was +1.86(1.12) which compared favourably with the adjusted final height SDS of +0.62(0.57) in the earlier cohort.

In summary, earlier treatment with growth hormone in Turner Syndrome has become conventional in the United Kingdom. Earlier and more aggressive treatment with growth hormone therapy confers a benefit in final adult height.

PO3-369 Turner, Noonan II

Effects of low-dose ethinyl estradiol (LDE) on LH/FSH concentrations during childhood in patients with Turner syndrome (TS): results of a double-blind, randomized, placebo-controlled clinical trial

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Objective: To evaluate natural history and effects of LDE on LH/FSH secretion during the prepubertal period in TS.

Methods: 149 patients with TS aged 5-12.5y were randomized to receive LDE or oral placebo (OP) ±GH, during childhood. Mean LDE/OP doses (ng/kg/d) for ages 5-8 and >8-12 were 24 (~0.3-0.5 mcg/d) and 35 (~1.0-1.7 mcg/d) respectively. LH/FSH (measured every 6m) were analyzed by age for % values >15 (LH) and 20 (FSH) IU/L (~2SD above normative means). Pretreatment data, reflecting natural history of LH/FSH in TS, are summarized for 123 patients who were prepubertal at baseline. Postbaseline data, reflecting effect of LDE during childhood, compare LDE-treated vs OP-treated patients.

Results: *Baseline.* Mean (±SD) age was 7.7±2.2y; 80% had 45,X karyotype. Age-related patterns differed for LH vs FSH: pretreatment LH values were elevated in only 1/30 (3%) age 5 & 1/40 (3%) ages 6-9 vs 22/41 (54%) age 10-12. In contrast, FSH values were elevated in 20/29 (69%) age 5 vs 12/40 (30%) age 6-9 vs 34/41 (83%) age 10-12 (statistical significance not tested).

Effect of LDE in childhood (Table). % elevated LH values was similar for LDE & OP groups ages 6-9, but was lower for LDE ages 10-12. For FSH, LDE effect was evident earlier, as % elevated values was lower for LDE than OP from age 6 onward. For both LH and FSH, % elevated values rose progressively from age 9-12 in both treatment groups, but was lower in LDE group at each age (statistical significance not tested). GH treatment had no detectable effect on LH/FSH.

% Elevated LH & FSH Values by Treatment Group and Age

Age (yr)	OP (n=54) %LH	LDE (n=57) %LH	OP (n=53) %FSH	LDE (n=57) %FSH
6	0	0	35	0
7	4	0	19	0
8	0	0	30	4
9	5	4	38	14
10	23	13	66	28
11	66	43	80	63
12	82	66	92	79

%=%values elevated within age/treatment group; OP=oral placebo; LDE=low-dose ethinyl estradiol

Conclusions: Different patterns were observed for LH vs FSH in untreated 5-12 yr-old prepubertal girls with TS. LH was normal until age 9, followed by elevated values ages 10-12; in contrast FSH values showed a triphasic pattern, with elevations in most girls aged 5 and 10-12, but normal values in most girls aged 6-9. Treatment with LDE during childhood at the doses used in this study maintained normal LH & FSH in most girls until the expected age of puberty (>9y), but apparently provided incomplete negative feedback on LH/FSH secretion once the hypothalamic-pituitary-gonadal axis was activated.

PO3-370 Turner, Noonan II

Early administration of growth hormone increases bone density in girls with Turner syndrome

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Introduction: High incidence of fractures has been reported in patients with Turner syndrome (TS). Low bone mineral density has been described as one of the potential causes of increased bone fragility in TS. Studies using volumetric techniques showed, that there is intrinsic, estrogen-independent deficit in cortical bone in TS. However, there are still limited data on the role of age, puberty and growth hormone administration on bone during childhood and adolescence.
Aims: 1. To compare bone density at the radius between prepubertal and pubertal girls with TS and healthy controls using peripheral quantitative computed tomography (pQCT). 2. To determine changes in bone density in relation to growth hormone (GH) administration.
Patients and methods: Bone mineral density was assessed in 67 patients with TS (mean age 13.9±3.3 yrs, range 6.0-18.4 yrs) using pQCT (XCT 2000, Stratec Medizintechnik) at the 4% and 65% sites of the non-dominant radius. At the time of measurement, 46/67 (69%) girls were treated with GH, the remaining 21/67 (31%) were off treatment for 1.5±1.0 years (mean±SD). Z-scores for density and bone mineral content were calculated using published reference data. For statistical evaluation, one-sample t-test was used. Multiple regression analysis was implemented to evaluate the influence of age, body height and age at GH treatment start.
Results: Prepubertal girls with TS had normal bone mineral content (BMC) and trabecular density at the distal radius (mean Z-scores -0.45 and -0.16, respectively, n.s.). At the proximal radius, BMC and cortical density were decreased (mean Z-scores were -1.15 and -2.03, respectively, $p<0.001$ vs. reference for both). In pubertal girls with TS, both BMC and trabecular density at the distal radius as well as BMC and cortical density at the proximal radius were decreased (mean Z-scores were -0.91, -0.98, -1.27 and -1.32 respectively, $p<0.001$ for all parameters). The regression analysis showed negative correlation between age at GH treatment start and cortical bone density after adjustment to age, body height and cortical thickness ($p<0.01$).
Conclusions: Both prepubertal and pubertal girls with TS have low cortical bone density that could be partially corrected by an early start of GH treatment. Trabecular bone density which is normal in prepubertal girls with TS decreases at puberty, probably due to subphysiological or delayed estrogen supplementation. Support: GAUK 47609, VZ MZ 64203, MSMT 21620819.

PO3-371 Turner, Noonan II

Auxological and metabolic effects of short period of growth hormone therapy in Turner's syndrome

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Treatment with growth hormone (GH) is an approved procedure improving final height in Turner's Syndrome (TS). Higher than physiological doses of GH used in therapy of TS may influence metabolism processes and change them.
The aim of the study was to assess the influence of growth hormone therapy on selected auxological and metabolic parameters in patients with TS in the initial stage of the treatment process.
Patients and methods: 19 female patients aged 8.6 ± 3.4 with diagnosed TS were assessed. 12 of them revealed monosomy of chromosome X, the others revealed its structural anomalies (1) or mosaicsm (6).
The girls were examined twice: the first examination was performed before beginning of growth hormone therapy and the second one in the middle of therapy with 50 microg/kg/day (mean 0.9 years). The following aspects were assessed: growth and body mass, BMI and BMI SD, lipids, glucose level, concentrations of HbA1c, insulin, leptin, adiponectin, interleukin 6 (IL-6), TNFalfa, IGF-1, IGFBP3. The control group comprised 12 girls chosen with regards to age and BMI.
Results: No significant statistical differences regarding the stated blood parameters between the group of female patients with TS before the start of the therapy and the control group of patients were found.
The growth rate of the patients with TS during the period of observation was 9.2 cm per year, which caused the change of SD in terms of height from 0.38 to 1.03. Despite the growing body mass, BMI SD did not change.
GH treatment, which lasted almost one year, was connected with significant growth of IGF-1 (from 110.3 ± 41.6 to 280.6 ± 182.8 ng/ml, $p = 0.008$). Other stated parameters did not change in a statistically significant way throughout the treatment. However, a decrease with regards to IL-6 (from 8.1 ± 8.3 to 6.4 ± 4.6 pg/ml) and an increase with regards to insulin (from 3.9 ± 2.0 to 6.9 ± 4.1 μIU/ml) were observed.
Conclusions: No significant metabolic differences between TS and the same age girls exist. IGF-1 is the growth response marker throughout GH therapy in TS. In the initial stage of administration GH has a positive influence on auxological parameters and does not change metabolic parameters significantly.

PO3-372 Turner, Noonan II

The association of karyotype and parental origin of the intact X chromosome with clinical phenotypes in girls with Turner syndrome

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Purpose : The various phenotype in Turner syndrome (TS) is caused by several factors such as karyotype and parental origin of the intact X chromosome. In TS patients with 45,X or X-isochromosome, we investigated the role of karyotype and parental origin of intact X chromosome on various phenotype in girls with Turner syndrome.
Methods : Sixty two patients with 45,X or X-isochromosome were eligible and 40 of them and their parents were enrolled in the genetic analysis using 9 highly polymorphic X microsatellite markers to reveal the parental origin of intact X chromosome. We evaluated phenotypic traits including baseline height and body mass index, the effect of growth hormone and existence of spontaneous puberty, autoimmune thyroid disease and/or hypothyroidism, renal anomaly, cardiovascular anomaly, glucose intolerance or sensorineural hearing loss (SNHL).
Results : No age differences was observed between patients with 45,X and X-isochromosme group. The 45,X group (n=35) had a higher frequency of renal anomaly (51.5% vs 20%, P=0.014) and cardiovascular anomaly (34.3% vs 11.5%, P=0.041) than X-isochromosome group (n=27). The frequency of thyroid autoimmunity was not different between two groups.
Twenty seven (67.5%) of 40 patients had an intact X chromosome with maternal origin (Xmat). There was no difference in the distribution of the Xmat or paternal origin (Xpat) between 45,X, 46,X,i(X) and 45,X/46,X,i(X) group. No unexpected hidden X mosaicism was found in any patients with 45,X. No significant differences in various phenotypic traits were observed between Xmat and Xpat group. Baseline height standard deviation score (HTSDS) of patients in both Xmat and Xpat group was not correlated with maternal and paternal HTSDS, respectively.
There was no significant effect of age, karyotype, and parental origin on various phenotypes except SNHL as a result of binary logistic regression analysis. As patients was older, subjects were likely to have SNHL (P=0.039).
Conclusion : Patients with 45,X seems to have a higher frequency of renal and cardiovascular anomaly than those with X-isochromosome. We found no evidence for X-linked genomic imprinting roles on effect of growth hormone and existence of spontaneous puberty, autoimmune thyroid disease, renal anomaly, cardiovascular anomaly, glucose intolerance or SNHL.

PO3-373 Turner, Noonan II

The influence of parental origin of X-chromosome on the physical phenotypes and growth hormone responsiveness of patients with Turner syndrome

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Aims: The effects of parental origin of X-chromosome on the certain phenotypic and cognitive profiles were suggested in Turner syndrome (TS) by previous studies. We investigated the possible parent-of-origin effects on the physical phenotypes and responsiveness to growth hormone (GH) in Korean patients with TS.
Methods: Twenty-nine TS patients with non-mosaic karyotype and their parents were studied. The parental origin of the normal X-chromosome was determined by comparing parental DNA polymorphisms using highly polymorphic microsatellite markers on the X-chromosome. For evaluating the parent-of-origin effects, we comparatively analyzed the typical phenotypic traits including congenital malformations, auxological and endocrinological profiles.
Results: In 62.1% patients, the retained X-chromosome was maternal (Xm) in origin and 37.9% was paternal (Xp). Cubitus valgus deformity was more frequently detected in the Xm group (P=0.048). However, significant parent-of-origin effects on stature, BMI, cardiac, renal, lymphatic, hearing or ocular systems were not observed. Height gain after GH treatment showed no difference. Patients' height was positively correlated with maternal heights in the Xm group (r=*0.48,* P=0.050) and paternal heights in the Xp group (r=*0.50,* P=0.140), respectively.
Conclusions: The parental origin of the X-chromosome seems to have some impact on several phenotypic traits of TS, and the importance of normal X-chromosome origin on patient's stature was suggested. Further study including a larger number of TS patients is needed to define a potential imprinting effect of an undetermined gene on X-chromosome.

PO3-374 Type 1 Diabetes III

Incidence of type 1 diabetes mellitus in the north-eastern Thailand

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Type 1 diabetes mellitus (T1DM) is the disease in children. The incidence is very low compare with T2DM in adults. We determined the incidence of T1DM in children under 15 years in 19 provinces in northeast Thailand. Data of new cases during 10 years from 1996 to 2005 were collected retrospectively by a mail survey from 275 hospitals in northeast Thailand. The total of 340 cases, 134 (39.4%) boys and 206 (60.6%) girls were identified. The incidence rate of T1DM was 0.64/100,000/year (95% confidence interval 0.57;0.71), 2 folds increased from the previous study during 1991 to 1995. More than half of the cases were diagnosed between the ages of 10 and 14 and the incidence rate in girls was 1.5 fold that of boys. Though the increased in incidence rate, the study indicated that the incidence of T1DM in northeast Thailand is still one of the very low incidence rates in the world.

PO3-375 Type 1 Diabetes III

Transitioning infants with monogenic diabetes to oral sulfonylurea therapy

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Background: Neonatal diabetes presents within the first year of life, and occurs at a frequency of 1 in 400,000 births. Dominant gain of function mutations in *KCNJ11*, the gene encoding the Kir 6.2 subunit of the ATP-sensitive potassium channel, and autosomal recessive and dominant mutations in *ABCC8* gene encoding the SUR1 subunit can cause both transient and permanent forms of neonatal diabetes. These ATP-sensitive potassium channel mutations lead to a persistently activated channel that often remains responsive to sulfonylureas and therefore can be treated with oral sulfonylurea therapy.
Objective: We present three cases of neonatal diabetes (NDM) that were initially treated with insulin and successfully transitioned to oral sulfonylurea therapy.
Design: Case reports of three patients with NDM, confirmed by mutational analysis performed by direct sequencing of the *KCNJ11 and ABCC8* genes.
Results: All three patients were diagnosed with diabetes prior to 1 year of age. All three presented with diabetic ketoacidosis. Diabetes autoimmune panel were all found to be negative, prompting mutational analysis to be sent. Case 1 and 2 were found to have mutations in *KCNJ11* (c.157G>A [p.Gly53Ser] and c.698T>A [p.Leu233His]) and case 3 was found to have mutations in the *ABCC8* gene (c.886 G>A [p.Gly296Arg] and c.89 C>T [p.Ala30Val]). Past medical history and developmental history were otherwise unremarkable. All three patients were initially managed with insulin and were admitted for inpatient observation during their transition; they were placed on an insulin drip to allow for insulin titration and to maintain adequate blood glucose control. Glyburide was initiated at a dose of 0.2 mg/kg/day and advanced by 0.2 mg/kg/day daily to a maximum dose of 1 mg/kg/day or until the patient no longer required insulin. C-peptide levels were drawn prior to beginning glyburide and periodically during transition to assess endogenous insulin secretion. Insulin was weaned off over 3-7 days. Initially C-peptide levels were undetectable in all cases, and rose to1.4 to 3.91 ng/mL by time of discharge. Average HgA1c was 8.8 % prior to transition, 6.4% after transition.
Conclusion: These cases illustrate the importance of genetic testing in children with new onset diabetes diagnosed at less than one year of age. Patients with *KCNJ11 or ABCC8* mutation can respond to oral sulfonylurea therapy and can often maintain improved glycemic control as well as improved quality of life on sulfonylureas.

PO3-376 Type 1 Diabetes III

Interstitial measurements of glucose, glycerol and lactate in adolescents with decompensated type 1 diabetes

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Objectives: The study was undertaken to assess the interstitial, adipose tissue concentrations of glucose, lactate and glycerol in teenagers with diabetes type 1 who suffered from the disease for a minimum of 5 years, in whom it was impossible to reach a satisfactory level of metabolic control of the disease.
Study design: Using microdialysis technique interstitial concentrations of glucose lactate and glycerol was measured in adipose tissue during 24-48 hours. Nineteen teenagers with poorly controlled type 1 diabetes (HbA1c 8.9 ± 2.85%) were compared with six adolescent control subjects.
Results: A statistically significant differences in concentration values of interstitial glucose between the investigated and control groups were found (10.4 vs. 4.26 mmol/l p=0.001174). The values of interstitial concentrations of lactates did not significantly differ in the two groups (2.96 vs. 2.54 mmol/l NS). The average daily glycerol concentrations in the investigated group were statistically significantly lower than those in the control group (258.26 vs. 397.88 µmol/l, p=0.018882). No such difference was detected in average night concentrations of glycerol (157.78 vs. 361.4 µmol/l, NS).
Conclusions: Authors conclude that microdialysis is the only one minimal invasive method for investigating adipose tissue metabolism in-vivo and provides a novel opportunity for glucose and lipids metabolism monitoring in adolescents with diabetes type 1.
In our observations interstitial glycerol concentrations, measured in abdominal subcutaneous adipose tissue as an index of lipolysis, were not significantly influenced by hyperglycemia in diabetic adolescents.

PO3-377 Type 1 Diabetes III

Dyslipemia and microalbuminuria in type 1 diabetes mellitus in children

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The high-density lipoprotein cholesterol (HDL Cholesterol), is a lipoprotein for the transportation of the cholesterol and triglycerides. Different researches have point out the relationship between lipid disorders with glycated haemoglobin and microproteinuria in type 1 diabetes mellitus. Others investigations have found the importance of decreased HDL Cholesterol levels and the presence of microproteinuria in these patients. We made a study with 52 male and female type 1 diabetes mellitus patients, aged 9 to 15 years, with normal weight and two years or more of duration of the disease. Dyslipidemia was observed in 30 patients (57%); the decrease of HDL Cholesterol was present in all the dyslipidemic patients. The increase of the LDL-.Cholesterol level, were observed in 27 (51, 9%), the total Cholesterol were increased in 12 (23%) and only in 4 patients (7, 6%) hypertriglyceridemia. We observed that microalbuminuria were present in 22 (42%) of the patients. It is important to point out, that 21 (70%) of the 30 dyslipidemic children had microalbuminuria. The dyslipemic group had a greater percent of microalbuminuria but there was not significative, The decrease of HDL Cholesterol were present in all dyslipemic patients. We also observed that the dyslipidemia had a positive correlation with the duration of the diabetes but there were no differences with the sex. Taking account the results of our laboratory evidence, we can conclude, that in the type 1 diabetes mellitus, the total cholesterol and triglycerides give no enough information, and is necessary to know about the level of HDL Cholesterol in order to made a prognosis of future renal injury. Finally, we consider that not only the glycaemic control but also the lipidic control are necessary to prevent, delay or ameliorate the diabetic renal injury.

PO3-378 Type 1 Diabetes III

The impact of baseline hemoglobin A1c levels prior to pump initiation on long term metabolic control

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Objective: To identify factors influencing long-term metabolic control patients with type 1 diabetes mellitus (T1DM) treated with pump.
Research design and methods: Data were obtained from retrospective chart review of 113 patients (52 males) with T1DM treated with insulin pump for up to 7 years. Their mean±SD age at diagnosis of T1DM was 9.7±5.1 years and at pump initiation 13.8±6.1 years. Linear trends and changes in HbA1c levels following pump insertion were performed by metabolic control prior to pump initiation, gender, age at pump initiation, duration between diagnosis of diabetes until pump initiation and duration on pump treatment.
Results: Mean HbA1c levels of patients with good baseline metabolic control (HbA1c level ≤7.5%) was significantly lower during the whole follow-up period (7.2±0.9%) compared to the moderate (baseline HbA1c level 7.5-≤9%) and poor (baseline HbA1c level >9%) control groups (8.1±0.9 and 8.2± 1.1 respectively p<0.001). However with time a significant trend for increasing HbA1c level was demonstrated in the group with good metabolic control (p for trend 0.004). HbA1c levels of patients with poor baseline metabolic control decreased significantly immediately after pump initiation (9.4±1.6 vs. 8.0±1.2, p=0.0001) and thereafter remained stable (p for trend=0.54). In the multivariable analysis, baseline HbA1c level ≤7.5%, duration of ≤1 year between diagnosis of diabetes and pump initiation, and younger age at pump initiation were independently associated with lower HbA1c levels during long-term follow-up.
Conclusions: Long-term response to pump treatment was dependent on baseline metabolic control. Early pump treatment had significant long-term impact on metabolic control.

PO3-379 Type 1 Diabetes III

Long term insulin pump treatment in girls with type 1 diabetes and eating disorders – is it feasible?

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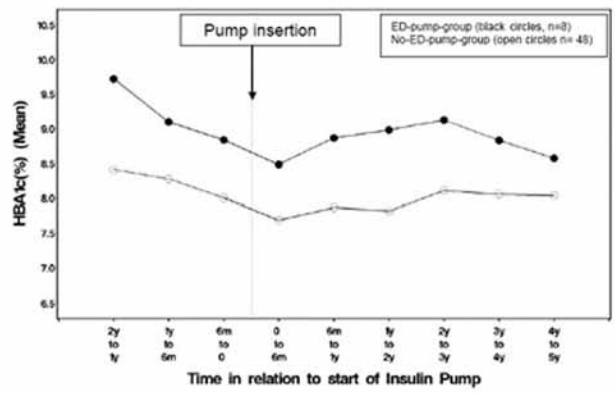
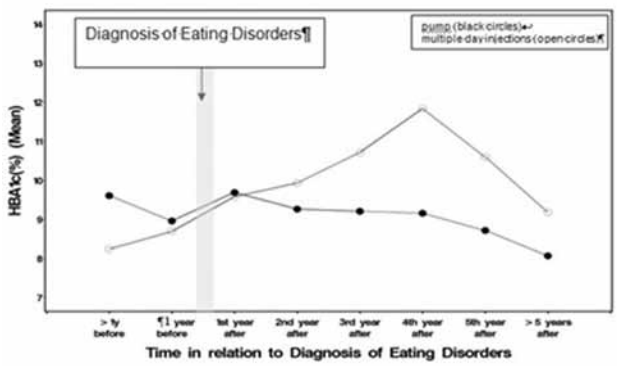
[1]Juvenile Diabetes Center, Maccabi Health Care Services, Raanana, Israel; [2]The Women and Children's Health Research Unit, Gertner Institute, Ramat-Gan, Israel; [3]Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

Aim: To evaluate the feasibility of insulin pump treatment in adolescent females with type 1 diabetes (T1DM) and eating disorders (ED).
Methods: 63 girls age >10 years were included in the study. 48 were treated with pump (no-ED-pump-group); 15 had ED, of whom 8 were treated with pump (ED-pump-group), and 7 were treated with multiple daily injections (ED-MDI-group). **Results**: Girls in ED-pump-group had higher HbA1c compared to those in the no-ED-pump-group (p=0.007).



In the ED-pump-group, levels 0 to 6 months after pump initiation were slightly but not significantly lower compared to baseline reference values (8.48 vs. 8.84 % respectively p=0.42), while in the no-ED-pump-group there was a significant decrease in HbA1c level 0-6 months after pump initiation (7.67 vs. 8.03% p=0.004). Thereafter HbA1c levels were not different from baseline reference in both groups. The rate of hypoglycemic episodes was similar in the ED-pump and no-ED-pump-groups (0.9 vs. 1.0 episodes per 100 patient years respectively). Girls with ED treated with pump had significantly lower HbA1c levels compared with to the ED-MDI-group 9.07±1.33 vs. 10.40±2.01 (p=0.04).



Conclusions: Treatment with pump was feasible in females with T1DM and ED, it was beneficial in lowering mean HbA1c levels for several months and was not associated with excess episodes of severe hypoglycemia or diabetic-keto-acidosis. Pump treatment appeared to maintain better control than treatment with MDI in females with T1DM and ED.

PO3-380 Type 1 Diabetes III

Microvascular complications in type 1 diabetes patients less than five years after diagnosis

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Background

In 1993 the Diabetes Control and Complications Trial (DCCT) demonstrated that intensive insulin therapy, with improved glycaemic control, resulted in a decreased risk of diabetic retinopathy and nephropathy.

Aim

To determine the prevalence of and trends in microvascular complications (microalbuminuria, borderline microalbuminuria and retinopathy) in a cohort of patients who underwent diabetes complications assessment at less than five years following diagnosis.

Method

Adolescents (11-20yrs) diagnosed with type 1 diabetes before age 15 yrs were assessed before 5 years duration. Incident groups were: Dx 1985-1990 (n=126); 1991-95 (n=276); 1996-2000 (n=305); 2001-2005 (n=176). Subjects were assessed for HbA1c, height, weight and blood pressure (BP); retinopathy by 7-field stereoscopic fundal photography (defined as microaneurysms or hemorrhages) and albumin excretion rate (AER). Borderline AER was defined as mean AER >7.5mcg/min and microalbuminuria as AER > 20mcg/min on at least 2/3 timed overnight urine collections.

Statistical Methods

The Mann-Whitney U test was used to compare median HbA1c, BMI SDS and systolic pressure centile between groups and logistic regression for outcome to adjust for time period of diagnosis, HbA1c, BP, gender, age and BMI.

Results

Comparing incident groups 1996-2005 vs 1985-1995, BMI SDS increased (0.77 vs 0.57, $p<0.05$) and HbA1c improved (8.3% vs 8.7%, $p<0.05$). Retinopathy was significantly reduced (14% vs. 6%, $p=0.0002$). Mean AER >7.5mcg/min did not change over time: 25%, 24%, 22%; 23%; nor did microalbuminuria: 2%; 3%; 4%; 4%. Predictors of retinopathy were age (OR 1.18, 1.04-1.34) and Dx 1996-2005 (OR 0.40, 0.25-0.65). Predictors of AER >7.5mcg/min were age (OR 1.13, 1.03-1.23), and diastolic BP (OR 1.02, 1.003-1.04).

Conclusion

Our current data shows no improvement in microalbuminuria over time, despite improvements in median HbA1c and reduction in retinopathy. This may have been counteracted by the increase in BMI which is a known risk factor for microalbuminuria independent of diabetes.

PO3-381 Type 1 Diabetes III

Skin AGEs measured via intrinsic fluorescence in healthy children

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[1]Pediatrics, Infants and Childrens Hospital at Maimonides, Brooklyn, NY, United States; [2]Kids Weight Down Program, Maimonides Medical Center, Brooklyn, NY, United States; [3]Pediatrics, New York Presbyterian Medical Center, New York, NY, United States; [4]Veralight, Albuquerque, NM, United States

Skin advanced glycation end-products (AGE) are a well-known biomarker of diabetes and an indicator of the integrated glycemic exposure that the body has endured. A novel noninvasive technology to measure skin AGE was developed using skin intrinsic fluorescence (SIF) and provides a quantitative result.
Objective: Cross-sectional study looking at SIF in healthy children to establish normal ranges.
Design/Methods: Cross-sectional study involving 6th to 8th grade children. SIF was measured by the SCOUT DM® instrument. Relevant clinical and biochemical parameters were also obtained.
Results: SIF indices was measured in 58 children (F: 32; Mean age: 12.8 +/- 0.7 yr.). Three different parameters are reported. Higher values indicated more advanced glycation end-products in the skin. The cohort was 52% Hispanic followed by 21% Asian. There was no correlation between the SIF indices and age, BMI%, waist circumference, total body fat or between genders. Two of three SIF parameters showed no difference between ethnicities, suggesting better utility for multi-ethnic groups. There was a tendency for all indices to be higher in obese vs. lean children but this did not reach significance.

SIF Indices Comparing Lean And Obese Children		
Characteristic	Group 1 (BMI<85th %) N=24 (F:12)	Group 2 (BMI>=85%) N=27 (F:15)
Age (years)	12.78 (0.76)	12.7 (0.6)
Waist circumference (cm)	91 (14)	100 (10.2)*
Total body fat (%)	56 (11.2)	65.8 (9.5)*
SAGE Index II (%)	0.0253 (0.003)	0.0264 (0.003)
SAGE Index III (%)	0.0348 (0.005)	0.035 (0.003)

*: Significant Difference when comparing Lean and Obese Groups

Conclusion:
SIF provides a quick, non-invasive and painless way of measuring skin AGEs. Measurements on larger cohort of children of different ages, ethnicities and BMI percentiles are required to establish normal ranges.

PO3-382 Type 1 Diabetes III

Skin AGEs as a marker of type 1 diabetes in children

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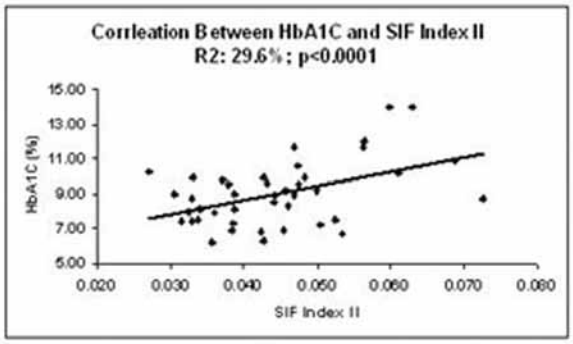
[1]Pediatrics, Infants and Childrens Hospital at Maimonides, Brooklyn, NY, United States; [2]Kids Weight Down Program, Maimonides Medical Center, Brooklyn, NY, United States; [3]Pediatrics, New York Presbyterian Medical Center, New York, NY, United States; [4]Veralight, Albuquerque, NM, United States

Skin advanced glycation end-products (AGE) are a well-known biomarker of diabetes, and an indicator of the integrated glycemic exposure that the body has endured. A novel noninvasive technology to measure skin AGE was developed using skin intrinsic fluorescence (SIF) and provides a quantitative result.
Objective: Cross-sectional study measuring SIF in children with type 1 diabetes (T1DM) and comparing them with known markers of diabetes control and also with same indices in healthy children.
Design/Methods: Cross-sectional study involving children with T1DM seen at a pediatric endocrine clinic. SIF was measured by SCOUT DM® and compared to the same in healthy children. Relevant clinical and biochemical parameters were also obtained.
Results: SIF indices was measured in 25 lean controls (F: 13; Mean age: 12.8 +/- 0.7 yrs) and 51 children with T1DM (F: 33; Mean age: 12.6 +/- 3.8 yr.). Three different SIF indices are reported, two of which showed no difference between ethnicities.
T1DM children had significantly higher BMI% and SIF indices when compared to lean controls.

	Lean Controls	T1DM	p-value
Mean age	12.79 (0.7)	12.6 (3.8)	0.15
BMI Percentile	53.4 (22)	69.7 (26.4)*	0.004
SIF Index I	12.88 (1.96)	16 (6.97)*	0.01
SIF Index II	0.0252 (0.003)	0.032 (0.004)*	0.0001
SIF Index III	0.0346 (0.005)	0.0428 (0.009)*	0.0001

There was no correlation between the SIF indices and BMI%, age at diagnosis of T1DM or time with diabetes. All SIF indices correlated significantly with HbA1C.



Conclusion: SIF indices provide a quick, non-invasive and painless way of measuring medium term glucose control in children with diabetes. It has potential as a screening tool for diagnosing diabetes and monitoring complications of diabetes.

PO3-383 Type 1 Diabetes III

Retinopathy in a paediatric diabetes clinic – more common than we thought?

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Introduction: In the UK, retinopathy screening is recommended in all children and young people with diabetes over the age of 12 (National Institute of Clinical Excellence guidelines, NICE, 2004). Before 2006, Nottingham University Hospitals (NUH) paediatric diabetes clinic advised patients to attend their optician to have this screening done and the results were rarely available for the clinicians. At the end of 2006, a formal retinopathy screening programme was introduced locally, with digital photographs taken and results being sent to the family doctor. Increasingly, reports have also been forwarded to our clinic and following receipt of a number of reports showing background retinopathy, a formal audit was conducted to look at the true incidence of retinopathy and maculopathy in our local paediatric diabetes population.
Methods: Using the NICE criteria, 119 patients attending QMC paediatric diabetes clinic aged between 12-18 should have had eye screening between Jan 08-Mar 09. Reports on 60 patients were available and were categorised as showing positive eye changes (PEC) or no eye changes (NEC). The ages, duration and type of diabetes, sex and average HbA1C over the past year for each patient were analysed and grouped according to presence or absence of eye changes. Statistical analysis was performed using the Student t test.
Results: 14 patients were found to have background retinopathy, 6 with both eyes affected, 8 with one. None had more advanced retinopathy or maculopathy. There were no statistical differences between sex, average age (15.7 vs 15.8) or HbA1Cs for the previous year (9.99 vs 9.52, p>0.1) in the two groups of patients. All the patients in whom retinopathy reports were available had type 1 diabetes. Duration of diabetes was significantly longer in the PEC group compared with NEC group (9.1 vs 5.7 yrs, p>0.001).Of the 59 patients in whom reports were missing, 32 lived in areas with separate screening programmes and results were not automatically forwarded to NUH, 5 had repeatedly not attended offered appointments (including 2 of the 3 patients with type 2 diabetes) and the remainder were either not known to the programme or the results were being chased up.
Conclusion: Retinopathy is detectable in teenagers with diabetes as young as 13 in our population. Duration of diabetes appeared more important than average HbA1C but improving the HbA1Cs in these teenagers will hopefully prevent progression.

PO3-384 Type 1 Diabetes III

Affect of fluid replacement therapy on complication and recovery time of diabetic ketoacidosis: comparison of two protocols

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Objectives: To evaluate the effect of two simultaneous interventions (I– protocol 1 which baseline deficit volume calculated as 10%, II– protocol 2 which baseline deficit volume calculated as 8.5%), in reducing recovery time and assessment of complications of different fluid replacement therapy in management of diabetic ketoacidosis.
Design: Two protocols (Sperling and Milwaukee) were choosed to evaluate recovery time and incidence of DKA complications in each group.
Measurement: Signs and symptoms of cerebral edema, hypokalemia, hypernatremia, sever blood glucose depletion, significant change in effective osmolality and recovery time in two protocols.
Results: During this 3-yr surveillance period, 18 subjects who had sever diabetic ketoacidosis (serum PH<7.1) were included in our analysis. 13 were females (72.2%), mean age of patients in protocol 1 were 7.5±3.7 yr (mean ±SD) and 10.8±4.4 yr in protocol 2, except of two cases all of them were new case of type 1 diabetes mellitus (TIDM). In the course of treatment hypokalemia happened in 5 cases in protocol 1 and 4 cases in protocol 2. Hypernatremia occurred in 2 cases of protocol 1 and 4 cases in protocol 2 and finally 1 patient in each group complicated by hypoglycemia. The mean recovery time in protocol 1 was 21±5.9 (mean ±SD) hours and 23±10.9 hours in protocol 2. None of them had any signs and symptoms of cerebral edema or mental status deterioration in the course of treatment.
Conclusions: According to our study different deficit volume calculated as basic dehydration which replaced in a different time (24 hours versus 36 hours) in similar circumstances (no difference in age and sex) and in a similar clinical setting (no difference in pretreatment serum glucose, sodium, potassium, effective osmolality, hemoglobin and serum urea nitrogen) had not added any risk of complications during treatment. There is also no significant difference in recovery time related to the different fluid replacement therapy.
Keywords: Cerebral edema, Diabetic ketoacidosis, Effective osmolality, Fluid replacement therapy, Hypernatremia, Hypokalemia, Recovery time

PO3-385 Type 1 Diabetes III

Basal-bolus vs. intensified conventional therapy for new onset type 1 diabetes in children: changes in clinical practice and length of stay

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Background. Insulin regimens for new onset type 1 diabetes mellitus (T1DM) include basal-bolus (BB) (e.g., with insulin glargine) and intensified conventional therapy (ICT) with intermediate-acting insulin (NPH). In mid-2007, we introduced a clinical practice guideline (CPG) to facilitate choice and management of these options by the family and diabetes team. We evaluated the impact of the CPG on regimen choice and clinical efficiency measured by length of stay (LOS).
Methods. CPG development occurred from 2006 to 2007. We analyzed all new onset T1DM patients admitted to a large pediatric academic medical center from 7/1/05 to 12/31/05 and from 10/1/07 to 9/30/08. Linear regression was used to evaluate the relationship between LOS and treatment, patient characteristics, and clinical course. P<0.05 was considered statistically significant.
Results. Among 126 patients (mean age 8.8±4.6 yrs) admitted from 7/1/05 to 12/31/05, 96% received ICT, 2% BB with insulin glargine, and 2% other. In contrast, among 221 patients (mean age 9.8±4.1 yrs) admitted from 10/1/07 to 9/30/08, 64% received ICT, 33% BB with insulin glargine, and 3% other (p<0.0001). Average LOS (ALOS) decreased from 2.9±0.9 days in 2005 to 2.6±1.1 in 2007/08 (p<0.01). BB was not significantly associated with ALOS in 2005, whereas in 2007/08 it was associated with a decrease of 0.32 days (p<0.05). Other significant predictors of shorter LOS in 2007/08, in bivariate analyses, included older age, lower HbA1c at diagnosis, male gender, absence

of DKA at presentation, no admission to intensive/intermediate care unit, shorter time from admission to first subcutaneous (SC) insulin injection by staff or parent/caregiver (P/C), more DNE teaching on first hospital day, and family history of T1DM. Insurance and attending physician did not predict LOS. In a multivariable linear regression model including insulin regimen and all other significant predictors, time to first SC injection by P/C remained the only significant predictor of LOS in 2007/08 ($p<0.0001$); ALOS increased by 0.61 days per day from admission to first SC injection by P/C. The relationship did not differ by insulin regimen.
Conclusion. Introduction of a CPG that facilitates choice of insulin regimen for T1DM at diagnosis contributed to increased use of BB and decreased ALOS at a large pediatric academic medical center. Regardless of insulin regimen, earlier administration of SC insulin by a P/C shortens LOS and improves clinical efficiency.

PO3-386 Type 1 Diabetes III

Use of a telehomecare program for young patients with type 1 diabetes

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There is increasing interest in telehomecare programs for health care delivery. A bilingual telehomecare program was introduced at the CHU Sainte-Justine, which allows for reviewing concepts, regular assessment and updating of the treatment plan, automatic downloading and transfer of blood glucose levels, and for email exchanges between families and diabetes professionals. The **purpose** of this randomized study was to determine the effects of a telehomecare (THC) program used for 3 months in families of children and adolescents with newly diagnosed type 1 diabetes. Outcomes of interest were patients and parents' health (reported number of hypoglycemias and sleep interruptions); knowledge of diabetes (using pre and post intervention questionnaires); organizational impacts (number and time for contacts with the physician on call) and family satisfaction with the software application.
Results: The sample now consists of 73 patients (29 girls) with new onset type 1 diabetes (recruitment to end May 2009). Ages range between 0.6-16.6 years (mean 9.0±4.6 yrs). Thirty six received randomly the software application. To date, 51 have completed the 3-month evaluation period (THC: 27). Results indicate 192 vs 50 (mean per pt 7.1±1.3 vs 2.0±0.7, p=0.001) reported hypoglycemias ≤3mmol/L, and 11 vs 0 (mean per pt 0.4±0.1 vs 0±0, p=0.01) reported sleep interruptions for THC users vs controls respectively. Results of questionnaires for knowledge of diabetes (total 20 points) are shown.

Performance on Diabetes Questionnaire						
	PATIENT (dx)	PATIENT (3mo)	MOTHER (dx)	MOTHER (3mo)	FATHER (dx)	FATHER (3mo)
TELE HOME CARE	15.9±1.9	15.6±2.2	16.4±1.6	16.8±1.7	16.1±1.4	16.3±2.0
CON-TROLS	15.6±2.2	15.6±2.0	15.9±1.6	16.1±1.5	15.8±1.8	16.2±1.1
p value	0.70	0.61	0.22	0.15	0.48	0.96

p value using unpaired Student T test

For THC users and controls respectively, there were 0.9±0.3 vs 1.1±0.6 (p=0.75) communications pt with the physician on call requiring 8.6±2.7 vs 7.8±4.5 min pt (p=0.88). THC users were satisfied and felt secure using the software.
Conclusions: Telehomecare for families of young patients with type 1 diabetes allows for more rigorous reporting of hypoglycemias and sleep interruptions with prompt adjustments in the treatment plan, compared to controls. THC use does not preclude appropriate contacts for emergencies and does not impair knowledge relevant to diabetes. The software application was found to be acceptable for the families.

PO3-387 Type 1 Diabetes III

Does continuity of care improve clinical outcomes, as expressed by change in HBA1c, in a paediatric diabetes clinic?

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Background: Studies have shown that continuity of care increases patient satisfaction, improves patient adherence to advice on behavourial risk factors and may have a positive effect on clinical outcomes. Within our diabetes clinic 4 consultants & 4 SpR's contribute to the provision of the service. Ensuring children see the same doctor at consecutive appointments is difficult.
Aim: To determine whether seeing the same or different doctors at consecutive hospital appointments had an effect on children's HbA1c.
Method: Retrospectively the records of a cohort of 154 children (82 male) with type 1 diabetes of >1 year duration were reviewed. Patients are routinely seen 3 monthly. Who saw the child over 3 consecutive clinic visits and their HbA1c was noted together with HBA1c at a further 4th visit 3 months later. Patients were divided into 3 groups, Group 1 saw the same doctor at all 3 appointments, Group 2 saw 2 different doctors over the 3 appointments and Group 3 saw a different doctor each time. Mean change in HBA1c from baseline was compared using ANOVA. We also compared the change in HBA1c at the 3rd visit for those patients who saw the same doctor at 2 previous consecutive appointments with those who saw different doctors each time.
Results: Complete data was available for 142 children. Over the 12 month period the mean change in HbA1c was: Group 1 (10/142) = +1.4%, Group 2 (75/142) = -0.10% & Group 3 (57/142) = -0.03 %. Review of the notes of the small number of children in group 1 suggested these were individuals with poor control who were offered targeted follow up with the same doctor and were not representative of the clinic population. Within Groups 2 & 3 when we examined the effect of seeing the same doctor at consecutive appointments (rather than the effect over the whole period where group 2 saw different doctors) there was a significant reduction in HbA1c of -0.32 in those seen by the same doctor consecutively compared to +0.02 in those who saw different doctors (p=0.03). Overall the mean COCI (Continuity of care index) was 0.23.
Conclusion: Continuity of care does seem to have a beneficial impact on HbA1c with a fall of 0.34% in those who saw the same person consecutively compared to children who saw different doctors each time. However this was a retrospective study over a short time span and it is not clear whether improved continuity would result in as sustained longterm improvement in HbA1c.

PO3-388 Type 1 Diabetes III

Genetic dependence of urinary enzyme, neutral alfa-glucosidase, in diabetic nephropathy in Uzbek children and adolescents with type 1 diabetes mellitus

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Aim: to assess clinical-diagnostic value of urinary neutral α-glucosidase activity in predicting chronic renal insufficiency in type 1 diabetes mellitus in Uzbek children and adolescents.
Materials and methods: we examined 152 children and adolescents of the Uzbek population, 122 children and adolescents with type 1 diabetes mellitus (51 male and 71 female). By proteinuria the patients were divided into 3 groups: with normoalbuminuria (NAU) (n=52), with microalbuminuria (MAU) (n=50) and marked proteinuria ((MPU) (n=20). 30 children and adolescents of matched age and sex were included into the control group. The urinary neutral α-glucosidase activity was measured by rate of glucose formation from maltose.
Results and discussion: in Uzbek children and adolescents with typ1 diabetes mellitus high incidence of D-allele was observed as diabetic nephropathy progressed. II Genotype was registered in 41.%% of cases on the MAU stage, but no cases were observed on MAU stage to be the evidence for high protection of the ACE gene genotype in chronic renal insufficiency progression and consistent with data of studies conducted in other ethnic populations. For the

first time the study on interconnection between activity of urinary neutral α-glucosidase activity and ACE gene genotype was performed. It was established that urinary neutral α-glucosidase activity depended not only on diabetic nephropathy (DN) severity, but on all DN stages in persons with ID and DD genotype it was confidently higher than in those with II genotype. That is the activity of the enzyme was turned out genetically determined by ACE gene. Conclusions: the findings broaden diagnostic opportunities in assessment of urinary neutral α-glucosidase activity not only in early diagnosing of pre-clinical DN in children and adolescents with type 1 diabetes mellitus, but also in predicting progression of terminal DN stages in patients with high susceptibility to chronic renal insufficiency. Registration of increase in the urinary neutral α-glucosidase in children and adolescents with type 1 diabetes mellitus on the pre-MAU stage as a marker of early pre-clinical DN stage will allow solving problem of identification of a group with confidently high DN predicting and progression risk as well as preventive therapy of the complication in question.

PO3-389 Type 1 Diabetes III

Insulin glargine treatment from diagnosis results in improved first year metabolic control in children with T1DM – a retrospective study

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Introduction: Insulin Glargine (Lantus) is thought to offer sustained insulin delivery for up to 24 hours. This may improve night-time insulin deficiency and improve GH generation of IGF-I in patients with T1DM. In accordance, we have found rapid normalization of the GH-IGF-axis in adolescents with T1DM started on Glargine. The aim of the present retrospective observational study was to analyze differences in metabolic control and daily insulin dose in children started on glargine or NPH insulin from diagnosis of T1DM, but otherwise treated according to the same multiple injection therapy (MIT).
Methodology: Metabolic control, total insulin requirements, basal/bolus relations and weight gain were analysed every 3 months during the first year in consecutive cases of T1DM children started on Glargine (n=49) or children of similar age started on NPH insulin (n=49). All children received MIT with direct acting analoges and NPH twice daily or Glargine once daily.
Results: There were no differences between groups regarding age or metabolic status at diagnosis.
In the Glargine group the mean HbA1c was lower at 3 months from start of treatment (5,5% ± 0,89vs 6.2% ±0,89,p<0,001) and at 6 months (5,6% ±1,1 vs 6,6% ±1,0;p<0,001). At 12 months the mean HbA1C was still significantly lower in the Glargine group (6,3%±1,56 vs 7,1%±1,17; p<0,001). The total insulin doses were similar (0.5 U/kg) at nadir in both groups. Interestingly, the mean total insulin doses at 12 months were significantly lower, 0,6 ±0,2U/kg BW^{-24h} in the Glargine group vs. 0,9 ±0,3U/kg BW^{-24h}; p<0,001, in the NPH group. There were no differences in basal/bolus relations, neither were there any differences in weight gain, between groups.
Conclusion: Metabolic control was significantly better during the first year on Glargine in children with T1DM. Furthermore, this was obtained with a lower mean insulin requirement at 12 months. The results could be explained by better preservation of endogenous insulin secretion and/or normalisation of the GH-IGF-axis resulting from sustained insulin delivery with Glargine. Prospective randomized studies which compare basal insulin analoges and NPH-insulin regarding their ability to preserve endogenous insulin and the effects on the production and changes in GH-IGFaxis, are needed, to understand the underlying mechanisms. Such a study is currently ongoing.

PO3-390 Type 1 Diabetes III

Improved glycaemic control twelve months after start of continuous subcutaneous infusion of insulin (CSII) in children with type 1 diabetes (T1D) is associated with HbA1c levels greater than 9% at baseline but not socio-economic factors

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Aim: To determine metabolic and socio-economic factors associated with improved glycaemic control after 12 months of CSII therapy in children with T1D.
Methods: Glycaemic control and demographic data were retrospectively collected on 51children on CSII therapy from six pediatric centres in NW England, comparing those who did and did not demonstrate improved glycaemic control 12 months after starting CSII. Social deprivation was scored using the National Index of Multiple Deprivation 2004.
Results: Age of start of CSII therapy was 11.3 years [Range 3.9-16.8] and duration of follow-up was 1.9 years [0.9-6.6]. After 12 months of CSII therapy, HbA1c levels fell from 8.6% (SD 1.3) by a mean of 0.6% [95%CI 0.3-1.0] (P<0.001). In those who did (n=22, 43%) compared with those who did not (n=29) demonstrate improved glycaemic control at 12 months; pre-CSII HbA1c levels were higher (9.1 (1.1) v 8.2 (1.3)%, P=0.01). In logistic regression analyses; a 0.5% lower HbA1c level after 12 months of CSII therapy was associated with a pre-CSII HbA1c greater than 9.0% (Hazard Ratio 12.5 [95% CI 1.3 – 116.2], P=0.026), after adjusting for known variables. Pre-CSII HbA1c levels between 7.5 to 9.0% and below 7.5% and indices of social deprivation including parental education did not affect the model.
Conclusion: Children with the worst glycaemic control pre-CSII demonstrate the most improvement after 12 months of CSII use. This improvement is unrelated to socio-economic indices, including parental education levels.

PO3-391 Type 1 Diabetes III

Challenges in transition to oral sulfonylurea from insulin in a case of *ABCC8* gene activating mutation causing neonatal diabetes

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Background: Activating mutations of the K_{ATP} channel in the pancreatic beta cell can lead to diabetes by preventing insulin release. Such mutations are a known cause of diabetes in patients diagnosed under 6 months of age. We describe a patient with neonatal diabetes who is a compound heterozygote for novel mutations of the *ABCC8* gene encoding the SUR1 receptor. In such patients, oral sulfonylurea treatment may be successful in restoring insulin secretion.
Methods: We present a 4 year old Chinese girl who presented at 1 day of age with persistent hyperglycemia due to neonatal diabetes. Her initial hemoglobin A1c (HbA1c) was 5.0% (normal upto 6.3) at 14 days of age and increased to 8.6% at 4 months of age. Autoimmune antibodies for diabetes were negative. She was treated with insulin for 4 years at a mean dose of 0.52 units/kg/day. While on insulin therapy, her HbA1c peaked at 9.7% at 1.2 years. She was found to be a compound heterozygote for novel *ABCC8* gene mutations (E1327K, V1523A /3127delACCins13). Molecular genetic analysis of the *KCNJ11* gene was negative. At age 3 years 3 months, a transition from insulin to the oral sulfonyluea glyburide was initiated. After 8 days, glyburide was discontinued secondary to urticaria, palmer erythema and diffuse maculopapular rash. At age 3years 11 months, glyburide was reintroduced at 1/100th of the total daily dose (1.7mg) and increased over 4 months.
Results: Normoglycemia [HbA1c 5.6%, mean blood glucose 123 mg/dl, C-peptide level 1.8 ng/mL (normal 0.8-3.1)] was achieved on glyburide 1mg/kg/day over 4 months with no further allergic reaction and no hypoglycemia. Concomitantly, insulin was weaned and discontinued. Treatment with glyburide led to normoglycemia by enhancing endogenous insulin secretion.

Conclusions: Correlations of the pathophysiology of neonatal diabetes with genetic mutations in the Kir6.2 or SUR subunits of the beta cell K_{ATP} channel has allowed the use of an oral sulphonylurea in place of injectable insulin therapy. Detailed genetic evaluation, including examination of the beta cell K_{ATP} channel (Kir6.2/SUR), is warranted in cases of neonatal diabetes. Patients treated with sulfonylureas should be monitored for known side effects such as hypoglycemia and hypersensitivity reactions.

PO3-392 Type 1 Diabetes III

The effects of telemedicine for the management of type 1 diabetes in children and adolescents: a systematic review and meta-analysis

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Background: Despite advances in insulin preparations and delivery mechanisms, glycemic control in children with type 1 diabetes (DM1) remains suboptimal. Differences in HbA1c levels between pediatric diabetes centres failed to show a correlation with insulin regimen, suggesting that other factors may be more influential on outcomes.
Objectives: To determine the effects of telemedicine (TM), the use of remote telecommunications for medical use, on the management of DM1 in children.
Methods: We searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL, and Web of Science up to Jan 2009. We also handsearched ClinicalTrials.gov, the Telemedicine Information Exchange, conference abstracts from relevant meetings 2007 to 2009, and article reference lists. We included randomized and controlled clinical trials that included participants with an established clinical diagnosis of DM1, age <19 years, in any outpatient setting evaluating a TM intervention involving transmission of blood glucose data with clinician feedback for the management of DM1. Study quality was assessed using the Cochrane Collaboration's tool for assessing risk of bias.
Results: Of 235 studies identified by our search, 9 studies involving 568 children were included. Participants were aged 12-17 years and had baseline HbA1c values between 8-10%. Interventions lasted from 3 to 12 months and 3 studies included an education co-intervention. All studies reported HbA1c measured at the end of the intervention. When these results were pooled in a meta-analysis, there was no effect of TM (weighted mean difference -0.13, 95% confidence interval -0.37 to 0.10). Heterogeneity measured with the I^2 statistic was 0%. There were no differences in frequency of severe hypoglycemia or diabetic ketoacidosis in studies that reported these outcomes. There were not enough studies that reported other outcomes to pool results.
Conclusions: TM did not improve glycemic control but did not result in increased complications. Although there was insufficient data to do a subgroup analysis on baseline HbA1c, studies with the lowest baseline HbA1c levels reported the smallest effects, suggesting that TM may have utility as a therapeutic adjunct in those with poor metabolic control. Future studies examining patient-important and other outcomes such as quality of life, complications, satisfaction, and costs-utility are needed to determine the potential usefulness of TM.

PO3-393 Type 1 Diabetes III

Insulin detemir use in T1DM children younger than seven years – our experience

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Background: Insulin therapy of children with newly diagnosed diabetes TYPE 1 (T1DM) up to 7 years old is very demandable task because of low incidence of remission phase and because of their unpredictable appetite and activity. Classic regular and intermediate--acting insulin therapy is not convenient for this age because of great variability of glycaemia and obligatory time for meals. Use of long-active insulin analogues like Detemir in this period is not recommended by authorities because of lack of clinical studies, but according their pharmacokinetic properties they could be useful for treatment of T1DM children of this age.
Aim: to use insulin Detemir in newly diagnosed T1DM patients in the period between 01.06.2007.-01.12.2008. who were younger than 7 years; to observe daily dose of insulin and scheme of application. Detemir and fast acting insulin; to estimate glucose variability, and metabolic regulation of T1DM by glycosylated haemoglobin level (HbA1c).
Methods: we had written consent of parents for introduction insulin Detemir; data concerning doses of insulin and glucose levels were collected from logbook of patients; HbA1c was measured very 3 months after diagnose (≤ 6,0 %, for non diabetic persons).
Results: From 01.06.2007.-01.12.2008.There were 10 newly diagnosed T1DM small children with mean age 4,8 (1,1-6,9) years. Mean time of Detemir introduction was 14th (7-28) day after diagnose. Average time of follow up was 8,5 (3-22) months. Mean total daily insulin dose was 0,34±0,14 IU/kg /body weight, and mean daily dose of Detemir was 0,29 ±0,11 IU/kg/body weight. Regular or fast-acting insulin was given meanly every second day. Detemir was injected once daily (in the morning) in seven, and two times daily in three patients. Self monitoring blood glucose (SMBG) results were in the range 2,8-18,7 mmol/l (average 7,6 mmol/l), and there was no severe hypoglycaemia episodes. Mean number of HbA1c measurements was 4 times for all patients after Detemir introduction, and results were in range 6,4-8,1% (mean 7,1%).
Conclusion: Insulin Detemir was good choice for therapy of newly diagnosed T1DM patients younger than 7 years. Total daily insulin dose indicates partial remission phase (< 0,5 IU/kg/body weight daily), children had stabile glycaemia and good results of metabolic control of T1DM.

PO3-394 Type 1 Diabetes III

The influence of the kind of therapy on growth hormone axis abnormalities in children with IDDM

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The abnormalities of functioning of growth hormone axis play a great role in IDDM patients in pathogenesis of diabetes complications, insulin-resistance, dawn phenomenon and fat disorders. The aim of the study was the evaluation of growth hormone in urine and GHBP, IGF-1, IGF-2, IGFBP-1,-2,3,6 and ghrelin in blood of prepubertal children with IDDM and estimate the influence of the kind of therapy. Material and methods: 67 patients and 15 age matched, healthy children were included into the study. All children were prepubertal(T<2), suffering from IDDM for more than twoyears, without any coexsisting diseases. All patients were divided into groups according to the kind of therapy:22 were treated with conventional insulin therapy, 21 with multiple insulin injections and 24 with continuous subcutaneus insulin infusion. Blood and urine samples were taken between 7.30and 8.30 a.m. in hospital in normoglycemia after the night without episodes of hypo or hyperglycemia. All analysis weremade by ELISA or RIA commercial kits. The results of the present study permitt to expression the following conclusions: 1.Metabolic control is the factor with the greates influence on the growth hormone axis. There have been proved the correlation of HbA1c and the concentrations of IGF-1, IGFBP-1 and IGFBP-6. 2.It was proved that even children with short diabetes duration and good metabolic control in pre-pubertal age show the growth hormone axis abnormalities without hipersecretion of GH and the lowering of IGF-1, IGF-2 and IGFBP-3 concentrations. However the decrease of GHB-Pconcentration, significant increase of IGFBP-1 concentration as well as the increase in concentrations of IGFBP-2 and IGFBP-6 were observed.It also were proved that the decrease in GHBP and the increase in IGFBP-1 concentrations are the earliest and the most sensitive markers of growth hormone abnormalities.3. Intensification of treatment influences the concentration variability of tested GH axis factors. The lowest severity of abnormalities were observed in the subgroup treated with the use of individual insulin pumps, where the GH, ghrelin and IGFBP-1 levels are the lowest, the highest GHBP and high IGF-1 and IGFBP-3.4. It was also further demonstrated that the age of children in the moment of diagnosis of diabetes may influence the GH axis.

PO3-395 Type 1 Diabetes III

A case of WHIM syndrome associated with diabetes and hypothyroidism

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Background: WHIM syndrome is a rare immunological disorder characterized by warts, hypogammaglobulinemia, infections, and myelokathexis. Myelokathexis manifests chronic neutropenia with marrow hyperplasia and inappropriate apoptosos of mature myeloid cells in the bone marrow. Dominant heterozygous mutations of the gene encoding CXC chemokine receptor 4 (CXCR4), a G-protein coupled receptor with a unique ligand, CXCL12, have been associated with this disorder. We reported a patient with genetically determined WHIM syndrome developed transient hypothyroidism and diabetes.
Case: A 5-year-old Japanese girl was referred for further evaluation and treatment for polydipsia and polyuria.
Past medical history and family history are remarkable: she is one of the dizygotic twin sisters with myelokathexis with a history of recurrent respiratory infections: her neutropenia was first documented at the age of 1 year old. Later a nonsense mutation (C®T) truncating the CXCR4 C-terminal cytoplasmic tail domain occurred at nucleotide position 1000(R334X) of the CXCR4 gene in one allele of the patient was identified and diagnosed as having WHIM syndrome. The parents and the brother are healthy and none of the family members nor relatives are similarly affected.
Pertinent laboratory findings were as follows: an erythrocyte count of 542 x 10^4/μl, Hb of 14.3 g/dl, Ht of 22.2 %, a leukocyte count of 1,800/μl, platelet 15.6 x 10^4/μl, glucose 136 mg/dl. The hemoglobin A1c level was 8.9%, urinary C-peptide of 15mg/day. Antibody to GAD was 92 U/ml and anti-insulin antibody was positive. Serum levels of thyroid hormones, such as T3 and freeT4 were 126.3 ng/dl and 1.96 ng/dl, respectively, and that of TSH was 1.54 μU/ml. Conventional insulin therapy controlled her serum glucose level normoglycemia. Euthyroidism has been archived without levothyroxine since 10 year old.
Discussion: An accelerated apoptosis of neutrophil precursors might account for the neutropenic phenotype in WHIM syndrome. The CXCR4 might be involved in the pathogenesis for type1 diabetes: the progression of the disease in non-obese diabetic mice was mainly caused by an autoimmune-destruction of the islet β-cells via apoptosis and CXCL12 and CXCR4 played important roles for the survival of β-cells.
In conclusion, the concurrence of two exceptionally rare disorders in a child with WHIM syndrome characterized by accelerated apoptosis of neutrophils may be clue to further understanding of the roles of these molecules.

PO3-396 Type 1 Diabetes III

Outpatient versus inpatient diabetes education for children diagnosed with new onset type 1 diabetes mellitus

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Initial education of newly diagnosed patients with type 1 diabetes mellitus (T1DM) is traditionally an inpatient process. Two commonly cited rationales for recommending inpatient education are the need to instill the seriousness of disease and the assured safety. Our objective was to investigate the relationship between teaching location (TL) and diabetes control in children with new onset type 1 diabetes mellitus. Additionally we explored the safety of outpatient education and the relationship between TL and frequency of patient contact in the first year post diagnosis.
METHODS: This retrospective study included children aged 0-18 newly diagnosed with T1DM in 2005-8 followed in our practice for at least 1 year. They were divided into 3 groups: G1 received all diabetes education as an outpatient; G2 received all initial education in the hospital; G3 started diabetes education inpatient and finished outpatient. Diabetes education was provided by the same group of diabetes educators for all groups. Hemoglobin A1C (A1C) values at 6 mos and 1 year post diagnosis were measured using the DCA 2000 analyzer.
RESULTS: We included 132 pts (83 boys and 49 girls) aged 1.2-17.9 years old (mean 10± 4.2). 55 (42%) pts were hospitalized initially: 17 were not in diabetic ketoacidosis (DKA), 8 had mild DKA and 30 had moderate-severe DKA requiring IV insulin.

Table

	Group 1/No Hosp	Group 2/ Hosp	Group 3/ Mixed	P value by ANOVA or t-Test
Number of pts	77 (59%)	19 (15%)	36 (27%)	
Age*	11±3.9	8.47±4.6	8.67±4.2	0.004
Hosp days	0	2.47±0.5	1.9±0.7	0.001
HbA1C - 6 months (%)	6.7±1.1	7.08±0.9	7.44±1.4	0.01
HbA1C - 1 year (%)	7.77±1.3	7.21±0.8	7.76±1.2	0.19
N of ph calls in the 1st year	8.1±7.0	14.4±13.3	9.8±7.7	0.01

*data given as mean ± SD

At 6 months A1C was lower in the outpatient group G1 than in G2 and G3. At 1 year, there were no significant differences between the groups as far as A1C, units/kg of insulin required, or type of regimen. No patients in G1 required admission after an outpatient education modality was chosen. G2 called our office more frequently during the first year. G1 was significantly older, but there were no differences in insurance type or ethnicity.
CONCLUSION: Outpatient T1DM education was not associated with worsened control at 1 year or need for admission. These data support the outpatient approach to initial education and may encourage patient and family independence.

PO3-397 Type 1 Diabetes III

Persistent reduction in hypoglycemia in children using continuous glucose monitoring (CGM): results of the JDRF-CGM extension study

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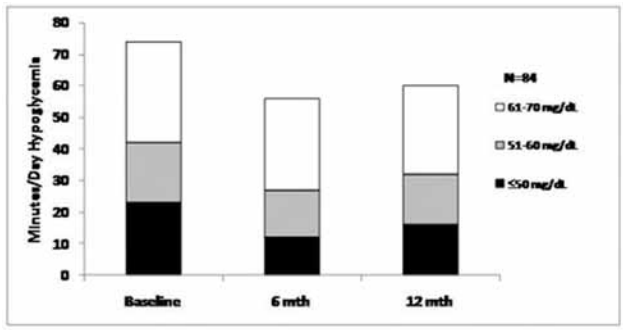
The JDRF CGM Study Group recently reported the results of 6 month randomized clinical trials of CGM vs standard meter monitoring in children (≥8 yrs) and adults with T1D and baseline HbA1c levels <10.0%. At the end of the 6 month randomization phase, all CGM subjects were invited to continue to use CGM in a 6 month extension study. In this report, we analyzed the effect of long-term CGM use on the frequency of biochemical and symptomatic hypoglycemia in children and adolescents. Among 105 subjects in the CGM group age 8 to <18 years who completed the 12 months of study, 101 were still using CGM at the end of the 6 month randomization phase (6 mth) and 91 at the end of the 6 month extension phase (12 mth). HbA1c levels were 7.6% at baseline, 7.4% at 6 mth, and 7.6% at 12 mth. Among the 84 subjects who had ≥24 hours of data in month 12, the duration of sensor glucose (SG) levels below several hypoglycemic thresholds (≤70, ≤60, and ≤50 mg/dL) decreased from baseline to 6 mth (p=0.02, 0.01 and 0.02 correspondingly) and 12 mth (p=0.06, 0.12, 0.18 compared with baseline) of CGM use, as illustrated in the Figure.



Reductions of SG levels in the hypoglycemic range were seen in pre-teens and teenagers and for both daytime and nocturnal time periods. Among the 12-month completers, severe hypoglycemia (seizure, loss of consciousness) decreased from 19.2 events per 100 person-years in the first six months to 3.8 in

the second six months (p=0.02). Notably, among children with baseline HbA1c <7.0% (n=25), a group at higher risk for severe or recurrent hypoglycemia, there were no episodes of severe hypoglycemia during the 6 month extension phase. CGM was found in this study to be effective in reducing the time spent in hypoglycemia and for markedly decreasing the rate of severe hypoglycemia without deterioration in overall glycemic control; and improvements in these areas were sustained over a 12-month duration.

PO3-398 Type 1 Diabetes III

Insulin analogues elicit atypical IGF-I receptor-mediated signaling events

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Insulin analogues have been developed in recent years by modifying the insulin molecule in order to achieve better pharmacokinetic properties able to mimic the endogenous secreted insulin. Long-acting analogues are characterized by their slow and constant release into the circulation, mimicking basal insulin secretion. This feature is due to modifications in the C-terminus of the insulin βchain. An emerging question is whether these changes affect the binding properties of the analogues to the insulin-like growth factor-I (IGF-IR) and/or insulin (InsR) receptors, and confer upon the analogues different biological properties than those of regular insulin, including an enhanced mitogenic potential. Recent in vitro studies in our laboratory revealed that two long-acting analogues, Glargine (Lantus, Sanofi Aventis) and Detemir (Levemir, Novo Nordisk), display increased mitogenic and antiapoptotic capacities in various cancer cell lines, strongly resembling those of IGF-I. The aim of this study was to investigate the signaling events elicited by glargine and detemir in comparison to regular human insulin and IGF-I in a colon cancer cell line.
Results of immunoprecipitation assays showed that glargine was able to phosphorylate both the InsR and IGF-IR. Activation of IGF-IR by glargine resembled its activation by IGF-I in terms of doses and time frame. Dose-dependent assays revealed that glargine activated the IGF-IR at 5-fold lower doses than those required to activate the InsR. In addition, glargine induced a sustained InsR phosphorylation whereas it activated the IGF-IR in a biphasic fashion. The ability of both analogues to activate the major signaling pathways, PI3K and MAPK, in terms of kinetics and intensity, are essentially different from those of insulin. Biological studies reveled that the analogues exhibit an IGF-I-like antiapoptotic effect and enhanced the proportion of cells in S-phase. Finally, confocal microscopy indicates that glargine led to IGF-IR internalization similarly to IGF-I.
In summary, our data is consistent with the notion that glargine and detemir exhibit IGF-I-like mitogenic and antiapoptotic activities in cancer cells through their ability to interact with the IGF-IR. The different binding characteristics of the analogues to the IGF-IR, in comparison to regular insulin, seems to promote atypical signaling events leading to different biological actions. The clinical implications of these findings remain to be established.

PO3-399 Type 1 Diabetes III

Subclinical impairment of the left ventricular diastolic function in adolescents with type 1 diabetes mellitus during puberty

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Diabetic cardiomyopathy, defined as functional and structural changes in cardiomyocytes and Interstitium, is one of the most essential causes of the chronic heart failure, independently from the complications of type 1 diabetes mellitus (DM1). The aim of the study was the echocardiographic assessment of selected parameters of the left ventricle (LV) in pubertal DM1patients and in their healthy peers, and the analysis of the dependence of the results from the duration, metabolic control as well as the model of DM1 treatment. The echocardiography investigation was performed (M - mode, 2DE, Doppler) in 60 DM1 patients (30 girls, 30 boys) aged 14-17 years without arterial hypertension, complications of diabetes nor other illness, that might influence on the results of the investigation and in 30 healthy volunteers. The metabolic control was estimated on the basis of the average value of HbA1c in whole treatment period (HbA1c1), last 2 years before investigation (HbA1c2) as well as in day of investigation (HbA1c3). In DM1 patients, both sexes, there was significant extension of the isovolumetric relaxation time (IRT), in comparison to control group (0,06 vs 0,056 s, $p < 0,05$), as well as not significant increase of the A wave value (in girls and in boys: 60,3 vs 58 cm /s and 56,12 vs 53,95 cm /s), the deceleration time (0,17 vs 0,15 s and 0,16 vs 0,15 s), and decrease of the E wave value (99,67 vs 101,8 cm /s and 97,35 vs 104,9 cm /s) and the E/A relation (1,7 vs 1,78 and 1,79 vs 2,0). There was no differences in the LV systolic function. There was a correlation between the IRT and daily insulin dose ($r = 0,39$, $p < 0,05$) as well as HbA1c1 and HbA1c2 ($r=0,42$, $r=0,46$, $p<0,05$), and also between the A wave value and the daily insulin dose ($r=0,4$, $p<0,05$), the time of DM1 duration ($r=0,46$, $p<0,01$) as well as HbA1c2 ($r=0,46$, $p<0,05$) in girls. Moreover there was the enlargement of the LV wall systolic dimension dependent from HbA1c1 and HbA1c2 ($r=0,45$, $r=0,41$, $p<0,05$). There was a correlation between the A wave value and HbA1c1 and HbA1c2 ($r=0,48$, $p<0,01$; $r=0,37$, $p<0,05$) in boys. Conclusions: In DM1 patients during puberty, without complications of diabetes independently from sex, there are observed early markers of the LV diastolic dysfunction. The LV diastolic dysfunction in girls depends from the degree of the insulin-resistance and from the DM1 duration and as well as in boys from metabolic control in long time period.

PO3-400 Type 1 Diabetes III

Does conventional therapy warrant earlier screening for microvascular complications among type1 diabetic adolescents in our population?

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Introduction: Screening for complications so as to decrease the morbidity and mortality in Type I diabetes mellitus among the adolescent population through clinical, laboratory parameters when they are subclinical, reversible and treatable is mandatory. There is a need to screen adolescent diabetics to identify chronic complications early, but poor review is available on Indian patients. Hence, it was decided to assess the glycemic status and screen for microvascular complications in Type 1 diabetic adolescents, since we are following conventional therapy due to economic constraints.
Materials and methods: Forty two Type 1 diabetics [23 females and 19 males] in the age group of 10-18 years with a mean age of 13.33 ± 2.82 years attending the Pediatric Endocrine Clinic were screened as per ADA protocol.
Results: The mean weight, height, BMI and mean insulin dose of the study group were 32.43±11.16kgs,138.45±14.42cms,16.42±2.87kg/m^2and1.12±0.268unit/kg/d respectively. The mean Systolic and Diastolic blood pressures were normal 108.875±8.93 and 69.12±7.17 mmHg. Duration of diabetes was 3.775±1.19 years and the mean glycosylated Hemoglobin (HbA1C) was 8.035±1.86. The lipid profile was in normal range. The complications observed were- Nephropathy in 5/42(11.9%) subjects, Retinopathy in 2/42 (4.76%) and Neuropathy in 3/42 (7.14%) and were more common among the pubertal children. The mean age of the seven children with complications (3 had both nephropathy and neuropathy) was 14.57±1.51yrs. with a mean diabetes duration of 5±3.1yrs which was significant. The average insulin dose was more-1.36±0.13 unit/kg/d. The systolic and diastolic blood pressures of this group were 117.43±9.78 and 75.71±4.54mmHg,with diastolic being significant.The mean HbA1C was 11.24±1.32%.Among18poorglycemicsubjectsHbA1C>8, 5(27.7%) had microalbuminuria and 3(16.6%) had Neuropathy which was significantly more than in good glycemic controls(<0.05).
Conclusion: To conclude, our adolescents started developing complications by the mean age of 14.5years and mean diabetes duration of 5years. Therefore, regular screening for microvascular complications as per ADA guidelines is vital for early detection of diabetic microangiopathy. Thus, stringent glycemic control and timely interventions instituted early, can play a pivotal role in prevention of blindness and end-stage renal failure in these adolescents with diabetes.

PO3-401 Type 1 Diabetes III

When to begin screening for diabetic nephropathy among type 1 diabetics in our population?

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Introduction:Diabetic nephropathy (DN) affects up to one third of patients with Type 1 DM. Microalbuminuria can occur after 2-5 yrs of diabetes before the development of overt disease.There are few studies from developing world especially where diabetic population is considerably undernourished as in our study population.Hence this study in adolescent Type 1 diabetic patients was done to screen for microalbuminuria.
Materials Methods: Thirty Type 1 Diabetic patients16 females and 14 males in the age of 10-18 years attending Pediatric Endocrine Clinic were screenedfor Microalbuminuria by quantitative immunoturbidimetric method on a total of three timed urine collections, performed within 6 months to reliably establish the presence of persistent microalbuminuria at least two of three tests show elevation.First voided early morning urine specimen was collected and abnormalities of albumin excretion were defined as- normal-<30,microalbuminuria-30-299and macroalbuminuria=>300 (values in microgram per milligram of creatinine).
Results: The mean age and BMI was13.33±2.44 years and15.87±2.52 kg/m^2.The mean insulin dose was 1.22±0.2 unit/kg/day. The mean diabetes duration and glycosylated Hemoglobin HbA1Cwas3.53±2.18yearsand9.37±1.89%. The mean Systolic and Diastolic blood pressures were111.47±8.02and71.67±5.01mmHg and the lipid profile were normal. It was observed that **none** of the participantsin the good control HbA1C<8.0% developed persistent microalbuminuria in the study period,**but** 5 of 14(35.7%) poor control patients developed persistent microalbuminuria with average urine albumin excretion of 67.38±32.78.However, no subject developed overt nephropathy during study period. The mean age, duration of diabetes and average insulin dose of these microalbuminuric patients was 14.6±1.8 years,5.4±3.6 years and 1.24±0.2unit/kg/day.The mean HbA1C was 11.6±1.23%.Both Systolic and diastolic blood pressures were higher 119.20±11.36 and 77.2±4.6 mm Hg p=0.011.The lipid profiles were not significantly different.
Conclusion: In our study population higher percentage of early nephropathy was observed due to poor glycemic control and two subjects were12years old and had diabetesfor3 years only. Therefore,screening after 5 years of diagnosis by ADA guideline may be rather late.Thus,in these very poorly controlled subjects, on conventional regime besides trying to achieve a tighter control one might have to consider screening for microalbuminuria after 2-3 yrs of diagnosis in our set up.

PO3-402 Type 1 Diabetes III

Soluble insulin receptor ectodomain level in patients with diabetes

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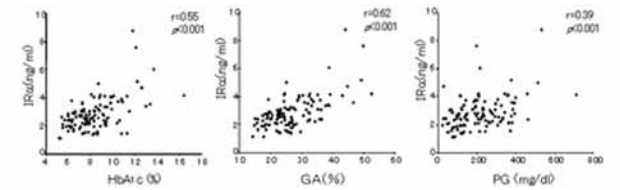
Objective: We identified soluble insulin receptor ectodomain (insulin receptor α-subunit, IRα) in human serum and developed enzyme-linked immunosorbent assay system that measured serum IRα. The aim of this study is to compare its level with other glycemic control markers, glycosylated hemoglobin (HbA1c), glycosylated albumin (GA), plasma glucose (PG), and evaluate clinical usefulness of IRα measurement in diabetic patients.
Subjects and Methods: Six-hundred thirty-six type 2 diabetic patients (T2DM) and 64 type 1 diabetic children (T1DM) were participated in this study. One-hundred twenty-three healthy volunteers confirmed to have normal glucose tolerance were enrolled as control. To evaluate the effect of diabetic control to IRα level, one-hundred-eighteen serum samples were randomly collected from type 1diabetic patients (male=20, female=44, duration of diabetes at sample collection 0-18 years) and the correlation between IRα and HbA1c, GA, and PG were studied. In 8 patients, the changes of these parameters from the onset of diabetes to 2 months later were compared. Long-time follow-up of these parameters was performed in a young T1DM patient.
Results: Both T1DM and T2DM group exhibited a significantly elevated serum level compared with control group (2.00±0.60, 2.26±0.80 vs. 1.59±0.40, p<0.001). More than 30% of the diabetic patients exhibited an IRα level higher than the cutoff value (2.39ng/ml, mean+2SD of control). In type 1 diabetic patients, serum IRα concentration was correlated positively with HbA1c (r=0.56, p<0.001), GA (r=0.62, p<0.001) and PG (r=0.39, p<0.001). Partial correlation analysis suggested that GA was predominantly correlated with IRα.



At onset, all 8 patients had elevated IRα level (5.5±1.8ng/ml). After starting insulin therapy, these declined relatively faster than HbA1c and GA and became almost nadir level at one month later (2.3±0.8ng/ml). During long-term follow-up of a patient, IRα level paralleled with GA and HbA1c levels and changed more dynamically than them.
Conclusion: Serum IRα level appeared to be a more rapid glycemic marker than HbA1c or GA.

PO3-403 Type 1 Diabetes III

The effects of family environment and parenting behaviors on diabetes care with type 1 diabetes in Korean children

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Objective : The aim of this study is to see the influence of metabolic control according to family environment, parenting behavior, and mental and psychological characteristic status of Type 1 diabetes children.
Methods : Among the children under the medical treatment who have diagnosed type 1 diabetes over 1 year, 11- to 17-year-old adolescents and their parents were studied for the Children's Depression Inventory(CDI), family APGAR(Affection, Partnership, Growth, Adaptation, Resolve), Parenting Behavior Inventory and Family Environment Scale(FES). The survey was invested and processed by statistic method.
Results : HbA1c was positive correlation with CDI score, the daily requirement of insulin dose and the duration of disease. However, it was negative correlation with the items of independence, intellectual-cultural orientation in the FES. In the binary logistic regression, the risk factors of inappropriate blood sugar control were increased by long period of diabetes suffering and low family APGAR score. Dysfunctional family which had low family APGAR score had lower score of paternal and maternal affection items, and maternal monitoring items and higher score of maternal intrusiveness items in the Parenting Behavior Inventory.
Conclusions : Improvement of family environment, family APGAR and parenting behavior also help metabolic control in type 1 diabetes children as well as proper insulin therapy, physical activities and dietary treatments which have been concerned. Moreover, this information can use education for parents who had children with diabetes.

PO3-404 Type 1 Diabetes III

The prevalence of polycystic ovary syndrome and hyperandrogenic disorders in adolescent girls with type 1 diabetes mellitus using intensive insulin treatment – the pilot study

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In adult women with type 1 diabetes mellitus (T1DM) increased prevalence of

hirsutism and polycystic ovary syndrome (PCOS) have been described. These conditions could be related to nonphysiologic insulin replacement therapy and poor glucose level control with hypo- and hyperglycemias.
The aim of our study was to estimate the prevalence of PCOS and its components in adolescent girls with T1DM with respect to their metabolic control.
Twenty four adolescent girls with T1DM (mean chronological age 15.8±1.2 years, mean gynecological age 33.0±10.7 months, mean HbA1c for the last 12 months 6.95±0.66%), treated with multiple insulin injections (15 girls, 62.5%) or with insulin pump (9 girls, 37.5%), were studied. In all girls, evaluation of hirsutism and menstrual disorders were performed and basal as well as GnRh analogue stimulated (Dipherelin 0.1 mg, s.c.) levels of androgens and gonadotropins were measured. PCOS was diagnosed according to ESHRE/ASRM criteria.
Six (25%) girls fulfilled the criteria of PCOS diagnosis. However some components of PCOS were found in another 6 (25%) girls [menstrual disturbances in 1 (4%), hirsutism in 1 (4%), polycystic morphology of the ovaries in 2 (8%), elevated testosterone and/or androstenedione level in 4 (16%)]. There was also a significant correlation between increase in testosterone level after stimulation and BMI z-score (r=0.54, p=0.026). Relationship between PCOS occurrence and the type of insulin therapy, T1DM duration, daily insulin dose per kg, mean HbA1c for the last 12 months and mean HbA1c for the T1DM's span was statistically insignificant. However, significant correlations between GnRh analogue stimulated testosterone level and mean HbA1c for the last 12 months (r=0.61, p=0.006) and mean HbA1c for the T1DM's span (r=0.47, p=0.04) was found. It is concluded that clinical and/or biochemical components of PCOS can be found in 50% of adolescents girls with T1DM that may be associated with the quality of metabolic control. Supported by MNiSW N407 015 32/0403.

PO3-405 Type 1 Diabetes III

Type 1 diabetes mellitus in children and adolescence in Ukraine

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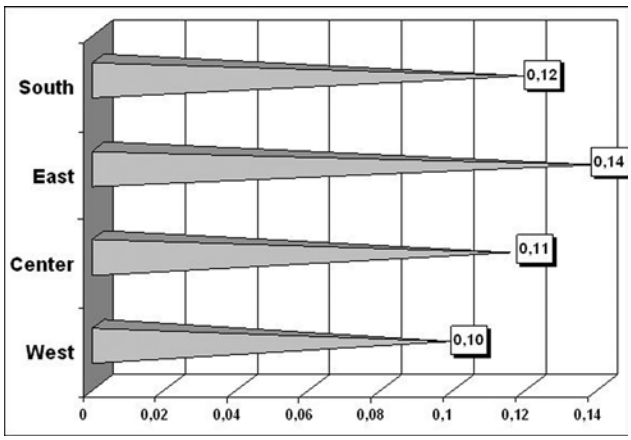
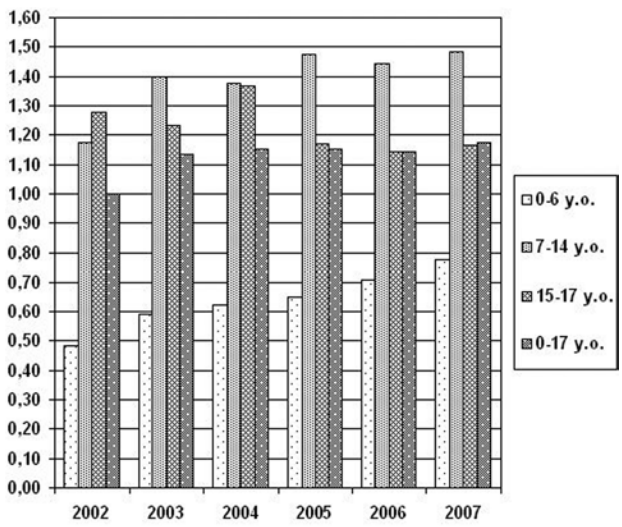
The aim of the study:
To determine a tendency of the first detected cases parameter of Type 1 Diabetes Mellitus (FDC T1DM) in children in different regions in Ukraine
Materials and Methods
Target group: children 0-17 y.o.
Methodology for selection: official statistical information on the FDC T1DM in children in Ukraine during 2002-2007
Results and Conclusions
During the analysis of different geographical areas of Ukraine the phenomenon of west-east "gradient" was discovered. The FDC T1DM per 10000 is highest in eastern region and lowest in western region.



In Ukraine 7932 children 0-17 y.o. with T1DM were registered in 2007.
The parameter of FDC T1DM in children 0–17 y.o. increased rapidly from 2002 (figure 2). From 2003 to 2006 it remained stable. In 2007 an increase of the FDC T1DM parameter was registered not only in children 0–17 y.o, but in all age groups.
The parameter of FDC T1DM is rapidly increasing in the age groups 0-6 and 7-14 that shows a trend toward earlier age of onset.



If we try to predict the number FDC T1DM during the next year, we would only be certain to say that the number FDC T1DM will increase, but are not able to predict the percentage of this growth, because this parameter changes differently each year.
During the last 6 years the absolute number of the FDC T1DM in children did not become higher compared with FDC parameter per 10000 of children population. This fact can be explained by continuing decrease of children population in Ukraine (from 10.3 million in 2002 to 8.5 million in 2007).
Because Ukraine is 5th in population in Europe the rise in T1DM suggests that the European population in general is experiencing an increase in T1DM.

LB-PO3-001 Late Breaking Submissions

Postpartum serum thyroid hormones in preterm neonates

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Introduction: The optimal ranges for serum thyroid hormones in preterm infants related to gestation and postnatal age has not been resolved, a need to determine preterm infant reference ranges is evident.
Objectives of the study: To clarify postnatal thyroid hormones levels preterm infants and determine reference ranges for this group. To analyze and interpret the data of infants with different gestation as compared to full term infants. To analyze the data of infants with different gestation in relation to their clinical condition.
Methods: A prospective controlled study including 100 preterm neonates, 27-36 weeks gestation, comparing them to the data from the national screening program of congenital hypothyroidism. History, clinical examination and *serum FT3, FT4 & TSH levels* were done.
Results: Significantly lower serum TSH levels in preterms compared to full terms. Significantly higher FT4 level in preterms compared to full terms, no significant difference regarding FT3 levels.
Significantly lower TSH level of the preterm gestational age subgroups when compared to full term (control group). No significant difference regarding FT3 or FT4 levels between subgroups and that of the full terms.
Conclusion: Preterms have significantly lower TSH and higher FT4 serum levels (measured between day 3 & 7) than full terms, with no significant difference regarding FT3 level.

It is recommended to evaluate the thyroid functions of the preterms in comparison to cutoffs specific to this group (in our study they are, FT3: 1.3- 4.1 pg/ml, FT4: 0.7- 2.3 ng%, and TSH: 0 - 10.2 uU/ml).
The TSH levels of the preterms may not have been elevated at 3 to 7 days of age, and a second screening or reevaluation specimen might have been needed at a later age to identify the elevation in TSH.

LB-PO3-002 Late Breaking Submissions

Frequency of hereditary medullary thyroid carcinoma and RET proto-oncogen polymorphisms in patients with apparently sporadic medullary thyroid carcinoma

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Medullary thyroid carcinoma (MTC) originates from C cells of the thyroid and represents approximately 10% of all thyroid cancers. Approximately 75% of all MTCs are believed to be sporadic, whereas the remaining 25% correspond to inherited cancer syndromes. Little is known about the etiology of sporadic MTC although previous reports suggest that the presence of inherited genetic variants of Ret are associated with the predisposition to or development of sporadic MTC. Several single nucleotide polymorphisms of the RET gene have been described in the general population as well as in patients with MTC. Aim: To evaluate the incidence of hereditary disease in patients with apparently sporadic MTC and to describe the frequency of inherited genetic variants in the RET proto-oncogen. Patients and Methods: Screenig of Ret exons 10, 11, 13, 14 and 15 was performed by direct sequencing of DNA from 74 patients with apparently sporadic MTC. The frequency of Leu769Leu (exon 13), Ser 804Ser (exon 14) Ser904Ser (exon 15) and Gly691Ser (exon 11) polymorphisms was studied in 36 of them. Results: 4/74 patients (5.4%) with apparently sporadic MTC presented hereditary disease. The mutations found were: Cys620Arg (2), Cys618Phe (1) and Cys634Tyr (1). 13/39 relatives (9> 20y and 4< 20y) were studied. In seven of them the Ret mutation was detected. 36/74 patients were studied for polymorphisms and the allelic frequencies were: 44.4% (16/36) Leu769Leu, 2 homozygous and 14 heterozygous, 33.3% (12/36) Ser904Ser, 2 homozygous and 10 heterozygous, 50% (8/16) Gly691Ser, 3 homozygous and 5 heterozygous. All patients were negative for Ser836Ser. Conclusions: The frequency of inherited disease that we found, among patients with apparent sporadic MTC, strengthens the routine application of Ret proto-oncogen DNA screening in all cases of this disease. The frequency of Ret polymorphisms detected in these patients may suggest a potential role of these variants in the development of sporadic MTC. A putative role of the polymorphism as genetic modifier remains to be established and further studies are necessary to characterize the clinical behavior of MTC.

LB-PO3-003 Late Breaking Submissions

Management and outcome of the children with congenital hypothyroidism followed in the Queen Fabiola Children's Hospital of Brussels

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Introduction
Congenital hypothyroidism (CH) is a major cause of avoidable mental retardation. Neonatal screening allows early diagnosis and treatment of CH in infants. Thyroid hormone therapy started within 2 weeks of age should normalize growth and cognitive development. The aim of this study was to assess the management and outcome of the children with CH referred to the Paediatric Endocrinology Unit of the Queen Fabiola Children's Hospital of Brussels.
Patients and methods
The study sample consisted of 104 patients with permanent primary congenital hypothyroidism born between 1978 and 2008 followed in the Queen Fabiola Hospital of Brussels. The following parameters were recorded: results of screening, initial management, TSH at 1 month of age, time of TSH normalisation, weight, length and head circumference at birth, at 1 and 6 years of age and school achievement.
Results
Of 104 patients, 18 had dyshomonogenesis and 86 had dysgenesis (47 ectopy, 35 athyreosis and 4 hypoplasia). The patients with athyreosis had higher TSH and lower T4 at diagnosis, more frequent absence of knee epiphyses on x-ray compared to those with ectopy.
7 % of the newborns were born post-term, compared with 1.5% in the general population. Birth weight was -0.4 ± 0.1 SDS.
Median age at treatment start was 11 days and median initial dose of levothyroxine was 10.6µg/kg/j. There was a statistically significant trend towards earlier treatment and earlier TSH normalisation with time.
The anthropometric data at 1 and 6 years were within the normal range except for a higher BMI at 6 years of age compared to the reference values (SDS 0.317±0.044; p=0.023).
15/45 patients had some school delay in primary school which was not different from the community results. Nonetheless, out of these 15 patients, 7 patients had a school delay ≥ 2years. These were born between 1982 and 1997, 7/7 had fT4<0.8 ng/dl at diagnosis, 6/7 had neonatal bone age delay, their LT4 treatment start ranged between 0 and 15 days of life, 5/7 patients had a TSH ≥5.5 uU/ml at 35 days of life. Finally, 4 patients required special schooling (3 for hearing impairment).
Conclusion
We observed earlier treatment and earlier TSH normalisation with time. Precociously treated CH patients have normal growth.
There was no significant school delay in our cohort, nonetheless, the long term outcome assessment remains primordial.

LB-PO3-004 Late Breaking Submissions

Discontinuation of thyroid hormone treatment among children in the United States with congenital hypothyroidism: findings from health insurance claims data

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BACKGROUND: Thyroid hormone treatment in children with congenital hypothyroidism can prevent intellectual disability. Congenital hypothyroidism guidelines recommend a trial off of therapy at 3 years of age for some children to evaluate for permanence of congenital hypothyroidism.
OBJECTIVE: To describe the rate at which children diagnosed with congenital hypothyroidism discontinue thyroid hormone treatment in early childhood.
METHODS: Retrospective analysis of the 2002-2006 MarketScan® Commercial Claims and Encounters research databases and the 2001-2005 MarketScan Medicaid databases. Children were classified as having congenital hypothyroidism in their birth year based on International Classification of Diseases, Ninth Revision codes and having filled a prescription for thyroid hormone treatment. Survival curve analysis was used to determine rates of discontinuation over time.
RESULTS: There were a total of 412 Medicaid-enrolled children and 292 privately-insured children with presumed congenital hypothyroidism included in this study. The overall birth prevalence of congenital hypothyroidism across both datasets was about 1 per 2,300. By 36 months, the overall proportion who had discontinued thyroid replacement treatment was 38% (95% Confidence Interval: 32%-44%). Although the discontinuation rate was similar by 36 months, Medicaid-enrolled children had a more rapid decline in the first 24 months of treatment compared to those with private insurance (P = 0.02). In the birth year, 53% of all cases of presumed congenital hypothyroidism were female. However, males were more likely to discontinue treatment (P = 0.04). By 36 months, 65% of those continuing treatment were female.
CONCLUSIONS: More than one-third of children treated for congenital hypothyroidism discontinued treatment within 36 months, which is inconsistent with current guidelines. It is not known how many of these children had transient hypothyroidism and no longer require treatment or milder forms of permanent hypothyroidism for which treatment should be continued but for which the effects of discontinuation may not be apparent.

LB-PO3-005 Late Breaking Submissions

High frequency of cardiac and behavioral complaints as presenting symptoms of hyperthyroidism in children

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Objective: Descriptive data to characterize the frequency of various chief complaints and presenting symptomatology in children with hyperthyroidism are lacking**.** Difficulties in recognizing atypical presentations may delay diagnosis and may increase morbidity in some children.

Patients and Methods: We performed a retrospective review of the medical records of 76 children with hyperthyroidism to characterize their chief complaints at initial presentation to care and document other presenting symptomatology**.** We recorded and categorized the patients' chief complaints as well as all other symptoms documented in the medical record. To determine whether the severity of hyperthyroidism differed among children presenting with specific symptoms, we compared initial free T4 concentrations between several sub-groups.

Results: Cardiac symptoms were the most frequent chief complaint, accounting for 23% of presenting complaints in the study population. Detection of goiter or proptosis on exam done for an unrelated purpose was also a frequent path to diagnosis (18%). Major mood and behavior disturbances (including criminal behaviors) were frequently present at diagnosis (21%), although these problems were infrequently cited as the chief complaint (6%). Weight loss, fatigue, worsening school performance and heat intolerance were the other symptoms most frequently noted on review of systems (>25% of patients). Mean free T4 concentrations did not differ between patients with and without cardiac symptoms and patients with and without major mood and behavior symptoms.

Conclusions: This retrospective study is the first to describe the chief complaints of hyperthyroid children at the time of presentation to medical attention and demonstrates that children with hyperthyroidism often present with cardiac complaints. In addition, disorders of behavior and mood occurred frequently in these children, including criminal behaviors. Clinicians should be aware of these presentations so that hyperthyroidism can be diagnosed more promptly, avoiding potential morbidity associated with delayed diagnosis.

LB-PO3-006 Late Breaking Submissions

Ultrasonographic description of the brain cortex and the cingulate sulcus in newborn and infants with congenital hypothyroidism

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Objectives

To demonstrate the presence of delay in brain cortex development in patients with CH.

Material and method

Transversal and prospective study in 31 newborn and infants with CH detected by neonatal screening and confirmed by thyroid scintiphotography, and thyroid hormonal profile, bone age at knee and with the assent of their parents to make the ultrasound record in the first 2 months of life. Children with genetic malformations or other congenital associate alterations were excluded.

Transfontanelar ultrasound transducer was used to explore three coronal sections (previous, middle and posterior) and three sagittal sections (left, way and right). The patterns of ripeness of the brain cortex were qualified by the modals of normality provided by the methods of Slagle, and Timor, and the cingulate sulcus was identified in the parasagittal sights between the thalamus and the anterior fontanel. The statistical program SAS's JMP, version 7.0, was used for the tabulation and elaboration of charts and graphics of results.

Results

A high percentage of infants with CH showed signs of immaturity in brain cortex. The contingences analysis shows association between Timor´s method and delayed bone age, but it did not allow identification by type. The study cluster (type of HC, thyroid hormonal profile, and bone age at treatment start) founds marginally significant association with Pearson 0.06 using Slagle´s method. In the results, the more sensitive ultrasound technique for demonstrating changes in the brain cortex ontogeny and the cingulated area was sagittal section registered by Slagle´s method. Other variables to consider were the age of treatment start and the time since treatment initiation though study performance, as average for both variables showed that children with neurodevelopment delayed started hormonal substitution at older age and has shorter periods of time since treatment initiation though study performance.

Conclusions

Preliminary discoveries reveal the frequent presence in the delay of the brain cortex development in children with CH, influencing the prediction of the patterns of normal ripeness in those with delayed bone age, athyrosis, lower plasma levels of T3 and T4, longer age at treatment beginning, and shorter period of treatment.

LB-PO3-007 Late Breaking Submissions

Timing of repeat newborn screening of congenital hypothyroidism

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Background: False positive rate of newborn screening for congenital hypothyroidism (CH) is high, which puts unnecessary physical and mental stress on patients and their families and creates large burdens to our medical system.

Objective: Our goal is to determine local incidence and positive predictive value (PPV) of newborn CH screening, exam the causes of false positives, and determine appropriate repeat screening time in order to avoid multiple repeating.

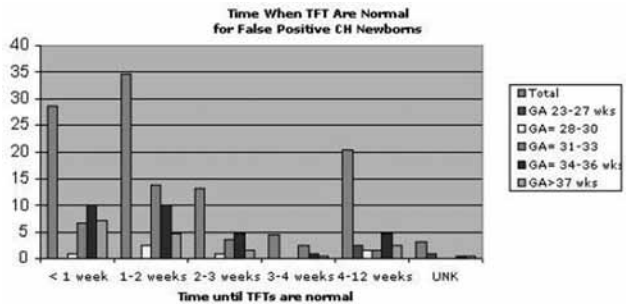
Design/Methods: All newborns with positive CH screening since 2006 were included in this retrospective study.

Results: 209 infants were identified. Of that group, 9 patients were determined to have congenital hypothyroidism for which they were treated. Clinically, those 9 patients were normal without any symptoms or abnormal signs. The remaining 200 patients were false positives. The PPV for CH screening test is 0.043. For the false positive CH newborns, 79% were premature (gestational age < 37 weeks) and 53% had low birth weights (BW < 2250 g). For full-term newborns we found the cause of false positives to be mainly early sampling, within first 24 hours of birth which represent 99% of the cases. The gestational ages and the timing of repeat thyroid function tests (TFTs) were analyzed. 66% (28) full-term newborn had repeated TFTs done within 4 days of life, and all had normal results. We also found that for most newborns with gestational ages above 31 weeks, TFT's become normal after the age of 1 month, while most newborns with extremely low gestational ages require up to 3 months for their TFT's to be normal.

Conclusions:

Our results show that the PPV of newborn CH screening is very low. Consistent with the previous reports from other states, prematurity and samples taken too early are the main causes of false positive results. We confirm that ideal sample time should be two to four days of life, as our newborn screening program suggests.

Children with very low gestational ages may require multiple tests up to 3 months while infants with higher gestational ages may require only 1 or 2 repeating tests up to age of 1 month for their TFT's to become normal.



LB-PO3-008 Late Breaking Submissions

Prevalence of celiac disease among females with Turner syndrome in midwest region of Brazil

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Background/Objective: Several recent studies have shown a higher prevalence of celiac disease (CD) among females with Turner Syndrome (TS) when compared to the general population. The aim of this study was to assess the prevalence of CD among a group of Brazilian females with TS, since to the present no such survey has been performed in Brazil. **Methods:** Fifty-six females with TS from Brasilia - Federal District (Midwest Region of Brazil) on gluten-containing diet were screened for CD utilizing immunoglobulin A antiendomysium (IgA-EMA) and immunoglobulin A anti-tissue transglutaminase (IgA-tTG) antibody assays and they were also genotyped for CD human leukocyte antigen (HLA) predisposing alleles. Patients showing positivity on serological testing were offered to perform small intestine biopsy for histological confirmation. **Results:** Mean age at diagnosis of TS was 5.5 ± 4.4 years; mean age at CD screening was 17.0 ± 9.3 years (range: 10 months to 52 years). Two symptomatic girls were positive for IgA-EMA and IgA-tTG and they also presented CD predisposing HLA-DQ2 alleles. One of the girls (karyotype 45,X) had the diagnosis of TS at 6 months of age and the other (karyotype 45,X/46,XX) at birth; CD was confirmed by jejunal biopsy at 10 months and at 7.5 years of age, respectively. This figure (2/56) represents a 3.6% prevalence of biopsy-proven CD in this group, which is ten times higher than that among females from the general population of the same geographical area, which is 0.34%. **Conclusion:** This outcome provides additional support to an association between these two disorders and reinforces that females with TS constitute a high risk population for developing CD, what may be acknowledged as a rationale for such screening.

LB-PO3-009 Late Breaking Submissions

Spontaneous puberty in Mexican Turner syndrome patients

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Objective

Describe karyotype and clinical characteristics of patients with Turner syndrome who presented spontaneous puberty development.

Cases description

From a total of 137 Turner patients only five presented spontaneous puberty.

Case 1: Mother's karyotype 46,Xt (X;20), The karyotype of the patient was 45,X/46,Xp-. Thelarche 12 years 10 months, pubarche 15 years, and menarche at 16 years 7 months, with breast Tanner-4 and pubic-2. At 17 years menstrual irregularities, followed by secondary amenorrhea and elevation of LH and FSH.

Case 2: Karyotype shows 45,X/46,X,rX, and hers mother has a normal karyotype. Thelarche and pubarche began at 10 years 6 months and menarche at 13 years 5 months, with mammary and pubic Tanner 4. One year lather she began with oligomenorrhea and no ovulation menstrual cycles.

Case 3: Karyotype 47,XXX/45,X. Mother's karyotype 46,XX/45,X/47,XXX, Pubarche and thelarche at 9 years and menarche at 10 years 8 months with breast and pubic Tanner-3. Currently she has regular menstrual cycles and progesterone levels for a physiological ovulation.

Case 4: Karyotype 46,XX45,X/47,XXX. Maternal karyotype is normal. Thelarche and pubarche at 9 years and menarche at 10, with Tanner breast-3 and pubic-2. Her menstrual cycles are regular and ovulation was hormonally present.

Case 5: Karyotype was 45,XO/47,XXX. Mother's karyotype normal. Thelarche 10 years, pubarche 11.5 years and menarche 12 years 2 months with breast Tanner stage-4 and pubic-3. Her menstrual cycles are regular and hormonal sequence during menstrual cycle is normal.

Results

All patients had karyotype mosaics, with an average onset of menarche at 150.2 months, thelarche at 120.2 months, and pubarche at 140.4 months. During puberty height increaseded 15 ±2 cm and final height was 17±3 cm below familiar epigenetic stature.

None of the 5 patients have webbed neck, cardiac or renal malformations, neonatal feet or hands lymphedema, or shield-shaped chest, but all present high palate, shortening of 4th metacarpal bone, moderate telethelia, moderate valgus of elbows, and multiple nevi.

Conclusions

Only 3.65% of our Turner girls presented spontaneous puberty, a percentage that is significantly lower than the reported in other studies.

Time elapsed from thelarche to menarche was similar than observer in normal population, and age of onset of menarche, thelarche and pubarche is similar to the general population.

LB-PO3-010 Late Breaking Submissions

Effectiveness of late growth hormone (GH) therapy (≥ 12 years CA) in Turner syndrome (TS)

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Background: Effectiveness of GH therapy in TS presenting late (≥12 years CA) is a matter of debate,[1] and no gain in final height after puberty onset has been reported.[2]

Methods: We analyzed first-year height velocity (HV) data and near-adult height data in 345 TS patients from the Pfizer International Growth Study Database (KIGS) registry who were treated with GH (mean dose, 0.26±0.08 mg/kg/week) after CA ≥12 years, BA ≥11 years.

Baseline characteristcs					
Variable	N	10th %ile	90th %ile	Mean	SD
Chronological age	345	12.56	16.81	14.42	1.62
Bone age	345	11.50	13.50	12.52	0.82
Height (cm)	345	130.00	146.00	137.67	6.51
Height veocity (cm/year)	129	1.30	5.46	3.40	1.69
Dose (mg/kg/week)	345	0.14	0.35	0.26	0.08

Table 1

Results: Baseline HV was 3.40±1.69 cm/year. First-year HV on GH was 5.51±1.92 cm. The increase in HV of 2 cm over baseline is lower than recently published first-year HV targets for patients with TS in the US-based NCGS database (fig 1).[3] This database, which included 370 TS patients of similar age at treatment onset, showed a first-year HV of 7.2 cm/year. The mean GH treatment dose in the NCGS database was higher at 0.35 mg/kg/week compared to 0.26±0.7 mg/kg/week in KIGS).

First Year GH Data					
Variable	N	10th %ile	90th %ile	Mean	SD
Chronological age	345	13.54	17.88	15.42	1.62
Bone age	225	12.00	14.00	12.82	1.06
Height (cm)	345	134.60	152.00	143.16	6.65
Height veocity (cm/year)	313	3.24	7.74	5.51	1.90
Dose (mg/kg/week)	333	0.16	0.37	0.27	0.09

Table 2

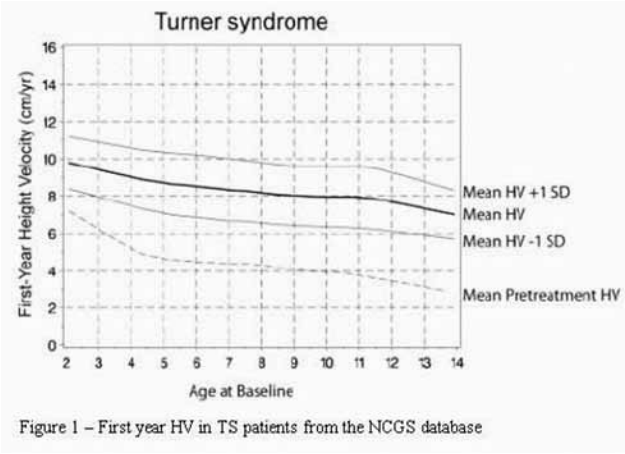
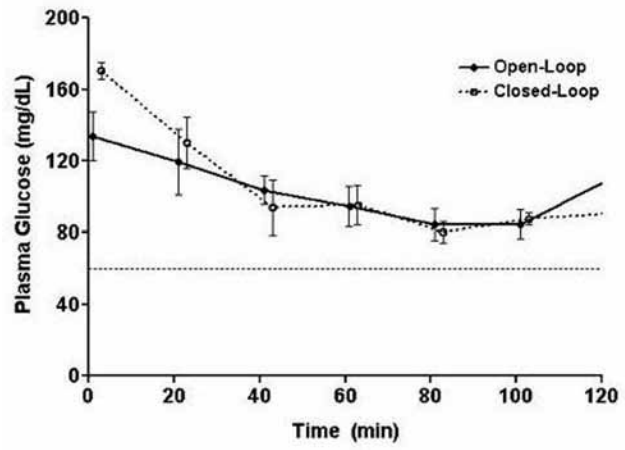


Figure 1 – First year HV in TS patients from the NCGS database



Conclusions: While there is still a significant increase in HV, the demonstrated GH dose effect suggests that physicians opt for a higher GH dose in older TS patients to maximize treatment outcome. Near-adult height data in 28 (45, X syndrome) late-starting patients (mean years on therapy, 3.96±0.66 years) show a syndrome-specific near-adult height SDS of 1.51±0.95. This clearly indicates that late initiation of GH therapy in TS shows robust height improvement that might be even more efficacious if a higher GH dose is used.

In five instances, oral carbohydrates were given for actual or impending hypoglycemia in the OL group, compared to two in the CL group. To date, automated CL control has shown promise in minimizing hypoglycemia during exercise in adolescent / young adult subjects with T1D without requiring manual pump suspension. Further studies are ongoing to determine whether CL control is effective in minimizing immediate and subsequent nocturnal hypoglycemia after exercise in youth with T1D.

LB-PO3-011 Late Breaking Submissions

Glucose control during exercise using a closed-loop artificial pancreas: preliminary results

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Children with type 1 diabetes (T1D) are at an increased risk for hypoglycemia during and immediately following exercise. We previously demonstrated an approximate 50 mg/dL fall in plasma glucose and 25% chance of hypoglycemia during a 60-minute treadmill test in youth with T1D using traditional open-loop (OL) insulin pump therapy. Avoidance of hypoglycemia was improved by manually suspending the insulin pump during exercise. Recently-developed closed-loop artificial pancreas systems, in which insulin delivery is automatically modulated every minute by real-time continuous glucose sensor data, have shown promise but have not been tested under exercise conditions. We have so far evaluated four subjects (age 13-26 y, mean HbA1c 7.5%, with T1D duration 2-12 y), during a 3-day closed-loop (CL) protocol that includes a structured 60-minute treadmill exercise session. Plasma glucose levels fell from mean baseline of 171±10 mg/dL to mean nadir of 70±9 mg/dL during CL, compared to a fall from 134±27 to 68±3 mg/dL during an identical OL protocol. Glucose excursions during the exercise session are illustrated below (closed-loop dotted line, open-loop solid line, hypoglycemia defined as 60 mg/dL):

LB-PO3-012 Late Breaking Submissions

Childhood immunizations and B-cell autoimmunity in the DAISY cohort

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We investigated whether the age or the number of vaccinations received during the first year of life are related to B cell autoimmunity.
Methods: Vaccination records of 1295 children enrolled in the Diabetes Autoimmunity Study in the Youth "DAISY", who were prospectively followed from birth to detect the appearance of autoantibodies were analyzed.
Records on all first DPT, Polio, HIB and HEPB vaccines were available in 94 subjects who developed autoimmunity (cases) and 1201 subjects with no autoantibodies (controls).
Results: Cases developed autoimmunity at age 54 ± 39 months and controls were followed during 88 ± 51 months, p <0.001. A higher percentage of cases were Non Hispanic White (80% vs. 70% p <0.05), had a first degree relative with Type 1 Diabetes (49% vs. 31% p<0.001), and carried the high risk DR3/4 HLA type (36% vs. 23% p<0.05) than controls.
Cases received their first polio vaccine slightly earlier than controls (2.09 ± 0.27 versus 2.20 ± 1.47 months, p <0.05); however, there was no effect of age of first polio vaccine on age of development of autoimmunity in adjusted analyses. There was no effect of any vaccination age or total doses of vaccine received before 6 and 12 months. Neither HEPB vaccine received during or after the neonatal period, nor Intramuscular instead of Oral Polio Vaccine were related to autoimmunity. In all models, a family history of Type 1 Diabetes and the presence of the high risk DR3/4 HLA genotype was associated with a greater risk of autoimmunity p<0.001.
Conclusion: Our study could not establish that age or number of doses of any vaccine received the first year of life is related with islet autoimmunity. Autoimmunity was significantly associated to DR3/4 HLA and family history, supporting the genetic predisposition to develop beta cell autoimmunity.

LB-PO3-013 Late Breaking Submissions

CSII (continuous subcutaneous insulin infusion) makes to increase a height velocity without glucose control improvement

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A case of the Type 1 diabetes mellitus that height velocity was marked increment after CSII introduction
[Introduction] It is known that growth in the child and adolescent of the Type 1 diabetes mellitus who blood glucose control is poor to recognize affecting that situation. After Continuous Subcutaneous Insulin Infusion (CSII) introduction, some case's growth was promoted following improvement of the blood glucose control were reported.
[Case] 14 years old boy. Chief complaint:short stature, the poor blood glucose control: Patient has developed Type 1 diabetes mellitus since 6 years old years and performed insulin therapy 4times a day, But HbA1c was always 9-10% level and aimed for compliance improvement of the Insulin and improvement of the blood glucose level and introduced CSII from 13 years old six months. Before the CSII introduction, HbA1c had kept 9-10% level and no significant change. Before, from the diabetes onset, height increase rate that was always 2 - 4cm/ year showed a growth rate showed +6SD of the standard velocity with 11cm/ year after CSII introduction. Though the spermary capacity started in 4ml and adolescence is not so progressive ateter CSII start one year ago.
[Consideration] Poor control case of type IDM, it is known for a patient of the disease that growth disturbance is obviously, but it is not clarified in the cause. There is the report with the IGF-1 production fall with the liver without being concerned with GH increase at the time of the blood glucose management exacerbation, and it is thought with the one of the causes. The CSII vnethoel did not have a change in HbA1c either, and the frequency of the hyperglycemia, but the height velocity increased, and that some kind of metabolic pathways concerned with growth were stable was supposed by stability supply of the basics of insulin infusion.
[Summary] The blood glucose control did not improve using the CSII proceeding, but we experienced that increase of the remarkable height velocity of +6SD with CSII.
[Concluding remarks] Because of CSII could supply basic insulin in a stable state, possibility to have a good influence was suggested for growth. The blood glucose control did not accept improvement as defectiveness, after CSII introduction, in this case height increase rate is remarkable improved.

LB-PO3-014 Late Breaking Submissions

Eating disorders and type I diabetes in adolescents – the tip of a very large iceberg

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It is estimated in several studies that 10-40% of females with Type I diabetes mellitus manipulate their insulin doses in order to lose or maintain weight. Bingeing is common, although typical purging methods are not utilized. Rather, intentional underdosing of insulin is utilized. Hemoglobin A1c levels > 10% are common and diagnostic in many cases. With the recent implementation of a residential treatment program for patients with diabetes and disordered eating at our center, it has become apparent that our patients are presenting with classic disordered eating (bulimia, anorexia nervosa) and are not misusing insulin as a part of their disorder. Hemoglobin A1c levels in these patients have been <10%. Generally, these patients are very conscientious of the long term effects of high blood sugars and use higher than average amounts of insulin to cover the food consumed during bingeing. This results in a very erratic pattern of blood sugars but with a reasonable overall blood sugar average or Hemoglobin A1c. These unremarkable A1c's do not instigate medical inquiry into or about eating disorders. Instead they may result in insulin adjustments, additional diabetes education or dietary consults all of which miss the mark and leave the eating disordered patient demoralized and isolated with her shame-based eating disorder an on-going and untreated secret.
The primary eating disorder in our patients is bulimia; however, some elements of restriction have been seen as well. Similar to patients without diabetes, these patients with bingeing and purging typically are of normal or slightly increased weight, and do not have bradycardia, orthostatic hypotension, hypothermia or lanugo. Menses may be abnormal. The incidence of classic disordered eating and Type I diabetes may be significantly higher than previously thought and more difficult to diagnose as the level of blood sugar control based on HbA1c may not be diagnostic. Little data is available to determine the rate of long term complications (nephropathy, neuropathy, retinopathy, heart disease) in this group of patients and how disordered eating may impact these. We present data on our patients in the residential treatment center to increase awareness of this combination of medical issues and offer strategies to screen and identify patients with an eating disorder and diabetes.

LB-PO3-015 Late Breaking Submissions

IL-12 and IL-18 serum levels and secretion pattern in juvenile patients with both diabetes mellitus type 1 and asthma (T1DM)

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Background: We recently demonstrated that patients with both Th1 mediated (type 1 diabetes mellitus, T1DM) and Th2 mediated (asthma) autoimmune diseases express a unique Th1 and Th2 cytokine pattern, different from patients with one disease only and healthy controls. Our aim was to determine the pattern of expression and production of the regulatory cytokines IL-12 and IL-18 in these patients.
Methods: Peripheral blood mononuclear cells (PBMC) were collected from 44 patients mean age 19.4± 4.7years, (10.5-28 years) divided to 4 paired groups: T1DM and asthma, asthma only, T1DM only and healthy controls. IL-12 and IL-18 mRNA expression from un-stimulated PBMC were determined using RT-PCR. IL-12 and IL-18 in serum and following stimulation of PBMC by LPS were determined by ELISA. Data was correlated to previously described clinical characteristics and Th1/Th2 cytokines IL-2, IL-4, IL-13 and INFγ levels.
Results: Patients with both T1DM and asthma but not patients with one disease only, had significantly higher serum levels of both IL-12 and IL-18 compared to controls: 146.2±69.2 pg/ml and 109.7±34.6 pg/ml, p=0.038 and 436.1±117.9 pg/ml, 320.2±99.1 pg/ml, p=0.028, respectively. Their peak IL-12 secretion was significantly lower compared to patients with asthma only, diabetes only and controls after 18 hours: 768.1±480.3 pg/ml, 2955.7±3500.1 pg/ml, 3188.1±2692.9 pg/ml, 2400.6±3329.5 pg/ml and after 48 hours : 956.3±489.3 pg/ml, 3405.6±3954.2pg/ml, 3544.4±2891 pg/ml, 3101.1± 4094pg/ml respectively, p<0.05 for all. No significant difference was detected in IL-18 and IL-12 mRNA expression between the groups.
Conclusion: Patients with T1DM and asthma demonstrate a different pattern of IL-12 and IL-18 regulatory cytokine secretion compared to healthy controls and patients with asthma or T1DM only.

LB-PO3-016 Late Breaking Submissions

The affect of carbohydrate counting ability on glycemic control of type 1 diabetics

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Objectives: We compared the ability of type 1 diabetics to accurately count carbohydrates. The ability was assessed by administering a short quiz, which was then scored. These scores were then analyzed and compared to the glycosylated hemoglobin (hgbA1c) for each respondent. We hypothesized that the more adept carb counters will have better HgbA1c.
Patients and Methods: During a visit with the pediatric endocrinologist, pediatric type 1 diabetics were asked to voluntarily participate in a survey and subsequent quiz which would help us ascertain demographic information of the patient, and quiz then on their ability to correctly count the number of carbo-

hydrates in a typical meal. The diabetics in the study were all type 1 diabetics who were either on insulin pumps or multiple daily injections of insulin. Each quiz consisted of pictures of typical meals along with written descriptions of the meal. The patient (of family members if patient was too young or not prepared to count carbs in meals) would then answer multiple choice questions regarding the number of carbohydrates in the meal. Each participant was also asked to complete a survey which asked basic demographic questions regarding patient age, gender, insulin administration (MDI vs Pump), and who does carb counting (parents, child or both).
Results: Our study shows a direct correlation between the ability to correctly count carbohydrates and a lower HgbA1c. We analyzed statistics in several categories based off our questionnaire. We broke down our data in several areas such as age groups, gender, MDI vs Pump, and parents vs child counting carbs. These areas were then analyzed with regards to their score on the quiz as well as their HgbA1c.
Conclusion: In terms of our analysis of age ranges the younger groups tended to have a lower HgbA1c as well as a higher score on the quiz. Patients on the insulin pump too had a better HgbA1c and better test scores as well. We were also able to conclude that patients who counted carbs together with parents had a better HgbA1c and better test score.

LB-PO3-017 Late Breaking Submissions

Growth in children with type 1 diabetes mellitus

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Objective
Determine growth and development pattern and its disorders in patients with DM-1.
Materials & Method
We included all patients with DM-1 with more than 6 months of follow-up. Age, gender, age at onset were compared with clinical, anthropometrical and biochemical variables analyzed quarterly: age, time of evolution, weight, height, growth velocity, Tanner puberty score, glucose, HbA1c, cholesterol, and triglycerides.
Differences in age at diagnosis, current age, pubertal stage, years of evolution as well as HbA1c, cholesterol and triglycerides averages were paired and analyzed for sex by T test for parametric variables and by Mann-Whitney U test for non-parametric variables. Anthropometrical parameters were compared with Mexican normal growth study. We also compared the dependent variables to identify factors associated with acceleration and deceleration of linear growth with X^2 test, Fisher's test and Spearman correlation. Multivariate analysis was used to identify the most important factor in the slowdown of linear growth and short stature with SPSS software version 10. Considered statistically significant a value less than 0.05
Results
264 patients were included: 116 (43.9%) male and 148 (56%) female, with an average age at diagnosis of 8.5±4.0 years and a mean of 6.1 years of evolution. Comparing our results with Mexican data, there are no significant differences at diagnosis in height or weight in boys, while in girls there is a trend to have a shorter height and higher weight, but comparing the diabetic patients, height at diagnosis was lower in girls than boys (-0.54 vs. 0.14 SD), and weight higher in boys than girls (0.8 vs. 0.2 ED). During evolution the height correlated inversely with HbA1c ($r = 0.8$, $p = 0.000$) while weight correlated with height ($r = 0.955$, $p = 0.000$). Z score of height decreased during follow-up while weight and BMI did not change significantly. Growth velocity in the group that maintain constant HbA1c <9% increased significantly after the seventh year of evolution compared with the group with constant HbA1c ≥ 9% (6.9±2.3 vs. 4.4±2.1cm/year) ($p = 0.003$) respectively. We found no significant correlation between growth velocity and changes in mean annual HbA1c if it maintained values lower o higher than 9%. When the patients reached final height, 5.1% of them had short stature.
Conclusion
Metabolic control has inversely correlated with growth velocity and final height in patients with type 1 diabetes mellitus.

LB-PO3-018 Late Breaking Submissions

Thyroid function in children with type 1 diabetes mellitus

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Precedents
Type 1 autoimmune diabetes mellitus (DM1A) is associated with other autoimmune diseases like Hashimoto thyroiditis (HT) (26.6%), celiac disease (6%), Addison syndrome (0.5%), and pernicious anemia (2.6%). The association between DM1A and HT is widely known, and the prevalence of positive antithyroid antibiodies is variable from 8 to 44%. The thyroid malfunction in patients with HT can manifest with subclinical hypothyroidism that affects metabolic control.
Objective
To determine the alterations in thyroid function and its impact in metabolic control in children and adolescents with DM1A
Material & Method
Transversal, descriptive and retrospective study in which was included 170 patients with DM1A.
In all the patients thyroid function test, including tyroglobulin, tiroglobulin antibodies, and thyroid peroxidase antibodies were performed twice a year.
Results
92 women (54.1%) and 78 men (45.9%) were studied. Normal thyroid function test was present in 41.1%. 100 patients (58.82%) were diagnosed with thyroid disease: thyroiditis in 43 patients (25.3%), and simple goiter in 57 patients (33.52%). In 11 of the 43 patients diagnosis of thyroiditis was demonstrated by fine needle aspiration biopsy (low cellular infiltration thyroiditis in 10 patients (5.9%) and Hashimoto thyroiditis in 1 patient (0.59%).
The thyroid malfunction was observed since the beginning of DM1A, as the prevalence of goiter at initial evaluation was 33.52%, and thyroiditis was present in 25.29% of patients. The presence of thyroid malfunction is associated with significant higher initial values of HbA1C in both sexes ($p=0.01$).
Thyroid malfunction is more frequent in older age at diabetes diagnosis, and with longer duration of DM1A in both sexes ($p=0.000$).
Conclusions
Systematically test thyroid function must be realized in patients with DM1A since diagnosis and then annually, in order to detect subclinical thyroid malfunction.

LB-PO3-019 Late Breaking Submissions

Potent non-IGFBP-3 binding analog of humanin improves glucose homeostasis in diabetic rodent models

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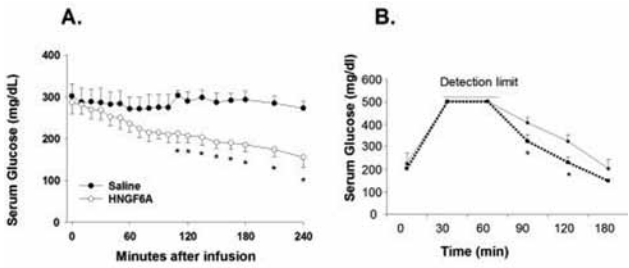
Humanin (HN) and its potent analogs are recognized for their neuro-protective role against AD-related neurotoxicity and other neuronal insults. HN when infused into the third ventricle dramatically improves hepatic and peripheral insulin sensitivity via the hypothalamus (ADA 2006). A potent, non-IGFBP-3 binding analog of HN, HNGF6A, when infused intravenously significantly improves insulin sensitivity. This is suggested by higher glucose infusion rates, lower hepatic glucose production and increase in skeletal muscle glucose uptake during hyperinsulinemic-euglycemic clamps.
We studied the effects of HNGF6A in 2 diabetic rodent models. Indwelling catheters were inserted into the right internal jugular vein and the left carotid artery in 3 month old, diabetic Zucker Diabetic Fatty (ZDF) (Harlan). Upon recovery, chronically catheterized rats were studied while awake, unrestrained and unstressed. 100ug HNGF6A bolus (0.5ug/uL) (*n=5) or 200uL saline (n*=4) was infused into the carotid artery. By venous sampling, glucose levels were determined (One Touch Ultra, LifeScan Inc.) for 4 hours. ZDF rats that received HNGF6A demonstrated a significant decrease in blood glucose by 90 minutes, and this persisted for the remainder of the study (Fig A, $p<0.05$).

Another diabetic mouse model, NONcNZO10/LtJ mice (Jackson Laboratory, Bar Harbor, ME), were injected IP with HNGF6A 25 microgram twice a day for 14 days. Mice were subjected to 6 hour fast followed by IP GTT on D15. Baseline glucose measurement was taken and the mice were injected IP with 0.5mg/g glucose. Glucose was sampled at 15, 30, 45, 60, 90, 120 min. Mice treated with HNGF6A displayed significantly better glucose tolerance; the glucose levels at 90 and 120 min were significantly lower than controls (Fig. B, $P<0.05$).

Our data demonstrates that treatment with HNGF6A lowers blood glucose levels in diabetic rodents. HNGF6A could be a potential therapeutic option for treatment of insulin resistance and type 2 diabetes.



LB-PO3-020 Late Breaking Submissions

The unbalanced equilibrium between insulin sensitivity and secretion predicts the development of IGT and T2D in obese children and adolescents

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Background: The relationship between insulin resistance and secretion is quantitatively described by the disposition index (DI), which is derived from a non-linear hyperbola-like curve and provides an integrated picture of both insulin sensitivity and insulin secretion. The aim of the present study was to evaluate whether the OGTT derived DI is associated with a higher risk of IGT and T2D in obese children and adolescents and whether it can be of any help in predicting the future development of IGT/T2D.

Subjects and Methods: We studied 1247 obese (mean age 13.0, 95%CI 8.3-17.6; mean z-score BMI 2.4, 95%CI 1.7-2.9) and 43 non obese (mean age 15.5, 95%CI 9.7-21.0; mean z-score BMI 0.3, 95%CI -0.9-1.0) children and adolescents, all of them underwent an oral glucose tolerance test (OGTT) and the DI was calculated by the product of the insulinogenic index (IGI) and whole body sensitivity index (WBISI, Matsuda Index). Ninety one NGT obese subjects were followed up for about 3 years (mean follow-up time 3.2; 95%CI 1.3-6.8). At baseline, out of the 1290 studied subjects, 217 obese (131 females; 101 Caucasians, 52 African Americans, 64 Hispanics) were IGT, and 33 obese (24 females; 10 Caucasians, 19 African Americans, 4 Hispanics) showed T2D; while of the subjects followed up 23 (15 females; 6 Caucasians, 6 African Americans and 11 Hispanics) became IGT and 2 (both African Americans males) developed T2D.

Results: The area under the Receiver Operator Characteristic (ROC) curve for the DI was 0.85 (95% CI 0.82-0.87) indicating a DI threshold of 5.8, as that which maximized sensitivity and specificity to identify IGT/T2D patients. Logistic regression analysis on the cross sectional group showed that a DI <5.8 is associated with about 14 times higher risk of having IGT/T2D (OR 13.922; 95% CI 9.060-21.393). Moreover, NGT subjects with a DI lower than 5.8 at baseline showed a three times higher risk of developing IGT or T2D at follow-up (OR 3.4; 95% CI 1.197-9.453) independently of their age, gender, ethnicity and BMI changes.

Conclusions: Our results suggest that the DI can be useful not only to study the physiopathology of glucose metabolism, but also as a measure to predict those obese children and adolescents at risk for future development of IGT or T2D.

LB-PO3-021 Late Breaking Submissions

The correlation between sulcular glucose blood level and capillary glucose blood level

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Background & Aim: Diabetes mellitus is the most common metabolic condition.

This Disease is not diagnosed in approximately 1/2 of the patients who suffering from the disease. Since, the prevalence of diabetes mellitus in patients with periodontitis is more in comparison to the periodontally healthy subjects; a high number of patients with periodontitis may have undiagnosed diabetes mellitus. The aim of this study was to evaluate the correlation between capillary glucose blood level and sulcular glucose blood level. In this study the blood that was gain from gingival tissue during routinely periodontal examination was used for determining sulcular glucose blood level.

Methods & Subjects: Thirty non-diabetics and thirty diabetic patients with moderate to severe periodontitis were included and subjected for the routinely clinical periodontal examination. Blood oozing from gingival sulcus of anterior teeth following periodontal pocket probing was collected with the stick of glucose self monitoring devices. As control, finger stick capillary blood was taken.Statistical analysis was performed by pearson,s correlation coefficient and t test.

Result: The patients glucose blood levels ranged from 58 mg/dl to 477mg/dl and the value of blood samples taken from gingival sulcus or finger tip showed a very high intra patient correlation ($r = 0.99$, $p < 0.0001$). The results suggested that blood oozing during routinely periodontal examination may be used for diabetes mellitus screening in dental clinics.

Conclusion: Since there is correlation between sulcular glucose blood level and capillary glucose blood level, may we can use this method for screening diabetic patients in dental clinic.

Read By Title

R-01 Adrenal

Final height in congenital adrenal hyperplasia associated with central precocious puberty

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Congenital adrenal hyperplasia (CAH) is a disorder of adrenal steroidogenesis. More than 90% of CAH are caused by 21-hydroxylase deficiency (21OHD) that presented in two forms: classic form, including saltwasting and simple virilising form, and non classic form.
One of the primary aims of 21OHD therapy is to guarantee a normal prepubertal and pubertal growth and to reach a final height (FH) similar to the genetic potential. In spite of this, short stature is a common feature in CAH and earlier diagnosis/start of therapy gives better height outcome. Non classic form of CAH is characterized by late onset and so in this group occur the most significant loss of FH.
Here we describe a boy with not salt wasting 21 hydroxylase deficiency, first child of not consanguineous healthy parents. He was born at term, by normal delivery after an uneventful pregnancy. When he was 6 year old he presented pubarche (PH3) and penis enlargement, with prepubertal testis (2 mls), and when he was 7 he was seen in our Clinic. At that time his weight was on the 97th centile, height more than the 97th centile, bone age corresponding to 13 years. CAH due to 21-hydroxylase deficiency was diagnosed with 17OHProgesterone measurement after ACTH stimulation test (52 ng/mL). Treatment with glucocorticoids was started. After screening for CYP21 gene mutations, his genotype showed compound heterozygosity for I172N (paternal allele) and IVS2-12A/C>G (maternal allele). This genotype determined a residual enzymatic activity of about 2%, and was concordant with the phenotype of non classic CAH.
When the boy was 7 year 10 month old he had central precocious puberty, characterized by: testis enlargement (5 mls bilaterally), increased growth velocity (7.3 cm/year) and LH peak of 12.6 mU/L after LHRH test, therefore GnRH analog was started. In spite of this, when the boy was 10 year 6 month old he had fusion of the metaphysis. His final height was 158 cm, against the genetic target of 168 cm.
This case give evidence to the fact that non classic CAH is cryptic, and late diagnosis/start of therapy can lead to significant loss of FH, as predicted by very advanced bone age. Moreover early activation of the hypothalamic-pituitary-gonadal axis, probably induced by the high androgenic levels that lasted a long time before puberty, can happen. Even if treatment with GnRH analog is quickly started, puberty progression can be stopped but it is very difficult to avoid low final height.

R-02 Adrenal

Paediatric coticosurrenaloma: a case report

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The occurrence of corticosurrenaloma in patients bearing a mutation of tumor suppressor gene P53 is classical in adults whereas poorly documented in children.
This case reports a 3-½-year-old girl who presented an adrenal tumor diagnosed on recent virilisation signs. Clinical examination noted a regular growth with a height of +1.5 SD, a pubertal score of P2S1A2 with advanced bone age of 6 yrs. In addition there was a clitoris hypertrophy with a length of 5 cm and erectile properties. There was no clinical nor hormonal signs of hypercorticism. This patient did not present personal or family histories of cancer. The initial assessment found a right adrenal mass (5 x 3,3 x 3cm) without invasion of the vessels nor right kidney, with a sDHEA secretion (initial level of sDHEA > 1000µg / ml and testosterone 120 ng/dl). After a multidisciplinary discussion, surgery was retained without additional treatment. Tumor appeared to be macroscopically very well limited, without no peri tumor adhesion. A total excision and adrenalectomy were performed. **Histology** showed the presence of very large cells with abundant eosinophilic cytoplasm, few mitoses, few atypical cells, no neoplastic embolism. The fragmentation of the tumor due to surgery by laparoscopy could not judge the quality of the surgery which seems macroscopically complete. We noted tumor nodules in pericapsular fat due to the fragmentation process. Sullivan's score was T1 N0 M0 and Weiss's score was 3.
We looked for susceptibility's genes : a P53-gene's mutation (c.374C > T, p. Thr125Met, exon 4) was found in the patient's leukocytes. Six months later, despite the lack of clinical regression of clitoris, biological (SDHEA < 10 µg / ml) and radiological (TEP X Ray: no fixation) parameters were normal.
This finding raises the question about the most appropriate treatment and follow-up of this young girl and the relevance of the family screening.

R-03 Adrenal

Increased growth pattern in the first six months of life in untreated infants with neonatal hyperandrogenism

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Introduction: It is well known that adrenal hypersecretion of androgens causes increased growth velocity even if previous reports suggest that it is not increased in the first years of life. The aim of this study is to investigate the growth pattern in the first six months of life in male children with neonatal hyperandrogenism and compare their data with data of normal boys of the same age.
Patients and Methods: We analyzed 30 male newborns with neonatal hyperandrogenism diagnosed by increased 17-hydroxyprogesterone (17-OHP) levels at neonatal screening for 21-hydroxylase deficiency (21-OHD) and confirmed by high androgen serum levels for the age. Babies affected by classic form of 21-OHD were excluded. For comparison we analyzed 52 healthy boys of the same age with normal 17-OHP values at neonatal screening. All infants were evaluated every three months from birth to six months of life: a complete physical examination was performed and the patients were submitted to blood tests to detect androgens' levels.
Results: Whereas no statistical differences were evidenced at birth for gestational age (38.7 ± 1.3 and 39.1 ± 1.2 weeks, respectively for newborns

with hyperandrogenism and healthy boys), birth weight (3255.0 ± 362.0 g and 3141.4 ± 460.3 g) and length (50.3 ± 1.8 cm and 50.6 ± 2.0 cm) between these two populations of newborns, during the clinical follow-up infants with hyperandrogenism showed a weight (6063.4 ± 673.7 g and 5076.0 ± 711.7 g; $p<0.05$ at the third month of life and 7742.5 ±746.2 g and 6981.9 ±1268.7 g; $p<0.05$ at the sixty month of life) and a length (60.6 ± 1.7 cm and 56.3 ± 3.0 cm; $p<0.001$ at the third month of life and 67.0 ± 1.5 cm and 65.4 ± 3.8 cm; $p>0.05$ at the sixty month of life) more elevated in comparison of controls' population. Androgens' levels decreased during the follow-up for all infants.
Conclusions: Untreated children with neonatal hyperandrogenism in the first six months of life presented an increased growth pattern in comparison with a population of control's boys. We hypothesize that neonatal hyperandrogenism influences the growth pattern already from the first months of life. Continuous follow-ups to be performed also during the childhood are necessary for these babies to confirm this result.

R-04 Adrenal

Testicular tumors in adrenogenital syndrome (AGS): case report

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Introduction: Adrenogenital syndrome due to 21-hydroxylase deficiency is an autosomal recessively inherited defect of steroid hormone biosynthesis, characterized by decreased cortisol, increased adrenocorticotrop hormone (ACTH) and accumulation of steroid precursors. Some male patients may develop clinically detectable testicular lesions, known as testicular tumors of AGS. Histologically, testicular tumors of AGS (TTAGS) consist of focal areas of endocrine cells that become hypertrophied after prolonged stimulation by increased ACTH.
Case report: A 19-year-old male patient suffering from prenatal virilization and salt wasting due to 21-hydroxylase deficiency was treated with hydrocortisone and fludrocortisone since birth. From the onset of puberty he had a poor compliance and repeat hormone measurements indicated high serum 17-hydroxyprogesterone (>20000 pg/ml; normal range, 610-3340), androstendione (>10000 pg/ml; normal range 300- 3100) and ACTH (96.7 pg/ml; normal range 5.4-27.7). Ultrasonography detected bilateral intratesticular hypoechogenic lesions with well-defined margins. During 6 weeks the diameter of the lesions showed a rapid increase in size from 12x9 mm to 35x10mm (right side) and from 9x2mm to 1,2x 3 mm (left side). Serum β-hCG (<1,2 IU/L) and AFP (5,2 IU/ml) were normal. Computed tomography indicated enlarged adrenal glands. Spermatogenesis was intact. Testicular biopsy was considered, but because of the rapid progression of the size of lesions the patient agreed with the urologist's suggestion to perform right orchidectomy. Histological examination of the lesions showed Leydig cell hyperplasia.
Conclusions: TTAGS caused by elevated levels of ACTH may pose diagnostic difficulties both clinically and pathologically. If AGS is not taken into account, a testicular mass is usually assumed to be a malignant lesion and, therefore, orchidectomy is perfomed as in our case.

R-05 Adrenal

Is alpha-feto protein involved in pathogenesis of infantile genital hair?

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Background: Although infantile genital hair is rare and benign condition, parents are worried about its etiology. Its etiopathogenesis has not been well understood. Recently, adrenal steroid levels were found in normal limit. Here I report five infants (three girls, two boys) aged between 3 and 10 months who had genital hair.
Methods: Serum prolactin, testosterone, 17-hydroxprogesterone (OHP), LH, FSH, estradiol, AST, ALT, beta-hCG and alpha-feto protein (AFP) levels obtained. Abdominopelvic and scrotal ultrasonographic (USG) examination were performed. None of the infants had any medication. All mothers were inquired about menstrual irregularities and gestational hirsutism.
Results: Congenital adrenal hyperplasia was excluded via performing ACTH stimulating test in a boy with higher 17-OHP. GnRH test was performed in three infants (2 girls and 1 boy) with higher LH levels and central precocious puberty was excluded. AFP levels of the all infants were increased for their age (16-57.9 ng/mL). Beta-hCG, AST and ALT levels were normal. USG examination was normal. None of the mothers have hirsutism and menstrual irregularities. One mother had infantile pubic hair. All infants have been followed for 6-12 months. Serum AFP levels were obtained in 3-months intervals. Genital hair of the four infants disappeared in conjunction with decreasing in serum AFP levels. One infant boy still has sparse scrotal hair.
Conclusion: Higher AFP levels may be involved in etiopathogenesis genital hair because of a decrease in its level associated with disappearing genital hair. However, if AFP levels of the infants with genital hair are measured, relation of genital hair and AFP would be proven.

R-06 Adrenal

Hypercortisolism due to excessive sport?

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We report on a 16 year old girl who presented with short stature and primary amenorrhea.
She was very sporty with several hours of sport every day (soccer, swimming, jogging, skipping). She didn't take any medicine, particularly no hormonal contraceptive.
During primary school she was relatively tall, later she grew worse.
Height was 151,6 cm (< 3rd perzentile), weight 47,5 kg (< 10th perzentile), bone age was 14 years, prospective final height 154,2 cm (MPH 161 ± 8,5 cm).
Pubertal development started with 12 years, Tanner stages B5 P5, but no menarche. Since she was 14 years old she had a round, red face, but no other clinical signs of Cushing syndrome.
Ultrasound of the internal genitals and the adrenals showed no pathology.
Hormonal evaluation showed basal low levels of gonadotropins und estradiol but good increase after stimulation with an LHRH analogon (Buserelin).
We found normal values for TSH, fT4, testosterone and prolactin. 17-OH-Progesterone was slightly elevated, the ACTH stimulation test revealed an abnormal increase. Sequencing of the 21-hydroxylase-gene however showed no mutations.
On all visits she had high levels of cortisol und ACTH. In timed urine sampling the cortisol excretion was markedly elevated. So we performed a 15h Cortisol- and ACTH- diurnal profile (8 -23 h) which showed highly elevated levels of Cortisol and ACTH that even increased in the evening hours. In a dexamethason suppression test with 1 mg suppression was poor, but sufficient with 2 mg.
In the cranial MRI there was no indication for an ACTH-producing adenoma of the hypophysis. The 24h-blood pressure monitoring revealed hypertonia but normal day-nigh-pattern. ECG and Echocardiography were normal. Hypertonia was treated with an ACE-inhibitor.
Although this patient is no competetive athlete we finally think that the high levels of cortisol and ACTH were related to the very intensive training this patient did as it is known that cortisol levels can rise in female endurance athletes as a physiological adaption to maintain adequate blood glucose levels.
So we recommended a life-style-change, i.e. less sporting activities, in order to reduce cortisol levels. The follow up data will be reported.

R-07 Adrenal

A case of X-linked adrenal hypoplasia congenita with central precocious puberty

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X-linked adrenal hypoplasia congenita (AHC) is caused by DAX-1 mutations and can occur as part of a contiguous gene deletion syndrome in association with glycerol kinase deficiency, Duchenne muscular dystrophy and IL1RAPL1 gene deficiency which is one of the multiple X-linked gene for nonspecific mental retardation. It is usually associated with hypogonadotropic hypogonadism, but central precocious puberty or even normal puberty have rarley been

reported.
Here I report a case of a six-year-old boy with X-linked adrenal hypoplasia congenita who has complete deletion of NR0B1, GK and IL1RAPL1 genes (Xp21 contiguous gene deletion syndrome) with central precocious puberty. Initially he was admitted for the managemnet of adrenal crisis at the age of 2 months and then managed with hydrocortisone and florinef. At 45 months of age, he was noticed to have testicular volumes of 4mL bilaterally, a penile length of 5cm, with Tanner stage 2 pubic hair. The LHRH stimulation test showed a LH peak of 8.26 IU/L, confirming central precocious puberty. During the treatment with GnRH agonist (Leuprolide acetate) for 1 year, the pubertal changes were regressed and after the temporary discontinuation of the injection for 6 months, the pubertal state was progressed. He is continuously on the treatment with GnRH agonist as well as steroid replacement.

R-08 Autoimmunity; Perinatal Endocrinology; Perinatal Programming

A case of panhypopituitarism compared with his normal twin brother

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Aims: We report a 29 2/12 year-old male case of idiopathic panhypopituitarism compared with his normal twin brother.
Methods: The laboratory and radiologic investigation with the cocktail test were done on the male complaining of short stature.
Results: He was born prior to his normal twin brother with some difficulties at home. His height was 151.5cm(<3 percentile), weight 49kg(<3 percentile) with Tanner stage I status. His father was 175cm, mother was 155cm and his normal twin brother was 173cm tall. His bone age was 14.8 year-old and the pituitary gland was small on MRI. T3 94.46ng/dL, T4 4.3µg/dL, TSH 6.08µIU/mL, IGF-1 96.0ng/mL, IGF-BP3 857ng/mL and testosterone was 0.1ng/mL. Mutation analysis of the transcription factors for pituitary formation including PROP1, Pit1, LHX3, Hesx1 and SOX3 was normal. The cocktail test results was as below on the tables.

Table 1. Cocktail test (at 29 2/12 year-old)

	GH (ng/mL)	Cortisol (µg/dL)	TSH (µIU/mL)	LH (mIU/mL)	FSH (mIU/mL)	Prolactin (ng/mL)
0 min	0.1	2.7	6.08	1.9	1.0	14.5
30 min	0.1	6.6	35.06	4.0	3.6	33.9
60 min	0.1	14.0	29.93	3.6	6.0	29.2
90 min	0.1	8.1	25.84			17.6
120 min	0.1	6.0	19.21			13.0

Table 2. Cocktail test (at 30 10/12 year-old)

	GH (ng/mL)	Cortisol (µg/dL)	TSH (µIU/mL)	LH (mIU/mL)	FSH (mIU/mL)	Prolactin (ng/mL)	ACTH (pg/mL)
0 min	0.1	0.7	0.08	1.2	0.1	5.8	10.8
30 min	0.1	4.4	0.27	2.5	2.4	30.9	51.8
60 min	0.1	9.5	0.25	2.8	3.8	33.3	136.9
90 min	0.1	11.5	0.21			23.6	70.4
120 min	0.1	11.6	0.17			22.8	55.8

Conclusions: We report a rare case of panhypopituitarism showing progressive loss of TSH response (on the coctail test) compared with his normal twin brother.

R-09 Bone, Calcium; Growth Plate

Solitary parathyroid adenoma presenting with hypercalcemic crisis in a pediatric patient

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Introduction:
Solitary parathyroid adenoma is a rare cause of primary hyperparathyroidism in children. While a common, more readily recognized endocrine disorder in adults, hyperparathyroidism in children often presents with vague complaints that persist for months before the diagnosis is made, often times with associated morbidity.
Methods:
We describe a 12 year old male with hypercalcemic crisis in the setting of a solitary parathyroid adenoma.
Results:
Patient presented with 4 months of increasing fatigue and long bone pain, and acute onset intractable emesis. Laboratory assessment showed serum calcium 19mg/dL (normal 8.8-10.1mg/dL), phosphorus 2.3mg/dL (3.3-5.4mg/dL), uric acid 9 mg/dL (normal 2.7 – 6.7mg/dL), creatinine 1.1 mg/dL (normal 0.2 – 0.5mg/dL). In context of vague, generalized complaints, a preliminary working diagnosis of malignancy was made. Subsequent laboratory investigation revealed iPTH 385pg/mL (normal 9-52 pg/mL), PTHrP <1.5pmol/L. Neck ultrasound showed a focal, solid hypoechoic, ovoid structure posterior to the left lobe of the thyroid measuring 2.3 x 0.8 x 1.1 cm. The patient underwent parathyroidectomy and pathology showed parathyroid adenoma. Patient's calcium reached a nadir of 7.5mg/dL 48 hours post-operatively.
Conclusion:
The diagnosis of hyperparathyroidism in the pediatric population is frequently delayed, is non-specifically symptomatic, and can result in significant morbidity. Serum calcium and iPTH levels should be checked in children presenting with nonspecific symptoms as parathyroid adenoma resection is curative and usually restores serum calcium levels quickly back to normal, thereby avoiding further complications.

R-10 Bone, Calcium; Growth Plate

Six year-old with autoimmune polyglandular syndrome: can genetics tell us the story?

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Children who have diabetes mellitus type 1 (DMT1) are at increased risk of developing other autoimmune diseases, and can be part of one of the polyglandular autoimmine syndromes. These associated diseases include Hashimoto's thyroiditis, Graves' disease, celiac disease, and Addison's disease. Since Addison's disease is potentially fatal if undiagnosed and untreated, it would be prudent to effectively screen individuals to determine if they are at risk of developing this disease. We present a case of a 6 year-old male with a history of DMT1, who presented in adrenal crisis and was subsequently diagnosed with Addison's disease. HLA-DRB1 404/DR4 is one of the genes involved in the development of Addison's disease in children with DMT1. Our patient later tested positive for this haplotype. Genetic testing is not routinely done in patients with DMT1 to determine if they will potentially develop other associated conditions. We propose using genetic testing of associated HLA haplotypes to screen children with DMT1 for Addison's disease. This shows the utility of genetic testing as a screen for Addison's disease in patients with DMT1.

R-11 Bone, Calcium; Growth Plate

Growth homone treatment in 5 patients with osteogenesis imperfecta

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Introduction : Biphosphonate treatment has greatly improved the bone status and reduced the occurrence of fractures and bone pain in children with osteogenesis imperfecta (OI). Short stature is often present in OI and growth hormone (GH) treatment can be indicated. We report our experience of GH treatment in 5 patients with OI.
Patients : 5 patients with OI and short stature (height under -2 SDS) were treated with GH in our department : 4 of them had also biphosphonate treatment. GH was indicated because of partial deficiency in 3 cases, or because of small for gestation age (SGA) status in 2 cases. GH dose was 0.23 mg/kg/day.
Results : Growth velocity increased in 4 cases: in 2 cases the growth velocity increased by 9 cm during the first year. It increased by 6 cm in one of these cases after 6 months and by 5 cm in the other case. In one patient growth velocity did not increase. No adverse events were noticed. Bone mineral density was not negatively affected by the treatment.
Conclusion : In case of growth delay in patient with OI, GH treatment can be used and seems efficient without adverse events.

R-12 Bone, Calcium; Growth Plate

Recurrent and worsening hypertrophic callus formation in a 9 years old girl with osteogenesis imperfecta type V

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Osteogenesis Imperfecta Type V is a rare clinical entity. It is a new form of brittle bone disease first reported by Glorieux FH et al in year 2000. Hypertrophic callus formation is the hallmark of Osteogenesis Imperfecta Type V. We would like to report a 9 years old Indian girl with recurrent and worsening hypertrophic callus formation that occured throughout the years with subsequent long bone fractures. There were 3 episodes of fractures with documented hypertrophic callus formations: right humerus fracture at birth, left femoral fracture at 2 year 6 months old and right femoral fracture at the age of 7 year 10 months. Hypertrophic callus formation at birth and 2 year 6 months old only evidence on radiographs but the hypertrophic callus formation that occurred with the fracture at 7 year 10 months old resulting in severe right thigh swelling that persisted to date associated with much pain and reduced mobility in this patient. This case illustrates that hypertrophic callus formation can result in significant morbidity and there is still no sign of callus regression in this patient.

R-13 Bone, Calcium; Growth Plate

Plasma alpha-tocopherol levels in patients with vitamin D deficiency

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Vitamin E have been shown to prevent oxidative stress. Interaction between vitamin D and other fat soluble vitamins was not clear. We planned to investigate alpha-tocopherol levels in in patients with vitamin D deficiency. Twenty patient with nutritional rickets were included to study. Before and after treatment (vitamin D and calcium), serum calcium, phosphorus, 25-hydroxyvitamin D (25(OH)D) and plasma alpha-tocopherol levels were measured. Plasma alpha-tocopherol levels were significantly increase from 4.41 µg/mL to 6.05 µg/mL ($p<0.05$). However, there was no correlation between alpha-tocopherol and 25(OH)D, PTH, Ca, P. Plasma alpha-tocopherol levels were low (<3 µg/mL) in 9 patients (45.0%) before treatment. After treatment 4 patient (20.0%) had still low alpha-tocopherol levels. This observation underlined alpha-tocopherol levels in vitamin D deficiency is parameter for follow-up. Further research is needed to find the role of alpha-tocopherol for vitamin D action.

R-14 Bone, Calcium; Growth Plate

Persistent nonfamilial hyperphosphatasemia as a cause of unexplained increase in plasma alkaline phosphatase

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Aims: Serum alkaline phosphatase represents a group of isoenzymes orginating mainly from bone, liver, intestine, kidney and placenta. Beningn transient hyperphosphatasemia is a syndrome charecterized by increased alkaline phosphatase activity in plasma early childhood who have no eidence of liver or bone disease. The etiology of beningn transient hyperphosphatasemia remains a mystery. The diagnosis of beningn transient hyperphosphatasemia is established after finding a transient but marked elevation of serum alkaline phosphatase level during rontine investigations for other unrelated conditions. Serum alkaline phosphatase levels return to normal in a few months.
Case Report: We report here, the case of 5 yearold male child persistent unexplained elevation of serum alkaline phosphatase to levels 14 times the upper limit of the reference range.
We call attention to this rarely observed condition to avoid unnecessary, extensive investigation of such cases.

R-15 Central Weight Regulation; Pancreas; Type 2 Diabetes, Insulin Resistance

A case of metabolic syndrome in an adolescent: diagnosis and management

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The widespread of obesity in childhood is a rising cause of metabolic syndrome (MS), the prevalence of which is clearly increasing.
We report the case of a 13-year-old boy with severe obesity (BMI> 99°p.le), polyuria, and polydipsia. Physical examination showed abdominal adiposity, acanthosis nigricans (neck, limb-folds and trunk) no sings of Cushing syndrome. Laboratory exams showed fasting hyperglycemia (293 mg/dl), HbA1c 12.8%, negative autoantibody screening concerning type 1 diabetes mellitus and glycosuria without ketonuria. Fasting C-peptide values and after glucose tolerance test were compatible with type 2 diabetes mellitus (T2DM). Lipid state was normal (total cholesterol 147 mg/dl, HDL cholesterol 35 mg/dL and triglycerides 159 mg/dl).
Further specific examinations performed showed: left ventricle hypertrophy, borderline arterial pressure, hepatic steathosis.
The stabilization of glycemic values was achieved with a metformin-based therapy of 500 mg three times daily and 2200 kcal diet.
A month later the blood glucose values improved (average blood glucose of 137 mg/dl) as well as the glycated hemoglobin (reduced to 9.1%) despite the weight had increased by 2 kg. It was therefore advised to continue the metformin-based therapy and to strictly follow the diet with regular aerobic activity.
Biguanides are the only oral hypoglicemic drug allowed for pediatric T2DM. They inhibit hepatic neo-gluconeogenesis and stimulate glucose uptake into the muscle. The resulting effect is an improvement of the glycemic profile, a slight weight reduction, an increase in fibrinolysis, an improvement of the lipid state, and the reduction of liver enzymes in patients with non-alcoholic steatohepatitis.
There is no pediatric study on the combination of metformin and anti-hypertensive drugs. However, indirect positive effects on blood pressure through weight loss and the improvement of metabolic state may be achievable through

metformin-related effects.
The complexity of the association between MS and T2DM, often requires a multidisciplinary approach (monitoring of all possible metabolic risk factors such as arterial pressure, glycemia, basal insulinaemia, HbA1c, lipid profile, and transaminasemia). Further studies are necessary to define new therapeutic approaches. However, the most effective therapy, but also the hardest to pursue involves behavioral and lifestyle changes.

R-16 Central Weight Regulation; Pancreas; Type 2 Diabetes, Insulin Resistance

Gliclazide-induced hypoglycemia: delayed presentation and successful treatment with octreotide

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Overdose ingestion of sulfonylurea drug (SUD) is a hyperinsulinaemic state that causes hypoglycemia. Although there are many reports on intoxication with various SUDs, gliclazide poisonings has been rarely reported. It has been suggested that, if hypoglycemia does not occur within 8 hours of SUD ingestion, asymptomatic children can be safely discharged. We present a case with gliclazide-induced hypoglycemia, which became symptomatic 24 hours after ingestion and successfully treated with octreotide.
Seventeen year-old girl with a history of suicide attempt was brought to the emergency department due to convulsion 36 hours after ingesting 20 tablets of gliclazide (1600 mg) and 4 tablets of citalopram (80 mg), an anti-depressant. Twelve hours prior to admission, she had suffered from hypoglycemic symptoms including feeling of hungry, fatigue, behavioral changes and confusion. Initial finger stick blood glucose was found to be 21 mg/dl. A 2 ml/ kg bolus of 20% dextrose was given and the infusion of 10% dextrose was continued at 100 ml/h. Because of refractory hypoglycemia, dextrose infusion increased to 13% via peripheral venous access. Despite that measure, blood glucose remained below 60 mg/dl. At that time, plasma insulin and C-peptide levels were 49 µU/mL and 9.95 ng/ml (N: 1.1–5.0), respectively. Octreotide (100 µg bolus) was administered intravenously followed by an infusion at 10 µg/h. Blood glucose was 103 mg/dl 1 hour later and remained stable without further symptoms of hypoglycemia. The dextrose infusion was weaned and euglycemia maintained. The octreotide infusion was ceased within 10 hours. Octreotide administration rapidly reversed hyperinsulinemic state without any adverse reactions. After stopping octreotide, hypoglycemia had not recurred until end of the 24 h.
Our patient ingested gliclazide and citalopram, appeared well for 24 hours, but then became symptomatic. Despite the fact that hypoglycemia usually develops within 8 hours of SUD ingestions, there is no sufficient data about gliclazide-induced hypoglycemia. In our patient, accompanying ingestion of citalopram may be a reason that delays the appearance of hypoglycemic symptoms. Consequently, we recommend that children ingesting gliclazide should be observed at least 24 hours. We also suggest octreotide as a first-line therapy together with dextrose for sulfonylurea-induced hypoglycemia.

R-17 Central Weight Regulation; Pancreas; Type 2 Diabetes, Insulin Resistance

Relationship between non-alcoholic fatty liver disease (NAFLD), and IGF-I, IGFBP-3 and DHEA-S in children and adolescence

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The aim of this study is to analyzed relationship between clinical factors associated with development of non-alcoholic fatty liver disease (NAFLD) and IGF-I, IGFBP-3, DHEA-S in children and adolescence.
We enrolled 43 patients who visited our hospital for routine examination or obesity in our study. We measured their anthropometry, blood pressure, bone age and fasting insulin glucose, triglyceride, HDL, transaminase, IGF-I, IGFBP-3, DHEA-S.
We classified subjects into 3 groups; Group 1 (n=18), no fatty liver with BMI less than 85th percentile; Group 2 (n=14), no fatty liver with BMI higher than 85th percentile; Group 3 (n=11), fatty liver with BMI higher than 85th percentile. Chronologic age, bone age and birth weight were not significantly different among 3 groups. Sex ratio was not significantly but the ratio of prepubertal/pubertal subjects was significantly different between fatty liver group and non-fatty liver group. Weight, height, BMI SDS and WC SDS were significantly higher in Group 2 and 3 than Group 1 and not significantly different between Group 2 and Group 3. Fasting insulin, glucose and FIGR, QUICKI were higher in Group 3 than Group 1 and not significantly different between Group 2 and Group 3. Plasma ALT and IGFBP-3 were higher in Group 3 than Group 1 and 2 and not significantly different between Group 1 and Group 2. Plasma TG, HDL, LDL cholesterol, IGF-Iand DHEA-S were not significantly different among 3 groups. Difference between BA and CA was positively correlated with BMI SDS, HOMA-IR and plasma IGF-I.
We suggest that IGFBP-3 may have a role in development of insulin resistance and NAFLD in obese children and adolescence but further studies are required considering other factors that regulate IGFBP-3.

R-18 Central Weight Regulation; Pancreas; Type 2 Diabetes, Insulin Resistance

Familial case of association of multiple endocrine neoplasia I and severe insulin-resistance with type 2 diabetes

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The components of MEN1 are hyperparathyroidism (90% of cases), pituitary adenomas (50%), non-active, as a rule, tumors of pancreatic B-cells, adrenal adenomas or hyperplasia, thyroid cancer (20%), rare concomitant disorders (lung cancer, lipomas, gastric polyposis, etc.) Tumor suppressed gene (11q13) mutations are established to be the cause of MEN1, with production of *MENIN* regulating cell proliferation being impaired. We present a girl, 17 yo, first seen as an outpatient at the age of 14 yo, with obesity (BMI>97p.c.), strong symptoms of insulin-resistance (IR) (WC-108sm, WC/HC 0,95, acantosis nigricans), arterial hypertension (>150/90), Tanner stage 5, and hyperadrogenia. Her mother's past history revealed severe type 2 diabetes since the age of 35 yo. At the examination, severe IR (HOMA = 16,6), type 2 diabetes mellitus (HbA1c=7%), and polycystic ovary syndrome were found. At the age of 15 yo, thyroid papillary cancer was detected and nodular adrenal hyperplasia was diagnosed. The mother was examined, too, and the both diagnoses were present as well. The both patients received combined therapy and are taking suppressive levothyroxine doses. Some months later, the girl complained of headache, and MRI scan revealed pituitary microadenoma (6*7*7). Her mother was also examined, and non-active hormonal adenoma was diagnosed. Furthermore, gastric polyposis and lung cancer were found in mother. During the follow-up period, we registered the severe course of T2DM with progressing complications and non-compensated hyperglycemia despite the modern approaches and drugs. Besides, the severe resistance to levothyroxine has developed in both cases. We conclude that combination of these pathologies can be caused by a dominant mutation, not described yet. The cases need additional genetic examination. The severity and impossible hyperglycemia control can result from the association with MEN I. Further screening of MEN syndrome components is required for the both patients.

R-19 Disorders of Sexual Differentiation (DSD)

Familial case of 46,XY disorder of sex development with normal androgen synthesis and action

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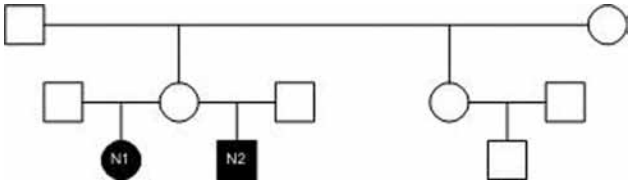
Introduction: A number of affected genes have been detected to cause 46,XY DSD. Nevertheless, molecular diagnosis remains unclear in about thirty percent of patients being searched for all causative-genes mutations.
Objective: To describe a family case of 46,XY DSD with X-linked or autosomal dominant inheritance (fig 1).



Patient N1: A child born to non-consanguineous parents with ambiguous genitalia (Prader III); she was assigned as a female. At the following examination at the age of 10 months the 46,XY karyotype was identified, the ultrasound test has shown the gonads in inguinal canals, the uterus was not found. The girl was suspected to have partial androgen resistance. She has undergone the gonadectomy and feminizing surgery at 5 yrs.
Patient N2: The uterine brother of the patient N1 was born with ambiguous genitalia as well but more virilized (Prader IV) with unilateral cryptorchidism. Assigned as a male the patient has underwent orchiopexy and multiple reconstructive procedures for hypospadia. The boy was referred to our clinic at the age of 11. His height was normal (SD=+0.5), the puberty stage was Tanner II. The data on his hormonal assessment is shown in table 1.

Hormonal evaluation of the 11 year 46,XY DSD boy (N2)			
	Basal	hGH stimulation test	Normal range
LH (U/L)	1.0		
FSH(U/L)	4.3		
Testosterone(nmol/L)	0.6	4,6	0.5-12
Androstendion (nmol/L)	1.2	8	0.6-8,4
Dihydrotestosterone (pg/ml)	35	305	30-170

Mutations in AR gene were not found.
At the next examination at 14 yrs he showed normal puberty with pubertal Tanner stage IV, testis volume 8 and 15 ml and normal levels of LH (3.0 U/L), FSH (9.0 U/L) and testosterone (11.1 nmol/L).
Conclusion: Familial cases of severe hypospadias and 46,XY DSD without defects of androgen synthesis and action are useful for distinguish new molecular mechanisms of this disorder. Mutations in chromosome X open reading frame 6 (CXorf6) gene (OMIM 300758) have recently been detected in patients penoscrotal hypospadias. Searching for CXorf6 gene mutations should be tried in our patients. Other candidate genes involved in the development of male genitalia still remain to be disclosed.

R-20 Disorders of Sexual Differentiation (DSD)

Growth, pubertal development and associated anomalies in 11 patients with XY partial gonadal dysgenesis

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Introduction: XY parcial gonadal dysgenesis (PGD), also known as dysgenetic male pseudohermaphroditism, is a rare cause of disorder of sex development (DSD). Its origin is unknown in most cases, and there are no information regarding growth, pubertal development and associated disorders in these cases. **Aim:** To evaluate the clinical features of pacients with PGD. **Patients and Methods:** Retrospective analysis of the clinical files of 11 patients with PGD reared as males who were diagnosed between 1989 and 1996. In all patients one of the gonads (a testis) was in the scrotum. All patients had been subject to molecular investigation (*SRY, WT1, DMRT1*), with normal results. **Results:** All patients achieved a final height within the growth channel. All had normal pubertal development, despite high gonadotropin levels. Associated disorders were hypothyroidism and mental deficiency (2 cases each), congenital dyserythropoiesis, schizophrenia, unilateral renal cyst and obesity (1 case each). **Conclusions:** Patients with PGD reared as males had normal growth and spontaneous pubertal development, although high levels of gonadotropins indicate high risk of premature gonadal failure. In most cases there was no cognitive deficit, and various associated disorders were found; however, other studies will be necessary to define if these were casual associations.

R-21 Disorders of Sexual Differentiation (DSD)

A rare male phenotype in a patient with 45.X/46.XY karyotype

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Mixed gonadal dysgenesis (MGD) is a rare form of male pseudohermaphroditism. The most frequent karyotype is 45.X/46.XY. The phenotype depends on the ratio of testicular tissue which induces virilization. The most of such patients have a short stature, stigma of Turner syndrome and female external genitalia. Some of patients could be present with different stages of incomplete masculinization. We report a 14-year-old boy, who was brought to our outpatient clinic on account of a short stature with decreased growth velocity and weight gain which started at the age of 5. Upon examination, his height was 134.5 cm (- 3.5 SDS), weight 50 kg (- 0.3 SDS), some Turner stigmata (widened neck and shoulders, increased internipple distance) and obesity (BMI > 97th centile) were revealed. External genitalia were presented with normal penis and unilateral testis of 4 ml (Tanner Stage II). According to the anamnesis the patient was presented at birth with unilateral right inguinal cryptorchidism, ablation of rudimentary testis was performed at the age of 4. The chromosome abnormalities were suspected. The karyotype was 45.X [22]/46.XY [40]. No mullerian structure was found at the ultrasound exam. Hormonal studies showed a prepubertal LH, FSH and testosterone. The patient was admitted to the growth hormone treatment (initial dose 0.05 mg/kg per day) in analogy to girls with Turner syndrome and gonadal dysgenesis. After 6 months of treatment acceleration of growth velocity (6.5 cm) and progress of puberty without worsening of target height was shown. Hormonal studies showed a pubertal serum LH (3.276 mIU/ml), FSH (14.79 mIU/ml) and testosterone (12.12nmol/l) levels. We concluded that unilateral cryptorchidism at birth without other external genitalia abnormalities in boys can be the only sign of incomplete virilization in patients with male phenotype on MGD. Such patients can have normal puberty; their fertility cannot be excluded. Boys with unexplained short stature should be screened with chromosomal analysis at any age. Growth hormone treatment should be started to boys with short stature and 45.X/46.XY mosaicism as early as possible.

R-22 Genetics of Growth; Genetics of Hormone Excess; GH Physiology

The Dubowitz syndrome – is growth hormone therapy an option?

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Backround: The Dubowitz syndrome is characterised by intrauterine growth retardation, severe postnatal growth failure, microcephaly, facial dysmorphism, clinodactyly of the 5th fingers, eczema, and a variable degree of mental retardation.
After its first description by Dubowitz in 1965, it became apparent that in 10 % of the cases the syndrome is associated with hematological or malignant disorders such as leukopenia, agranulocytosis, aplastic anemia, acute lymphoblastic leukemia, lymphoma, and neuroblastoma. The pathogenesis of the Dubowitz syndrome and the true incidence of malignancy and immunologic deficiency in this syndrome are unknown. An autosomal recessive inheritance is postulated, the underlying genetic defect, however, is unknown.
Methods: Clinical and genetic evaluation of a child with suspected Dubowitz syndrome and review of the literature.
Case report: We report on a two-year-old girl with severe short stature (body height -5.1 SDS) and dystrophy (BMI -3.7 SDS). In view of her birth weight (- 2.3 SDS), birth length (- 2.8 SDS) and head circumference (- 2.2 SDS) at 37 weeks of gestation she was classified as born small for gestational age. The child shows marked microcephaly (- 5.4 SDS), characteristic dysmorphic features, high-pitched voice, mild mental retardation, and eczema. In view of this combination of clinical symptoms the diagnosis of Dubowitz syndrome was made. Alcohol abuse during pregnancy, growth hormone deficiency, and

further causes of severe growth failure were excluded. Cytogenetic analysis revealed a normal 46,XX karyotype. To identify genomic microdeletions and duplication we started the Array-CGH.
Discussion: As the girl was born SGA, she might profit from growth hormone treatment from the age of four years in terms of skeletal growth, metabolic effects and neuropsychological development. However, in view of the reported association of Dubowitz syndrome with malignant and haematological diseases the use of growth hormone in this syndrome must be carefully discussed. Although growth hormone therapy does not increase the malignancy rate in general, the predisposition to neoplasias in Dubowitz syndrome and a potential harmful effect of growth hormone cannot be excluded.
Conclusion: The Dubowitz syndrome is a rare cause of severe intrauterine and severe postnatal growth retardation. In view of the predisposition to haematological or malignant disorders growth hormone treatment should only be considered with caution.

R-23 Genetics of Growth; Genetics of Hormone Excess; GH Physiology

Effects of growth hormone treatment in Noonan syndrome – case report

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Noonan syndrome (NS) is a genetic disease with many features similar to Turner syndrome. It has been recently found that NS is caused by mutations in the PTPN11 gene on chromosome 12. Molecular genetic abnormalities have been found only in about 50% patients with clinical diagnosis of NS. The diagnosis is clinical using a special scoring system. One of the major diagnostic criteria in the scoring system is short stature.
There is still discussion about advantages and safety of GH treatment in NS. It is suggested that the presence of a mutation in PTPN11 gene can cause a GH resistance and impair the response to GH treatment. The aim of the study is to present a boy with NS and GH deficiency treated with GH.
Case report: the patient from twin pregnancy, born at 36 gestational week, with 2570 g birth weight. The target height according to parents = 170,5 cm. The twin brother presented a normal growth (final height = 180 cm). The mother was operated because of pulmonary stenosis, also the mother's brother was short and had the same congenital heart defect. The pulmonary stenosis was diagnosed and operated in the boy as he was 2 y. old. In the age of 3 y. he was operated because of cryptorchismus. Due to the mental retardation the boy attended a special school. In the age of 10 y. the subclinical hypothyroidism was diagnosed. The karyotype was normal. As he was 12 y. old the malabsorption syndrome was recognized and the gluten-free diet was recommended. Because of growth deceleration the stimulation tests were performed and the partial GH deficiency was diagnosed (GH peak = 5.4 ng/ml). In the age of 16 y. the GH treatment was started. The bone age was 10 y., the height SDS was -6.1, parental adjusted height was -4.7. In the first year of treatment the height velocity was 6 cm/year. The height SDS after one, two and four years of treatment was respectively -5,6; -4,7; -2,7. The average GH dose = 0.026 mg/kg/day. During the GH treatment no adverse effects were observed. The IGF-1 concentrations were within normal range. Due to dysmorphic features and past medical history the patient was analyzed genetically once more. The NS was confirmed in molecular diagnostics. The mutation 1510A>G in 13 exon of PTPN11 gene in one allele has been found. Currently the patient is 21 years old and is 165 cm (-2,3SD) high. The GH therapy is finished. The presented case is an example of a positive response to substitution dose GH treatment in NS with confirmed PTPN11 mutation.

R-24 Genetics of Growth; Genetics of Hormone Excess; GH Physiology

Marasmic kwashiorkor in a patient with lysinuric protein intolerance. Case report

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Lysinuric protein intolerance is a rare, autosomal recessive metabolic disorder caused by the defective transport of the cationic amino acids lysine, arginine and ornithine at the basolateral membrane of renal tubular cells and enterocytes. The clinical presentation lysinuric protein intolerance includes gastrointestinal symptoms, such as vomiting and diarrhoea, failure to thrive, episodes of coma, hepatosplenomegaly and osteporosis soon after weaning. In lysinuric protein intolerance, growth failure and delayed skeletal maturation have been reported as a consequence of protein malnutrition.
Here we report a girl who first presented with an acrodermatitis enteropathica-like eruption, malnutrition and vomiting eventually had the diagnosis of Lysinuric protein intolerance. We discuss the probable cause of her skin lesions and the differential diagnosis with malnutrition.

R-25 GH and IGF Use

The European Increlex® growth forum database (EU IGFD): a European subject registry for long-term safety and efficacy monitoring

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Mecasermin [rDNA origin] injection (recombinant DNA-derived human insulin-like growth factor-1 [IGF-1]; Increlex®) was approved by the EMEA in August 2007 for the long-term treatment of growth failure in children and adolescents with severe primary insulin-like growth factor-1 deficiency (SPIGFD). As part of the marketing authorisation, the manufacturer agreed to implement a registry of treated patients to obtain additional data on long-term safety and efficacy. **Design/Methods**: The Registry is a descriptive, multicentre, observational, prospective, open-ended, non-interventional, surveillance registry utilizing a web-based electronic Case Report Form to collect information from 11 European countries initially. The goal is to enrol 60% of Increlex®-treated patients. Short children with SPIGFD beginning therapy with Increlex® (or previously treated) are eligible for inclusion, unless they are participating in a clinical trial, including trials with Increlex®. The data collected are those that exist in the patient's medical record as part of standard medical care.
The Registry collects data on serious adverse events (AEs), targeted adverse events, whatever their relationship to Increlex®, and AEs related to Increlex® administration; it also monitors the efficacy of Increlex® therapy outside the setting of a clinical trial. The Registry follows a similar design to the US IGFD Registry to facilitate analysis of pooled US and European data. In the US IGFD Registry, which started in May 2006 (610 patients enrolled by March 2009), no new safety issues or unexpected adverse events have been observed and Increlex® is generally safe and well tolerated in the Registry population. Higher Increlex® doses are associated with better growth rates. **Results**: Eleven sites from 5 countries are ready to enrol patients in the Registry. The first patients were enrolled in January 2009 and by 14 April 2009, the Registry contained 6 patients. Similar to the US experience, site initiation and inclusion of the first patients has been a lengthy process due to complex administrative processes. **Summary**: This abstract aims to raise awareness of the EU IGFD. This Registry is now open for web-based patient registration, to collect long-term safety and efficacy data on Increlex® therapy in children affected by growth failure in Europe.
Abstract submitted on behalf of the EU IGFD Group

R-26 GH and IGF Use

Growth hormone therapy improves growth and muscle performance in a boy with mitochondrial encephalomyopathy

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Introduction: MELAS (myopathy, encephalopathy, lactic acidosis, and stroke) is a progressive mitochondrial disorder for which there is at present no cure. Onset is usually between childhood and adolescence and its clinical presentation and severity are highly variable. Typical features consist of stroke-like episodes with neuromuscular symptoms. Additional symptoms are diabetes mellitus, dilative cardiomyopathy, hearing loss, short stature, and exercise intolerance. Recombinant human GH has been shown to have anabolic effects on protein metabolism and to reduce muscle wasting in various diseases.
In literature there are few data about the relationship between short stature, growth hormone deficiency (GHD) and therapy with recombinant human GH in mitochondrial encephalopathy.
Case report: We describe a prepubertal 11 year-old boy with short stature, growth's failure, atypical fatigue and language disturbs who was initially diagnosed as idiopathic GHD. MR revealed no abnormalities of the hypothalamo-pitiutary area but showed an increased signal in the white substance and in the midbrain. The boy was found to have a mitochondrial encephalomyopathy (lactic acidosis, exercise intolerance, EEG anormalities, myopathy with c-cytochrome oxidase deficit, and neurosensorial hypoacusia). GH treatment was started at dose of 0.2 mg/kg/week. After the first year of GH therapy, the patient was still prepubertal, presenting a significant acceleration of growth velocity (8 cm/year), a significant gain in height SD scores (SDS) (-2,94 vs –2,4 SDS) and a better exercise tolerance.
Discussion: The patient presented a GHD associated with mitochondrial defects like neurosensorial hypoacusia, EEG's abnormalities, myopathy, histological muscular anomalies and language disturbs. All the elements that could lead to consider the GHD as a manifestation of the mitochondrial disease are quite uncertain. Neuropsychiatric and endocrinologic features will be studied and followed for a long time in order to understand how to classify the manifestations which came from the match of both the disease.
Conclusion: The mechanisms involved in the relation between GHD and mitochondrial disease are not known. Further investigation is needed to clarify whether a possible pathogenetic linkage may exist between mitochondrial myopathy and impairment of GH secretion and whether a mitochondrial disorder may be a disease in which the known protein anabolic effect of GH has a long-term therapeutic benefit.

R-27 GH and IGF Use

Efficacy and safety of the treatment of growth hormone deficient children (GHD) with the recombinant hGH Omnitrope®: 84-month long-term data

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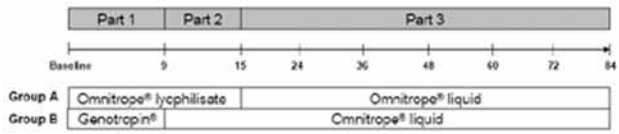
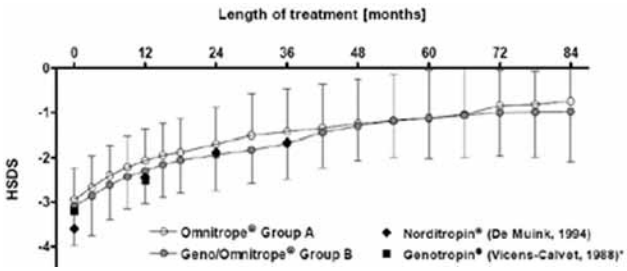
AIM:
This phase III clinical study in GHD children with growth retardation was designed to compare efficacy and safety of Omnitrope® with Genotropin® and to assess long-term safety and efficacy of Omnitrope®. Results on 84-month of treatment with Omnitrope® are presented.



METHOD:
89 treatment-naïve, prepubertal children (HSDS -3.0 ± 0.8, height velocity (HV)SDS -2.3 ± 1.1) with GHD (peak < 10 ng/ml, in 2 tests) were randomised (Part 1) to Omnitrope® lyophilisate (Group A, n=44) or Genotropin® (Group B, n=45) for 9 months and received subcutaneous dose of 0.03 mg/kg/day. In Part 2, patients receiving Omnitrope® lyophilisate continued the same treatment for a further 6 months, while patients on Genotropin® were switched to Omnitrope® liquid for the subsequent 6 months. In Part 3, patients in both groups received Omnitrope® liquid for a period up to 69 months.
RESULTS:
The development of height, HSDS, HV, HVSDS and IGF-1, IGFBP-3 levels were comparable between both groups of patients and confirmed the well-known growth response of GHD children to rhGH treatment. IGF-1 levels increased in both treatment groups to mean levels in the upper normal range. Transiently developed anti-GH antibodies during treatment with Omnitrope® liquid were acceptably low and of no clinical significance. Omnitrope® was well tolerated and safe over 7 years of treatment.
CONCLUSION:
The clinical comparability between Omnitrope® and Genotropin® was demonstrated. Long-term safety and efficacy of treatment with Omnitrope® were proven.

Efficacy results

	Baseline		84 months	
	Group A	Group B	Group A	Group B
HSDS	-2.95 ± 0.72	-3.10 ± 0.88	-0.78	-1.01
HV (cm/yr)	3.83 ± 1.21	3.94 ± 0.82	5.53	5.53
HVSDS	-2.27 ± 1.23	-2.27 ± 0.93	-0.18	0.11



R-28 GH and IGF Use

Bioequivalence studies of the recombinant hGH Omnitrope®: two comparative phase I randomised studies and population pharmacokinetic analysis

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METHOD:
Two randomized, double-blind, crossover phase I studies in healthy volunteers were conducted to compare three different formulations of recombinant human growth hormone (rhGH): Omnitrope® lyophilisate, Omnitrope® liquid and Genotropin® (lyophilised powder for injection). Both pharmacokinetics (area under the curve [AUC], C_{max}, t_{max} and $t_{½}$) and pharmacodynamics (IGF-1,

IGFBP-3 and NEFA) were assessed after a single subcutaneous injection of 5 mg rhGH.

RESULTS:

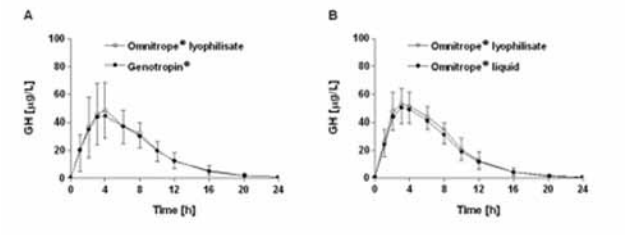
The three formulations had comparable pharmacokinetics and pharmacodynamics. All the ratios (90% confidence intervals) for AUC and C_{max} treatment means were within the predefined FDA and EMEA acceptance range of 80%-125% for bioequivalence. In addition, a comparative population pharmacokinetic analysis further supports that Omnitrope® lyophilisate, Omnitrope® liquid and Genotropin® can be regarded as equivalent in terms of pharmacokinetics.

CONCLUSION:

Omnitrope® lyophilisate was demonstrated to be bioequivalent to both Genotropin® and the Omnitrope® liquid formulation.

Pharmacodynamic parameters of rhGH in studies 1 & 2 (mean ± SD, unless otherwise stated)				
IGF-1	Study 1		Study 2	
	Omnitrope® lyophilisate	Genotropin®	Omnitrope® lyophilisate	Omnitrope® liquid
AUC [h µg/L]	31974 ± 10766	29893 ± 9569	18806 ± 4381	19087 ± 4684
Cmax [µg/L]	458 ± 34	428 ± 152	260 ± 53	264 ± 58
tmax [h] Median [range]	24 [12;97]	24 [12;96]	24 [12;48]	24 [12;24]]
ANOVA				
Ratio of LSM, [90% CI]	AUC: 1.07 [0.99; 1.14]		AUC: 0.98 [0.99; 1.14]	
	Cmax: 1.07 [0.95; 1.20]		Cmax: 0.98 [0.95; 1.20]	

AUC = area under the serum concentration-time curve from zero to the last measurable concentration, Cmax [µg/L] = maximum observed serum concentration, t1/2 = elimination half-life, tmax = time to reach peak or maximum concentration following drug administration, ANOVA = analysis of variance, LSM = least square means, CI = confidence interval



R-29 GH and IGF Use

Pseudoachondroplasia and growth hormone deficiency

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Pseudoachondroplasia is a well described skeletal dysplasia with short stature and a waddling gait as major features linked to mutation in the COMP gene on chromosome 19. We report 2 unrelated individuals with pseudoachondroplasia, who also had growth hormone deficiency on stimulation test. There are no other reported cases of pseudoachondroplasia and growth hormone deficiency, however many individuals with skeletal dysplasias are not evaluated for growth hormone deficiency as there is a clear explanation for their short stature. We suggest that growth hormone deficiency may be an associated part of this entity and evaluation of more individuals is needed.

R-30 GH and IGF Use

The effect of nutritional support on the endocrine system, in two growth hormone (GH) treated patients – a nutritional lesson

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Although nutritional counseling is an integral part of the management of rapidly growing children, few studies have focused on the importance of nutritional supervision during growth-hormone (GH) therapy. We have previously reported that optimal nutritional support improves growth before and during GH therapy (Pediatrics 2005; 116:68). Based on this experience our treatment protocol includes nutritional supplementation (per RDA) based on a 3 day nutritional recall. On 3 monthly bases nutrition is monitored and when needed iron, and blood count.

Case study: A GHD patient, treated with GH was referred for uncontrolled primary hypothyroidism and poor growth. TSH levels 10-25, borderline low FT4 and hemoglobin 9.5 gr/dl. Oral iron supplementation had no significant effect on blood iron (<25 mcg/l) and hemoglobin. Nutritional recall revealed (% RDA): calories-88%, Protein 110%, iron 50%, Vitamin A 10%. Serum Vitamin A 10 mcg/l. Post Vitamin A supplementation blood iron normalized. TSH became < 5 and the growth velocity improved. This improvement can be explained by a cascade of events:Improving vitamin A status generally increases hemoglobin concentrations and reduces anemia. Vitamin A was reported to enhance non-heme iron absorption to improved iron-deficient erythropoiesis. During vitamin A deficiency, iron is retained in the liver and spleen, and it is less available for erythropoiesis. In vitamin A–deficient rats, iron uptake by the bone marrow is impaired, and erythrocyte incorporation of ^{59}Fe is decreased. In deficient animals, repletion with vitamin A increases the use of iron in bone and spleen. In humans, vitamin A deficiency is associated with a low percentage of transferin saturation and low iron binding capacity. In children, consumption of vitamin A-fortified sugar increases serum iron concentration, ferritin concentration, and percentage of transferrin saturation. Retinoids may stimulate erythropoiesis through a direct effect on the later stages of red cell development. Iron is needed for the normal function of the thyroid peroxidase.A heme like enzyme needed for thyroxine production. So normalizing vitamin A, normalizes iron and hence thyroid function and last but not least growth. To conclude, rapid growth needs nutritional monitoring and in many cases nutritional supplementation

R-31 Gonads and Puberty

Favourable outcome with tamoxifen in juvenile breast hypertrophy

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Juvenile breast hypertrophy is rarely seen and characterized by excessive breast enlargement in the peripubertal period. The clinical entity is thought to result from increased sensitivity of mammary tissue to normal levels of circulating hormones. Here, we report a twelve-year and six-month-old premenarchal female patient suffering from juvenile breast hypertrophy, who presented at the third month of symptoms and dramatically benefited from tamoxifen treatment.

R-32 Gonads and Puberty

Precocious puberty associated with congenital myxedema in a child with Down syndrome – morbid association – case report

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Background: Neonatal thyroid screening could allow the precocious treatment in congenital myxedema, reducing neuropsychic troubles and other secondary endocrine troubles occurrence. Down syndrome can focus all the attention at birth on this genetic disease, while neglecting the adjunct pathology.
Case: BI, female, aged 2 years and 10 months, 92 cm height and 10.5 kg weight is admitted for vaginal bleedings. Clinical exam: dysmorphism with height hypotrophy, macroglossia, no dentition, ombilical hernia, anterior fontanella opened (2/2 cm); hyperpigmented external genital organs with slight clitoridian hypertrophy. Anamnesis: a supermatured pregnancy, birth karyotype 47XX+21, mother's age at the conception 38 years.
Hormonal exams: TSH = 41.6 µUI/ml (N = 0.4-7.0); FT4 = 0.3 µg/ml (N = 5.0 - 13.0); FSH = 12 mUI/ml (N <2.5); LH = 0.6 mUI/ml (N <2.5); estrogens = 21.5 pg/ml (N <10). Echocardiography: medium quantity of pericardial fluid. Abdominal and pelvic echography: homogenous hepatomegaly; hypotonal bended colecyst; hypoplasial uterus; paramedian right trans-sonic formation, septed, with transversal section of 64/41 mm. Another paramedian left hypoechogenous formation, of 31/32 mm. Exploratory laparotomy and biopsy: both ovaries with increased volume: fibrothecoma type stroma, with rare primordial follicles. Diagnosis: Down syndrome, congenital myxedema, true precocious puberty.
Treatment: L-thyroxine 75µg/day and medroxyprogesterone acetate 10 mg/day. After 12 months, the baby's height = 94 cm; weight = 11 kg. Hormonal exam: TSH = 2.2 µUI/ml; FT4 = 6.2 µg/ml; FSH = 3.1 mUI/ml; estrogens = 13.2 pg/ml; bone age under 2 years. The mother refuses hospitalization and treatment with Gn-RH agonist (Diphereline®), continues the treatment with L-thyroxine. In the next year the child develops type I sugar diabetes, mother refuses therapy and the child dies.
Discussions: High occurrence of Down syndrome - 1/800 births - occurrence that increases with mother's age, needs TSH screening, taking into consideration the association of cca. 30% cases with congenital myxedema. In Down syndrome, usually the menarche is late, but in this case the primary hypothyroidism with raised TSH level can be the cause of precocious puberty, through FSH-like effect of α subunit in TSH. Later association with type I sugar diabetes and parent's refusal of collaboration conducted to early death of the child.

R-33 HPG Axis; Reproductive Endocrinology; Testis

The antagonistic effects of traditional Chinese medicine on estrogen-like activity of environmental endocrine disrupptors

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Objective:
The children with precocious puberty, who have exposed to higher pollution of environmental endocrine disruptors (EEDs), are treated with traditional Chinese Medicine (TCM). It is varified that the entagonistic effects of TCM on estrogen-like activity of EEDs.
Methods:
73 girls with precocious puberty, whose serum levels of EEDs were higher, were treated by TCM. The formula consisted of *Radix rehmanniae, Carapax et Plastrum testudinis, Cortex phellodendri, Rhizoma anemarrhenae*, etc. All medicines were extracted and concentrated (1 ml mixture contained approximately 2.5 g crude extract). The dosage was 60 ml / day. The therapeutic course was three months. The volume of uterus and overy, bone mineral density were measured, serum E_2 and osteocalcin(OST) were determined before and after therapy. The animal model contaminated with 4-nonylphenol(4-NP) and bisphenol A(BPA) were fed the above mentioned formula of TCM. The dosage was 5 ml / day. The therapeutic course was 14 days. Uterine wet weight, height of the luminal epithelium, thickness of the myometrium and the level of protein expression of proliferating cell nuclear antigen(PCNA) in rat uterine were determined before and after therapy. The data were analysed by SPSS 11.5.
Results:
After therapy, in the girls with precocious puberty, the volume of uterus decreased from 4.0±0.5 ml to 2.6±0.4 ml ($p<0.01$), serum E_2 descended from 174.84±16.40 pmol / L to 85.91±9.65 pmol / L ($p<0.01$), BMD decreased from 0.537±0.067 g / cm^2 to 0.417±0.056 g / cm^2 ($p<0.01$), serum OST decended from 16.85±3.16 µg / L to 10.06±3.37 µg / L ($p<0.01$). In the contaminated animal models, the uterine wet weight decreased from 0.081±0.009 mg to 0.055±0.008 mg($p<0.05$), height of the luminal epithelium decreased from 23.27±5.64µm to 17.45±4.30µm ($p<0.05$), thickness of the myometrium decreased from 82.29±13.92µm to 55.20±10.20µm ($p<0.05$), positive cell area of PCNA descended from 5490.25±678.58µm^2 to 4301.59±464.02µm^2($p<0.05$).
Conclusion:
The present therapeutic regime of TCM could effectively against the estrogen-like activity of EEDs.

R-34 HPG Axis; Reproductive Endocrinology; Testis

PHACE-syndrome and panhypopituitarism

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Case report: We are reporting an 11-year-old girl with growth retardation since her 4th year of life (actual height 5cm < 3. perc., weight 3. perc.) and hypothyroidism diagnosed 4 months before presentation. MRI of the brain revealed complex structural and vascular anomalies with cerebellar dysplasia on the right side, deformation and dislocation of the brain stem, arachnoidal cysts, and an empty sella. Our patient had a congenital right-sided facial hemangioma of plaque-morphology, treated in infancy with interferon followed by laser therapy. The girl is of normal intelligence, stage of pubertal development was prepubertal (Tanner PH1, B1).
Laboratory studies: Endocrine function testing revealed growth hormone deficiency, no cortisol response to hypoglycemia, as well as central hypogonadism. A diurnal profile of cortisol secretion was not obtainable, but repeated measurements of ACTH and cortisol showed constantly low levels for both parameters (cortisol max. 2,7 µg/dl, ACTH max. 12 pg/ml). 24h urinary cortisol excretion was also markedly lowered. Free thyroxine (fT4) was normal under continued thyroxine replacement. Measured thyrotropin levels never exceeded 0.024 mIU/l confirming the diagnosis of central hypothyroidism. In summary, our patient suffered from panhypopituitarism.
Imaging studies: MRI of the brain confirmed the result of an empty sella, presumably caused by dorsal dislocation of the infundibulum by arachnoidal cysts. Cranial of the posterior pituitary gland a structure was identified consistent with a considerably reduced pituitary gland. Angiography revealed an occlusion of the right internal carotid artery and a doubled middle cerebral artery.
Conclusion: We diagnosed our patient with **PHACE-syndrome** (OMIM Nr. 606519). PHACE-syndrome is a neurocutaneous disorder that refers to the association of large, plaque-like, "segmental" haemangiomas of the face, with one or more of the following anomalies: **Posterior fossa brain malformations, arterial cerebrovascular anomalies**, cardiac anomalies, and eye anomalies. The development of panhypopituitarism, which in our patient led to diagnostic work-up and finally to the diagnosis of PHACE-syndrome, has not been reported in association with PHACE-Syndrom until now. Treatment with hydrocortisone and growth hormone was initiated. We conclude that testing of the hypophysal function in patients with PHACE-Syndrom and intracranial malformations is recommendable, both at diagnosis and during follow-up.

R-35 HPG Axis; Reproductive Endocrinology; Testis

Osteosarcoma after androgen replacement therapy in a patient with acute lymphoblastic leukemia (ALL)

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Primary hypogonadism is often seen after treatment of malignant disease and the replacement of sex steroid is essential for those cancer survivors. A patient with ALL diagnosed at the age of 6 years achieved a remission after chemotherapy and 18 grays (Gy) of cranial irradiation. When he was 12 years old, ALL relapsed in his right testis, then he received chemotherapy and local irradiation with 26 Gy in bilateral testes. He was then treated with autologous peripheral blood stem cell transplantation at the age of 15 years after cyclophosphamide and total body irradiation with 12 Gy. He was diagnosed as primary hypogonadism at the age of 16 and replacement therapy of androgen was started with intramuscular injections of testosterone enanthate (TE) since then. The initial dose of TE was 50 mg monthly, and the dose was gradually increased. Androgen replacement was discontinued at the age of 21 because he did not visit the hospital. At 26 years of age, he suddenly visited our clinic, when he was diagnosed as diabetes mellitus (DM). Intensive insulin treatment normalized his blood glucose levels, and a reduction of body weight after diet therapy and exercise made insulin therapy unnecessary for him during a one-month admission. After the remission of DM, androgen treatment was restarted biweekly with the dose of 250 mg of TE. Four months after the initiation of androgen therapy, he complained discomfort and pain in his left buttock. An MRI examination revealed a large lobulated tumor around the left sacroiliac joint that extended to the left ilium and the gluteal muscles. The common iliac vein and the inferior vena cava were occluded with thrombus or tumor. There were a few peripheral vessel occlusions in the right pulmonary arteries on chest CT scan. A needle biopsy of the tumor made the pathological diagnosis of chondroblastic osteosarcoma. Biweekly TE was continued until he died due to pulmonary thrombosis 5 weeks after the diagnosis of osteosarcoma. Immunohistochemical analysis on the biopsy specimen showed androgen receptor and estrogen receptor beta in the tumor cells, whereas immunoreactivity of estrogen receptor alpha and aromatase was not detected. Although the osteosarcoma of this patient was apparently a second malignancy after the treatment for ALL, it was possible that exogenous testosterone might be involved with the proliferation of osteosarcoma cells. Attention is necessary when to treat hypogonadism in cancer survivors.

R-36 HPG Axis; Reproductive Endocrinology; Testis

Cholestasis, hypoglycemia, and micropenis: manifestations of congenital anterior hypopituitarism

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Introduction
Cholestasis occuring in infancy should be evaluated completely to exclude hepatic as well as endocrine or metabolic causes. Panhypopituitarism should be considered in any infant who presents with cholestasis, hypoglycemia, and other manifestations of pituitary malfunction.
Case
A 40 day old male was referred to our hospital for evaluation of cholestasis. He was the first child of nonconsanguineous parents. On physical examination he had pallor, dysmorphic face, micropenis and postaxial polydactily. He was icteric. Laboratory findings revealed; direct bilirubin 7,29 mg/dL (0-1,2) with a total bilirubin level of 5,98 mg/dL (0-0,3) with AST 82 U/L (0-40); ALT 33 U/L (0-41), GGT level of 189 U/L (8-61). Serum aminoacids and urine metabolic screen, alpha 1 antitrypsin was normal, serum bile acids were elevated. Throughout his hospitalization, the patient was noted to have bradycardia, and apneic episodes associated with hypoglycemia as low as 17 mg/dL that responded to infusions of intravenous dextrose. At the time of hypoglycemia; cortisol level of 1 mg/dl including growth hormone level of 0,059 ng/mL, insulin: 2 IU/mL, LH: 0,1 mIU/mL, FSH: 0,05 mIU/mL, PRL: 1,99 ng/mL, keton: positive, and ACTH: 6,0 pg/mL (9-46) sT4:0,4 ng/dL (0,7-1,9) and TSH:0,05 UIU/mL. Congenital anterior hypopituitarism was diagnosed and magnetic resonance imaging of pituitary gland revealed pituitary hypoplasia, ectopic neurohypophysis, and absent pituitary stalk. There was no septo-optic dysplasia. Hydrocortisone and Na-L Thyroxine replacement therapy was started. His blood glucose levels stabilised and cholestasis resolved within 2-3 weeks after initiation of therapy.
Conclusion
An infant presenting with cholestasis accompaning to hypoglycemia and micropenis must alert the clinician to the possibility of anterior hypopituitarism. At the time of the hypoglycemia, studying anterior pituitary hormone levels are crucial for determing the main cause.

R-37 HPG Axis; Reproductive Endocrinology; Testis

Evolution or coincidence of long-term gonadotropin-releasing hormone agonist treatment for central precocious puberty in girls

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Introduction: Very few abnormalities in endocrine function have been reported during long-term gonadotropin-releasing hormone agonist (GnRHa) treatment in girls with central precocious puberty (CPP). Most authors agree that this therapy is save and effective.
Aim of the study: To evaluate the effects of long-term GnRHa therapy in girls with CPP of idiopathic or organic origin.
Methods: We monitored thyroid function and glucose levels in 40 girls with CPP. Nine of them (19,5%) were with an organic CPP and 31 (80,1%) with idiopathic CPP. They have received monthly depot injections of triptorelin acetate for a time period of 6 months to 8,5 years. Thyroid function was examinated by measuring serum levels of thyrotropin (TSH), thyroxin (T4), thyroid antibodies, and ultrasound of the thyroid gland.
Results: We found 4 girls (12,9%) with idiopathic CPP who had elevated levels of TSH, one of them having also elevated thyroid antibodies, mild goitier and an abnormal ultrasound of the thyroid gland suggesting Hashimoto thyroiditis at the age of 8 years. This child had a familiar history of thyroid cancer in the mother. Another girl with hamarthoma developed diabetes mellitus type 1 at the age of 8,5 years. Both of these girls were very early diagnosed for CPP, at six months and 8 months respectively, and the complications occurred after almost 7-8 years of GnRHa therapy.
Conclusions: We are not certain that these autoimmune diseases are related to the GnRHa treatment or are simply a coincidence. Although, we suggest a closer monitoring of thyroid function and glucose levels especially in children who have had a long period of GnRHa treatment.

R-38 HPG Axis; Reproductive Endocrinology; Testis

The prevalence of PCOS, between 2004-2008 in adolescent girls from the South – West of Romania

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Introduction: The clinical pictures of PCOS is variable and may be underdignose.
Aim : To study the prevalence of PCO in adolescent girls in our region.
Material and method: Between 2004-2008, we follow up the adolescent girls admitted in our clinic, from south west region of the country, for hirsutism, acne, irregular menses and obesity. We perform, clinical examination (anthropometrics dates, BMI, Galaway in hirsute girl), laboratory exams (lipids, OGTT, insulinemia -HOMA index, PCR, testosterone, SHBG-FAI, 17 OH – progesterone, DHEA, and DHEA-S, LH, FSH), imagistic exams (genital ultrashall, MRI in selected cases).

Results: From 6314 adolescent girls admitted in our clinic 2704 was diagnosis with POCS according to Rotterdam Consensus. 77.89% (4918) were overweight and 42.96% (2713) were obese. FAI were height (according to the age). LH/FSH ratio was between 3.04-4.01; 17OH progesterone positive girls were exclude from the study. In the obsesses group the fasting glucose reveal diabetes mellitus in 2 cases, OGTT impairing glucose in 9.61% (206 cases). HOMA index were high then 95 percentiles in 178 cases (6.98%). Dislipidemia were found in all obsesses girls. The imagistic analyses confirmed the ovarian involvement. The treatment for the obsesses patient were changing the life stile and metformin 2mg/day (in obsesses patients) and Diane 35 for non obese girls.

Conclusions:

1. The prevalence of PCOS in adolescents in our region is important.
2. The incidence of obesity is height and we must follow-up the metabolic disturbances especially in obsesses girls.
3. There are no differences between the cases from urban and rural area.

R-39 HPG Axis; Reproductive Endocrinology; Testis

Prevalence of the metabolic syndrome in Algerian adolescent women with PCOS

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Polycystic ovary syndrome and the metabolic syndrome have many features in common and have probably the same pathogenesis.

OBJECTIVE

This descriptive study have to determine the prevalence of the metabolic syndrome (defined by the NCEP-ATPIII criteria) in PCOS adolescent (defined by the rotterdam criteria).

METHODS

Ninety adolescents with PCOS (mean age 17.2+/-108 years and mean BMI =28.87+/-3.2 kg/m²) were evaluted for features of the metabolic syndrome.

RESULTS

9.09% of them had a metabolic syndrome.4.8% had IGT, 28.5% had a decreae HDL-C (HDL-C< 1.30mmol/l), 6.25 % had a high triglyceridemia (TG>1.70 mmol/l) and 42.8 % had a waist circunference > 88cm.None of them had increased blood pressure and neither diabetes mellitus.

CONCLUSION

The metabolic syndrome is not frequent in our young population .although high wais circunference and low HDL-C have to be screened in this population as they are relatively frequent.

R-40 HPG Axis; Reproductive Endocrinology; Testis

Vaginal synechia

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Vaginal synechiae (labial adhesion) is paper thin attachment or fusion between the inner walls of the opening of the vagina. The etiology of labial adhesion is unclear, but conditions that lead to chronic irritation of the vulva in the hypo estrogenic girl are believed to be important. It may be caused by infections, inflammatory conditions, or dermatological conditions, as well as local irritants. FA, a 15 month-old-girl, 10 kg, 75 cm with her parents chief complain was fusion in her genital area that covering her vagina. There was no history of genital trauma, surgical intervention or sexual abuse. On genital examination found a thin, flat, pale membranous line between the labia. Visual inspection revealed the labia minor fused from the superior end (clitoris) to the inferior (vaginal introitus) apex of the vulva. The urinary meatus was not visible, but the patient denied any urinary difficulties. There was no evidence of infection.

The diagnosis was vaginal synechiae. Estrogen cream was administered twice daily for 2 weeks., The fusion was released after two weeks. Her vulva was healing anatomically well. On her next visit one month later, the clinical evaluation showed no recurrent of adhesion.

R-41 New Technologies; Systems Biology

Ectopia lentis as a presenting feature of homocstynuria type 1

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Background: Homocistynuria type 1 is an autosomal recessive inheritance metabolic disorder with prevalence of 1/200000. It is due to the deficiency of the enzyme cystathionine beta synthase that transforms the homocysteine in cysteine, for what it needs pyridoxal phosphate as a co-factor.

The enzyme deficiency creates an accumulation of homocysteine in serum and urine, the methionine in serum is usually found with an increase, and cystationine and cysteine with a decrease.

Case report: 8 year-old boy patient was referred by myopia magna and ectopia lentis. He was the first born from Moroccan consanguineous parents. Father prensenting mild mental retardation. Pregnancy and delivery were normal. Normal psychomotor development, bad school performance and altered behaviour. Physical examination: weight and eight + 0.3 SDS, TA: 123/73 mmHg (>P90), peculiar facies and scoliotic attitude. He did not show laxity of the ligaments neither arachnodactyly.

Biochemical findings: serum levels of homocysteine: 50 mmol/l (N <15).Blood amino acid determination (cromatography of ionic exchange): methionine: 228 (21 ± 6.4) and homocystine: 17.7 (1 ± 0.2). Homocystine urinary excretion: 34.9 (1.8± 1.3) and methionine: 20.7 (7.4 ± 5.4)

Organic acid plasma (liquid cromatography of high resolution): very high levels of homocysteine 263 (6.3 ± 2.4) and decrease of cysteine 87(190 ± 50)

Chest and spinal column RX, echocardiography, electrocardiogram and abdominal ultrasound were normal.

He was treated with high oral doses of Pyridoxine, Folic Acid and vitamin B12.

Subsequent laboratory: blood homocysteine 0.5 and no urinary excretion.

Given the good response it is decided a moderate restricion of high biological value, with further controls within normal values.

At 11, he had bilateral ectopia lentis surgery implanting an intra-ocular lens.

Last analytic showed a slight and asymptomatic deficit of factor VII.

Determination of homocysteine to the family: father: homocysteine 15.2mmol/l (carrier status)

Comments:

Homocystinuria is the second most common cause of heritable ectopia lentis.

Most of the patients require high doses of vitamin B6.

Patients who respond to treatment with pyridoxine show adiminished tolerance to methionine, thus, they should keep a certain restriction of methionine in their diet.

The best method to detect homocystine in urine is the chromatography of ionic exchange.

Do not forget the prevention of the tromboembolic phenomenon in these patients in case they need surgery.

R-42 New Technologies; Systems Biology

Structuring a working model for researchers – lets get S.T.A.R.T.E.D.

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A Principal Investigator's involvement in their research can vary widely, with many hiring postgraduate students or nurses to carry out the actual gathering of data. Organising the practicalities of a new research project can be daunting for the inexperienced researcher. Here we outline the problems encountered by two research nurses undertaking a large longitudinal study and the subsequent development of the S.T.A.R.T.E.D model.

There are a variety of patient care nursing models and frameworks already in existence; however their relevance to population based research is minimal. The need for a structured approach became apparent to the authors when issues involving protecting data across multiple sites arose. The need to access and update data in many different environments (home, office, subjects' houses or hospital wards for example) was highlighted. This, in line with maintaining confidentiality and ensuring researcher safety by having access to visiting schedules, required much thought.

Working through these and other issues encountered, led to the creation of a framework to guide the planning needed to progress with any given study. The seven most pertinent issues that make the data collecting process safer for both the researcher and the subject were identified as solo visiting, technology, approval, responsibility, training, equipment, and data protection. Each of these areas has been explored fully to include elements such as home visiting safety and assessing training needs (for example sample collection and child protection). These seven categories can be remembered using the mnemonic S.T.A.R.T.E.D.

Within clinical trials there are protocols to facilitate the trial and provide structure for the researchers involved, where as in population based research there is no such framework. S.T.A.R.T.E.D. was developed as a consequence of specific issues faced in the authors' study and its benefits can clearly be seen. This framework helps ensure a safe, well thought out approach to data collection outside of the hospital environment. It can be applied to new projects, giving structure to the initial setting-up phase of a research assignment, and is adaptable for use in many research and home visiting environments. We propose the S.T.A.R.T.E.D. model as a useful framework for those new to research and research planning.

R-43 Obesity, Fat

Clinical effect and mechanism of Shouti oral liquid in simple obesity with hyperinsulinism in children

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Objective To study the clinical effect and the mechanism of Shouti Oral Liquid in the therapy of simple obesity with hyperinsulinism in children.

Methods Forty-three patients were randomized into treatment group (TG) and control group (CG). Patients in TG took Shouti Oral Liquid and the patients in CG took Metformin tab (dimethyldiguanide). The therapy course lasted six months. The plasma lipid, body fat, insulin resistance index (IR) and some hormones correlated with obesity were measured before and after therapy and the changes were analyzed.

Results The weight, F%, BMI, LP, TNF-α, FINS, IR were all declined after therapy than pre-therapy in both group. The proportion of remarkable effective, normal effective and total efficiency were 22.7%, 59.1%, 81.8% in TG and 9.5%, 52.4%, 61.9% in OG respectively. The therapeutic effect in TG was superior to OG. The short-term therapy with Shouti Oral Liquid did not appear evident adverse effects.

Conclusion Lipid metabolism disorder, insulin resistance and high level of LP and TNF-α were observed in children with obesity and hyperinsulinism. The total effect using Shouti Oral Liquid was superior to control medicine. The mechanism involved in the therapeutic effect of Shouti Oral Liquid may possibly act by regulating lipid metabolism, decreasing LP and TNF-α and ameliorating insulin resistance.

R-44 Obesity, Fat

Evaluation of suspected monogenetic forms of obesity in childhood

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Obesity has become an increasingly prevalent public health problem and represents the complex interaction of genetic, developmental, behavioral, and environmental influences. Although rare, some children have single gene disorders associated with either syndromic or nonsyndromic obesity that can now be diagnosed with modern molecular genetic techniques. Characteristics of some monogenic forms of obesity include specific patterns of growth including short statures, cognitive impairments, dysmorphic features, birth defects and a family history of similarly affected individuals or consanguinity. We will review distinguishing clinical characteristics and methods of molecular diagnosis for Prader Willi syndrome, Bardet-Biedl syndrome, melanocortin 4 receptor mutations, and other genetic causes of pediatric obesity. Many of the genes for monogenic forms of obesity relate to the development and function of the hypothalamus and central control of food intake and energy homeostasis and provide insight into molecular pathways for study the genetic control of energy homeostasis in the more common multifactorial forms of obesity.

R-45 Obesity, Fat

Impact of bi-parental gastric bypass on the family unit and child with obesity: one case report

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Background: Parental obesity is a risk factor for childhood obesity. For some patients, weight loss surgery has improved health outcomes. However, research regarding the short and long-term psychosocial outcomes of weight-loss surgeries has yielded variable results. There is little information on the effect of gastric bypass on subsequent generations. We are interested in the impact of parental weight loss surgery on the family unit and the intergenerational transmission of weight-related beliefs and behaviors.

Case Description: MC is a 12y7m White male who presented fortreatment of dyslipidemia. History revealed a motivated 12-year-old with interest in increasing physical activity and reducing caloric intake. Exam showed an obese mid-pubertal male with blood pressure of 134/80 mmHg, height of 159.94 cm, weight of 98.60 kg, and calculated BMI of 38.5 kg/m2. He had acanthosis nigricans on neck and striae on abdomen. Both parents had undergone gastric bypass surgery and achieved successful results with weight loss. They subsequently regained a significant amount of weight. Parents indicated that the lack of change in their cognitions and behavioral tendencies ensured that they could not be successful at maintaining their weight loss in the long-term. Parents reported great concern about the possibility of transmitting their maladaptive mindset, behaviors related to food and physical inactivity to their children.

Discussion: Parents who have had weight loss surgery may inadvertently continue to have a negative impact on their children's health outcomes if they maintain unhealthy cognitions and behaviors with regard to food and activity. Lifestyle modification with decreased energy intake and increased energy expenditure related behaviors leads to weight loss and improvement in hypertension, dyslipidemia and insulin resistance. Children are dependent on their parents for changes in environment that facilitate such behaviors. Genetic predisposition for weight gain and food addiction may also play a role in success of lifestyle modification.

Conclusion: Given that children of obese parents may be genetically vulnerable to developing obesity, it is essential that the implications of failed gastric bypass surgery on the family unit be further researched. The long-term impact of weight-loss surgery needs to be examined, with consideration for the unhealthy weight-related cognitions and behavioral tendencies that are modeled to the children

R-46 Obesity, Fat

Autism and Prader-Willi syndrome

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Background: Abnormality in the chromosomal region 15q11-q13, involved in Prader-Willi syndrome (PWS) is the most frequent chromosomal defect reported in autistic syndromes. Phenotypic similarities in behaviour and psychiatrist traits between these 2 syndromes were poorly reported in the literature.

Aim of the study: we conducted a study in 13 PWS patients followed in the French Reference Centre for PWS to evaluate the prevalence of clinical signs of autism in this population.

Methods: 13 patients (7 male), median age 11.8 years (7-16.5), median BMI 3.7 Z-score (0-15) were included. The following tests were used: WISC (IQ evaluation), Child Behaviour Check List (CBCL), Childhood Autism Rating Scale (CARS) and Social Communication Questionnaire (SCQ).

Results: Mean IQ was 53, verbal IQ was 60 and performance IQ was 56. None of the patients reached a CARS score >29.5 (threshold for autism). CBCL scores were clinical or borderline in 50% of the patients for the item "anxious/depressed", in 60% of the patients for the items "withdrawn/depressed" and "activities" and in 70% of the patients for the items "thought problems" and "social". All patients had an abnormal score to the item "School". Using SCQ, 2 patients had pervasive development disorder and 3 patients had pervasive development not otherwise specified. These 5 patients presented atypical features for PWS such as motor stereotypes, unusual fears…

Conclusion: Search for autistic traits is important in children with PWS in order to implement a specific management and particularly psychiatric care.

R-47 Obesity, Fat

Abstract Withdrawn

R-48 Obesity, Fat

Four weeks administration of miglitol elevated the ghrelin level in high-fat fed mice. That's why miglitol was effective in the diabetes of Prader Willi syndrome?

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Miglitol, a 1-deoxynojirimycin derivative is an alpha-glucosidase inhibitor. In the present study, chronic (4-week) oral administration of miglitol in high-fat fed mice was investigated. High-fat diet increased the body weight by 11% compared with the control diet mice. Three mg/kg, once a day miglitol administration increased the fasting ghrelin level to 51.0 +/- 4.2 fmol/ml with a slight reduction of body weight, whereas that of saline-treated, high-fat fed mice was 38.3 +/- 5.9 fmol/ml. We experienced that miglitol was effective for the control of their diabetes in 2 cases with Prader Willi syndrome. Miglitol is absorbed in the upper region of the small intestine and may alter the gut-brain peptide, ghrelin level. This might be one reason why miglitol was effective in the diabetes of Prader Willi syndrome.

R-49 Obesity, Fat

Pradder-Willi syndrome in a newborn infant

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Introduction: Prader Willi syndrome is a genetic disease transmitted by non mendelian heredity. Loss of active genes in a specific part of chromosome 15 (15q11-q13 region) causes the syndrome, the typical features of which may not be visible at birth.
Aim: Case of infant with Prader Willi syndrome.
Materials and methods: Female,full term neonate,with birth weight 2620gr,born via cesarian section(due to breech presentation) by deuteripara mother,presents during clinical examination right after birth with hypotonia (without muscular inability), absence of Moro and tendinous reflexes, reduction of frontal wrinkling with dextral ptosis of the angle of the mouth, overriding of cranial sutures (CH 33cm) and feeding difficulties. Obstetrical history: Reduced fetal mobility noticed by the mother during gestation. Family history: family members without myopathy / neuropathy,previous child without developmental (kinetic or learning) retardation. Laboratory testing (blood, biochemical, hormonal, metabolic, TORCH), cerebral imaging evaluation (U/S, CT, MRI), echocardiography, kidney-urinary bladder U/S, fundoscopy and genetic evaluation were performed. Biochemical testing revealed CPK: 2933 U/L, ALS 68: U/L, while the rest of the test results were within normal ranges. Imaging evaluation and fundoscopy showed no pathological findings. Genetic evaluation showed no genetic stigma. Total parenteral nutrition (TPN) was administered for 19 days, followed by gradual refeeding. While CPK and ALS values decreased the infant remained hypotonic. Suspecting Prader Willi syndrome, Karyotype analysis and molecular control were performed.
Results: Molecular analysis revealed two allelomorphs (maternal origin only) of GABRB3, CYP19 and DI5S87, finding which is compatible with the presence of uniparental disomy. MSP (Methylation Specific PCR) revealed no physiological standard methyliosis – compatible finding with Prader Willi syndrome.
Conclusions: Patients with Prader Willi syndrome may be slightly dysmorphic and disease would not be diagnosed if the suspicion of diagnosis is not laid during evaluation of hypotonia. Problems during neonatal period such us hypotonia and difficulties in food intake can be proved to be signs of the syndrome.

R-50 Obesity, Fat

Evaluation of bariatric surgery for morbid obesity adolescents: a preliminary study using laparoscopic adjustable gastric banding

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INTRODUCTION:
Severe obesity is a chronic condition difficult to treat through diet and exercise alone. A study carried out by the Israel ministry of health included Jewish and Arab population, 6% of adolescents included in the study were obese with BMI>95% adjusted for age and gender.Bariatric surgery(BS) is considered as a treatment option for adolescents who have developed extreme obesity.(BMI > 40).after life behavioral changes have failed or suffer from serious obesity-related health problems such as type 2 diabetes, severe sleep apnea. Laparoscopy Adjustable Gastric Band surgery **(LAGB)**works by decreasing food intake by placing a bracelet-like band around the top of the stomach **.**Data on outcomes, complications of **LAGB** in adolescents will be presented.
AIMS
To evaluate co morbidities before surgery and the benefits and risks of LAGB. To determine if it is an appropriate treatment option for teens under 18 years with comorbidities an a BMI >40. To establish clinical recommendations to perform LAGB in adolescents.
METHODOLOGY
Data was obtained by revision of charts. Patients were interviewed and examined at the bariatric clinic using a special validated questionnaire including pre surgery: biochemical and hormonal profile status, body fat, cardiovascular risks, sleep apnea episodes, diabetes indicators, depressive symptoms, quality of life, eating habits, and nutritional status.Out of 100 adolescent patients who underwent **(LAGB) by an expert team,**20 agreed to participate in the study after Helsinki approval was obtained
RESULTS
Preliminary results from 20 patients after LAGB compare to a control group of obesity patients with an BMA>.40 that did not have LAGB,will be reported regarding BMI, pre surgery biochemical and hormonal profile,quality of life and surgical complications.
CONCLUSIONS
In adolescents LAGB is a safe procedure, may prevent or improve complications of metabolic syndrome and the development of type 2 diabetes.May be considered after failure of life behavioral change and should be performed by a team of expert surgeons with a wide experience in BS. Further clinical studies are necessary to establish clinical guidelines for performing LAGB in adolescent population.

R-51 Obesity, Fat

Two cases of non-alcoholic fatty liver disease (NAFLD) in childhood

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[**Introduction**] NAFLD associated with pathological findings similar to those of alcohol-induced liver disease has been reported among non-alcoholic and young subjects suffering from lifestyle-related diseases such as diabetes, obesity, and hyperlipidemia.Various factors that cause hepatocellular damage, such as insulin resistance, are involved in some cases of NAFLD. Moreover, in adults, NAFLD may progress to hepatic cirrhosis, increasing the risk of developing liver cancer. [**Case 1**] A 9-year-old boy. His height and weight were 144.6 cm and 54.9 kg, respectively; the patient was obese (BMI 26.3kg/m^2) and showed symptoms of acanthosis nigricans. Urinalysis performed while the child was at school indicated glycosuria. The patient's casual plasma glucose level, HbA1c level, and urinary C-peptide level were 233 mg/dl, 11.0%, and 82.0 μg/day, respectively. Moreover, anti-GAD antibodies were not detected in the patient, leading to the diagnosis of type2 diabetes mellitus. Furthermore, the abdominal ultrasound examination revealed the presence of a fatty liver, and the levels of AST and ALT were 131 IU/l and 244 IU/l, respectively, indicating liver dysfunction. Consequently, liver biopsy was performed for diagnostic purposes.
[**Case 2**] A 15-year-old boy. His height and weight were 162.0 cm and 57.0 kg, respectively; the patient's BMI (21.7kg/m^2) was within the normal range. However, a random blood test revealed elevated levels of serum triglyceride (327 mg/dl), γ-GTP (87 IU/l), AST (70 IU/l), and ALT (137 IU/l), indicating hyperlipidemia and liver dysfunction. The patients fasting plasma glucose level, serum insulin level, and homeostasis model assessment of insulin resistance (HOMA-IR) score were 98 mg/dl, 33.50 μIU/ml, and 8.11, respectively, indicating hyperinsulinemia and insulin resistance. The findings of imaging studies indicated the presence of a fatty liver, and the liver biopsy revealed pathological findings that were consistent with those of NAFLD, supporting the diagnosis of this.
[**Summary**] In some cases of NAFLD, the onset of the disease is caused by hyperlipidemia and insulin resistance, even when there is no indication of obesity. In addition, patients with type2 diabetes in the childhood or liver dysfunction and fatty liver are considered to be high-risk groups, and in order to ensure early detection and management of this disease, it is important to perform liver biopsy at an appropriate time.

R-52 Obesity, Fat

Metabolic syndrome in Prader-Willy syndrome

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Metabolic syndrome - consists of multiple interrelated risk factors which increase the risk for atherosclerotic cardiovascular disease, and raises the risk for type 2 diabetes. Metabolic Syndrome criteria are three of the following, obesity, hypertrigyseride, hypercholesterol, hypertension and hyperglycemia. Prader–Willi syndrome (PWS), the most common genetic cause of marked obesity in humans, is usually due to a derived chromosome 15q11–q13 deletion or maternal disomy 15 uniparental disomy (UPD).
A 7-year-old girl weighing 41.5 kilograms, height 110 cms (P<3), BMI 34.3 kgs/m2 (P>97). She snored and had acanthosis nigricans. Physical examinations found: obesity, waist circumference 97 cm, blood pressure 120/90 mmHg. Laboratory findings: fasting glucose 119 mg/dl, total cholesterol 187 mg/dl, HDL 11 mg/dl, LDL 153 mg/dl, triglyceride 197 mg/dl, FT4 1.16 ng/dl and TSH 2.026 mU/L
The electrocardiography showed cardiomiopathy dilatation, pulmonary hypertention and left ventricle hypertrophy. The chest radiography indicated cardiomegaly and hyperlordosis vertebrae lumbalis (lateral). Results of G-Banding technique chromosome analyse was 46, XX, del(15)(q11.2q12), i.e. there was a delesion in the long arm 15 chromosome, in q11.2q12.
The patient was treated with furosemide, ACE inhibitor, metformin, simvastatin, a diet and exercise.

R-53 Obesity, Fat

Effect of alloprinol on hyperuricemia in male obese children

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There are some reports that serum concentrations of uric acid are elevated in obese children and adolescents. It is well known that a xanthine oxidase inhibitor is effective for adult with hyperuricemia. We investigated the effect of alloprinol on three obese children with hyperuricemia. On the start of the treatment, serum uricate levels of them were higher than 9.0 mg/dl. First case (BMI 45.6) was 13 7/12 years old, complicated with type 2 diabetes mellitus, sleep apnea syndrome and fatty liver, second case (BMI 31.7) was 14 6/12 years old with mild hypercholesterolemia and third case (BMI 30.5) was 14 10/12 years old, associated with hypertension. Alloprinol 100mg twice or three times daily resulted in reduction of serum uricate levels in 2-4 weeks, but they had to be continuously treated with maintenance dose of 100mg once or twice daily. It is the most important to correct overweight for normalizing serum uricate level in obese children, but it is hard to reduce the body weight because of their clinical asymptomatic manifestations. Alloprinol is effective for obese children with hyperuricemia to prevent gout and the development of hypertension, and coronary heart disease nevertheless.

R-54 SGA; Turner, Noonan

Mutation analysis of a mother and son with Noonan syndrome: first report from Turkey

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Noonan Syndrome is characterized by autosomal dominant inheritance, short stature, typical craniofacial features, skeletal abnormalities, congenital heart defects, and predisposition to malignant tumors. The genetic defects underlying the disorder were not clear until 2001. This report is the first to describe the mutation analysis and associated clinical features of a Turkish mother and son, who were clinically diagnosed Noonan Syndrome when the boy was referred to our department due to short stature. The analysis revealed an A®G transition at position 923 in exon 8 indicating an Asn308Ser substitution.

R-55 SGA; Turner, Noonan

Treatment with the combination of GnRH analogue and rhGH in 3 patients with Turner mosaicism diagnosed after menarche

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BACKGROUND Turner syndrome is characterised by short stature, ovarian dysgenesis and infertility. However, between 5 to 30-40% of cases experience late but spontaneous puberty and 4% progress to menarche. In those patients with spontaneous menarche it is possible that treatment with GnRH analogue, with the aim to delay bone maturity, in association with recombinant growth hormone (rhGH) could improve final height.
AIM of our study was to describe the effect of the combination of GnRH analogue and rhGH treatment on the growth of 3 patients with Turner mosaicism which was diagnosed after menarche.
POPULATION AND METHODS All the 3 patients were diagnosed with

Turner mosaicism after menarche because of short stature (range between -0.4 and -3.3 standard deviations (SD) of bone age) minor facial dysmorphisms, or a history of recurrent otitis. At diagnosis mean age was 13.8 years (range 12.25-14.5), mean bone age 13.2 years (range 12-14.5) and mean height 145.5 cm (range 141.6-151.4). They were all treated with rhGH (mean dosage 0.35 mg/kg/week) in association with GnRH analogue (3.75 mg/every 28 days). Mean duration of therapy was 20.3 months (range 10-33).
RESULTS All 3 patients recuperated in terms of SD of height compared both to bone age (with a mean of +0.7 SD of height from diagnosis, range +0.1-+1.6) and chronological age. Two achieved a final height above 155 cm, respectively 156.6 and 155.2 cm. The third case had a final height of 145.4 cm, but started treatment when bone age was already of 14.5 years. All 3 patients presented regular menses after having stopped the combination treatment.
CONCLUSIONS Combined treatment with GnRH analogue and rhGH could be an option in those cases of Turner syndrome with spontaneous menarche with the aim of improving final height. Our three cases seem to suggest a certain improvement, although bone age may represent a limiting factor. A comparison between rhGH alone and the combination treatment with GnRH analogue on a larger number of patients could be subject for future research. From the clinical practice point of view, our case-series highlight the importance of considering Turner syndrome diagnosis also in girls with menarche.

R-56 SGA; Turner, Noonan

Turner syndrome: a particular case report

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Our case report regards a 10 years old female with M2 P1-2 pubertal stage, short stature, phenotipic features suggestive to Turner Sindrome (TS). At first, she was defined as a short staure patient with Hypochondroplasia (H) and TS diagnosis was made only afterwards. Family history demonstered similar clinical features in paternal grandmother; more, both families showed a short stature! When she was observed for the first time in other department to have a surgical therapy for interatrial defect (DIA), short stature with short limbs were noted: it was performed a X-ray total body with final diagnosis of H. Further patient evaluation in our department, showed weight to 50°centile and height < 3° centile for age, short neck with a webben appearance, low hairline at the back of the neck, low set ears, cubitus valgus, synophrys, shortness of fourth metacarpal and metatarsal, disproportionate shortness with alterated trunk/limbs ratio (trunk > limbs). More, blood exams showed growth hormone deficiency (GHD) (GH = 3.9 ng/ml at basal time, 0.7 ng/ml at 60' and 90' after Clonidine test), bone age value of 8 yrs 10 m old (less than chronological age but higher than statural age) and XO cariotype; all other exams performed do not result significantly alterated. These findings permitted a TS diagnosis in association with GH deficiency. We began GH therapy replacement (1 UI/Kg/week; 12 mg/week) with good patient compliance and height gain. Our case report underlines the importance of anamnesis but at the same time clinical evaluation of patient: short stature in family history is non sufficient alone for a correct diagnosis!

R-57 SGA; Turner, Noonan

The characteristics of Turner's syndrome in the Republic of Uzbekistan

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TS is a chromosomopathy caused by complete or partial monosomy of X-chromosome. The main constant features of TS are short stature and gonadal dysgenesis. The growth variation is depended from chronological age, karyotype and ovarian failure.
Aim: retrospective analyze of frequency of age of diagnosis, growth, phenotypic and karyotype characteristics of TS in Uzbekistan.
Material and methods: 29 female with TS were examined in our clinical from 1997 until 2007. All patients underwent by karyotype. Level of night GH secretion on urine, IGF-1, IGFBP-3 has been measured by RIA.
Results: We have found that 90,7% of patients were in pubertal age period (11-16 yr); mean age of diagnosis was 14,6 ±0,3 yr; growth delay was-4,5±0,26SDS; differences between chronological and bone age were 4,5 ± 0,1 yr. Analysis of karyotype has been showed frequency of monosomy was 62,1 %; mosaicism -37, 9% (45X/46XX-24,1%; 45X/ 46XY-13,8%). Level of night GH secretion on urine was 1,4± 0,16ng/ml; level of IGF-1 was 69,3±7,3 ng/ml and IGFBP-3-1726,4± 98,5 ng/ml.**Conclusion:** The diagnosis of TS was established in pubertal age period, with the deficiency of GH and IGF-1. The main causes of medical aid appealability was short stature.

R-58 SGA; Turner, Noonan

Final height in Turner syndrome treated with rhGH

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Short stature is one of the main features in Turner syndrome /TS/. At present appropriate treatment for girls with TS may include growth promoting therapy and pubertal induction with the dual aims of optimizing adult height and facilitating psychosocial adjustment.
We treated 82 girls with TS aged 4 -18 years with recombinant human Growth Hormone /rhGH/ for 2 to 12 years.23 of them reach final height / reach 20years of age and bone age - 18 years /. The growth velocity increase from 2,5 sm - 1 year before terapy till 7,03 sm in the first year, 5,17sm for the second year and 4,49sm till 2,98sm from 3 - d to ten - d years of treatment. The growth retardation decrease from SDS h = - 2,56 till SDS h = - 1,26. The final height was 151,2 sm, with 11,2 sm more than final height of untreated girls. The final height depends on retardation of growth and bone age in the beginning of therapy, on the duration of treatment and dose of rhGH.
In conclusion it was underlined that the early diagnosis and early rhGH therapy is of a greath importance for the best final heght and good social prognosis of TS.

R-59 Thyroid

Thyroid hormone resistance syndrome: case report

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Introduction
Pituitary resistance to thyroid hormone is defined by high levels of hormones and TSH inappropriately normal/high and clinical hyperthyroidism
Phenotype is variable, depending the severity of the mutation, the diversity of response and tissue-specific factors unrelated to the mutation
Case Report
Female, 1y2m, born at term by cesarean. Mother presented hypertension in pregnancy, birth weight=3150g (-0.51SDS), length=50cm (0.28SDS). Mid parental height 155.9cm (-1.15SDS), family history of hypothyroidism. Presented failure to thrive, and despite her nutritionally balanced diet, she has been losing weight. Neuropsychomotor development adequate. Chronological age=1y2m, weight=6895g (-3.71SDS), height=73cm (-0.99SDS), BMI=12.9kg/sqm, irritability, tachycardia, Tanner 1. No phenotypic abnormalities detected and thyroid examination normal.

See figure 1 for hormonal profile at baseline.

	Case 1	Reference values
TSH	3.50	0.3-5.0 uIU/mL
FT4	2.66	0.80-1.40 ng/dL
T4	20.3	7.2-15.6 ng/mL
FT3	0.75	0.2-0.4 ng/dL
T3	334.0	94.0-269.0 ng/dL
TBG	29.9	2-70 ng/dL
Antithyroglobulin Ab	<20	<40 IU/L
Antiperoxidase Ab	5.2	<12 IU/L

TBG=thyroid binding globulin; Ab=antibody

Thyroid US revealed thyroid increased in size, volume 3.3cm3 (2.3 ± 0.7cm3)
Diagnostic hypothesis: Thyroid Hormone Resistance Syndrome
Follow-up: Treated propilthiouracil therapy at age of 1y2m, dose of 3.8 mg/kg/day. After treatment, increase in both weight and height, and improvement in irritability and tachycardia. Decrease in FT4 levels and increase in TSH. Propilthiuracil was discountinued. However, as FT4 gradually increased over again, she started methimazole 0.62 mg/kg/day. She is aged 3y and has been treated for 2y5m, showing satisfactory linear growth and weight gain, weight=13.9kg (+0.02SDS), height=93 cm (-0.23SDS), BMI=16.1 kg/sqm (+0.27 SDS), Tanner 1.

See the following figure 2 for hormonal profile after treatment.

	Case 1	Reference values
TSH	3.25	0.4-4.0 mIU/mL
FT4	2.01	0.70-1.48 ng/dL

Molecular analysis: Thyroid hormone receptor revealed a mutation.
Nucleotide changes: 1243 CGC-GGC
Códon changes: R320G, Arginin to glicin position 320
Parent´s and brother DNA without mutation
Conclusion: Children with amendment in molecular receptor TSH, has great difficulty of monitoring clinical and laboratorially

R-60 Thyroid

Prevalence of associated birth defects in congenital hypothyroidism

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Introduction: A high prevalence of congenital anomalies have been reported to be associated with congenital hypothyroidism. Prevalence is variable in different ethnic groups and there is scant data from India.
Subjects and Methods: This is data from an ongoing cross sectional study conducted among children with confirmed primary hypothyroidism having an early infantile or neonatal onset of symptoms. Pertechnetate thyroid scintigraphy, echocardiography, ultrasonography of abdomen and radiography of spine were performed.
Results: Among 8 males and 6 females studied, 10 had congenital malformations. Dysmorphic features were quite prevalent. Five subjects had cardiac malformations [osteum secundum atrial septal defect (ASD) 3, patent ductus arteriosus (PDA) 1, both osteum secundum ASD and PDA 1]. Six subjects had neural tube defects in the form of spina bifida occulta. No renal anomalies were detected on ultrasonography of abdomen.
Conclusions: High prevalence of cardiac and neural tube defects is seen in congenital hypothyroidism. Meticulous clinical examination and appropriate screening is required in each subject.

R-61 Thyroid

A case of thyroid hemiagenesis

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Thyroid hemiagenesis is a rare congenital anomaly in which one thyroid lobe fails to develop. We report 26-month-old female who presented compensated hypothyroidism. She visited our hospital for growth retardation and bowleg. Thyroid scan revealed the absence of left lobe. Ultrasonography confirmed the left lobe agenesis. She had no goiter and parathyroid hormone deficiency. No other associated congenital anomaly. The purpose of this report is to present a rare case of thyroid hemiagenesis in toddler.

R-62 Type 1 Diabetes

Competitive sports and diabetes

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The fact is the athletes do better academically than their no athletic counterparts; and, regular physical activity can enhance mental health, reducing symptoms of stress and depression and improving self-esteem.
However, In diabetes patients there may be special considerations if interested in certain adventure sports, like rock climbing, hang gliding, or scuba diving Factors such as muscle contraction, increased blood flow and increased body temperature cause the body to more responsive or 'sensitive' to insulin during exercise.
Some lectures show no correlation between the degree of activity and HbA_{1c} levels, or hypoglycemic events. HbA_{1c} levels were less than 6,8% in non-active active patients versus of active patients and A negative correlation was found between physical activity and daily insulin usage, We conclude that patients' attitude towards exercise was not improved by our educational methods and that physical exercise was not necessarily associated with good blood glucose control. $HbA1_c$ levels, used to measure long-term glycemic control, might soon be replaced with average blood glucose levels; a change experts say will add clarity to diabetic patients looking to manage their disease. Its true base on high level of glucose effects and lactic effect of DM.

R-63 Type 1 Diabetes

Good result for betamethasone added to an insulin analog, as treatment for insulin analog induced lipoatrophy. Report of a case

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Introduction
Insulin analog induced lipoatrophy is a rare complication during the treatment for type 1 diabetes mellitus (T1DM) and is characterized by the loss of subcutaneous fat at insulin injection sites. The exact pathofysiology of insulin analog induced lipoatrophy is not completely understood. Immunological reactions causing lipoatrophy have been described. We demonstrate a seven-year-old girl with T1DM who developed lipoatrophy during treatment with insulin-aspart by continuous subcutaneous insulin infusion (CSII), and who was successfully treated by the addition of betamethasone to the insulin analog.
Patient
A seven-year-old girl was diagnosed with T1DM at the age of one year. Treat-

ment by CSII (insulin-aspart) was started at the age of five years. The insulin infusion set was changed every third day. Metabolic control of the diabetes was poor, mean HbA1c was 9.5%. Besides the T1DM, the patient was otherwise healthy. At the age of seven years she developed progressive lipoatrophy on both buttocks and upper legs, at the insulin infusion sites. At first, insulin-aspart was replaced by insulin-lispro. The CSII-needle was inserted at the edges of the lipoatrophic sites. However, the lipoatrophic process did not improve. Then, betamethasone was added to the insulin-lispro (1 microgram betamethasone per IU insulin). During the following months the lipoatrophy almost completely resolved. Metabolic control of the diabetes remained poor. Unfortunately, because of fluctuating blood glucose values the betamethasone/insulin-lispro solution had to be stopped and only insulin-lispro was continued. At the age of nine years the lipoatrophic process remains stable.

Conclusion

The addition of betamethasone to an insulin analog may be helpful in the treatment of insulin analog induced lipoatrophy. In our experience, success is determined by early recognition of the lipoatrophy and immediate start of the treatment. Also, a prolonged infusion of the bolus during meals, or the administration of the betamethasone/insulin analog solution by single needle injection, seem to give better results.

R-64 Type 1 Diabetes

Potential role of sorbitol pathway in diabetes type-1 complications and activation of poly (ADP-ribose polymerase) (PARP) pathway

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Complications common to Type-1 Diabetes, such as cataract, cardiovascular disorders and polyneuropathy have been associated with activation of sorbitol pathway, non-enzymatic glycosylation and activation of protein kinase-c. When there is hyperglycemia, excess glucose is shunted to polyol pathway, Aldose reductase(AR) competes with glutathione reductase for their co-factor NADPH leading to decrease in GSH, altered NAD^+/ NADPH ratio causes NOx to produce ROS. Sorbitol accumulates in endothelial and retinal pigment epithelial cells, causes osmotic stress and decrease in Na^+-K^+ ATPase which lead to damage of retinal pericyte and endothelial cells. Fructose-3 phophate and 3-deoxyglucosone (3-DG) metabolite of fructose increases AGE formation. Binding of AGE with receptors on monocytes, endothelium etc increases oxidative stress. This results in increased formation of Peroxynitrite (Product of superoxide anion radical with nitric oxide). Increased Nitrosative stress has been implicated in DNA strand breakage, followed by PARP activation which depletes NAD^+ and results in increased formation of methylglyoxal (AGE), and death of retinol pericyte and insulin producing Islet cell in Type-1 Diabetes. NAD^+ depletion increases the glycolytic flux towards the formation of diacylglycerol, an activatior of Protein Kinase-C. PARP activates a number of transcription factors (Nuclear factor kappa-B, APIST, P-53 Protein), this in turn up-regulates numerous genes like endothelin-1, COX-2 and inflammatory genes those implicated in pathogenesis of peripheral diabetic neuropathy, retinopathy and nephropathy.

R-65 Type 1 Diabetes

Report of an adolescent boy with Marfan syndrome associated with type 1 diabetes

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The association between Marfan syndrome and type 1 diabetes is very rare. Herein we report a 14 year-old boy with type 1 diabetes and Marfan syndrome. This patient is the fifth case in the literature of Marfan syndrome associated with insulin-dependent diabetes mellitus, although the relation between them remains unclear.

A 14- year-old boy was admitted to hospital with polyuria, polydipsia and mouth dryness. His blood glucose level was 339 mg/dL. Following the diagnosis of diabetes mellitus, he received insulin treatment. His laboratory findings:HbA1c:8.8%, low C-peptide level 0.96 ng/mL (0.9-7.1), insulin antibody (-), islet cell antibody (+), antiGAD antibody (-). He had tall sature (182 cm, >97 percentile), arachnodactylia of the hands, fingers, feet and toes, myopia, bilaterally subluxation of the lenses. Echocardiography revealed aortic rooth dilatation, tricuspid regurgitation, sligth aortic regurgigation. A negative urinary cyanide nitropruside test and normal blood homocysteine level was found to eliminate homocysteine metabolism defects. These symptoms and findings were compatible with Marfan syndrome. It was learned that his brother (18-year-old) had an operation for aortic dissection and Marfan syndrome diagnosed in another center.

Marfan syndrome is a common, autosomal-dominant, systemic disorder of connective tissue. It is caused by mutations in FBN1, the gene encoding fibrillin-1, associated with a disregulating of transforming growth factor-β activating and signaling. Transforming growth factor-β (TGF- β) plays a crucial role in regulating cell proliferation and in modulating immune cell function. Type 1 diabetes is an autoimmune condition resulting from a cell-mediated immune destruction of pancreatic β cells. So transforming growth factor-β deficiency may contribute to the pathogenesis of the Marfan syndrome associated with type 1 diabetes.

R-66 Type 1 Diabetes

Infantile-onset diabetes mellitus in a case of Wolcott-Rallison syndrome: a 1-year follow-up

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The incidence of Infantile-onset Diabetes Mellitus (IODM) is increasing, with multiple syndromic associations rather than a single perspective. A 6-month -old girl of consanguineous marriage was admitted to the hospital with a history of failure to thrive for the last month duration. She was diagnosed diabetes mellitus because of her hyperglycemia (425mg/dl). She had no acidosis. After with insulin treatment, she had full recovery but remained on regular insulin injections. Mutations were identified in the gene encoding the eukaryotic translation initiation factor 2-α 3 (EIF2AK3). After Wolcott-Rallison Syndrome (WRS) was diagnosed, epiphyseal dysplasia also became evident. At the 9th months of her follow up, her liver functions tests (LFT) were markedly elevated (ALT: 3976 U/L, AST: 3100 U/L), no cause was identified and her LFTs resolved spontaneously. In conclusion, any infant with diabetes mellitus should be screened for epiphyseal dysplasia and mutation analysis shoul be performed, so that diagnosis of WRS and implications for management planning can be made early. Family support is mandatory and close attention should be paid to psychosocial issues.

R-67 Type 1 Diabetes

Clinical – biological study concerning the association autoimmune thyroid disease – type 1 diabetes mellitus in children and adolescent

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Introduction. Type 1 Diabetes Mellits (type 1 DM) might be associated on a variable degree with other autoimmune diseases (endocrine and/or non-endocrine).

Aim. Detection of the association type 1 DM - autoimmune thyroid disease (ATD) in type 1 diabetic children and adolescents followed-up in Clinic II Pediatrics between 2000 – 2009.

Material and method. We evaluted clinically, biologically and by ultrasound exam 95 patients (55 girls, 40 boys) aged between $1^9/_{12}$ - $17^9/_{12}$ (at first visit). The duration of evolution of type 1 DM varied between 1 day (onset) and $15^4/_{12}$ years. In the cases suspected of ATD the titre of specific antithyroid antibodies: antithyroxinperoxidaze (antiTPO) and antithyreoglobulin (antiTG).

Results. Even since the onset of diabetes we found the following associated diseases: 2 cases with vitiligo, 1 case with alopecia areata and 3 cases (2 girls, 1 boy) with goitre. In evolution 8 cases (7 girls, 1 boy) developed difuse goitre**.**

All 8 cases also presented poor glycemic control and clinical signs of hypothyroidism (7 cases) or hyperthyroidism (1 case). In 10 cases we found increased TSH with decreased FT4. The single case with signs of hyperthyroidism presented positive titres of TPO and TG antibodies, increased FT4 with decreased TSH, to almost zero. Thyroid echography found morphological anomalies in all cases, even in those without clinical evident goitre. TPO and TG antibodies were positive in significant titre. In our screening we found also 5 cases without goitre – with normal FT4 but with slightly increased TSH and negative titres of TPO and TG antibodies, which are also monitored periodically.
***Conclusions*.** 1. Autoimmune diseases might be associated with type 1 DM in the child even since its onset. 2. Our results are concordant with the literature data showing that ATD is the most frequent autoimmune disease associated in type 1 DM.

R-68 Type 1 Diabetes

Multi-organ system dysfunction in an adolescent female with longstanding poorly controlled type 1 diabetes mellitus

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Objective: To highlight the potential for severe and rare multi-organ system complications secondary to poorly controlled Type 1 Diabetes Mellitus (DM).
Setting: An urban tertiary Medical Center.
Patient: A 14 year old female with Type 1 DM diagnosed at age 8 years with chronic nonadherence to prescribed medication and diet (17 episodes of diabetic ketoacidosis). Hypothyroidism developed at age 9 years following radioactive iodine therapy for Graves' Disease present at DM diagnosis. The patient developed several severe complications of poor glycemic control 5 years after diagnosis around the time HbA1C and TSH levels peaked 5 at 16.4% (normal <6.3) and 219 microIU/L (normal <4.8), respectively. Glycogenic hepatopathy occurred manifesting as acute elevations in serum liver transaminases and massive tender hepatomegaly. Workups for autoimmune and infectious hepatitis were negative. A liver biopsy demonstrated deposition of glycogen in hepatocytes with glycogenated nuclei without steatosis. Two months later, the patient exhibited involuntary choreo-athetoid hand movements with a concomitant blood sugar > 600 mg/dL and ketonuria. In addition to poorly controlled DM, chronic hypothyroidism contributed to severe dyslipidemia (LDL 445 mg/dL, triglycerides 1607 mg/dL) despite prescribed statin therapy. There was coexisting chronic vaginal candidiasis and microalbuminuria.
Intervention: Liver function tests and hepatomegaly improved over 3 months with insulin therapy. Involuntary movements resolved within 48 hours of hospital admission with achievement of near normoglycemia. The vaginal candidiasis improved with normalization of blood sugars and fluconazole. Microalbuminuria resolved with ACE inhibitor therapy. Dyslipidemia persisted with continued TSH elevation.
Conclusions: Both common and unusual acute and chronic severe multiorgan system complications of poor glycometabolic control can manifest in young patients with longstanding uncontrolled Type 1 DM. Glycogenic hepatopathy is an often underrecognized form of diabetic liver disease which may mimic steatohepatitis and requires biopsy for definitive diagnosis. Movement disorders are rare complications of nonketotic hyperglycemia in adults but are less commonly reported in Type 1 diabetics. Both complications are avoidable and largely reversible with good glycemic control. In addition, our patient demonstrates that improved glycemic control alone cannot correct dyslipidemia in the face of coexisting hypothyroidism**.**

R-69 Type 1 Diabetes

Integrated and comprehensive management of type-1 diabetes in Indonesia

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Despite the huge children population which reach more than 80 million, prevalence of type-1 DM is still un-known. The very small number of identified cases found through hospital records has not express the real burden as escalation of cases was found through out the years. Awareness of people including physicians and other health workers are still lacking which resulted in children die un-diagnosed. Recent data shows that 87% of newly diagnosed patients come with diabetic keto-acidosis (DKA), 77% of patients have DKA at least once and 30% of them have more than 3 episodes of DKA.
To improve current type-1 DM management situation in Indonesia, specific project was developed by the Indonesian Paediatrics Society with support from World Diabetes Foundation.
Projects' goals are:

- To increase awareness among various stake holders about type-1 DM problems in Indonesia
- To improve the capacity of detecting and managing type-1 DM in Indonesia through training of health care personnel, families of type-1 DM patients as well as providing peer-based and role model support in diabetes management for the patients through diabetes camp.

To achieve the goals several activities during the three years projects' duration were planned; which include:

1. Public Awareness campaign on type-1 DM
2. Revision of guidelines for management of type-1 DM and development of training materials
3. Training on the management of type-1 DM for paediatricians which will be conducted in 7 cities (Jakarta, Yogyakarta, Semarang, Surabaya, Denpasar, Medan and Manado)
4. Training of nurses as type-1 DM educators which will be conducted in Jakarta and Surabaya
5. Training on the management of diabetes at home for families of children with diabetes, to be conducted in Jakarta, Semarang, Yogyakarta, and Surabaya
6. Diabetes Camp

Expected outcomes are:

- 10 million people exposed to the cause per year through campaigns and events
- 360 paediatricians trained in the detection and management of type-1 DM
- 60 nurses trained as diabetes educators for type-1 DM
- 25 - 30 hospitals in 12 cities will have special diabetes educators for type-1 DM
- 150 families trained in the management of type-1 DM at home
- 100 children with type-1 DM experience peer-based and role model support in diabetes management through diabetes camp
- At least 375 already identified type-1 DM patients and their families have access to continuous structure education provided by diabetes educators

R-70 Type 1 Diabetes

Hypernatremia and hyperosmolarity in new onset diabetes – the potential role of sugar and salt containing fluids

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Background: The classic signs and symptoms of Type 1 Diabetes Mellitus (T1DM) include polyuria, polydipsia, and weight loss. Prior to presentation, consumption of sugar and salt containing fluids may result in exaggerated hyperglycemia, hyperosmolarity, and complicate the clinical picture at presentation. and subsequent medical management. There are rare reports describing such cases in children with T1DM
Objective: To describe 2 cases of new-onset T1DM presenting with hyperosmolarity, hypernatremia, and hyperglycemia after consumption of large amounts of sugar containing soft drinks and to quantify the amount of sugar and sodium ingested via these liquids.
Results: Case 1 is a 9 year old male with a 1 week history of classic symptoms. He drank approximately 4 liters of fluids high in sugar and salt (208 grams carbohydrates, 376 mg sodium) within a few hours prior to presentation. His initial serum glucose was 1520 mg/dL, serum sodium 145 mmol/L (169 mmol/L corrected), serum osmolarity 405 mmol/L, and bicarbonate 10 mmol/L. He was arousable but disoriented and confused. He received IV fluids over 5 days and electrolytes slowly normalized. Head CT was normal with no signs of cerebral

edema. At discharge, he was stable and back to baseline. Case 2 is a 13 year old male with a 5 day history of symptoms. He consumed approximately 8.4 liters of high sugar and salt containing fluids (499 grams carbohydrates, 529 mg sodium) over several hours prior to presentation. His initial blood sugar was 1024 mg/dL, serum sodium 137 mmol/L (151 mmol/L corrected), serum osmolarity 333 mmol/L, and bicarbonate 24 mmol/L. Electrolytes normalized with IV fluids and oral rehydration over his 3 day hospitalization. His mental status was normal throughout his inpatient stay.

Discussion: Hyperosmolarity at presentation of T1DM may be due to several causes. Hyperglycemia causes osmotic diuresis resulting in hyperosmolarity. The ingestion of fluids high in sugar and sodium may exacerbate preexisting hyperglycemia and promote hypernatremia. Although Case 2 drank a larger volume of fluids, the hyperglycemia and hyperosmolarity was more pronounced in Case 1, who was also more acidotic and symptomatic at presentation. Interestingly, both individuals ingested only a fraction of the recommended daily allowance of sodium. These observations suggest that osmotic diuresis due to hyperglycemia may be the primary cause of hyperosmolarity and hypernatremia in these cases.

R-71 Type 1 Diabetes

Is complementary and alternative medicine an attitude among type 1 diabetic children?

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The use of complementary and alternative medicine (CAM) is an area of interest for research people recently. In the present study we conducted a survey using a questionnaire completed by childhood onset type 1 diabetic subjects or by their parents depending on age. A total of 195 subjects (age 14.02+/-4.7, mean+/-SD; F/M 103/92) attended the survey. Eighty-five subjects (43.6%) reported any kind of CAM use of which 64 (75.3%) was herbal medicine. Sixty-nine subjects (81.2%) informed the diabetes specialist about CAM use. Thirty-eight subjects (44.7%) evaluated CAM as efficious. In less than 10% of subjects was the physician who was recommended CAM and only 3 subjects (3.5%) interrupted insulin injections for the sake of CAM. We obtained detailed information about family status, disease status and disease-family interrelations. We did not find any relation of CAM use with parental education and insulin dose. There was significant relationship between CAM use and higher family income (p=0.027), urban residance (p=0.05), presence of complications (p=0.03), medical therapy dissatisfaction (p=0.034) and prior CAM use among parents (p=0.001). In conclusion CAM use is a considerable practice among diabetic children which is not always shared with visiting physicians and sometimes even giving rise to treatment cessation. Therefore the questioning of CAM use in routine case report forms is imperative. Moreover there is need for prospective studies comparing metabolic control between CAM-users and others.

LB-R-72 Late Breaking Submissions

Retroperitoneal functional ectopic adrenal carcinoma

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Three years 10 months old boy, whom started since 4 months old with progressive weight gain, generalized hirsutism, apocrine sweat, pubic hair, enlarged penile, and frequent erections.

Weight 26.8 kg (percentile >97), height 109 cm (percentile >97), BMI 24.7 (percentile >95), TA 120/80 (percentile >90), full moon face, hump back, abdomen with increased fat mass and purple striae; a mass of 4-5 cm was found in the left iliac fosse, of hard consistency, mobile and regular surface, Tanner scale was 4 for genitals and 3 for pubic hair, penile volume index 26.9 cc (+8.89 SD = 14 years), testis volume was 2 cc (+0.09 SD = 3 years).

Adrenal androgens, cortisol and renin were increased and ACTH was suppressed. AP chest radiograph was normal, abdominal CT scan shows heterogeneous mass in left iliac fosse (4.4 x 5.8 cm, lobed contours, and irregular areas of necrosis without calcifications). Both adrenal glands were small and were localized in the upper zone of the respective kidney.

Surgical resection was performed, and the tumour was located in the left retroperitoneal region, adjacent to the spermatic left cord, it has nodular appearance, and it has highly vascularyzed wall part. During surgery, a control CT showed adrenal glands, retroperitoneum and liver without evidence of macroscopic metastasis tissue. Hormonal determinations performed after surgery showed normalization of adrenal androgens precursors. Two months after the surgery a control CT shows multiple metastasis lesions in both lung and in bilateral lymph nodes, and bone scintigraphy was normal.

Immunohistochemistry analysis demonstrated that all malignant cells were positive for Viventina, SNE, synaptophysin, citoquertinas (AE1/AE3) and EMA, with the following oncogenes and proliferation markers: Ki67 (MIB-1) (45), p27 (85%), p16 (40%), p15 (75%) and PRB (90%) and negative for PS-100, chromogranin and cyclin D1.

Histological final diagnosis was extra-adrenal tumour of adrenal cortex 70g, adrenal gland-type carcinoma with capsule invasion, vascular permeation, and tumour cells thrombus in the hiliar vessels. The biopsy of both adrenal glands was normal.

Conclusion

This patient with adrenal carcinoma in ectopic adrenal rests, adjacent to the spermatic cord with clinical and biochemical overexpression of the three hormonal types of adrenal function and presence of metastases in, as we know, the first case reported in the literature.

LB-R-73 Late Breaking Submissions

Growth hormone deficiency in patients with pituitary lesions

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Aim. We here report growth data and pituitary function tests in patients with short stature and abnormal morphology of pituitary on magnetic resonance imaging (MRI)

Results. Four patients with different initial diagnosis (Congenital Hypothyroidism –CH, Facial Genital Dysplasion- FGD, Uveitis, and Pubertas Tarda –PubT) referred to endocrinologist examination because of growth failure. Auxological data are presented in the table, as well as growth hormone response in Clonidine and insulin provocation tests. MRI of pituitary was indicated and discovered pituitary lesions in all of them.

Variable	Pat No 1	Pat No 2	Pat No 3	Pat No 4
Age (years)	4.3	9.0	14.3	13.25
Bone age	1.5	7.5	9.5	10.0
Height SD	-3.2	-2.4	-2.6	-2.7
GH peak mU/l				
Insulin test	0.29	12.5	3.9	0.6
Clonidine	2.4	5.0	17.0	3.18
IGF1ng/ml	25	121	270	169
Diagnosis				
At admission	CH	FGD	Uveitis	PubT
Final	Panhypopit.	Hypopit	Hypopit	Hypopit

MRI documented hypoplastic pituitary in No1, stalk thickening in No2, and hypodense intrasellar mass in No3 and 4, suggestive of pituitary microadenoma or hypophysitis.

Conclusion. Careful functional and imaging investigation is necessary in all patients with growth failure, despite of initial diagnosis.

LB-R-74 Late Breaking Submissions

Biliary pancreatitis and non alcoholic steatohepatitis in an obese adolescent female on oral contraceptives, metformin and spironolactone

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Introduction: It is a well known fact that gall stones, Non Alcoholic Steato Hepatitis (NASH) and biliary pancreatitis are more prevalent in obese females. The association of biliary hepatitis, pancreatitis and OCP s has been published in literature but the association of biliary pancreatitis with Spironolactone treatment is not well published in pediatric population.
Case presentation: 15 yr old obese Hispanic female was admitted to the pediatric floor with right upper quadrant pain, vomiting and weakness for three days. Her past history was significant for dyslipidemia, PCOS, Metabolic syndrome and left ovarian cystadenectomy. She was on treatment with OCP and Metformin for PCOS for a period of 2yrs prior to admission. Spironolactone was added just 9 weeks prior to admission due to her uncontrolled hirsuitism. On examination she was found to be obese with acanthosis nigricans, hirsutism, tanner 5 puberty and tenderness in the right upper quadrant. She had elevated levels of amylase (1593 IU/L), lipase (2062 IU/L) and liver enzymes (LFTs)(AST-868/ALT-1573). Imaging studies showed multiple (cholesterol) gall stones and thickened gall bladder. She had elective cholecystectomy with liver biopsy that showed micro and macro vesicular steatosis and mild portal inflammation with early fibrotic changes.
Discussion: Comorbidities of obesity includes fatty liver that later progresses to non alcoholic steatohepatitis (NASH). Although the exact mechanisms responsible for the pathogenesis are unknown, insulin resistance probably plays a role. NASH may progress to cirrhosis in up to 20% of patients. NASH is now recognized to be a leading cause of cryptogenic cirrhosis. PCOS is frequently associated with obesity in adolescent girls. Metformin, OCP are commonly used to manage the condition. Our patient was on Metformin and OCP for a period of 2 years. Just 9 weeks prior to admission, she was started on Spironolactone to improve her hirsuitism.
Coclusion: OCPs and Metformin are known to induce hepatotoxicity but are not known to cause NASH. Spironolactone, as a hepatic microsomal enzyme inducer affects the cholesterol saturation thereby resulting in gall stones and biliary pancreatitis. It is unclear if the disease status itself, medications, or their combination has triggered gallstones and biliary pancreatitis in our patient. We speculate the association of Spironolactone with her hepatitis and biliary pancreatitis. More studies need to be done.

LB-R-75 Late Breaking Submissions

Transcranial magnetic therapy in the treatment of obese children

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Aim: to evaluate the efficacy and safety of transcranial magnetic therapy (TMT) in the program of treatment of children with obesity. Patients and methods: 125 obese children 10-16 y.o. were examined. Tests: physical evaluation; height, weight, BMI, WC, evaluation of fat mass (FM%) –BIA; blood pressure; HRV analysis; rheoencephalography; EEG. Design: randomized, placebo-controlled study. Therapy included conventional treatment: diet, physical activity, educational training for all children and TMT in 89 children; 36 patients of control group went through placebo procedures. TMT was carried out with application of travelling magnetic field of frequency 1-10 Hz, induction - 45 mTl during 8-12 minutes every day, 10-12 procedures course. Before investigation and treatment all patients and parents signed informed consent form. Investigation was approved by local Ethical Committee. Results: the main clinical features of the patients: excessive appetite (87,5%), headache (81,7%), arterial hypertension (59%), menstrual problems (25,6% of girls), abnormal body temperature regulation (14%). Dysfunction of autonomous nervous system was found in 63,4% of patients and included sympathetic predominance – 10,7%, parasympathetic – 67,8% of patients. Disturbance of regulation with central nervous system hyperactivity was revealed in 16% of patients. All patients were evaluated in 1 and 3 months. The headache ceased in 77,6% of children, BP normalized in 80,2% of patients. Improvement of autonomous nervous system function was registered in 72% of children. In the control group the headache ceased in 24,6% of patients, BP decreased in 33,8% ($p< 0,001$). Improvement of autonomous nervous system function were not registered in control group. No adverse effects were registered in both groups. Conclusion: transcranial magnetic therapy is the efficient method for correction of autonomic nervous dysfunction in children with obesity.

LB-R-76 Late Breaking Submissions

Comparing the therapeutic effects of spirinolactone + cyproterone compound with metformin on polycystic ovarian syndrome

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Polycystic ovarian disease is one of the most common causes of hirsutism, irregular menstruation, obesity, anovulation or infertility in women. More than 50% of these women become obese, a half of them hypertensive, and $^1/_3$ of them diabetic at a time during their life.
Compound contraceptive pills are the usual treatment of this disorder as they suppress the androgen secreted by the ovaries. Administrating Spirinolactone as an androgen antagonist together with a contraceptive pill such as Cyproterone compound (Dian) can be effective in the treatment of the sings & complications of the patients. Metformin is also useful to improve the insulin resistance, hirsutism and oligomenorrhea of PCOS patients.
This study was performed on 113 women, 20 to 30 years old, diagnosed to have PCOS, visited in Imam Hosein and Loghman outpatient clinics during three years. They came with complaints such as obesity hirsutism oligomenorrhea or amenorrhea. All of them were randomly divided in 6 statistically equal groups (the obese with and without insulin resistance and the non-obese taking Metformin and the obese with and without insulin resistance and the non-obese taking Spirinolactone with Dian).
In conclusion we found that Metformin is effective in improving obesity, hirsutism and oligomenorrhea of obese PCOS patient (regardless of their insulin resistance)($P< 0.005$).
Insulin resistance as an important factor increases the Metformin efficacy in weight reduction of patients ($P< 0.05$). Metformin's therapeutic effect on the hirsutism and oligomenorrhea of non-obese PCOS patients is not valuable. Spirinolactone in conjunction with Dian is effective in improving the hirsutism, obesity and oligomenorrhea of the obese (regardless of their insulin resistance) ($p< 0. 05$) and non-obese patients ($p< 0.005$). Metformin is more effective than Spirinolactone together with Dian on weight reduction in obese PCOs ($p< 0.005$), and Spirinolactone together with Dian is more effective on hirsutism and oligomenorrhea of non-obese patients than Metformin alone ($p< 0.05$).

LB-R-77 Late Breaking Submissions

Ophthalmological diseases in patients with type 1 diabetes mellitus

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Objectives
To know the moment in which ophthalmic diseases developed and its associated risk factors in patients with DM1.
Material & Methods
Clinical history, anthropometrical, demographic, metabolic and clinical variables were analyzed at diagnosis and twice a year: age, and gender were consigned at diabetes start, and during evolution the following data were consigned quarterly: time of evolution, Tanner puberty development, weight, height, BMI, A1c hemoglobin, glucose, triglycerides, cholesterol, urinary protein content, systemic arterial tension, number of ketoacidosis events. Ophthalmologic examination was done by two investigators who were standardized for

the examination of the following form: evaluation of the visual sharpness with Snell's record; primary ametropia detection; extra ocular muscles evaluation, mono and binocular by pursuit of luminous stimuli; microscopy with lamp of crack for annexes evaluation, conjunctive, corneous, previous camera, rainbow, pupil and crystalline; Direct ophthalmoscopy for retina, optical nerve, arterial and venous characteristics and passages as well as spot with its brightness was performed annually, as well as indirect ophthalmoscopy mydriatic exploration of the eye bottom; intra ocular ramming intake with tonometry of Shootz and fluorangiography with retinal camera and intravenous fluorescein in those patients with abnormal indirect ophthalmoscopy.

Results

25 (49%) women and 26 (51%) men. At the time of diagnosis there were 48 healthy patients and 3 with myopia; in the first year, 2 more ametropias were detected; at second year, one more ametropic patient was diagnosed, and in the third year another patient presented ametropia (7 ametropic and 44 healthy patients). After the third year of evolution, two with nonproliferative retinopathy and one with nonproliferative retinopathy and cataract. The time of evolution of the DM1 in the cases with ametropia was of 57±34 months and in the serious alterations 113±54 months (p=0.04). In the patients with serious alterations it was observed a persistent elevation of the HbA1c (p=0.00) and triglycerides (p=0.00).

Conclusions

The ophthalmologic evaluation in patients with DM1 is of great importance since the diagnosis time, with an annual pursuit because ophthalmic alterations were observed since the beginning of the disease. Continuous monitoring by multidisciplinary equipment must be done to facilitate early detection of ocular complications.

LB-R-78 Late Breaking Submissions

Evaluation of periodontal disease in diabetic patients (NIDDM, IDDM) by using CPITN index

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Among the late complications associated to the diabetes mellitus, periodontal disease has been highlighted, and it can be more severe and refractory to treatment than in healthy subjects. Our objective is to determine the prevalence of gingivitis and periodontitis as well as the Community Periodontal Index of Need of Treatment (CPITN) in diabetic population compared with a control one and analyze the histological characteristics in the gingiva of diabetic patients. Therefore an analytic case-control study was conducted among 264 IDDM, NIDDM patients attending a diabetes clinic in Tehran, Iran as case group and 246 persons attending Oral Disease Department of Dental Faculty in Tehran, Iran as control group. The study groups included 233 males and 277 females (112males, 134females in control group; 121males, 143females in case group). The severity of periodontal destruction has been shown to be related to the direct and indirect effects of glycemic control, with other factors also being implicated. Periodontitis incidence was 75.5% in NIDDM, 52.9% in IDDM and 26.4% in control group. Also gingivitis incidence (score1,2) was 24.5% in NIDDM,47.1% in IDDM and 66.3% in control group. In comparison to the females, males had more periodontitis but less gingivitis in all groups. This difference was statistically significant (P < 0.0001). Periodontitis incidence increased by age in all groups; also periodontitis severity increased by age in control group. Diabetes duration had no effect on gingivitis and periodontitis incidence before 4 years; however, they increased by duration more than 4 years. Duration of insulin consumption was considered as diabetes duration. Periodontal abscess, xerostomia, fungal infections were more prevalent in case groups (IDDM, NIDDM) and it was statistically significant (P <0.0001). The analysis of the data also shows that diabetes is two times more prevalent in patients with periodontitis than non-periodontitis patients (12.5% versus 6.3%) and this difference is also statistically significant (P<0.0001).

Thank You

HORMONE
RESEARCH

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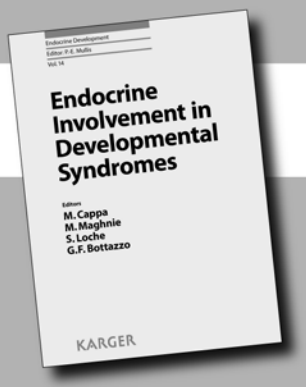

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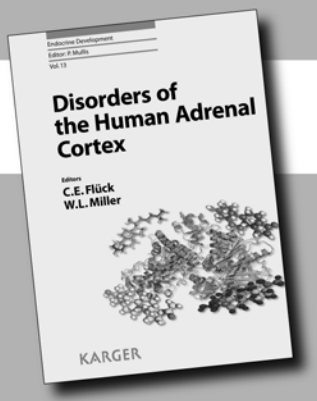

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