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As announced by the President of ESPE, Dr. Atilla Büyükgebiz, it was the first time in ESPE’s history that the meeting is being held in a city having land in both Europe and Asia and having speakers from China and India, as well as a Japanese Society for Pediatric Endocrinology (JSPE)/ESPE joint lecture. The theme of the meeting was “Paediatric Endocrinology and Public Health”. Dr. Büyükgebiz said that efficient public health systems should allow a prompt detection and management of severe childhood diseases which can compromise future health: pediatric endocrine diseases can result in childhood death or severe physical and neuropsychological consequences that can be prevented by proper diagnosis and management. Moreover, childhood and adolescent obesity is an essential public health concern that was discussed at the ESPE 2008 meeting. The “Symposium Istanbul” was dedicated to quality of child care and health in countries of various levels of development and to the challenge that developing countries are facing when trying to provide every born child with the best endocrine care. More than 800 abstracts from 60 different countries were submitted for presentation at the meeting. More than 2400 attendees were registered at the Meeting. The activities directed to clinical practitioners, as well as clinical and basic researchers, included 6 Plenary Lectures, 10 Symposia, 14 Sessions of 6 Oral Free Communications each (total 84), 3 Sessions of Poster Presentations (total 750 communications), 6 Satellite Symposia, 6 ESPE Working Group Sessions, 2 interactive sessions, 2 Year Book Presentation Sessions, 2 New Technology Sessions and 7 (x2) Meet the Expert Sessions.

As in previous years, the ESPE annual meeting was the most important international event of the year in the field of Pediatric Endocrinology.

Summaries and comments of some of the presentations follow:

PLENARY LECTURES
Summaries and comments on most Plenary Lectures, some Symposia and Free Communications follow:

PL1 Genes and Environment
PL1-1 - Epigenetic inheritance in medicine
Eva Jablonka
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Abstract.
The importance of epigenetic inheritance to medicine is beginning to be appreciated. I briefly review some existing lines of research and focus especially on the effects of environmental factors such as abnormal nutrition, endocrine disruptors and social stress on chronic diseases. When such factors are introduced during critical periods of
early mammalian development they often lead to chronic diseases that can be transferred to descendant generations. Although many of the studies are on animal models, they have obvious implication for human health. The study of the epigenetic mechanisms that underlie persistent developmental effects is important for understanding the distribution and causes of many diseases, and for our ability to intervene and control them.

**Comment.**

Dr. Jablonka explained that in evolutionary theory, heredity became identified with genetics, and variation was seen in terms of combinations of randomly generated gene mutations. She argued that this view is now changing, because it is clear that a notion of hereditary variation that is based solely on randomly varying genes that are unaffected by developmental conditions is an inadequate basis for evolutionary theories. Such a view not only fails to provide satisfying explanations of many evolutionary phenomena, it also makes assumptions that are not consistent with the data that are emerging from disciplines ranging from molecular biology to cultural studies. These data show that the genome is far more responsive to the environment than previously thought, and that not all transmissible variation is underlain by genetic differences. In Evolution in Four Dimensions (2005), she identified four types of inheritance (genetic, epigenetic, behavioral, and symbol-based), each of which can provide variations on which natural selection will act. Some of these variations arise in response to developmental conditions. She argued that a better insight into evolutionary processes will result from recognizing that transmitted variations that are not based on DNA differences have played a role. This is particularly true for understanding the evolution of human behavior, where all four dimensions of heredity have been important.

On the other hand, in most discussions of the evolution of sex chromosomes, it is presumed that the morphological differences between the X and Y were initiated by genetic changes. An alternative possibility is that, in the early stages, a key role was played by epigenetic modifications of chromatin structure that did not depend directly on genetic changes. Such modifications could have resulted from spontaneous epimutations at a sex-determining locus or, in mammals, from selection in females for the epigenetic silencing of imprinted regions of the paternally derived sex chromosome. Other features of mammalian sex chromosomes that are easier to explain if the epigenetic dimension of chromosome evolution is considered include the relatively large number of X-linked genes associated with human brain development, and the overrepresentation of spermatogenesis genes on the X. Both may be evolutionary consequences of dosage compensation through X-inactivation.
suffer from these conditions and the pandemic continues to grow without any economic or geographical boundaries. This disease cluster exemplifies a mismatch between the biological system and its ability to cope with environmental challenges introduced in recent history of humans. Nutrient or energy surplus, as is the case in obesity, is associated with chronic, low grade, inflammatory responses in metabolically active sites, most notably, in adipose tissue. This heightened inflammatory status is a critical link between obesity and other associated pathologies, such as insulin resistance and type 2 diabetes. In recent years, emerging evidence support a new concept that nutrients directly engage in specific stress signaling pathways to trigger these responses. Among the mechanisms uncovered are the inflammatory JNK and IKK pathways, Toll Receptors, Endoplasmic Reticulum (ER) stress response pathways, and the STAMP molecule, all of which play a critical role in the metabolic function and its deterioration in cellular models as well as whole animals, leading to type 2 diabetes, dyslipidemia, cardiovascular disease, and many other complications. In this discussion, I will present new evidence demonstrating the role of JNK and ER stress pathways and the function of STAMP molecule in the interactions between nutrients and cellular signalling pathways as they relate to systemic metabolism. I will also discuss how these pathways fail to adapt to environmental insults and could be modified by a variety of interventions for preventive or therapeutic purposes.

Comment.

Over the last decade discoveries in the metabolism field, starting with the association of increased tumor necrosis factor alpha (TNF-α) and other inflammatory cytokines in obesity, have demonstrated the strong inflammatory underpinnings of obesity and associated metabolic diseases. Obesity leads to elevated production of pro-inflammatory molecules such as TNF-α, IL-6, IL-1β, and MCP-1 in experimental murine models and in humans, notably in adipose tissue. Furthermore, migration of inflammatory cells to obese adipose tissue, particularly at later stages of the disease, may contribute to and possibly propagate inflammatory responses. Alterations in lipids and lipid mediators represent another potential component of both inflammatory responses and insulin resistance in obesity. Recently, several mechanistic models have been proposed to explain the emergence of inflammatory and stress responses in obesity and type 2 diabetes, including organelle dysfunction influencing mitochondria and endoplasmic reticulum (ER) and associated stress signaling pathways.

It is evident that many of these harmful responses have common targets in regulating insulin receptor signalling. One possible target is insulin receptor substrate 1 (IRS-1) serine phosphorylation which is mediated by inflammatory kinases such as c-Jun N-terminal kinase (JNK) and IκB kinase beta (IKKβ) and consequently modulates insulin action. Pharmacological inhibition or genetic ablation of either JNK1 or IKKβ is effective in the treatment of experimental insulin resistance and diabetes. JNK, a member of the mitogen-activated protein (MAP) kinase family, is activated by a wide variety of stimuli, including cytokines and environmental stress. It has been shown that JNK1 is necessary for TNF-α induced serine phosphorylation of IRS-1 and insulin resistance in cells and animals. Whole body genetic deficiency of JNK1, but not JNK2, results in marked protection against insulin resistance and hepatosteosis induced by obesity. JNK activity has also been linked to adverse metabolic outcomes in several critical cellular models and tissues. Additionally, inhibition of JNK activity in liver cells using either dominant negative JNK1 or shRNA against JNK1 lowers circulating glucose and insulin levels and increases insulin sensitivity in obese models. In contrast, JNK1 activity has little effect on muscle glycogen levels or the protein levels of key
molecules involved in glucose metabolism, suggesting that enhanced skeletal muscle glucose metabolism may not underlie the direct beneficial effects of JNK1-deficiency in mice. The combined results of these studies reveal that JNK1 activity has differential effects on metabolic disease depending upon tissue and cell type examined.

Adipose tissue inflammation is a critical pathophysiological mechanism underlying obesity-induced metabolic changes and immune cells infiltrate adipose tissue during the late stages of obesity. It remains to be determined, however, whether contributions of bone marrow-derived cells or those of the parenchymal elements are primarily responsible for triggering the inflammatory changes and dictate the detrimental metabolic outcomes of obesity. Since JNK1 action has been shown to lie at the interface of obesity and inflammation and the results of several studies indicate that macrophage activity may contribute to insulin resistance in diet-induced obesity, bone marrow transplant experiments were performed to test whether myeloid JNK1 regulates the development of insulin resistance. Specifically, they transplanted JNK1-deficient bone marrow into wild type (WT) mice and examined the metabolic impact in the resulting chimeras. Their results showed that parenchymal JNK1 plays a predominant role in mediating insulin sensitivity in non-immune cells and that JNK1 activity in myeloid cells is not sufficient to alter systemic glucose metabolism. Therefore, parenchymal JNK activity is the dominant determinant for metabolic homeostasis (Vallerie et al PLoS ONE. 2008; 3(9): e3151).

On the other hand, Hotamisligil’s group (Cell, 2008 134:933-44) also identified a lipokine as a lipid hormone linking adipose tissue to systemic metabolism. Indeed, their search to find how metabolic alterations in adipose tissue are linked to whole-body metabolism through lipid signals led to identification of C16:1n7-palmitoleate as an adipose tissue-derived lipid hormone that strongly stimulates muscle insulin action and suppresses hepatosteatosis. Their data revealed a lipid-mediated endocrine network and demonstrated that adipose tissue uses lipokines such as C16:1n7-palmitoleate to communicate with distant organs and regulate systemic metabolic homeostasis.

PL2 From Gene to Disease
PL2-3 - RNA-mediated epigenetic heredity: From a white-tipped tail to familial diseases
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Abstract.
Paramutation, first observed in maize (Brink, R. A. Genetics 1956, 41, 872-879) and subsequently in a variety of plants (Chandler, V. L. & Stam, M. Nat Rev Genet 2004, 5, 532-44), is a heritable epigenetic change of the phenotype of a "paramutable" allele, initiated by interaction in heterozygotes with a "paramutagenic" form of the locus. Often referred to as an exception to the law of Mendel, which states that genetic factors segregate unchanged from heterozygotes, paramutation is meiotically stable and inherited in the absence of the inducing allele. To date, the closest observations in an animal species were changes in the DNA methylation profiles directed by the allelic locus in the mouse that we and others described as "paramutation-like" effects. We now report a modification in the phenotypic expression of the wild type allele of the Kit receptor gene in the progeny of heterozygotes with a null insertion mutant (Rassoulzadegan, M. et al. Nature 2006, 431, 469-474). In spite of a wild type genomic
structure, the modified homozygotes maintain the "White Spotted" phenotype characteristic of Kit mutants, in this case a white tip of the tail and white feet. This epigenetic modification is efficiently inherited in the absence of the mutant allele. It was related to a decreased level of Kit mRNA, concomitant with the accumulation of RNA molecules of abnormal sizes. On the other hand, transcription of the locus was upregulated in heterozygotes. Sustained expression at the postmeiotic stages, at which the gene is normally silent, led to the accumulation of RNA in late spermatids and in the spermatozoon. Microinjection into one-cell embryos of RNA from Kit tm1Alf/+ heterozygotes, or of Kit specific microRNAs efficiently induced a heritable White-Spotted phenotype. Consistent with converging evidence of a role of RNA in the establishment of epigenetic states and with the detection of RNA in human spermatozoa (Miller, D. et al. Trends Mol Med 2005, 11, 156-63), our results reveal an unexpected mode of epigenetic inheritance by the zygotic transfer of RNA molecules.

Comments
By contrast with a wide definition of the 'epigenetic variation', including all changes in gene expression that do not result from alteration of the gene structure, a more restricted class had been defined, initially in plants, under the name 'paramutation'. It corresponds to epigenetic modifications distinct from the regulatory interactions of the cell differentiation pathways, mitotically stable and sexually transmitted with non-Mendelian patterns. This class of epigenetic changes appeared for some time restricted to the plant world, but examples progressively accumulated of epigenetic inheritance in organisms ranging from mice to humans. Occurrence of paramutation in the mouse and possible mechanisms were then established in the paradigmatic case of a mutant phenotype maintained and hereditarily transmitted by wild type homozygotes. Together with recent findings in plants indicative of a necessary step of RNA amplification in the reference maize paramutation, the mouse studies point to a new role of RNA, as an inducer and hereditary determinant of epigenetic variation. Given the known presence of a wide range of RNAs in human spermatozoa, as well as a number of unexplained cases of familial disease predisposition and transgenerational maintenance, speculations can be extended to possible roles of RNA-mediated inheritance in human biology and pathology.

There has been a recent resurgence of interest in the notion that DNA is not the sole determinant of our inherited phenotype. The strongest evidence for transgenerational epigenetic inheritance has come from studies of paramutation in plants. But few examples have been reported in other species, and the molecular basis for the process has been unclear. A recent paper by Rassoulzadegan and colleagues have now identified the Kit locus as the first example of a paramutable gene of the mouse. Kit(+/+) homozygotes born from Kit(tm1Alf)(+/+) heterozygotes maintain and transmit to their progeny the white-spotted phenotype characteristic of the mutant heterozygote. The observation of unusual amounts of RNA in the sperm of the paramutated (Kit*) males had led us to consider the possibility of RNA-mediated inheritance. A role for RNA was supported further by the efficient establishment of the epigenetic modification following microinjection in one-cell embryos of either sperm RNA of the paramutated males or of the Kit-specific microRNAs miR-221 and -222. Paramutation may be considered to be one possibility of epigenetic modification in the case of familial disease predispositions that are not fully accounted for by Mendelian analysis.

**PL2 From Gene to Disease**
**PL2-4 - Signal-transduction in type 1 diabetes mellitus**
Per-Olof Berggren  
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The insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors. Besides the action of other nutrients, incretin factors, innervation and systemic growth factors, also autocrine and paracrine regulatory loops within the islet of Langerhans modulate function of the insulin-producing β-cell. Although this modulatory role is well appreciated, the underlying molecular mechanisms involved remain poorly understood. The action of the actual factors is mediated by β-cell membrane receptors coupled to either G-proteins or tyrosine kinases, which subsequently activate respective second messenger cascades. Due to differences in cytoarchitecture between rodent and human islets, the human β-cell may be subjected to a unique extracellular milieu which may have implications for signal-transduction and thereby β-cell function and survival. Exposure of the pancreatic β-cell to stimulatory glucose concentrations leads to the activation of a chain of events, which culminates in the release of stored insulin. This complex of processes starts with the uptake of glucose by the β-cell glucose transporters and proceeds with the conversion of glucose into glucose-6-phosphate by the β-cell isoform of glucokinase. Metabolism of glucose in glycolysis and the Krebs cycle results in the generation of ATP. The coupling of glucose metabolism to electrical activity remains central in all models of β-cell stimulus-secretion coupling. The resting membrane potential of the pancreatic β-cell is set by the ATP-sensitive potassium (KATP) channel. Elevation in the ATP/ADP ratio leads to closure of KATP channels, which in turn results in depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type Ca^{2+} channels leads to an increase in cytoplasmic free Ca^{2+} concentration, [Ca^{2+}]_{i}, which promotes insulin secretion. It is of interest to note that [Ca^{2+}]_{i} is not only increasing but is actually increasing and decreasing in an oscillatory manner, which may be crucial for both β-cell function and survival. Unphysiological increases in [Ca^{2+}]_{i} have been linked to cell death in a number of experimental systems. Ca^{2+} coming from the extracellular space, through the voltage-gated L-type Ca^{2+}-channel, is an important determinant of [Ca^{2+}]_{i}. Hence, any alterations in the capability of the voltage-gated L-type Ca^{2+}-channels to conduct Ca^{2+}-influx will have major effects on [Ca^{2+}]_{i}. We have shown that serum from patients with type 1-diabetes increases L-type voltage-gated Ca^{2+}-channel activity in insulin-producing cells. The subsequent increase in [Ca^{2+}]_{i} is associated with DNA fragmentation typical of programmed cell death or apoptosis. These effects of the serum are prevented by adding verapamil, a blocker of voltage-gated L-type Ca^{2+}-channels. A serummediated increase in Ca^{2+}-influx may thus work in concert with the autoimmune reaction associated with type 1 diabetes and contribute to the destruction of β-cells in vivo and thereby aggravate the disease progression.  
In this context we have shown that serum from type-1 diabetic patients contains increased concentrations of apolipoprotein CIII (apoCIII). This factor increases [Ca^{2+}]_{i} and promotes β-cell death. The effects of type-1 diabetic serum and apoCIII on [Ca^{2+}]_{i} and β-cell death are abolished when β-cells are co-incubated with antisera against apoCIII. Signal-transduction will be discussed in light of pancreatic β-cell function under normal conditions and how changes in this process affect the β-cell in type 1 diabetes.  

Comments.  
The abstract is self-explanatory.
**PL4-5 - Disorders of sex development: new genes and new mechanism**

Tsutomu Ogata  
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**Abstract.**  
External genital abnormality in genetic males accounts for the majority of disorders of sex development (DSD), and results from a variety of genetic and environmental factors. Here, I report our recent results on a new gene and a new mechanism leading to male genital abnormality. We identified three different nonsense mutations of MAMLD1, which underwent nonsense mediated mRNA decay, in patients with hypospadias (HS). 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, without demonstrable DNA-binding capacity. Transient knockdown experiments showed significantly reduced testosterone production in murine Leydig tumor cells. These findings indicate that MAMLD1 is a new gene for HS. The prevalence of HS and cryptorchidism (CO) appears to have increased gradually, probably because of deleterious effects of environmental endocrine disruptors (EEDs). Since Most EEDs exert estrogenic effects via estrogen receptors, we performed haplotype analyses of ESR1 and ESR2. While no significant results were obtained for ESR2, we identified a ~40 kb haplotype block in the 3′f region of ESR1 and revealed marked association of homozygosity for the specific haplotype with CO (P=0.0040, OR=7.55) and HS (P=0.000057, OR=13.75). Similar results were reproduced in Italian patients with CO and HS (a collaboration study with Dr. Massart). These results suggest the relevance of genetic susceptibility to male genital abnormalities in response to estrogenic EEDs. Furthermore, I will present our update data on the molecular mechanism involved in the susceptibility.

**Comment.**  
This interesting presentation raises the important clinical question of the incidence of mutations in the MAMLD1 (CXorf6) gene in idiopathic hypospadias. Mutations in this gene have also been found in patients with complex 46,XY DSD including micropenis, bifid scrotum, and penoscrotal hypospadias. Moreover, a recent paper, (Kalka et al., Eur J Endocrinol 2008, 159:453-8), reported that mutations of this gene are associated with a range of severities of isolated hypospadias. In 41 patients with glandular to perineal hypospadias, four mutations (9.7% of cases) were identified. To clarify the molecular function, they first examined CXorf6 protein structure, identifying homology to mastermind-like 2 (MAML2) protein, which functions as a co-activator in canonical Notch signaling. Transactivation analysis for wild-type CXorf6 protein by luciferase assays showed that CXorf6 significantly transactivated the promoter of a noncanonical Notch target gene hairy/enhancer of split 3 (Hes3) without demonstrable DNA-binding capacity. To explain the functional consequences of the mutations Ogata et al. carried out transactivation analysis for the previously described three apparently pathologic nonsense mutations, indicating that E124X and Q197X proteins had no transactivation function, whereas R653X protein retained a nearly normal transactivation function. Subcellular localization analysis revealed that wild-type and R653X proteins co-localized with MAML2 protein in nuclear bodies, whereas E124X and Q197X proteins...
were incapable of localizing to nuclear bodies. Thus, further studies were performed for R653X, revealing the occurrence of nonsense mediated mRNA decay in vivo. Next, transient knockdown of CXorf6 was performed using small interfering RNA, showing reduced testosterone production in mouse Leydig tumor cells. Furthermore, steroidogenic factor 1 (SF1) protein bound to a specific sequence in the upstream of the CXorf6 coding region and exerted a transactivation activity. These results suggest that CXorf6 transactivates the Hes3 promoter, augments testosterone production, and contains the SF1 target sequence, thereby providing the first clue to clarify the biological role of CXorf6.

PL5 Water Electrolyte System
PL5-7 - Central water and electrolyte homeostasis
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Serum osmolality is controlled within a range of 2-3% because of the vital functions it serves. This control is mediated by osmosensors and thirst sensors at the blood-brain barrier near the hypothalamus, which regulate the secretion of vasopressin (VP) from the posterior pituitary, and water drinking behavior, respectively. In the setting of high osmolality, VP secretion limits future water losses, whereas thirst/drinking corrects prior water deficits. In analyzing primary or iatrogenic disorders of water balance, it is important to determine whether VP secretion or water intake is abnormal before deciding upon therapy. Central diabetes insipidus (DI) is most often caused by surgical or accidental trauma, germinoma neoplasms, infiltrative diseases such as histiocytosis X, or brain malformations such as septo-optic dysplasia. Rarely, mutations of the VP gene can cause central DI, probably by causing degeneration of VP neurons due to endoplasmic reticulum stress. Oral or intranasal dDAVP is an effective treatment of central DI. In infants, subcutaneous dDAVP can be an effective therapy but must be closely monitored. Nephrogenic DI is most commonly caused by drugs such as lithium, but can be due to mutations in the VP2 receptor or aquaporin 2 water channel. Nephrogenic DI is treated with diuretics and by limiting solute intake. VP secretion is stimulated not only by hyperosmolality, but also by hypovolemia or hypotension. The latter stimuli cause VP secretion and hyponatremia when accompanied by excessive water intake. Treatment includes restoration of blood volume to normal. In children, SIADH is a rare cause of hyponatremia. Treatment includes water restriction, and perhaps VP receptor antagonists in the future. Severe hyponatremia must be treated with caution, as rapid restoration of serum sodium can cause myelinolysis, neurodegeneration, and death. Thus only symptomatic hyponatremia should be treated emergently, and at a rate of rise of no greater than 0.5 mEq Na/hour.

Comments
The abstract is self-explanatory

PL5 Water Electrolyte System
PL5-8 - The aquaporin water channel
Søren Nielsen
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Abstract.
Abstract text has not been submitted.

Comments.
(Taken from Fenton RA et al., Proc Natl Acad Sci U S A. 2008; 105:3134-9).
A major physiological response of the kidney collecting duct (CD) to AVP is to initialize the trafficking of intracellular AQP2-containing vesicles to the plasma membrane, thus increasing water permeability and urinary concentrating capacity. Although phosphorylation of aquaporin 2 (AQP2) at S256 is known to play a critical role in this exocytic pathway, very little is known about the additional role of phosphorylation at S261, S264, and S269. In the current study, we used a phospho-specific antibody to examine both the acute and long-term effects of AVP on the regulation of pS264–AQP2. Our results show clearly strong increases in S264–AQP2 phosphorylation in response to AVP throughout the CD system, providing clear evidence that dynamic phosphorylation of AQP2 may play divergent roles in its subcellular distribution.

The anti-pS264 antibody used in this study successfully recognized a synthetic AQP2 peptide phosphorylated at S264 and did not cross-react with either unphosphorylated or S256-phosphorylated peptides. Immunohistochemistry determined that pS264–AQP2 was present in principal cells of all CD segments from inner medulla collecting duct (IMCD) through to the cortical collecting duct, as well as cells of the connectin tubule (CNT). In normal animals with free access to water, pS264–AQP2 distribution was consistent, with the majority of labeling being associated with the apical plasma membrane domain. An analysis of subcellular distribution demonstrated that pS264–AQP2 is not located in the ER, Golgi, or lysosomes but is associated with both the plasma membrane and endocytic retrieval compartments. The high degree of colocalization between total AQP2 and pS264–AQP2 suggests that, even under normal conditions, a large percentage of total AQP2 may be phosphorylated at this position. In our previous study, the subapical punctate distribution of the pS261–AQP2 form is completely different from the distribution of the pS264–AQP2 described here, suggesting the presence of both distinct subcellular pools of AQP2 and that phosphorylation may be involved in regulating the subcellular localization of AQP2.

A major finding in the present study is the divergent regulation of pS264–AQP2 by AVP. Acute AVP exposure resulted in increased abundance of pS264–AQP2 within 30 min that remained elevated throughout the time period examined. In addition to this, we observed a time-dependent redistribution of the subcellular localization of pS264. Fifteen minutes after a single IV injection of 1 ng dDAVP, pS264–AQP2 labeling was not apparent in intracellular vesicles but was highly abundant in both the basolateral and apical membrane domains. Sixty minutes after a single i.v. injection of dDAVP, the majority of pS264–AQP2 was associated with the apical plasma membrane and early endosomes, a small percentage was localized to recycling endosomes, and none of the pS264–AQP2 was localized to lysosomes. The colocalization of pS264–AQP2 with the clathrin-coated pit marker adaptin-β after 60 min suggests that a fraction of total pS264–AQP2 is present in coated pits, presumably in preparation for endocytic retrieval of AQP2 by a clathrin-mediated process. This endocytic retrieval of pS264–AQP2 was evident from its internalization into EE1-positive early endosomes, a process that has been shown previously to occur for total AQP2 by a phosphatidylinositol 3-kinase-dependent mechanism. Furthermore, the appearance of pS264–AQP2 in Rab11-positive recycling vesicles suggests that S264 phosphorylation may be involved in AQP2 recycling after internalization.
In conclusion, the study presents the first direct evidence demonstrating AVP regulation of AQP2 phosphorylation at S264. It also shows that differentially phosphorylated forms of AQP2 can have drastically different intracellular distributions. Our findings suggest that phosphorylation of AQP2 at distinct sites may influence both AQP2 trafficking and compartmentalization; providing the clues for extensive future research into aquaporin function.

**PL6 ESPE Award Session & Activities 2 (Henning Andersen Prize - Clinical)**

**PL6-9 - European registry for congenital hyperinsulinism**

Klaus Mohnike1; Khalid Hussain2; Susann Empting1; Thomas Meissner3; Oliver Blankenstein4; Tim M. Strom5; Christine Bellanné6; Claire Nihoul-Fekete7; Pascale De Lonlay8

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**Abstract.**

Congenital hyperinsulinism (CHI) is a devastating medical condition characterised by marked clinical, histological and genetic heterogeneity. The molecular basis of CHI is still unknown in about 60% of patients and the outcome in diffuse type is still unsatisfactory, with the risk of diabetes mellitus and hypoglycaemic brain damage. To integrate the clinical approach and research in CHI a registry is required. Prospective data acquisition has been approved by the institutional ethical board. In addition, historical pseudonymous data were included. Since 1976 841 children (age 1day-19 years) were referred to participating paediatric centres in France, Great Britain and Germany for evaluation and treatment of CHI. Pancreatic venous sampling (PVS; n=234), arterial calciumstimulation (n=46) or 18F-DOPA-Positron-Emission-Tomography (18F-Dopa-PET) resp. 18F-Dopa-PET-CT (n=136) were used for search of a pancreatic focus. 138 focal (FF), 256 diffuse (FD) and 22 atypical CHI were diagnosed. Results in histological proven CHI: 113 FF, 131 FD, 33 atypical and 38 unclassified. Diazoxide resistance (95/103 FF vs.103/108 FD, age at presentation, FF: 61 neonatal, 52 infantile (=day 8-180) vs. FD: 112 neonatal, 11 infantile, 2 > 6 months), 6 missing data. Birth weight range: 2.3–5.6 vs. 2.3–6.4 kg, insulin levels during hypoglycemia, mean glucose requirement (16.1 vs. 16.9 mg/kg*min) were not different in FF and FD. Using sequencing technique a mutation in ABCC8 or KCNJ11 was present in 38 of 40 FF. Out of 51 FD 26 were heterozygote, 15 were compound-heterozygote or homozygote. Conclusions: 1. focal CHI occurs not only in neonatal but in 46 % at infantile age and in 8/108 diazoxide-responsive CHI. 2. No differences were found between diffuse and focal CHI for birth weight, length, minimal glucose, maximal insulin concentrations and glucose infusion rate. 3. Mutational analysis has still been characterized by a low detection rate of mutations in K-ATP channel.

**Comments**

The abstract is self-explanatory
Sotos syndrome is characterized by overgrowth (height and/or head circumference ≥ 98th percentile), facial dysmorphism and mental retardation and is caused by haploinsufficiency of NSD1. NSD1 is involved in the transcriptional regulation of chromatin and is thought to act as both a co-activator and co-repressor of nuclear hormone receptors such as the retinoic acid (RA) receptor. However, the functional roles of NSD1 largely remain to be elucidated. To identify downstream effectors and to map NSD1 into a causative signaling pathway, genome-wide expression arrays (Affymetrix hgu133plus2) were performed on RNA of dermal fibroblasts of 9 Sotos syndrome patients with a confirmed NSD1 abnormality and 9 age-sex matched controls cultured +/- RA. Differentially expressed genes were identified using the Linear Models for Microarray Data package (Bioconductor R). The GlobalTest package was used for analysis of KEGG signaling pathways and Gene Ontology terms. Comparing the groups of Sotos versus Control, either +/- RA, a total of six differentially expressed genes (p.value <0.05) were identified: RASIP1, PKP3, RBM47, MCOLN3, KIAA1128 and KIAA0895. The Ras Interacting Protein 1 (RASIP1) and the RNA Binding protein Motif 47 (RB47) were found to be differentially expressed both +/- RA. Furthermore, the KEGG Mitogen-Activated Protein Kinase (MAPK) pathway and the MAPK Kinase Kinase cascade GO-Term showed a significant difference between the Sotos and Control group after stimulation with RA (FDR-adjusted p.value of 0.023 and 0.0032, respectively). This is the first study to show that deregulation of the MAPK signaling pathway is associated with Sotos syndrome. This pathway is well-known to be involved in other growth disorders, albeit resulting in short stature, such as Noonan and Costello syndrome. Furthermore it confirms the role of NSD1 as a co-factor during RA signaling. In vitro studies are currently performed to further delineate the influence of the NSD1 on this pathway.

Comments.
It has been published that overgrowth and advanced maturation in infancy to early childhood, mental retardation, hypotonia, hyperreflexia, and characteristic minor anomalies are present in patients with intragenic NSD1 mutations predicted to form a truncated NSD1 protein, whereas major anomalies in the central nervous system (agenesis or hypoplasia of the corpus callosum), cardiovascular system (patent ductus arteriosus and atrial septal defect), and urinary system (vesicoureteric reflux, hydronephrosis, and small kidney) were exclusively exhibited by patients with a fairly common ~2.2 Mb deletion involving the entire NSD1 gene. The results suggested that clinical features in Sotos syndrome are classified into two major categories, those primarily caused by NSD1 haploinsufficiency and those primarily ascribed to some factors, such as the dosage effects of genes other than NSD1, involved in the deletion.
intragenic NSD1 mutations predicted to form a truncated NSD1 protein and in 21 patients with a fairly common ~2.2 Mb deletion involving the entire NSD1 gene. The results also serve to identify clinical features primarily ascribed to some factors other than NSD1 haploinsufficiency. Agenesis or hypoplasia of the corpus callosum, cardiovascular and urinary anomalies, neonatal jaundice, and recurrent convulsions would primarily be the result of loss of specific disease genes, other than NSD1, with variable penetrance and expressivity, because they were absent in patients with mutations and frequently exhibited by patients with deletions. In support of this, the deleted region is known to harbour at least 21 genes, although characterisation of these genes remains poor in terms of clinical effects.

The MAPK signaling pathway. The cytoplasmic serine/threonine kinases transduce extracellular signals into regulatory events that impact cellular responses. The induction of one kinase triggers the activation of several downstream kinases, leading to the regulation of transcription factors to affect gene function. This arrangement allows for the kinase cascade to be amplified, and integrated according to the cellular context. An upstream mitogen or growth factor signal initiates a module of three kinases: a mitogen-activated protein (MAP) kinase kinase kinase (MAPKKK; e.g., Raf) that phosphorylates and activates a MAP kinase kinase (MAPKK; e.g., MEK) and finally activation of MAP kinase (MAPK; e.g., ERK). Thus, this MAP3K-MAP2K-MAPK module represents critical effectors that regulate extracellular stimuli into cellular responses, such as differentiation, proliferation, and apoptosis all of which function during development. Some of these MAP3K effectors may have redundant functions, and also serve as unique nexus depending on the context of the signaling pathway.

S1 ESPE-ISPAD Symposia: A cure for Diabetes
S1-11 - Closed loop insulin delivery
Tadej Battelino
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Abstract.
Continuous subcutaneous insulin infusion (CSII) has become a routine treatment modality preferred by an increasing percentage of people with type 1 diabetes mellitus (T1DM). CSII proved to be safe and efficient in all age groups. Continuous glucose monitoring (CGM) significantly decreased HbA1c without an increase in hypoglycaemia in a randomised controlled trial (RCT), and increased the time spent in normoglycaemia. However, patients’ compliance is still mandatory in order to achieve good metabolic control. An automated closed loop between continuous insulin delivery and continuous glucose monitoring would diminish the need of permanent compliance of the patients and probably enable more patients to reach the target HbA1c. Several centres reported preliminary results of pilot clinical trials with external and internal closed loops. Results of glycaemia seem to be acceptable in periods without eating or increased physical activity, but are less satisfactory whenever a fast change in glucose concentration is induced. This problem is caused in part by the delay between the glucose concentration in the blood and the glucose concentration in the subcutaneous tissue, by the delay of insulin action and by the delay of computer algorithms. Several strategies are being investigated to overcome these obstacles, with the use of more sensitive and faster algorithms, improved prediction models and possibly improved sensors. It is likely that semi-closed loops including automated discontinuation of
insulin delivery at hypoglycaemia and/or automated insulin delivery in combination with manual meal boluses will precede a fully automated closed loop system. Any technical solution that will diminish the need of constant patient compliance in maintaining good metabolic control is expected to facilitate the management of T1DM and enable more patients to reach current targets of glycaemia without the threat of severe hypoglycaemia.

Comments
Taken from the abstract of another recent publication by the author on this subject (Battelino T, Bolinder J. Clinical use of real-time continuous glucose monitoring. Curr Diabetes Rev. 2008 218-22):
Maintaining near-normal glycaemia in all patients with diabetes mellitus (DM) has become a standard and a well accepted recommendation. Unfortunately, most people with DM do not achieve this clinical goal because of marked glycaemic fluctuations and hypoglycaemia. Real-time continuous glucose monitoring (RT-CGM) has been introduced recently into clinical practice offering more knowledge about current glucose concentration and trend and enabling people with DM to intervene and prevent unwanted glucose excursions by acting upon real-time and predictive alarms. Several RT-CGM devices proved to be sufficiently accurate and feasible for routine use. Observational reports with The Guardian and Paradigm RT by Medtronic, the STS by DexCom, FreeStyle Navigator by Abbott and GlucoDay by Menarini established initial clinical benefit. Five randomised controlled trials (RCT) demonstrated significantly improved glucose variability or metabolic control, one of them showing a statistically significant and clinically meaningful decrease of HbA1c with a 3 months use of the Guardian RT (Medtronic, Northridge, CA). The great potential of RT-CGM devices to improve daily glucose control and quality of life in people with DM can only be developed further through RCTs, clarifying in more details the optimal clinical use and the most beneficial indications for this novel technique.

S1 ESPE-ISPAD Symposia: A cure for Diabetes
S1-12 - Insulin-Producing stem cells
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Abstract.
A major challenge in the treatment of Diabetes is to provide patients with an insulin source that regulates glucose levels on a mandatory minute-to-minute basis. Conceivable approaches to achieve this are restoring an endogenous source and/or implanting an autologous- or non-autologous-derived source. At present there are different strategies under investigation such as stimulation of regeneration of the residual islet-cells in the diabetic pancreas, increasing the regenerative capacity by infusion of precursor cells, and islet-cell transplantation. The principle application of these approaches has been shown in both experimental animals and humans. The studies addressing the implantation of an autologous- or non-autologous-derived source for treatment of diabetes have always been of limited clinical relevance due to shortage of sufficient supply of islet-cells. This has been the main rationale for researchers to design means to generate inexhaustible islet-sources. During recent years many have focussed on application of stem cells as an inexhaustible source. Stem cells are self-renewing progenitor cells that can differentiate into one or more specialised cell
types. Stem cells have a number of characteristics that qualify them as a potential inexhaustible source for islet-cells. Theoretically they have the capacity for unlimited replication and with adequate differentiation they can become fully mature and functional islet-cells. Several strategies and different stem cell sources for islet-cell substitution have been proposed. Not all have shown the same degree of success. In the present lecture we will discuss the successes and failures of the different approaches in view of future clinical application. Also, we will discuss the present insights in developmental biology of the pancreas since this knowledge is mandatory for understanding and designing strategies to create fully functional islet-cells from stem cells of both in non-pancreatic and pancreatic origin.

**Comments.**
Recent work in directing embryonic stem (ES) cell differentiation has primarily involved investigators striving to recapitulate the signaling and growth factor progression of normal pancreatic endocrine development in vitro. In 2006, Xu et al., demonstrated that human ES cells could be differentiated into PDX1-positive pancreatic progenitors by passing these cells through an embryoid body formation step. Later that year, D’Amour et al. described a 5-step process that, using growth factors and various culture conditions, allowed progression from undifferentiated ES cells to definitive endoderm to hormone-expressing endocrine cells. This group used factors such as FGFs, hedgehog inhibitors, and RA to promote differentiation, and they assayed the development of endocrine cells by measuring the expression levels of transcription factors shown to be critical at each step in development, such as PDX1, NGN3, and PAX6. The insulin-positive cells thus generated had insulin content similar to that found in adult islet cells, as well as functional ATP-sensitive potassium channel and voltage-dependent calcium channels. Notable limitations of this achievement include the lack of glucose-responsive insulin secretion and the relatively low rate of transdifferentiation; insulin-positive cells made up only about 7% of the differentiated population.

Therefore, although researchers have already begun to capitalize on the findings of developmental biology to direct ES cell differentiation, much of the mystery of the specification and maturation of endocrine cells remains to be deduced by research in model organisms so that the process can be recapitulated in ES cells. Work in human ES cells must always be considered with the caveats that their use and generation is restricted in the United States, and that the cell lines currently in use have been manipulated in culture for several years. The recent demonstration that human somatic cells can be reprogrammed to a pluripotent stem cell fate suggests that these ethical and legal issues may be circumvented. Two groups of investigators used genes known to be enriched in ES cells and involved in the establishment of pluripotency to reprogram human somatic cells. Using slightly different protocols, both groups established pluripotent lines that were capable of differentiating into all 3 germ layers and forming teratomas when injected into mice. The limitations of this finding are considerable, given that the reprogramming involved the use of integrating lentiviral and retroviral vectors and in 1 case, the expression of the proto-oncogene c-Myc. However, if we assume that alternative methods of cell transduction can be developed, somatic cell-derived insulin-producing cells for diabetes therapy may be possible in the not too distant future.

**S8 Reports from the Consensus Meeting and ESPE Research Unit Lecture**

**S8-32 - Idiopathic short stature**

Jan M. Wit

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Abstract.
On October 17-20, 2007 a workshop was organized by the Growth Hormone Research Society, ESPE and LWPES to review and weigh available evidence related to the evaluation and management of children with idiopathic short stature (ISS). Prior to the workshop, two discussion documents had been prepared by the organizing committee. The consensus draft was critically reviewed by all participants in a plenary forum, and a final version has been submitted (Cohen et al). ISS is defined by a height <-2 SDS without detectable cause, and can be subcategorized in familial short stature and non-familial short stature, with normal or delayed puberty. This definition includes constitutional delay of growth and puberty (CDGP). Short stature may be a risk factor for psychosocial problems, but true psychopathology is rare. In the US and several other countries GH treatment is approved for children shorter than -2.25 SDS. Successful first year response to GH treatment in includes an increase in height SDS >0.3-0.5. The mean increase in adult height attributable to GH therapy after 3.5-7 years is 3.5-7.5 cm. Responses are highly variable and depend on dose, length of therapy and other, but presently unknown, factors. During therapy IGF-I levels are helpful in assessing compliance and GH sensitivity; levels that are consistently elevated (>2.5 SDS) should prompt consideration of GH reduction. If height prediction is below -2 SDS at the time of pubertal onset, the addition of GnRH analogues may be considered. GH therapy in ISS has a similar safety profile to other GH indications. Oxandrolone and low dose testosterone therapy promote the short-term acceleration of growth with no decrease in adult height potential, and low dose testosterone is the appropriate therapy for males with mild short stature and CDGP. GnRH analogue therapy alone is not recommended in children with ISS. Psychological counseling is worthwhile to consider

Comment.
As stated in the article, many of the 32 contributors to the document are consultants and/or receive grants from several Pharmaceutical Companies. They are, notwithstanding, distinguished leaders in the field of pediatric endocrinology and growth. Consensus participants reviewed discussion-summaries, voted and reached a majority-decision on each document-section. (Cohen et al., J Clin Endocrinol Metab. 2008 Sep 9 [Epub ahead of print].

Some of the statements of the Consensus follow. ISS should be sub-categorized, principally based on auxological criteria. The main distinction is between children with a familial history of short stature, whose heights are within the expected range for parental target height and those children who are short for their parents. ISS should also be classified by the presence or absence of bone age delay, indicating the probability of delayed growth and puberty. Short individuals with no family history of short stature generally have a lower adult height in comparison to target height. In patients for whom the history and physical exam do not suggest a particular diagnosis, screening laboratory tests are indicated. These include a complete blood count, ESR, creatinine, electrolytes, bicarbonate, calcium, phosphate, alkaline phosphatase, albumin, TSH and FT4 and IGF-I levels. Screening for celiac disease is also recommended. A karyotype should be performed in all girls with unexplained short stature, and in short boys with associated genital abnormalities. A bone age X-ray should be obtained and reviewed by an expert. This gives an indication of the child’s remaining growth potential and may narrow the differential diagnosis. A skeletal survey should be reserved for patients with suspicion of a skeletal dysplasia, such as those with abnormal body proportions or a height SDS substantially below midparental height SDS, and should be read by an
expert in bone disorders GHD must be excluded to make a diagnosis of ISS. This requires both clinical and biochemical evaluation, as no single test or set of tests can define GHD. GH testing should be performed in any patient with a compatible history and physical examination, a low height velocity or in whom low IGF-I levels are observed. The majority of experts concur that a patient who is short, with normal height velocity, no bone age delay and a plasma IGF-I level above the mean for age does not require GH testing. At the present time, a new GH reference standard is being introduced which may require a downward adjustment of the lower limit of normal. In addition, changes in assay methodology influence choice of cut-off values for the diagnosis of GHD. It is acknowledged that there is a wide variability in GH and IGF-I values and in their interpretation among currently available commercial and inhouse assays. If a diagnosis of ISS is made, an MRI is not indicated. Although it is clear that there is variable GH sensitivity among children with short stature, the IGF-I generation test, while capable of documenting severe GH insensitivity, cannot currently detect more moderate degrees. In situations where a specific genetic diagnosis associated with short stature is expected (such as Noonan syndrome or GH insensitivity syndrome), the gene(s) of interest should be examined. Online resources exist such as Genetest (www.genetests.org), which identify laboratories capable of performing these tests. Although routine analysis of SHOX should not be undertaken in all children with ISS, SHOX gene analysis should be considered for any patient with clinical findings compatible with SHOX haploinsufficiency. ISS should be under the auspices of pediatric endocrinologists and management decisions should be evidence based. The interest of the child is the primary concern. One must discourage the expectation that taller stature is necessarily associated with positive changes in quality of life. Growth-promoting measures should be effective and should take into consideration the risks, benefits, and treatment alternatives including counseling. The shorter the child, the more consideration should be given to treatment with GH. The FDA approved cut-off in the US (and seven other nations) is -2.25 SDS, while in other countries lower cut-offs are proposed. Children whose heights are below -2.0 SDS and who are more than 2.0 SDS below their midparental target height and/or have a predicted height below -2.0 SDS are also believed by some experts to warrant treatment consideration. Serial IGF-I measurements during GH therapy are useful to assess efficacy, safety and compliance and have been proposed as a tool for adjusting the GH dose. Children treated with GH should be monitored for height, weight, pubertal development, and adverse effects at 3-6 month intervals. Dosage is usually selected and adjusted by weight. If the growth response is considered inadequate, the dose may be increased. There are no definitive data concerning the long-term safety of doses higher than 50 ug/kg/day in children with ISS. The upper limit of GH dosage used in other pediatric conditions is approximately 70 ug/kg/day, but the possibility of using such doses varies in terms of national health economics. There are two schools of thought about the duration of treatment. One is that treatment should stop when near adult height is achieved (height velocity < 2 cm/year, and/or bone age > 16 yrs in boys and > 14 yrs in girls). Alternatively, therapy can be discontinued when height is in the “normal” adult range (above – 2 SDS), or has reached another cut-off for the reference adult population. Stopping therapy is influenced by patient/family satisfaction with the result of therapy, on-going cost-benefit analysis or when the child wants to stop for other reasons. The expected result of GH treatment in ISS is an increase in height SDS and height velocity resulting in increased adult height. Since there is a continuum of GH responses, the definition of nonresponsiveness is arbitrary. Suggested criteria for poor first-year response include
height velocity SDS less than +1 or change in height SDS less than 0.3-0.5, depending on age. ISS represents a significant clinical entity within the pediatric endocrinology practice and multiple therapeutic interventions may be considered for these patients after appropriate evaluation has been conducted. Further clinical research and development is warranted to optimize the management of these children and to ensure that treatments are safe and beneficial.

S8 Reports from the Consensus Meeting and ESPE Research Unit Lecture S8-33 - The use of GnRH agonists (analogues) in children
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Abstract.
Development of long acting analogues of gonadotropin releasing hormone (GnRHa) revolutionized the treatment of central precocious puberty (CPP) worldwide. However, despite a favorable track record of safety and efficacy significant questions remain regarding their use in children. A consensus conference, jointly sponsored by the LWPES and ESPE, was convened to address issues pertaining to GnRHa therapy in children and adolescents, to derive consensus or evidence-based recommendations and to identify areas deserving further study. Several important observations emerged from this conference. Perhaps most important is that despite a considerable body of literature, few rigorously conducted and controlled prospective studies are available from which to derive evidence-based recommendations. Most of the conclusions reached a level of evidence that underscores the need for further research in key areas. The safety of GnRHa is well established, and their efficacy to increase adult height is undisputed only in early onset progressive CPP. This highlights the need to increase our knowledge of the normal limits of puberty and of the physical and psychosocial consequences of precocious puberty and of GnRHa treatments. The available data suggest that, after GnRHa treatment, gonadal function is not impaired in girls and development of PCOS or polycystic ovary morphology is not clearly different from that in the general population. However, few patients have reached child-bearing age and therefore long-term data on fecundity and ovarian reserve of treated patients with CPP are needed. The conference’s systematic review also highlighted the lack of objective support for commonly voiced concerns such as the propensity for GnRHa to promote weight gain or to affect BMD in adulthood. It should be clear that there is still much to learn from collaborative and carefully executed investigation into the pathophysiology as well as the short and long term consequences of treated and untreated CPP.

Comment.
No publication of the outcome of the consensus is available as yet.

WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-59 - The development of ESPE DSD registry
Faisal Ahmed
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Abstract.
Abstract text has not been submitted

Comment.
Information on the ESPE DSD Working Group is available in the ESPE web site.

ESPE DSD Working Group. Aims: Individuals with disorders of sex development (DSD) constitute a special group of persons with multi-faceted medical and social problems leading to increased care needs. In addition, due the complexity of DSD, special educational programmes need to be developed for the diverse range of professionals involved in the care of patients with DSD. The aim of the Group is to bring together basic scientists and clinical physicians involved in this field to: 1) promote active research with special attention to cross collaboration between basic and clinical aspects; 2) develop and maintain a registry as a resource for research; 3) promote knowledge and education; 4) set standards of holistic care of patients with DSD. Formation of group was proposed by Faisal Ahmed, UK, Silvano Bertelloni, Italy, Sten Drop, The Netherlands, Olaf Hiort, Germany, Ieuan Hughes, UK.

Organization and Coordination. The Working Group Board is composed by Coordinator, Secretary and three more members. The working group organizes a yearly symposium during ESPE Annual Meeting where techniques and results are presented. ESPE Research Unit grant: Exploring the utility of a secure web-based register of disorders of sex development (2006). Planned activities for the next 2 to 3 years. European Register for DSD (Chair F. Ahmed), E-learning program (Chair S. Drop), Collaborative clinical and basic projects steered through EuroDSD (Chair O. Hiort), Exchange of material and, eventually, personnel. Formulate guidelines for more appropriate and earlier intervention aiming to better management of DSD individuals in childhood and adulthood. Collaboration with patient support group. The ESPE DSD Group has the potential to become the forum for guiding ESPE in issues relating to DSD; oversee and manage the ESPE DSD Register which will benefit ESPE members as well as others; link with other specialist groups in the field of DSD; strengthen the standard of collaborative research in DSD in Europe. In addition, because a DSD presenting as ambiguous genitalia of the newborn is one of the most challenging clinical problems not only for the pediatric endocrinologists but also for neonatologists, genetists, and all pediatricians, the Euro DSD group can act in a qualified supporting role for all these professionals as well as for families.

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WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-60 - Development of an e.learning web portal on DSD
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Abstract.
Based on experience gained with an interactive program: "Pediatric Endocrinology IN ter ACTION, Growth and Puberty" (ErasmusMC;2004), presently available on the website of ESPE, LWPES and SLAP and containing core modules aimed at medical students and advanced modules aimed at post-doc students, fellows etc, an editorial and technical staff has been formed to develop an e.learning web portal to give entrance to an interactive learning environment for an up to date program on DSD including normal development, patho-physiological mechanisms and current views on diagnostic and therapeutic interventions, psychological counseling, outcome. Furthermore, study results will be implemented and evidence based guidelines provided. It will be a meeting place of experts, teachers and students at various levels, to gain, share, contribute and develop knowledge in an accessible and flexible way. It will allow for assessment of several competencies. The "audience" will consist not only of ESPE members but the program will also reach out to health care workers globally. The e-learning portal will contain several functionalities: a) Personal login, account and access rights; b) Theoretical information offered in a number of chapters covering relevant theory and cases and will include images, animations, video and questions aimed at developing several competencies; c) Mediclopedia’, offering the user the option to lookup specific subjects in an alphabetically ordered list; d) Forum, enabling the users to post comments and remarks, to discuss and to share knowledge; e) Assessment, allowing for assessment of various competencies; f) Administrative general information, content management, search. A board of medical editorial, educational and technical contributors has been formed.

Comment.
"Pediatric Endocrinology IN ter ACTION, Growth and Puberty" (ErasmusMC;2004), is presently available on the website of ESPE, but only to members of ESPE.

WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-61 - From national networks to a European collaborative study on DSD
Olaf Hiort
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Abstract.
Disorders of Sex Development (DSD) constitute an array of rare to very rare disorders affecting the genito-urinary tract and in most instances also the endocrinereproductive system. To date, the aetiology of DSD in many of the patients remains unresolved. Therefore, novel approaches to the diagnosis are crucially needed and are very likely to provide important novel insights into the aetiology of DSD, which will translate into clinical benefits by improving the diagnostic assessment of patients, the basis for prognostic predictions and eventually by groundbreaking for novel treatments and individually tailored therapeutic approaches. The German government established a programme for networks on rare diseases, funding 10 nationally operating networks from 2003 until 2008. The German Network on DSD (www.netzwerk-is.de) addresses the development of structures for research on rare disorders of sex development with regard to basic research related to defining genetic disorders, assessment of intracellular steroid metabolism in well characterized disorders, and a large clinical evaluation study. However, several issues of genetic, biochemical and functional studies cannot be addressed due to the restriction of the network to the necessarily smaller, national patient cohorts. In addition, the availability of specialised expertise in DSD is limited in comparison to the options that cross-European collaboration offers in the DSD field.
This has led to a collaborative approach of six European countries for a structured research project called EuroDSD to gain funding within the 7th European Framework Programme. EuroDSD starts in May 2008 for a period of 3 years by linking a European patient-based data collection and analysis tools with research on development of novel diagnostic strategies to identify new causes of DSD in conjunction with a strong programme on functional molecular biology of the androgen receptor, thereby allowing for an in-depth analysis of a key factor in the pathogenesis of DSD.

Comment.
The EuroDSD is a collaboration of doctors and scientists from all over Europe. Within the 7th Framework Programme and its call for a collaborative project on the natural course and pathophysiology of rare diseases, EuroDSD is supported by the EU Commission. Disorders of Sexual Development (DSD) constitute a group of rare to very rare, mostly heritable disorders affecting the genito-urinary tract and in most instances also the endocrine-reproductive system. Long-term outcome studies on various DSD entities are desperately needed in order to establish a basis for evidence-based medicine regarding sex assignment and conservative and surgical treatment options. The project combines unique strengths by linking a European patient-based data collection and analysis tools: (WP01), Virtual Research Environment (VRE) with research on development of novel diagnostic strategies to identify new causes of DSD; (WP02), Identification of novel genetic markers for DSD; (WP04), Characterization of the "androgen-memory"; and (WP05), Steroid Metabolomics; in conjunction with a strong programme on functional molecular biology of the androgen receptor (WP03) Functional assessment of androgen action; thereby allowing for an in-depth analysis of a key factor in the pathogenesis of DSD.

WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-64 - Testis development: update
Olle Söder
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Abstract.
Abstract text has not been submitted

Comments.
Taken from Söder O., Best Practice & Research Clinical Endocrinology & Metabolism Vol. 21, No. 3, pp. 381–391, 2007.
Sex differentiation in vertebrates is by definition sexually dimorphic and starts at the level of the sex chromosomes. The sexual dimorphism of gonadal differentiation is discussed with a focus on human development. In the embryo, the indifferent gonadal anlagen harbours four different cell lineages with bipotential fates dependent on the sex of the individual (Supporting cells: Sertoli, Granulosa; Steroidogenic cells: Leydig, Theca; Stromal cells Peritubular, Stromal; Germ cells: Gonocytes, Spermatogenesis, Oogenesis; and Unknown; Macrophages). The gonadal anlagen appear by 32 dpc of human embryonic development. These paired bipotential structures are located at the ventromedial surface of the mesonephros and arise from the mesoderm by contributions from somatic mesenchymal cells from the mesonephros and epithelial cells migrating from the coelomic surface of the gonadal ridge. At this stage no sexual dimorphism can
be distinguished morphologically, and germ-cell precursors (gonocytes) are not yet present. The mesonephros also constitutes the primordium of the adrenal glands and the urinary system. Interestingly, several peptide growth factors have also been implicated in gonadal development from the indifferent gonadal anlagen, most notably the insulin-like growth factor superfamily. Two important transcriptional regulators involved in formation of the urogenital ridge are the tumour suppressor gene Wilms’ tumour-associated gene-1 (WT1) and the orphan nuclear receptor steroidogenic factor-1 (SF-1). Disruption of WT1 in mice leads to lack of formation of kidneys, gonads and adrenals. In humans distinct but not identical phenotypes arise after WT1 loss-of-function (LOF) mutation, resulting in urogenital and other malformations in boys with WAGR, Deny–Drash or Frasier syndromes. By the end of the 5th week pc of human embryonic development the gonadal anlagen are composed of somatic cell types of three different lineages with a bipotential fate, dependent on their future paths (see below). At this stage the indifferent anlagen are colonized by immigrating primordial germ cells (PGCs), which are termed gonocytes when permanently resident in the gonad. In males, lack of germ cells still allows differentiation of somatic cells, including Leydig cells, with steroidogenic activity. Affected males will undergo pubertal development but are infertile due to the Sertoli-cell-only syndrome. In the female, the presence of gonocytes is mandatory for further differentiation of the gonadal anlagen along the female path. In the absence of gonocytes, follicular cells degenerate resulting in non-functional streak gonads. The cell-division path taken by the gonocytes is another sexually dimorphic event in the gonadal anlagen. In males, gonocytes will continue mitotic proliferation and then become mitotically quiescent. In female embryos, however, gonocytes are recruited into meiosis soon after arrival into the gonadal anlagen where they will be blocked at an early stage until further differentiation is initiated. The supporting cell lineage is critical for sex determination and further gonadal differentiation. In the testis it gives rise to Sertoli cells which are nurse cells for spermatogenesis, supplying the developing germs cells with nutrients and growth factors. Thus, control of Sertoli-cell proliferation in the developing testis is of major importance for future production of male germ cells. Sertoli-cell differentiation and proliferation are critical first steps of male sex determination and are vulnerable targets of disruptive actions of endogenous factors and xenobiotics such as inflammatory mediators and endocrine disrupting chemicals. Production of anti-Müllerian hormone (AMH) starts at an early stage of Sertoli-cell maturation and is required for regression of the anlagen for female internal genitalia. In the male, Leydig cells develop from steroidogenic precursor cells immigrating from the coelomic epithelium and the mesonephric mesenchyme to contribute to the indifferent gonad. Their differentiation starts in the 7th week pc of human development and is dependent on signals from Sertoli cells. Desert hedgehog (DHH) and fibroblast growth factor-9 (FGF9) are Sertoli-cell-derived factors mandatory for the proliferation and differentiation of functional Leydig cells. Fetal-type Leydig cells start to produce androgen during the 8th week of human gestation and are at first regulated by the placental human chorionic gonadotropin (hCG). On Leydig cells, this hormone shares a receptor with pituitary luteinizing hormone (LH), which appears much later in development when the HPG axis is established in the fetus at the beginning of the second trimester of human pregnancy. In addition to androgen, which is crucial for male differentiation of external and internal genitalia, Leydig cells also produce SF1 required for steroidogenesis and insulin-like factor-3 (INSL3). INSL3 and its receptor LGR8 are needed for the first transabdominal phase of testicular descent, occurring at weeks 8–16 of fetal age in humans. Interestingly, adrenocortical and gonadal steroidogenic cells
seem to share an embryonic origin in the coelomic epithelium and may exist as one lineage before divergence into the gonadal and adrenocortical paths. In line with this, adrenocorticotropic hormone (ACTH) has been implicated as a regulatory factor for fetal Leydig cells expressing ACTH receptors in the early phase of gonadal differentiation. Connective-tissue or stromal-cell lineage cells are important constituents of the testis as they are needed for early histogenesis of the seminiferous cords. These cells are termed peritubular cells (PTCs) or myoid cells in the testis. They form a basal layer surrounding the seminiferous cords where they give support to the Sertoli cells. In the postpubertal testis they may add contractile forces thought to be required for tubular fluid and cell flow. Pre-PTCs migrate directly from the adjacent mesonephros on direct orders from Sertoli cells via chemotactic signals. Also cells contributing to the vasculature of the testis migrate via the same paths. This migration process is an important step in sex determination and is SRY-dependent. PTCs are highly proliferative cells, a feature that seems important for male gonadal development. The testicular interstitium of adult individuals also contains a population of resident macrophages, constituting up to 20% of the interstitial cells. These macrophages can be identified by specific markers. Their physiological role is not fully understood but has been suggested to be associated with testis immune functions and the role of the testis as an immune sanctuary.

WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-66 - Molecular diagnoses in a series of 122 index patients with 46,XY disorder of sex development
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Abstract.
A series of 122 index patients, referred from our centre and 30 other Spanish hospitals, presenting a 46,XY disorder of sex development (46,XY DSD) were analysed for a molecular diagnosis in the last six years. Genes analysed were: AR (androgen receptor), SRD5A2 (5α-reductase type 2), HSD17B3 (17β-hydroxy-steroid dehydrogenase type 3), CYP17A1 (17α-hydroxylase and 17,20-lyase) and LHCGR (LH/CG receptor). A molecular diagnosis was reached in 61.5% of patients, with anomalies corresponding to the following genes or chromosomes: AR (45.9%), SRD5A2 (6.5%), HSD17B3 (3.3%), CYP17A1 (0.9%), LHCGR (0.9%), Denys-Drash syndrome, other testicular dysgenesis syndromes (9-del-p23-pter and 18p-del) (3.2%) and septo-optic dysplasia (0.8%), while in 38.5% of patients no molecular diagnosis was obtained. Among the phenotypes, 38.5% were completely female and 61.5% ambiguous. Among patients with female genitalia, a molecular diagnosis was reached in 91.5% of cases, with 89.4% carrying one or two (in 2 cases) AR gene mutations, 2.1% CYP17A1 mutations, and no affected gene was detected in 8.5%. Among the ambiguous genitalia, a molecular diagnosis was obtained in only 42.6% of cases, with 18.6% carrying an AR mutation, 10.6% SRD5A2 mutations, 5.4% HSD17B3 mutations, 1.4% LHCGR mutations, 5.3% testicular dysgenesis of descriptible origin and 1.3% septo-optic dysplasia. In conclusion, detection of the molecular cause of 46,XY DSD was
successful in 61.5% of patients, with AR gene being the most frequently affected; however, the percentage was higher in the completely female phenotype, as was that of an affected AR gene. Thirty-eight percent of patients remained without a molecular diagnosis owing to lack of adequate biochemical and/or candidate gene orientation or to the lack of analysis of new candidate genes still to be described.

Comments
The abstract is self-explanatory

WG6 ESPE Disorder of Sex Development (DSD) Working Group
WG6-67 - RNA-array technology and its use in defining sex-related gene expression
Paul-Martin Holterhus
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Abstract.
Normal male external genital differentiation is dependent on normal androgen action via the androgen receptor (AR). Mutations in the AR-gene inhibit AR-function and in turn lead to androgen insensitivity syndrome (AIS) in 46,XY individuals, one of the most common disorders of sex development (DSD). Clinical management would benefit from markers of individual AR-function. Moreover, many patients with presumed AIS on clinical grounds don’t seem to have mutations in the AR-gene. This talk will review our recent genome-wide studies on AR-function and sex-specific gene expression in genital skin fibroblast cultures using cDNA-microarrays. Within the external genitalia, fibroblasts contain a stable gene transcription signature which represents their origin of biopsy. Therefore, only homologous genital fibroblasts may be compared with respect to AR-function and sex-specific gene expression. If performed this way, fibroblasts derived from labia majora in 46,XY CAIS patients having documented AR-mutations and from normal scrotum (both originated from the embryonic urogenital swellings) differ significantly in mRNA transcription of 612 transcripts. Inclusion of independent samples derived from complete and partial AIS (CAIS and PAIS, respectively) patients confirms that this gene transcription signature represents a "transcriptional memory" of prenatal genital androgenization. The identified genes will be helpful to decipher mechanisms of external genital development. In a very recent unpublished microarray - and RT-PCR study, DHT-treatment of normal scrotum fibroblasts revealed significant up-regulation of Apolipoprotein D, a regulator of senescence and an important pheromone transporter in humans. CAIS patients did not respond, PAIS did respond marginally while normal scrotum-like up-regulation was present in labia majora of 17βHSD type III deficiency indicating AR-specificity. Therefore, in addition to analysis of individual androgen memory, functional analysis of APOD in genital fibroblasts could provide promising insights into AR-function of an individual with DSD.

Comments
The abstract is self-explanatory

FC2 Gonads and Puberty
FC2-74 - 10-years experience: Mutations in the luteinizing hormone receptor (LHR) gene and the genotype phenotype correlation
Annette Richter-Unruh1; Miriam Verhoef-Post2; Jörg Gromoll3; Ute Gross4; Axel Themmen5
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**Abstract.**

Not only plays the LHR an important role in male sex differentiation as shown by Leydig cell hypoplasia (LCH), the receptor is also key to the occurrence of precocious puberty in boys who carry activating mutations. Since 1998 we have analysed more than 400 patients suspected to have mutations in the LHR gene and received blood samples from Germany, Canada, Israel, Italy, the Netherlands, Poland, Turkey, United Kingdom, USA. In 46,XY females we found 14 new inactivating mutations in 11 patients: an insertion of 9 amino acids in exon 1, two splice site mutations in intron 1, intron 4 +38 bp, V144F, Q190*, T461I, W491*, A589fs, Y623S. We presented the first 3 patients with mutations in a new exon of the LH receptor gene (exon 6a; A557C, A558G) that redefines the genomic organisation of LHR gene. Actually we have found a fourth compound heterozygous patient with new mutations in exon 6a (A580G) and in exon 11 (I415T). Following new alterations were identified assumed to be polymorphisms: Intron 1 -28 bp, intron 2 +11 bp, Y113N, R124Q, E187E, L204L, P224S, P341P, C439R, V553L. To evaluate if delayed puberty in boys and primary amenorrhoea in 46,XX girls can be due to partial LCH we have studied 31 patients resulting in the identification of 7 new heterozygous LHR gene changes in 3 girls and in 5 boys. On the other allele no DNA changes in the LHR gene were identified, therefore diagnosis of LCH is not free from doubt. In 11 families with FMPP we identified the activating germline mutation I542L mutation and in a Canadian boy the A373V mutation. In 6 boys a somatic mutation (D578H) was found in a Leydig cell adenoma or Leydig cell hyperplasia. In conclusion, the absence of causative mutations in the majority of the LCH patients suggests that other receptor regions may be responsible for a number of cases. Alternatively, the displayed phenotype is caused by other genetic defects. Phenotype-genotype correlations will help to understand genetic variability of gonadotropin action and indicate new regulatory pathways of receptor regulation.

**Comments**
The abstract is self-explanatory

**FC2 Gonads and Puberty**

**FC2-75 - Successful treatment of unilateral cryptorchid boys risking infertility with LH-RH analogue**

Faruk Hadziselimovic
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**Abstract.**

**Background:** Infertility is the primary concern for boys with uni- or bilateral undescended testes. An early and seemingly successful orchidopexy does not improve fertility in a substantial number of cryptorchid males. We confirmed that LH-RH analogue (LH-RHa) treatment induces an increase in and maturation of the germ cells; however, it was uncertain if treatment would improve the chance of fertility later in life. 

**Patients and Methods:** Thirty unilateral cryptorchid boys, with an average age of 3 years at the time of surgery, were included in the study. Testicular biopsy showed that
they had impaired testicular maturation and were therefore at high risk for infertility. Fifteen of the 30 unilateral cryptorchid boys were treated with 10 µg LH-RHa (Buserelin) nasal spray, administered on alternate days for a period of 6 months, following orchidopexy. The control group consisted of 15 cryptorchid boys who had been treated by Schoemakers type of orchidopexy, only. After puberty, the ejaculates of both groups were analyzed. **Results:** All males in the untreated group were severely oligospermic, with 20% being azoospermic. In contrast, 86% of the treated ex-cryptorchid males had a sperm concentration within the normal range; this was significantly different from the sperm concentration found in the untreated group. (p < 0.000008). **Conclusion:** For the first time, we demonstrate that infertility in cryptorchidism can be successfully corrected when suitably treated with a LH-RHa. Sperm parameters normalized following therapy in the majority of cryptorchid males who, untreated, would have remained infertile. This innovative hormonal treatment will have a profound effect on the current recommended surgical treatment of boys with undescended testes.

**Comments.**
This is an astonishing report showing that treatment with LH-RHa after orchidopexy, during early pre-puberty, is able to improve sperm production in adulthood in patients with cryptorchidism. It would be nice if this finding were to be confirmed by another group, but it is certainly a difficult study to be carried out. The speaker published this results in the Int Braz J Urol. 2008 May-Jun;34(3):319-26; discussion 327-8. Successful treatment of unilateral cryptorchid boys risking infertility with LH-RH analogue. Hadziselimovic F., Kindertagesklinik, Liestal, Switzerland. praxis.oris@bluewin.ch
The summary is the same as the one transcribed above.

**FC2 Gonads and Puberty**
**FC2-76 - Two KISS1 mutations associated with gonadotropin-dependent precocious puberty**
Leticia FG Silveira; Mariza AG Santos; Vinicius N Brito; Acacio P Silveira-Neto; Berenice B Mendonca; Ana Claudia Latronico
Hospital das Clínicas, Universidade de Sao Paulo, Laboratorio de Hormonios e Genetica Molecular, São Paulo, Brazil

**Abstract.**
Kisspeptin, encoded by the KiSS1 gene, is an excitatory neuroregulator for the secretion of hypothalamic gonadotropin-releasing hormone (GnRH). The kisspeptin actions are mediated by a G protein-coupled receptor (GPR54). Recently, a GPR54-activating mutation was implicated in the pathogenesis of gonadotropin dependent precocious puberty (GDPP). The aim of this study was to investigate KiSS1 gene mutations in children with sporadic or familial idiopathic GDPP. Sixty nine Brazilian children (65 girls and 4 boys) with GDPP were selected. All children had advanced bone age, pubertal levels of basal and/or GnRH-stimulated LH and FSH and normal central nervous system MRI. A group of 150 individuals who had puberty at adequate age were used as controls. Genomic DNA was extracted from peripheral leukocytes and the 3 exons of the KiSS1 gene were amplified and automatically sequenced. Two novel KiSS1 missense mutations (P74S and H90D) were identified in two unrelated children with sporadic GDPP. The P74S mutation was identified in heterozygous state in a boy who developed puberty at 1 yr of age. His mother and maternal grandmother also carried the P74S mutation in heterozygous state, suggesting incomplete sex-dependent
penetrance. The H90D mutation was identified in homozygous state in a girl who developed puberty at 6 yr of age. Her mother, who had menarche at 10 yr of age was heterozygous for the H90D mutation. Both KiSS1 mutations were absent in 300 control alleles and are located in the amino-terminal region of the kisspeptin-54, which may be involved in protein stabilization and protection against proteolytic digestion. We conclude that KiSS1 mutations appear to be associated with the phenotype of GDPP in a complex mode of inheritance.

Comments
The abstract is self-explanatory

FC7 Disorders of Sex Development
FC7-104 - Genetic defects in 255 patients with 46, XY DSD and complete female phenotype
Delphine Mallet1; Ingrid Plotton1; Claire Nihoul-Fekete2; Marc Nicolino3; Anne-Marie Bertrand4; Michel David3; Laurence Michel-Calemard1; Yves Morel1
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Abstract.
Mutations of genes involved in sex development have been largely reported in the literature, but few data are available to evaluate the proportion of each gene defect in DSD. In our cohort of 255 patients with 46, XY DSD and complete female phenotype, two groups have been established according to hormonal data and presence or absence of a uterus: group 1: disorders of testicular development (low AMH, n=31); group 2: disorders in androgen synthesis or action (normal AMH, n=224). In group 1, our strategy to detect a molecular defect is as follows: SRY is first sequenced, and then SRY/SOX9 deletion or WNT4/DAX1 duplication are searched by MLPA. Afterwards, SF1, WT1, DHH and FGF9 are sequenced. When syndromic features or mental retardation are associated with the testicular defect, a CGH-array study is performed.

The molecular defect has been identified in 12 patients: seven SRY, one SF1 and two WT1 mutations, one DAX1 duplication and one 9p deletion. In group 2, two subgroups have been determined based on testosterone level. In subgroup 2a (low testosterone n=47), almost each time hormonal data (ACTH/hCG test) suggested a defect in biosynthesis, the mutations have been identified: 3beta-HSD (n=1), 17alpha-hydroxylase/17,20-lyase (n=4), 17beta-HSD deficiency (n=28). Nevertheless, in our 14 patients suspected of lipoid adrenal hyperplasia (9 with StAR mutations, 3 with CYP11A mutations), 2 have no mutation. In subgroup 2b (normal/high testosterone), AR gene mutations have been identified in 165 patients; 5alpha-reductase deficiency, evoked by high T/DHT ratio, has been confirmed in 4 patients. Nevertheless, in 8 patients, no mutation has been found and supplementary studies are in progress (mRNA, 5'UTR, 3'UTR and protein study). In summary, our screening was successful for patients with disorders in androgen synthesis or action (95%) but less efficient in patients with gonadal dysgenesis (39%). We thank all clinicians who send DNA of their patients.

Comments
The abstract is self-explanatory
FC12 Top Rated Clinical Abstracts
FC12-133 - DHEA (dehydroepiandrosterone) replacement in adolescent girls and young women with central adrenal insufficiency is beneficial: A randomized, double-blind, placebo-controlled multicentre trial
Gerhard Binder1; Sophia Weber1; Marion Ehrismann1; Nicole Zaiser2; Christoph Meisner3; Michael B. Ranke1; Ludwig Maier4; Stefan A. Wudy5; Udo Heinrich6; Markus Bettendorf6; Helmut-G. Doerr7; Roland Pfaeffle8; Eberhard Keller8
1University-Children's Hospital, Paediatric Endocrinology Section, Tuebingen, Germany; 2University of Tuebingen, Center for Coordination of Clinical Studies, Tuebingen, Germany; 3University Tuebingen, Department of Medical Biometry, Tuebingen, Germany; 4University of Ulm, Pharmacy, Ulm, Germany; 5University of Giessen, Steroid Laboratory, Giessen, Germany; 6University-Children's Hospital, Paediatric Endocrinology, Heidelberg, Germany; 7University-Children's Hospital, Paediatric Endocrinology, Erlangen, Germany; 8University-Children's Hospital, Paediatric Endocrinology, Leipzig, Germany

Abstract.
The efficacy of oral DHEA in the treatment of atrichia pubis and psychological distress in young females with central adrenal insufficiency is unknown. We aimed to evaluate this therapy. 23 young females (mean age 18 yrs, range 13-25) were enrolled in a double-blind RCT. Inclusion criteria: ACTH deficiency plus ≥2 additional pituitary deficiencies, serum DHEA <400 ng/ml and puberty >B2. Exclusion criteria: cerebral radiation, tumour remission <1 yr, amaurosis, hypothalamic obesity, psychiatric disorders and instable hormone medication. Patients were randomized to placebo or 25mg HPLC-purified DHEA/d for 12 months after stratification into a non-tumour (n=7) and a tumour group (n=16). Efficacy evaluation: clinical scoring of pubic hair stage at 0,6,12 months (primary endpoint) and psychometrical evaluation (symptom checklist SCL-90-R) at 0,12 months (secondary endpoint). Androgen levels and safety parameters were measured at 0,6,12 months; 24h-androgen urinary excretion at 0,12 months. With placebo, 4 patients dropped out because of change of residence (n=2), recurrence of craniopharyngeoma, and manifestation of type I diabetes; with DHEA, one patient because of recurrent anxiety attacks. DHEA substitution resulted in normalization of DHEAS and androstanediol glucuronide serum (P<0.006) and of its urinary metabolite levels (P<0.0001), placebo had no effect. Serum levels of androstenedione increased significantly in the DHEA group, but did not normalize (P < 0.02). The DHEA group exhibited a significant progress in pubic hair growth from Tanner stage 1-3 to 2-5 (in mean: +1.5 stages) while the placebo group did not (P<0.0019). Importantly, eight of the ten SCL-90-R scores including those for depression, anxiety, interpersonal sensitivity and the global severity index improved in the DHEA group significantly in comparison to the placebo group (P<0.048). DHEA was well tolerated. In adolescent girls with central adrenal insufficiency daily replacement with 25 mg DHEA is beneficial: atrichia pubis vanishes and well-being improves significantly.

FC12 Top Rated Clinical Abstracts
FC12-134 - Genetic heterogeneity in permanent neonatal diabetes
Sarah Flanagan; Emma Edghill; Ann-Marie Patch; Jayne Minton; Sian Ellard; Andrew Hattersley
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Abstract.
Permanent neonatal diabetes (PNDM) is diabetes presenting in the first 6 months of life requiring life-long insulin therapy. Isolated PNDM can result from mutations in the KCNJ11 and ABCC8 genes which encode the Kir6.2 and SUR1 subunits of the ATP-sensitive (KATP) potassium channel and the Insulin (INS) and Glucokinase (GCK) genes. Syndromic PNDM, where there are clinical features in addition to hyperglycaemia, can result from mutations in IPF-1, PTF1A, FOXP3, GLIS3 and EIF2AK3. Defining the genetic aetiology of PNDM is an important to guide clinical management as patients with KCNJ11 and ABCC8 mutations can be successfully treated with high dose oral sulphonylureas rather than insulin. The relative prevalence of genetic subgroups is unknown as no study has investigated all known genetic subtypes. Our aim was to define the genetic aetiology in a consecutive, international series of 281 PNDM patients. In all patients KCNJ11 was sequenced. Further investigation depended on whether other clinical features were present. In isolated hyperglycaemia ABCC8, INS and GCK were sequenced. In patients with syndromic PNDM IPF-1, PTF1A, FOXP3, GLIS3, NEUROD1 and EIF2AK3 were sequenced depending on the additional clinical features. Mutations were identified in 182 patients (65%). KCNJ11 mutations were the commonest cause of PNDM, accounting for 32% (n=90) of all cases. Other mutations were INS 37 (13%), ABCC8 30 (11%), EIF2AK3 9 (3%), GCK 7 (2%), FOXP3 6 (2%), PTF1A 2 (0.7%) and NEUROD1 1 (0.3%). No mutations were identified in IPF-1 or GLIS3. In this largest series we have shown that a genetic diagnosis is possible for 65% of patients with PNDM with 43% having a KATP channel mutation which can be treated with sulphonylureas. These two genes should be tested in all PNDM patients diagnosed under 6 months. Additional genes for PNDM remain to be identified.

FC12 Top Rated Clinical Abstracts
FC12-136 - SF1 gene mutation is a frequent cause of 46,XY complete gonadal dysgenesis: Identification of five new mutations among 22 XY sex reversed girls
Pascal Philibert1; Françoise Audran2; Muriel Houang3; Veruska Molini4; Anne-Marie Frances5; Eliane Tarral6; Lidka Lisa7; Durval Damiani8; Françoise Paris1; Charles Sultan1 1CHU, Service D'Hormonologie et Endocrinologie Pédiatrie, Montpellier, France; 2CHU, Service D'Hormonologie, Montpellier, France; 3CHU, Service D'exploration fonctionnelle, Paris, France; 4Université, Département de Pédiatrie, Turin, Italy; 5CH, Service de Génétique Médicale, La Seyne sur Mer, France; 6CH, Service de Pédiatrie, Le Mans, France; 7CH, Département de Pédiatrie, Prague, Czech Republic; 8Université, Service D'Endocrinologie Pédiatrie, Sao Paulo, Brazil

Abstract.
According to the consensus statement, disorders of testicular development in XY patients include complete gonadal dysgenesis (Swyer syndrome), partial gonadal dysgenesis and gonadal regression. The usual presentation of complete gonadal dysgenesis (CGD) is adolescent primary amenorrhea with a lack of breast development and unambiguous female phenotype. Genetic analyses have shown that only 15-20% of 46,XY CGD are due to mutation or deletion of SRY. This work was undertaken to evaluate the frequency of SF1 gene abnormalities in this syndrome. In the last 3 years, we had the opportunity to analyse SRY and SF1 gene mutations in a group of 22 XY
adolescent girls with CGD. In all cases the phenotype was unambiguously female: 19 adolescents were referred for primary amenorrhea and 3 newborns for discrepancy between prenatal karyotype and neonatal female phenotype. The mean basal plasma testosterone was 21.9 ±19.9 ng/dl, LH: 20.8 ±19.9 UI/l, FSH: 50.2 ±29.9 UI/l and AMH: 15.5 ±22.7 ng/ml. Genomic DNA was obtained from peripheral blood leukocytes: SRY and SF1 gene sequences were analysed. Among the 22 DNAs, we identified:
- 3 SRY gene mutations: p.Y129X, c.71delA, c.1XX-1XXdelins [TAAAGTATCATGTGAAAAAGTAAAG] - 5 new SF1 gene mutations: p.M1V, p.R39P, p.M78I, p. Q316X, c.151delG These data confirm the known frequency of SRY gene abnormalities (13.6 %) in patients with 46,XY CGD. Most interesting was the high frequency of SF1 gene mutation (22.7 %): The finding of 5 new SF1 gene mutations out of 22 patients points to the key role of this gene in testis determination. It also underlines the need to systematically analyse SF1 gene sequence in adolescents with XY primary amenorrhea and low testosterone/AMH levels, as well as in newborns with 46,XY sex reversal.

FC12-138 - Unusual virilisation in 10 girls with juvenile granulosa cell tumours (JGCT) is related to intratumoral aromatase deficiency
Nicolas Kalfa1; Gery Médury2; Pascal Philibert3; Catherine Patte4; Brigitte Boizet-Bonhoure5; Marc Fellous6; Elisabeth Thibaut7; Catherine Pienkowski8; Micheline Misrahi9; Charles Sultan10 1Hopital Lapeyronie, Service d'Hormonologie, Montpellier, France; 2AP-HP, University Paris Sud 11, Laboratory of Molecular Genetics, Pharmacology and, Paris, France; 3Hopital Lapeyronie Chu Montpellier, Service d Hormonologie, Montpellier, France; 4Institut Gustave Roussy, Département d'Oncologie, Villejuif, France; 5Institut de Genetique Humaine, CNRS UPR1142, Développement et Pathologie de la Gonade, Montpellier, France; 6Institut Cochin, Université Paris Descartes, U567, Paris, France; 7Hôpital Necker, Service d'Endocrinologie Pédiatrique, Paris, France; 8Hôpital des Enfants, CHU Toulouse, Service d'Endocrinologie Pédiatrique, Toulouse, France; 9AP-HP, University Paris Sud 11, Le Kremlin Bicêtre, Laboratory of Molecular Genetics, Pharmacology and, Paris, France; 10Hôpital Arnaud de Villeneuve, CHU Montpellier, Unité d’Endocrinologie-Gynécologie Pédiatriques, Montpellier, France

Abstract.
In a previous nationwide study including the French Society of Children Cancer, we focussed on the physiopathology of juvenile ovarian granulosa cell tumours (OGCT). Most of the 33 JGCT were revealed by hyperoestrogenism responsible for precocious pseudopuberty in the prepubertal period or an abdominal tumour in the postpubertal period. Nevertheless, we noted unusual hyperandrogenism in 10 patients both before and after puberty. This study aimed to determine whether this hyperandrogenism could be related to: - granulosa-thecal cell hyperactivity (activated Gαs) - abnormal expression of SOX9, a male determining gene, in gonad - low aromatase expression in tumour tissue. Clinical symptoms of hyperandrogenism included pubarche and/or litoromegaly. The mean plasmatic testosterone level was 1.1ng/dl vs. control <0.5ng/dl. Tumour tissue specimens were studied using nested PCR with EagI digestion to detect activating mutation of Gαs, immunofluorescence of SOX9 and immunochemistry of aromatase (rabbit polyclonal antibody, 1:500). All results were compared with those of patients with JGCT without hyperandrogenism. - An R201C activating mutation of Gαs was identified in 3/10 patients with hyperandrogenism vs. 5/16 patients without such symptoms. - SOX9 was only expressed in the nucleus in 5/10 cases. Thus, no
correlation between its expression or nuclear localisation and hyperandrogenism was established. In 9/10 patients, the intratumoral expression of aromatase was absent (n=7) or dramatically reduced (n=2). In contrast, 14/23 patients without virilisation exhibited conserved aromatase expression in their tumour (p=0.02). Neither hyperactivity of Gαs nor unusual expression of SOX9 causes virilisation in girls with JGCT. On the other hand, a localised defect of aromatase expression in granulosa cell tumours is involved in hyperandrogenism. Clinical and biological evidence of hyperoestrogenism does not however exclude some degree of peripheral aromatase activity.

FC12 Top Rated Clinical Abstracts
FC12-139 - FGFR1: A genetic etiology for delayed puberty
Elka Jacobson-Dickman1; Apisadaporn Thambudit1; Yisrael Sidis1; Taneli Raivio1; Andrew Dwyer1; Lindsay Cole1; David Schwartz2; William F Crowley Jr.1; Paul A. Boepple1; Nelly Pitteloud1 Massachusetts General Hospital, Reproductive Endocrine Unit, Boston, United States; 2University of South Carolina School of Medicine, Pediatric Endocrinology, Columbia, United States

Abstract.
Delayed puberty (DP) is characterized by lagging reactivation of pulsatile GnRH secretion during adolescence. Genetic studies in patients with Idiopathic Hypogonadotropic Hypogonadism (IHH), a disorder characterized by absent or partial puberty, have shed light on genes controlling puberty. Among them, FGFR1 mutations underlie 10% of IHH cases with variable phenotypes within and across families carrying the same gene defect, suggesting an influence of modifier genes and/or environment. We hypothesized that DP lies at the mild end of the IHH reproductive spectrum and can therefore be caused by FGFR1 mutations. Two cohorts of individuals with DP were screened for FGFR1 mutations. The first cohort was identified by detailed family histories of 40 IHH probands carrying a heterozygous FGFR1 mutation (29M, 11F). The second cohort included 7 individuals with Constitutional Delay of Puberty (CDP) who were not from families with IHH (6M, 1F). FGFR1 missense mutations were studied in vitro using an FGF luciferase reporter bioassay. Among 40 IHH pedigrees, we identify 9 DP subjects from 8 families (5M, 4F) carrying a heterozygous FGFR1 mutation (Y99C, N117S, R250Q, E274G, L342S, R470L, R622X, Q680X). Interestingly, in 5 families the IHH proband carries an additional gene defect (either GnRHR or NELF), which has an additive effect with the FGFR1 mutant to produce a more severe phenotype. We also identified FGFR1 mutations in 2 individuals with CDP (both P772S). In vitro data reveal that FGFR1 mutants have reduced function. FGFR1 mutations were identified in subjects with DP within IHH families, indicating that DP lies at the mild reproductive spectrum of IHH. Intriguingly, our 2 subjects with CDP and FGFR1 mutations share the same reproductive phenotypes to our DP subjects from IHH families, suggesting that FGFR1 mutations may underlie some cases of CDP. These genetic findings can potentially provide insight into the variability of the timing of normal puberty.

FC12 Top Rated Clinical Abstracts
FC12-140 - Prevalence of DUOX2 mutations among children affected by congenital hypothyroidism and dyshormonogenesis
Francesca Cortinovis1; Ilaria Zamproni2; Luca Persani3; Maria Cristina Vigone4; Stefano Mora2; Giovanna Weber1; Giuseppe Chiumello1 Vita-Salute San Raffaele
DUOX2 has been identified as the catalytic core of H2O2 generator in thyroid cells. The generation of hydrogen peroxide is a crucial step in thyroid hormones synthesis and homozygous or compound heterozygous mutations in DUOX2 gene are reported to cause permanent congenital hypothyroidism (CH), while heterozygous mutations are described in association with transient CH. We selected 20 unrelated patients affected by CH (blood TSH at birth: 25-395 mU/L) with partial iodide organification defect (PIOD), documented by a positive perchlorate test. The DNA of the patients was analyzed for mutations in the DUOX2 gene. We identified 8 mutations, 4 nonsense (W414X, Y425X, R842X, Q1023X), 1 frameshift (S956fsX994) and 3 missense (R376W, Y475C, D506N) mutations, in seven patients (4 M, 3 F) out of 20. The mutations W414X, Y425X, Y475C and Q1023X are firstly reported. Compound heterozygosity was observed in 2 of these cases (discharge: 28% and 39%): both of these cases were affected with mild permanent hypothyroidism as documented by hyperthyrotropinemia at revaluation at 3-5 years (TSH 6.3 and 5.5mU/L, respectively). Simple heterozygous mutations were observed in the other 5 PIOD cases (discharge: 63%, 20%, 22%, 26%, 26%). At diagnostic revaluation, only one male of these heterozygous cases displayed a normal thyroid function confirmed by serum TSH of 1.87 mU/L at 2 years after l-thyroxine withdrawal. In the remaining 4 heterozygous patients TSH rose above the upper limit of normal range after 1-2 months of l-thyroxine withdrawal (range TSH 5.5-36.27 mU/L). Analysis of the relatives revealed a variable expression of the thyroid defect in the carriers of the genetic alterations in three families suggesting the existence of genetic/environmental factors able to act as modifiers. In conclusion, mutations in DUOX2 appear to be the most prevalent genetic defect among children with CH and PIOD.