Marco A. Rivarola and Alicia Belgorosky
Servicio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires, Argentina

The general theme of Meeting was BRIDGING CLINICAL CARE and BASIC RESEARCH. Attendance was estimated in approximately 3500 registrants from most European countries and from many countries of the world. This Meeting is, in practice, a true international congress in pediatric endocrinology.

PLENARY LECTURES

PL1 - Autoimmunity and Diabetes
PL1-1 - Type 1 diabetes epidemiology and genetics in central Europe
Ondrej Cinek
Charles University in Prague, Second Medical Faculty, Department of Pediatrics, Prague, Czech Republic

Background: The rapid rise in the incidence of type 1 diabetes (T1D) in Central and Eastern Europe observed over the past two decades cannot be attributed to genetic factors. Changing environmental exposures are likely responsible for this trend, though no single major environmental factor has been identified.

Objective and hypotheses: The presentation will discuss T1D occurrence and etiology, with particular attention given to studies aimed at populations of Central and Eastern Europe.

Results: Since late 1980s, populations in this part of Europe have experienced rapid changes in exposures to some of the environmental factors which had been previously implicated in T1D etiology in Western populations. These factors may be identical in their nature to what was observed elsewhere, but they undoubtedly differ in their swiftness of change. This may be exemplified by the recent increase in the average maternal age, or the varying patterns of crude natality observed in some populations. Further changes in exposures may be connected with the rapid increase in hygiene standards, with the rising proportion of cesarean sections, with changes in infant feeding patterns or in attendance to kindergartens. However, studies performed in Central and Eastern Europe have not revealed any unique patterns of associations with these putative risk factors.

Conclusions: No data indicate that Eastern or Central European countries form any special entity with respect to T1D epidemiology; however the T1D incidence trends should remain actively observed, as such observations are a potential source of information on disease etiology.

Related publication
Overview of the Type I Diabetes Genetics Consortium
SS Rich,1,2 B Akolkar,3 P Concannon,1,4 H Erlich,5 JE Hilner,6 C Julier,7 G Morahan,8 J Nerup,9 C Nierras,10 F Pociot,9 and JA Todd11
The Type I Diabetes Genetics Consortium (T1DGC) is an international, multicenter research program with two primary goals. The first goal is to identify genomic regions and candidate genes whose variants modify an individual’s risk of type I diabetes (T1D) and help explain the clustering of the disease in families. The second goal is to make research data available to the research community and to establish resources that can be used by, and that are fully accessible to, the research community. To facilitate the access to these resources, the T1DGC has developed a Consortium Agreement (http://www.t1dgc.org) that specifies the rights and responsibilities of investigators who participate in Consortium activities. The T1DGC has assembled a resource of affected sib-pair families, parent–child trios, and case–control collections with banks of DNA, serum, plasma, and EBV-transformed cell lines. In addition, both candidate gene and genome-wide (linkage and association) studies have been performed and displayed in T1DBase (http://www.t1dbase.org) for all researchers to use in their own investigations. In this supplement, a subset of the T1DGC collection has been used to investigate earlier published candidate genes for T1D, to confirm the results from a genome-wide association scan for T1D, and to determine associations with candidate genes for other autoimmune diseases or with type II diabetes that may be involved with β-cell function.

PL1-2 - Endocrine autoimmunity: lessons from autoimmune polyendocrine syndrome type I
Abstract. Autoimmune polyendocrine syndrome type I (APS-I) is a monogenic model disease of autoimmunity. Its hallmarks are chronic mucocutaneous candidosis, hypoparathyroidism and adrenal insufficiency, but many other autoimmune disease components occur less frequently. The first components usually appear in childhood, but may be delayed to adolescence or early adult life. There is enormous variation in presentation and phenotype, which makes the diagnosis difficult. Antibodies against interferon-omega and -alpha have recently been shown to be sensitive and relatively specific markers for APS-I, and mutational analysis of the autoimmune regulator gene gives the diagnosis in >95% of cases. The treatment and follow-up of patients is demanding and requires the collaboration of specialists of several fields. However, the literature is especially sparse regarding information on treatment and follow-up; hence, we present here a comprehensive overview on clinical characteristics, treatment and follow-up based on personal experience and published studies.


PL2 - Novel Avenues in Energy Metabolism
PL2-3 - How the fetal hypothalamus senses maternal glucose metabolism
Jens C. Brüning
University of Cologne, and Center of Molecular Medicine Cologne, Department of Mouse Genetics and Metabolism, Institute for Genetics, Cologne, Germany
Abstract text has not been submitted.


Abstract. The central nervous system (CNS) is capable of gathering information on the body's nutritional state and it implements appropriate behavioral and metabolic responses to changes in fuel availability. This feedback signaling of peripheral tissues ensures the maintenance of energy homeostasis. The hypothalamus is a primary site of convergence and integration for these nutrient-related feedback signals, which include central and peripheral neuronal inputs as well as hormonal signals. Increasing evidence indicates that glucose and lipids are detected by
specialized fuel-sensing neurons that are integrated in these hypothalamic neuronal circuits. The purpose of this review is to outline the current understanding of fuel-sensing mechanisms in the hypothalamus, to integrate the recent findings in this field, and to address the potential role of dysregulation in these pathways in the development of obesity and type 2 diabetes mellitus.

**Concluding Remarks.** The global prevalence of obesity and diabetes mellitus is increasing at an alarming rate and dysregulation of the central control of energy homeostasis has been posited as a causative mechanism of these metabolic diseases. Besides the capability of selective brain regions, especially the hypothalamus, to integrate a variety of neuronal and hormonal signals, increasing evidence indicates that fuel sensing also plays a pivotal role in the central regulation of food intake, energy expenditure, and glucose homeostasis. Recent progress has confirmed the presence of distinct hypothalamic neuron populations that respond to changes in circulating nutrient concentrations by either activating or inhibiting their firing activity. At the molecular level, some proteins required for neuronal fuel sensing have already been identified. Among them, K$_{ATP}$ channels and the well-known fuel sensor AMPK represent critical points of interaction between hypothalamic glucose and lipid sensing. A wide range of data further implicates the modulation of neuronal fuel sensing by a variety of hormones such as insulin, leptin, and ghrelin, leading to an increased degree of complexity in the response of these neurons to changes in whole-body energy level. Beyond the scope of this review is another relative new concept in central nutrient sensing involving the role of amino acids in the regulation of energy homeostasis. Studies have demonstrated that increasing or decreasing the dietary intake of leucine, a branched-chain amino acid, as well as ICV administration of this amino acid affects glucose metabolism and food intake. The latter was attributed to changes in hypothalamic Agouti-related protein (AgRP) expression and mechanistically linked to mammalian target of rapamycin (mTOR).

A better understanding of the complex interaction of nutrients, hormones, and neuronal circuitries in the modulation of central behavioral and metabolic output signals might point to novel interventions to prevent or treat obesity and type 2 diabetes mellitus.

**PL2-4 - A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms**

Gerard Karsenty
*Columbia University Medical Center, Genetics & Development, New York, United States*

Our laboratory has been testing the hypothesis that bone mass and energy metabolism are co-regulated. This led us first to show that leptin that is made in adipocytes inhibits both bone mass accrual and appetite by inhibiting serotonin synthesis and release by brainstem neurons. Having identified this regulatory loop and many of its molecular components we then asked whether bone was an endocrine organ regulating energy metabolism. This line of research allowed us to show that the osteoblast-specific secreted molecule osteocalcin favors, when undercarboxylated, insulin secretion, insulin sensitivity and energy expenditure. We further showed that osteoblasts express another gene, Esp, that encodes a tyrosine phosphatase and acts as an
inhibitor of osteocalcin decarboxylation. How an intracellular tyrosine phosphatase could regulate the carboxylation statues of a secreted molecule like osteocalcin is unknown. Likewise, we do not know what is the human equivalent, if it exists, of Esp that is pseudogene in humans. We have embarked in a systematic study, using biochemical, molecular biology and genetics as tools to answer these two questions.


Abstract. The serotonin molecule has some remarkable properties. It is synthesized by two different genes at two different sites, and, surprisingly, plays antagonistic functions on bone mass accrual at these two sites. When produced peripherally, serotonin acts as a hormone to inhibit bone formation. In contrast, when produced in the brain, serotonin acts as a neurotransmitter to exert a positive and dominant effect on bone mass accrual by enhancing bone formation and limiting bone resorption. The effect of serotonin on bone biology could be harnessed pharmacologically to treat diseases such as osteoporosis.


Abstract. The broad expression of the insulin receptor suggests that the spectrum of insulin function has not been fully described. A cell type expressing this receptor is the osteoblast, a bone-specific cell favoring glucose metabolism through a hormone, osteocalcin, that becomes active once uncarboxylated. We show here that insulin signaling in osteoblasts is necessary for whole-body glucose homeostasis because it increases osteocalcin activity. To achieve this function insulin signaling in osteoblasts takes advantage of the regulation of osteoclastic bone resorption exerted by osteoblasts. Indeed, since bone resorption occurs at a pH acidic enough to decarboxylate proteins, osteoclasts determine the carboxylation status and function of osteocalcin. Accordingly, increasing or decreasing insulin signaling in osteoblasts promotes or hampers glucose metabolism in a bone resorption-dependent manner in mice and humans. Hence, in a feed-forward loop, insulin signals in osteoblasts activate a hormone, osteocalcin, that promotes glucose metabolism.

PL4 - ESPE Award Session and Activities 2 (Henning Andersen Prizes - Basic)

PL4-5 - Hypophosphatasia: enzyme replacement therapy for affected children using bone-targeted, tissue-nonspecific alkaline phosphatase
Michael P. Whyte1; Cheryl R. Greenberg2; Deborah Wenkert1; William H. McAlister3; Katherine L. Madson1; Amy L. Reeves1; Karen E. Mack1; Lise Bourrier4; Aziz Mhanni5; Alison M. Skrinar6; Hal Landy7
Abstract. Hypophosphatasia (HPP) is the inborn-error-of-metabolism caused by deactivating mutation(s) within the gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP). Natural substrates for TNSALP accumulate extracellularly and include inorganic pyrophosphate (PPi), a mineralization inhibitor, and pyridoxal 5'-phosphate (PLP), the principal form of vitamin B6. Rickets and osteomalacia occur because PPi blocks hydroxyapatite crystal growth within the skeletal matrix. Diminished PLP hydrolysis reveals TNSALP to be an ectoenzyme. HPP severity spans stillbirth from profound skeletal hypomineralization, to osteomalacia late in life. There is no established medical treatment. ENB-0040 is a bone-targeted, human recombinant, TNSALP fusion protein that preserved skeletal mineralization and survival when injected daily into a mouse model of infantile HPP (JBMR 2008; 23:777). Patient trials began in '08. In a 6-mo, phase I/II study of 6 pts (£3 yrs) with life-threatening HPP, marked skeletal remineralization, weaning from respiratory support, and improved motor development occurred with IV infusion of ENB-0040 2 mg/kg, followed by 1-3 mg/kg SC 3x/wk. Here, we report observations from a phase II, open-label, multicenter assessment of ENB-0040 for 13 HPP children, ages 5-12 yr, receiving either 2 or 3 mg/kg SC 3x/wk for 6 mo. All pts have completed Wk 6, some Wk 18. Transient injection-site erythema was common, but well tolerated. There have been no SAEs. At Wk 6, serum ALP activity was 2,300-10,700 U/L (93-309 NI), and elevated plasma PLP levels decreased, normalizing in 6/8 pts. Increases in serum PTH from enhanced skeletal mineralization occurred, but without hypocalcemia from 'hungry bones'. Skeletal radiographic improvement was observed in all pts at Wk 6, with further gains at subsequent visits. All pts reported increased strength, endurance, and mobility within weeks of therapy. The 7 pts at Wk 12 walked ~50-110 m further in a 6-min walk test. Bone-targeted ENB-0040 is a promising enzyme replacement therapy for childhood HPP.

PL4-6 - Enhancement of the canonical Wnt pathway in Rathke’s pouch results in pituitary tumours reminiscent of human adamantinomatous craniopharyngioma
Carles Gaston-Massuet1; Cynthia Andoniadou1; Tom Jaques1; Paul Le Tissier1; Mehul Dattani2; Juan Pedro Martinez-Barbera2
1Institute of Child Health, UCL, Neural Development Unit, London, United Kingdom; 2Institute of Child Health, UCL, Clinical Molecular Genetics, London, United Kingdom
Abstract. Wnt/beta-catenin signalling pathway is required during embryonic development for normal cell proliferation, differentiation and for organ homeostasis in adulthood. Over-activation of this pathway has been implicated in human cancers such as colon or skin cancers. Here, we demonstrate that
enhancement of the Wnt pathway in the embryonic Rathke’s pouch causes over-proliferation of progenitor cells and severe differentiation defects in the Pit1-lineage, which results in extreme growth retardation and hypopituitarism. Mutant mice mostly die perinatally but those that survive weaning develop lethal pituitary tumors. Histopathological analysis revealed that these murine tumors most closely resemble human adamantinomatous craniopharyngioma rather than any other pituitary tumor, including pituitary adenomas, Rathke’s cleft cysts, xanthogranulomas, posterior pituitary tumors (e.g. pituitocytomas) or even the adult (papillary) form of craniopharyngioma. This tumorogenic effect only occurs when Wnt pathway over-activation occurs in the early Rathke’s pouch progenitors, but not when committed or differentiated cells are targeted. Finally, we demonstrate that genetic overexpression of the paired-like homeobox repressor Hesx1 is able to delay tumor formation, by partially antagonising the activation of Wnt signalling downstream of beta-catenin. Together, our findings provide new insights into the roles of the Wnt pathway in the control of pituitary cell proliferation and demonstrate, for the first time, a causative role the Wnt pathway in an undifferentiated multipotent pituitary progenitor in the genesis of murine pituitary tumors that are reminiscent of human craniopharyngioma.

PL5 - G-Protein Coupled Receptors/Adrenal Diseases
PL5-7 - Specificity and promiscuity of G-protein coupled receptors
Gilbert Vassart
Université Libre de Bruxelles, IRIBHM, Brussels, Belgium

Abstract. With few exceptions specificity of recognition and activation by their respective agonists is a major characteristic of G protein-coupled receptors (GPCRs). For protein/peptide hormones, specificity is the result of tight co-evolution of the receptor-agonist couples. In this respect, glycoprotein hormone receptors (GPHRs) constitute an interesting subfamily with the recent addition, in primates only, of an additional agonist, chorionic gonadotropin (CG). During human pregnancy, circulating CG reaches levels several orders of magnitude higher than the pituitary hormones, thus challenging the specificity barriers of GPHRs. The dichotomy between hormone recognition, by the ectodomain, and activation of the G protein, by the rhodopsin-like serpentine portion, is a well established property of GPHRs. The specificity barrier avoiding promiscuous activation of the FSH receptor by hCG during pregnancy was thus believed to lie in the ectodomain. Mutations responsible for rare spontaneous cases of ovarian hyperstimulation syndromes have partially modified this view. A series of naturally occurring mutations have been identified which cause increase in sensitivity of the FSH receptor to hCG. Surprisingly the large majority of these mutations were located in the serpentine portion of the receptor. In addition to their effect on sensitivity to hCG, they also increased sensitivity to TSH, and were responsible for activating the receptor constitutively. Together, the available information indicates that the ectodomain and the serpentine domain of the FSH receptor each contribute to the specificity barrier preventing its illegitimate activation by hCG. While the former is responsible for establishment of binding specificity, the latter introduces a novel notion of functional specificity. Studies of a large panel of primate and non primate FSHRs demonstrate a direct relation between promiscuous activation by CG and basal activity,
suggesting that silencing of the FSHR has been selected by evolution to cope with the increasing pregnancy levels of CG during evolution of primates.

**PL5-8 - Molecular basis of adrenal insufficiency in children (Kenji Fujieda memorial lecture)**
Kenji Fujieda1; Toshihiro Tajima2
1Asahikawa Medical College, Pediatrics, Asahikawa, Japan; 2Hokkaido University, Graduate School of Medicine, Sapporo, Japan
Defective production of adrenal steroids due to either primary adrenal failure or hypothalamic-pituitary impairment of corticotrophic axis cause adrenal insufficiency. These can be subgrouped into three categories: 1) adrenal dysgenesis; 2) adrenal destruction; and 3) impaired steroidogenesis. Depending on the etiologies, adrenal crisis may occur in early infancy or symptoms of adrenal insufficiency may insidiously develop in childhood/adolescence. Adrenal crisis represents an endocrine emergency, and thus the rapid recognition and prompt therapy for adrenal crisis are critical for survival even before the diagnosis is made. Recent molecular-genetic approach for adrenal disease gives valuable insights into the adrenal organogenesis, the regulation of steroid hormone biosynthesis, and the developmental and reproductive endocrinology. The recognition of various disorders that cause adrenal insufficiency at molecular level often has implications for the management of the patient. Of those, lipoid congenital adrenal hyperplasia presents with various severity of pathogenesis. Human disease due to mutations of NADPH-cytochrome P450 oxidoreductase (POR) was identified in 2004. The mutations of POR caused partial deficiencies in steroid 17-hydroxylase, 17, 20 lyase, and 21-hydroxlase with Antley-Bixler syndrome (OMIM 207410) (ABS). ABS is characterized by craniosynostosis, radio/humeral synostosis, ambiguous genitalia, and other congenital anomalies. In addition, some patients with ABS showed abnormal steroid hormone profiles of elevated serum 17-hydroxyprogesterone and low cortisol levels. In this plenary lecture I would review recent progress of the molecular pathogenesis of lipoid congenital adrenal hyperplasia and P450 Oxidoreductase deficiency with a brief overview of the latest progress on congenital adrenal diseases.

**PL6 - PROP1 and the Bridge to Krk**
**PL6-9 - PROP1 and the Bridge to Krk**
John Parks
Emory University, Pediatric Endocrinology and Diabetes, Atlanta, United States

Krk is the largest island in the Adriatic Sea. Formerly reachable only by boat, it is now linked to the world by a bridge and an international airport. It has been home to an extended kindred of 24 men and women with severe hypopituitarism. They were born between 1864 and 1976. Successive waves of medical visitors have written of these "Little People of Krk" since von Juaregg's report in 106. Each generation has seen them from its own perspective. They have been seen as medical curiosities and then as examples of what happens when newly recognized hormones of the anterior pituitary are lacking. As young adults, they look like young children. With time, there is a coarsening of features, followed by a wrinkled and prematurely aged appearance. From a genetic perspective, they reflect homozygosity for a recessive mutation inherited from a common ancestor. The mutation consists of a single base pair
deletion in codon 150 of the pituitary transcription factor, PROP1. It is the second most common mutation in PROP1, exceeded only by a two base pair deletion in codon 99. PROP1 abnormalities are by far the most common genetic cause of multiple pituitary hormone deficiency (MPHD) in Europeans. Dr. Lenartoska has identified 39 cases in Poland and Dr. Lebl has reported on 17 in the Czech Republic. While the two deletions were first considered to indicate hot spots for mutation, our work has pointed to a founder effect. Virtually all cases of one and two bp deletions from the Western hemisphere share common SNP haplotype environments. The Krk subjects also provide insight into the effects of MPHD on longevity. In contrast to Swiss subjects with IGFD IA, and in common with MPHD mice, they tend outlive their siblings. The past century has changed Krk from an island of fishing villages and scholarly monasteries to a destination resort with beaches, clubs, condos and timeshares. It has brought equally changes in our understanding of the causes and consequences of hypopituitarism.

WORKING GROUPS

WG2 - ESPE Disorder of Sex Development Working Group

WG2-52 - The European DSD Registry
Martina Rodie1; Jipu Jiang2; Richard Sinnott2; Syed Faisal Ahmed1
1Royal Hospital for Sick Children, Department of Child Health, Glasgow, United Kingdom; 2University of Glasgow, National e-science Centre, Glasgow, United Kingdom

Disorders of sex development (DSD) are a rare group of conditions which require further research. Effective research into understanding the aetiology as well as long-term outcome of these rare conditions requires multicentre collaboration across countries. The EuroDSD programme (www.eurodsd.eu) is one such collaboration. It includes doctors and scientists from all over Europe and is funded by the EU Commission under the 7th Framework Programme. The European DSD Registry is central to the project within the EuroDSD programme and provides a means of connecting the research centres and the clinical centres within a Virtual Research Environment (VRE). The objective of the registry is to provide as much information as possible about DSDs and to gather data in order to perform long term outcome studies. There are currently 548 cases on the Register. The majority of cases are from the Netherlands and United Kingdom, 32% and 31% respectively. The median year of birth is 1993 (range 1927-2009) and the age of presentation ranges from <1month to 62 years. 371 cases are assigned female sex (68%) and 177 are assigned male sex (32%). Disorders of androgen action are the commonest disorder type with 186 cases (34%). Of these cases 144 (77%) are complete androgen insensitivity syndrome, 40 (22%) are partial androgen insensitivity syndrome and 2 cases (1%) have a diagnosis of other. In summary, the European DSD Registry is a live, web-based platform that can act as a resource for research into a number of aspects of DSD. The future direction of the Registry is to facilitate DSD research across the globe. The work on this project is still very much on going both from a software development and a clinical research/usage perspective. The lessons that have
been learnt can be applied to collaborative research in a number of other rare conditions.

**WG2-53 - E-learning and its application to DSD**

Stenvert L.S. Drop  
*Sophia Children’s Hospital/ErasmusMC, Endocrinology, Rotterdam, Netherlands*

The aim of the webportal is to provide entrance to an interactive learning environment for an up-to-date program on DSD including normal development, patho-physiological mechanisms, diagnostic and therapeutic interventions, psychological counselling, outcome. Target groups are medical students, residents, fellows, specialists, consultants, teachers around the world. The portal is developed in the English language with two levels of learning: a. basal (medical student): the focus is the understanding of the normal development and its patho-physiology with clinical and social implications. b. advanced (post-doc, etc) : the fellow is additionally invited to analyze and diagnose disorders, to solve problems, to appraise scientific evidence, and to communicate with professionals and patients (parents). A forum functionality enables the users to post comments and remarks and to discuss certain topics. The forum will be used for specific discussions on a case or study results or knowledge sharing. Following an intensive phase of construction the webportal has gone live. Currently authors have been invited to contribute content on DSD to the portal. The main emphasis is on data entry, i.e text for chapters and case descriptions. Editorial working groups have been formed, consisting of a group of medical editorial contributors. Further extension of these groups with experts from several pediatric endocrinological societies is foreseen. As author and/or editor they will be responsible for the content of the program. A technical staff is involved in the construction, lay out and function of the web portal. The development of the functionality for scoring of questions and the development of formative assessment of competencies of students and portal users is a separate entity requiring specific expertise. The educational background and objectives have been summarised recently (1). (1)Grijpink-van de Biggelaar K, Drop SL, Schuwirth L. Development of an ESPE e-learning portal: educational considerations. Horm Res Paediatr 2010; 73:223-30

**WG2-54 - Technological advances in steroid analysis for DSD diagnosis**

Nils Krone  
*Centre for Endocrinology, Diabetes and Metabolism, School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, United Kingdom*

For about 40 years, disorders of steroidogenesis have been primarily studied through quantification of selected analytes by immunoassays. Structural similarity of steroids compromise specificity of immunoassays by crossreactivity, and immunoassays are notoriously inexact in low concentration ranges as seen in paediatrics. During recent years laboratory test of patients suffering from potential endocrine conditions have become more accurate. High performance liquid chromatography/ tandem mass spectrometry (LC-MS/MS) is the most successful approach to improve specificity problems inherent in many immunoassays and has become the method of choice for clinical steroid analysis. LC-MS/MS is increasingly replacing immunobased techniques
because of its high sensitivity, greater specificity, high reproducibility and ability to analyse multiple steroids from small sample volumes simultaneously. LC-MS/MS is a highly valuable second-tier test in CAH newborn screening and is enhancing diagnostic capabilities of routine testing particularly when steroid profiles are available. However, LC-MS/MS is of limited use in defining novel metabolomes. Gas chromatography mass spectrometry (GC/MS), in contrast, is unsuitable to rapid high sensitivity analysis of specific compounds, but is the most powerful discovery tool for defining steroidobolomes. GC/MS has defined several metabolomes with the most recent being apparent cortisol reductase deficiency and P450 oxidoreductase deficiency. Almost all steroidogenic conditions are defined by the absolute concentration of steroid metabolites, many are diagnosed between ratios of substrate metabolite to product metabolite of enzymatic reactions. A particular benefit of GC/MS is its non-selective nature; a scanned run will contain every steroid excreted, providing an integrated metabolome. The analysis of such "undefined" metabolomes using i.e. biocomputational analysis by supervised machine learning tools provides the opportunity for identification of novel metabolomes and characterisation of novel steroidogenic disorders causing DSD.

WG2-55 - A new look at androgen insensitivity
Paul Martin Holterhus
Christian-Albrechts-University, Pediatrics, Division of Pediatric Endocrinology, Kiel, Germany
Sex-specific development of the human comprises irreversible sexual differentiation of the external genitalia during embryogenesis, extra-genital sexual dimorphism during puberty as well as sex-specific development of the brain. Both, genetic factors and sex hormones are crucial. The presence or absence of androgen biosynthesis and androgen action via the androgen receptor play key roles in sex specific development. Androgen insensitivity syndrome (AIS) due to inactivating mutations of the androgen receptor gene may be considered a „human disease model“ supporting this concept. The androgen receptor is a ligand activated transcription factor of androgen regulated genes. At the single cell level, androgens induce specific reversible changes of the transcriptome being responsible for the biological effects. We have shown global differences in the orchestration of androgen receptor coregulators as a likely mechanisms contributing to tissue specificity of androgen action (1). Apolipoprotein D (APOD), a cell cycle regulator and a human pheromone transporter is androgen regulated in genital skin fibroblasts which can be used as a new functional test for AIS (2). Microarray analyses have shown that during sensitive windows of embryogenesis androgens also imprint long-term changes of the basal transcriptome in these cells thus representing a "transcriptional androgen memory" (3). The same phenomenon occurs in peripheral blood mononuclear cells giving rise to male, female or even intersex basal transcription patterns (4). More recently, as part of EuroDSD research, our genome-wide methylation studies have given first insights into the role of the methylome in androgen memory. The lecture will present these data in the context of AIS. (1) Bebermeier et al. 2006, Journal of Molecular Medicine 84:919-931, (2) Appari et al. 2009, Journal of Molecular Medicine, 87:623-632; (3) Holterhus et al. 2007, BMC-Genomics 8:376; (4) Holterhus et al. 2009, BMC-Genomics 10:292.

WG2-56 - Issues in management of 46,XY DSD
A/ Normal 46,XY development: To simplify, one can distinguish:
1. Formation of undifferentiated gonads
2. SRY gene
3. Timely synthesis of AMH and androgens (testosterone and dihydrotestosterone)
4. Androgen receptor gene
B/ DSD: Are either complete or partial deficiency of any one of these factors. Gene mutations producing complete deficiencies result in female genitalia, partial deficiencies in ambiguous genitalia.
C/ Sex of Rearing: At birth, female sex of rearing is advisable in 46,XY DSD with complete androgen deficiency, complete AIS, and complete gonadal dysgenesis. Some CGD women have been pregnant through in vitro fertilization. Decision about gender in cases of ambiguous genitalia is more difficult and based on multiple factors:
1. Degree of ambiguity: length of phallus and vagino-utricular pouch.
2. Leydig and Sertoli cells function.
3. Karyotype. 45,XO/46,XY. 46,XX with or without SRY.
Following appropriate discussion, parents make the final decision.
D/ Long-range follow-up: This is most important. During childhood, contacts with parents will help answering questions and consider problems related to the specific DSD. In early or late adolescence, patients must be given information about their condition. Psychological help might be included. Surgical correction is advised in infancy to harmonize the appearance of the external genitalia and sex of rearing. Additional correction is expected later. DSD can be helped greatly by follow-up. This question and others related to 46,XY will be discussed by the panel of experts.

WG2-57 - Gonadal pathology and tumor risk in relation to clinical characteristics in patients with 45,X/46,XY mosaicism
Martine Cools1; Jana Pleskacova2; Katja P Wolfenbuttel3; Remko Hersmus2; Hans Stoop2; Stenvert LS Drop4; Piet Hoebeke5; Leendert HJ Looijenga2
1University Hospital Ghent, Ghent University, Pediatric Endocrinology, Ghent, Belgium; 2Josephine Nefkens Institute, Daniel Den Hoed Cancer Center, Erasmus MC - University Medical Center, Department of Pathology, Rotterdam, Netherlands; 3Erasmus University Hospital Rotterdam, Department of Urology, Division of Pediatric Urology, Rotterdam, Netherlands; 4Erasmus University Hospital Rotterdam, Department of Pediatrics, Division of Pediatric Endocrinology, Rotterdam, Netherlands; 5University Hospital Ghent, Ghent University, Department of Urology, Division of Pediatric Urology, Ghent, Belgium

Background: Individuals with 45,X/46,XY DSD and mild undervirilisation or ambiguous genitalia are increasingly raised as males. However, gonadal management remains controversial since data on tumor risk and hormonal function in this specific population are unavailable.

Methods: We combined detailed pathological studies with clinical data of 45,X/46,XY individuals (n=47, 83 gonadal specimen, obtained after biopsy or prophylactic gonadectomy), divided into 3 groups based on the external masculinisation score (EMS): Group I: mild undervirilisation, EMS ≥7, n=15;
Group II: ambiguous genitalia, EMS<7, n=22; Group III: female phenotype, EMS 0-1, n=46). Tumor risk was assessed by the presence of malignant or premalignant (immunohistochemical staining for OCT3/4, TSPY, stem cell factor) characteristics. In males with at least one preserved gonad, available data on hormonal function were recorded.

**Results:** A type II germ cell tumor (GCT) was found in 4/83 samples (4.7%), indices for premalignancy in 13 samples (15.3%). However, the risk for cancer was significantly higher in group II (ambiguous phenotype) as compared to groups I (p=0.01) and III (p<0.001) (Table I). Information on hormonal function was available in 10/18 males with at least one preserved gonad. Five boys are prepubertal, all had a moderate to good HCG-induced testosterone response. Five males are (post)pubertal, none of them needs androgen suppletion, but FSH is raised in all five.

**Conclusions:** In conclusion, in 45,X/46,XY individuals, tumor risk is significantly higher in patients with marked genital ambiguity as compared to patients with mild undervirilisation or with a female phenotype. Leydig cell function is at least partially preserved in 45,X/46,XY males and often allows spontaneous pubertal development. These data are highly relevant for clinical decision making with regard to gonadectomy in patients with 45,X/46,XY DSD.

**TABLE I**

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<thead>
<tr>
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<th>Mild undervirilisation</th>
<th>Ambiguous genitalia</th>
<th>Female</th>
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<tbody>
<tr>
<td>Premalignant changes</td>
<td>1/15</td>
<td>11/22</td>
<td>1/46</td>
</tr>
<tr>
<td>Type II GCT</td>
<td>0/15</td>
<td>2/22</td>
<td>2/46</td>
</tr>
<tr>
<td>Tumor risk</td>
<td>1/15 (6.7%)</td>
<td>11/22 (50%)</td>
<td>2/46 (4.3%)</td>
</tr>
</tbody>
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**WG2-58 - Long term results in XY DSD raised as females**

Karinne Gueniche1; Mélanie Jacquot1; Elisabeth Thibaud2; Michel Polak2
1Hôpital Necker, Université Paris Descartes, Institut de Psychologie, LPCP EA 4056, Pediatric Endocrinology and Gynecology, Paris, France; 2Hôpital Necker, AP-HP, Université Paris Descartes, Pediatric Endocrinology and Gynecology, Paris, France

Few studies have addressed the question of the long term outcomes including well-being in DSD patients. Our study, aimed to assess cognitive, psychic functioning, sexual behavior and quality of life in a cohort of XY DSD raised as girls, treated at a single institution.

**Subjects and methods:** 15 patients aged 18 to 41 with a 46 XY DSD, whose gender was assigned as female, whose treatment included genital surgery and raised as girls, were recruited and studied.

The protocol included a gynecological examination, a semi-directive interview, a WAIS III-R intelligence test, the WHO quality of life 26 and a questionnaire of sexual behavior to be compared to normative data from the same French population.

**Results:** The global cognitive abilities of the cohort are in the lower half of the normal (average IQ = 90). The assessment given by the women to their quality of life, from a psychological point of view (57.5%) and as concerns social relationships (56.4%), was significantly lower than that of the reference population (RP) (p = <0.05). Six of the fifteen women had never had sex ; a proportion much higher than average among women of their age (40% versus 5.2%) (p= <0.05). Homosexual attraction (38.3%) and genital sexuality (30%) are much more frequent than in the RP (respectively 6.5% and 1.9%) (p =
<0.05). The question of sexuality was addressed as a medical issue (27.7%, against 5.2% for the RP) (p = <0.05).

**Conclusions:** The quantitative analysis revealed these women’s dissatisfaction with their emotional and social life. We underscored the fact that these women are in great psychic pain. Their quality of life is greatly altered, their sexual and love life inhibited; they are not subjectively satisfied with the surgery (despite satisfactory gynecological examinations) and with the knowledge of their disorder.

**WG2-59 - How ESPE members manage testicular DSD: a survey**

Nathalie Josso

**INSERM, U782, Clamart, France**

Management of disorders of sex differentiation due to abnormalities of either testicular tissue or testicular hormones and receptors has much evolved during the last decade both from a scientific and a psycho-social viewpoint. New techniques in molecular biology, cytogenetics, radiology and laparoscopy have improved the percentage of successfully diagnosed cases. Better surgical approaches have improved the outcome, although longterm studies are not yet feasible. Due to lobbying by patient support groups, the wishes of the parents are now paramount for sex assignment and for timing of reconstructive surgery. General guidelines have been drawn up by a group of ESPE and Lawson Wilkins society members. However, most of them hail from Anglo-Saxon countries and it is well known that sex and its abnormalities is strongly influenced by ethnicity. The DSD Work group has emailed a questionnaire to all ESPE members asking those caring for DSD patients to answer detailed questions pertaining to diagnosis and management. The first question relates to nomenclature, asking whether the old terminology, for instance male pseudohermaphroditism, true hermaphroditism, has been abandoned in favour of the terms coined by the ESPE-Lawson Wilkins work group (Hughes et al, 2006). The next section explores the diagnostic hormonal and molecular tools available to ESPE members and the following one deals with the parameters of sex assignment and communication with the family and the child as he/she grows older. The last section is designed to be filled out by the surgeon, not necessarily him/herself an ESPE member, and deals with the techniques and timing of genitoplasty. Obviously, there are wide individual differences between cases and therefore spaces for comments have been generously inserted throughout the document. Responses to the survey will be analyzed at the meeting of the DSD work group.

**SELECTED SYMPOSIA**

- **S1 Ovarian Development**

**S1-10 - FOXL2 and downstream events in ovarian development**

Marc Fellous1; Reiner Veitia2

1University of Paris 7/Cochin Institut/Inserm, Genetic, Paris, France; 2Université Paris-Diderot/Paris 7 and Institut Universitaire de France, Genetic, Paris, France

FOXL2 is one of the earliest somatic markers of ovarian development and encodes a protein belonging to the Forkhead/Winged-Helix family of
transcription factors. Its mutations are responsible for a genetic disorder characterized by craniofacial abnormalities, either isolated (BPES II) or in association with premature ovarian failure (BPES type I). Mouse embryonic XX gonads lacking the forkhead transcription factor Foxl2 form meiotic prophase oocytes, but partially activate the genetic program for somatic testis determination. Mouse inducible deletion of Foxl2 in adult ovarian leads to upregulation of testis-specific genes including the critical Sox9 and reprogramming of granulosa and theca cell lineages into Sertoli-like and Leydig-like cell lineages occurs with testosterone levels comparable to normal XY male. As predicted by our Z model ovarian phenotype is an active process throughout life. We have shown that cell stress up regulates FOXL2 expression in an ovarian granulosa cell model. Upon oxidative stress, was observe an increased recruitment of FOXL2 to several stress-response promoters, notably that of the mitochondrial manganese superoxide dismutase (MnSOD).

Moreover, mutations of FOXL2 induce aberrant regulation of MnSOD promoter. Our results establish that FOXL2 is an actor of the stress response which may be linked to ovarian aging in BPES patients. Recently, he somatic mutation in the FOXL2 gene (p.Cys134Trp) has been identified in the vast majority of adult ovarian granulosa-cell tumors (OGCTs) studied. In addition, this mutation seems to be specific of adult OGCTs and is likely to be a driver of malignant transformation. Foxl2 could be considered as a tumor suppressor. Further studies on the molecular pathogenesis of BPES and OCGTs will have important medical implications for the understanding disorders of sexual development in children and premature menopause in women.


**S1-11 - WNT4 and R-Spondin 1 signalling in ovarian development**

Anna Biason-Lauber

*University Children’s Hospital, Endocrinology/Diabetology, Zurich, Switzerland*

In sex development we can distinguish two different processes: sex determination and sex differentiation. The former is the developmental decision that directs the undifferentiated embryo into a sexually dimorphic individual. The second process, sex differentiation, takes place once the sex determination decision has been made through factors produced by the gonads that determine the development of the phenotypic sex. Generally speaking, factors influencing sex determination are transcriptional regulators, whereas factors important for sex differentiation are secreted hormones and their receptors. Although factors involved in male sexual development have been well studied, the pathways that regulate female sexual determination remain incompletely defined. To date, no genes have been shown to play a role in ovarian development equivalent to that played by the SRY gene in testicular development. Wnt4, one of a few factors with a demonstrated function in the ovarian-determination pathway, has been found to be involved in sexual differentiation by suppressing male sexual differentiation, promoting Müllerian ducts differentiation and maintaining oocyte health. WNT4 expression in the ovary seems to be regulated by R-spondin 1 (RSPO1), a trombospondin family member protein. Mutations in RSPO1 cause palmoplantar hyperkeratosis with squamous cell carcinoma of skin and 46 XX, DSD with “functional” testis. The presence of ‘functional’ testes in the sex-reversed individuals was confirmed by the absence of mullerian derivatives and by the masculinization of
the internal and external genitalia, presumably induced by functioning Sertoli and Leydig cells, respectively. All sex-reversed individuals were sterile. Notably, the data suggested that normal RSPO1 is not required for testis differentiation and function and may synergize with WNT4 in XX gonads to stabilize beta-catenin. The role of WNT4, RSPO1 and of other new factors, such as FOXL2 and CBX2 in ovarian development will be discussed.

**S1-12 - NR5A1 (SF1) and premature ovarian failure**

Ken McElreavey  
*Institut Pasteur, Human Developmental Genetics, Paris, France*

NR5A1, also termed steroidogenic factor 1, is a member of the nuclear receptor superfamily and functions as a key transcriptional regulator of many genes involved in the hypothalamic-pituitary-gonadal steroidogenic axis. Until recently, NR5A1 mutations were described in association with 46,XY DSD with or without adrenal insufficiency. We identified 4 families with individuals presenting with 46,XY DSD as well as primary ovarian insufficiency (POI) including primary and secondary amenorrhea as well as premature ovarian failure. Missense or frameshift mutations in NR5A1 were identified in each family. Further analysis of sporadic cases of POI has revealed additional mutations in approximately 4% of cases. These mutant proteins are unable to transactivate downstream gonadal promoters. The mutant proteins however retain their ability to synergise with a known NR5A1 protein partner, GATA4 to modulate gene expression. Our more recent work has identified some of these mutations in men with normal development of the external genitalia but who unexplained infertility. Some of these mutations are population specific and thus suggest founder mutations. Our data expand the range of phenotypes associated with mutations in NR5A1. These findings pose considerable challenges in understanding the relationship between the mutation and the associated phenotype not only in unrelated individuals but also within families. Phenotypic variability may be due to intrinsic properties of the mutant NR5A1 protein itself and/or it may be a consequence of genetic modifiers that may include the variants in known/novel partners of NR5A1. In addition, environmental factors may also contribute to the severity of the phenotype. Many of these issues could be addressed by understanding the precise molecular role of NR5A1 in cellular and temporal contexts and understanding the genetic variation(s) in individuals who carry NR5A1 mutations using next generation sequencing approaches.

**S8 - Epigenetics in Paediatric Endocrinology**

**S8-31 - Introduction to epigenetics – implications in paediatric endocrinology**

Miguel Constancia  
*University of Cambridge, Metabolic Research Laboratories, Cambridge, United Kingdom*

Epigenetics is an exciting and rapidly moving field that impacts basic biomedical research and clinical medicine. It is defined as the study of heritable changes in gene function that occur without a change in the DNA sequence. The main epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNAs that affect a number of processes such as chromosome architecture and function, and gene expression. During cell division, cells acquire the same epigenetic modifications as the parent cell, resulting in the
heritable transmission of these epigenetic states and a ‘memory’ of a cell’s identity. Epigenetic states, however, have inherent flexibility because they can undergo regulated change in response to particular stimuli in order to modulate gene expression as the need arises; for example, during the development of stem cells into particular organ systems, or during reprogramming events in germ cells and early embryo to restore pluripotency, or in response to external environmental factors, such as diet and environmental chemicals. Epigenetic modifications, therefore, can render the genome functionally flexible and adaptable, but at the same time render it ‘vulnerable’. Functional epigenetic asymmetry that exists between the genomes of the parents has important influences during pregnancy and throughout life. These imprinting effects include contributions to the allocation of maternal resources – especially to the control of key aspects related to growth and adaptations to feeding and metabolism. If these naturally occurring epigenetic processes occur improperly, adverse health and behaviors can ensue, with important implications for pediatric endocrinology and programming of adult disease. During the talk, I will review the key concepts of epigenetic mechanisms of gene regulation and give an overview of the importance of epigenetics in the context of pediatric endocrinology.

S8-32 - Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease
Karen A. Lillycrop
Southampton, United Kingdom
Abstract text has not been submitted.

Related Review.
Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale?
Graham C Burdge1, Karen A Lillycrop2, and Alan A Jackson1
1Institute of Human Nutrition, University of Southampton, Southampton, UK
2Developmental and Cell Biology, University of Southampton, Southampton, UK
Abstract
There is substantial evidence which shows that constraints in the early life environment is an important determinant of risk of metabolic and cardiovascular disease. There is emerging evidence that higher birth weight, which reflects a more abundant prenatal environment, is associated with increased risk of cancer, in particular breast cancer and childhood leukaemia. Using specific examples from epidemiology and experimental studies, this review discusses the hypothesis that increased susceptibility to cardiovascular, metabolic disease and cancer have a common origin in developmental changes induced in the developing fetus by aspects of the intra uterine environment including nutrition which involve stable changes to the epigenetic regulation of specific genes. However, the induction of specific disease risk is dependent upon the nature of the environmental challenge and interactions between the susceptibility set by the altered epigenome and the environment throughout the life course.

S8-33 - Epigenetic changes after prenatal exposure to the Dutch famine of 1944-45
L. H. Lumey
Epidemiologic studies suggest that adult disease risk may be associated with adverse environmental conditions early in development but the biological mechanisms behind these relations are unclear. Our group used the circumstances of the Dutch famine of 1944-45 to evaluate epigenetic changes in men and women at age 60 with prenatal famine exposure. We expected that famine exposure could be associated with a decrease in methylation of the differentially methylated region of the imprinted insulin-like growth factor 2 (IGF2) gene. We defined exposure around the time of conception or in very early pregnancy as a critical period as this is a sensitive period for setting DNA methylation levels. We also examined methylation patterns at other loci associated with growth and development. The study included adults born in selected hospitals in the western Netherlands to mothers who were either exposed to famine around the time of conception or early pregnancy (n=60) or exposed during the last trimester of pregnancy (n=62). Timing of famine exposure was defined based on available emergency food rations in relation to mother’s last menstrual period. To minimize potential confounding by maternal genes, early family environment or gender, all study subjects were matched to an unexposed same-sex sibling control. In all, 244 study subjects were examined. We obtained appropriate institutional ethics committee clearances for study. Men and women exposed during early gestation showed a decrease in IGF2 methylation compared to unexposed same-sex sibling controls. No associations were seen with famine exposure in late gestation or with birth weight. Methylation patterns of additional loci were varied and sometimes sex-specific. We suggest that persistent changes in DNA methylation may be a common consequence of prenatal famine exposure and that changes may depend on fetal sex and the timing of the exposure.

Free Communications

FC4 - Defects in the GH/IGF-I Axis

FC4-94 - Mutations in the Fibroblast Growth Factor 8 (FGF8) gene are associated with complex midline and hypothalamo-pituitary defects
Mark McCabe1; Vaita Tziaferi1; Juan-Pedro Martinez-Barbera2; Louise Gregory1; Joanna Walker3; Pei-san Tsai4; Nelly Pitteloud5; Mehul Dattani1
1Institute of Child Health, Clinical and Molecular Genetics Unit, London, United Kingdom; 2Institute of Child Health, Neural Development Unit, London, United Kingdom; 3St Mary’s Hospital, Department of Paediatrics, Portsmouth, United Kingdom; 4University of Colorado, Department of Integrative Physiology, Boulder, United States; 5Harvard Reproductive Endocrine Sciences Center and Reproductive Endocrine Unit, Department of Medicine, Boston, United States

FGF8 is important for GnRH neuronal development and may also be critical for normal hypothalamo-pituitary development. Loss-of-function mutations in FGF8 in humans have been associated with Kallmann syndrome (KS), as characterised by hypogonadotrophic hypogonadism and anosmia. Other phenotypic features include cleft lip/palate and sensori-neural hearing loss. Hypomorphic Fgf8 mutant mice demonstrate poor telencephalic development
with deletions of midbrain tissue, absence of olfactory bulbs and optic chiasm, and holoprosencephaly (HPE) with an abnormal corpus callosum. We aimed to investigate the role of FGF8 in the formation of midline forebrain and raniophal structures in humans. Patients with congenital hypopituitarism and midline forebrain/craniofacial defects (n=600) were screened by direct sequencing for mutations in FGF8. Two novel missense mutations were identified in FGF8 in regions that are highly conserved across species: i) homozygous p.R189H and ii) heterozygous p.Q216E. The p.R189H mutation was identified in a female patient with semi-lobar HPE, diabetes insipidus and ACTH insufficiency born to consanguineous parents. The heterozygous parents were unaffected. The p.Q216E mutation was identified in a female patient with an absent corpus callosum, hypoplastic optic nerves and Moebius syndrome. Functional analysis of these mutations is ongoing. Human embryonic tissue analysed by in situ hybridization revealed FGF8 expression in the ventral diencephalon and anterior forebrain, but not the developing Rathke’s pouch. This pattern is similar to that described in mice. To conclude, we demonstrate for the first time a recessive FGF8 mutation in a patient with HPE, a condition previously being associated with heterozygous mutations in SIX3 and the Sonic Hedgehog signalling pathway. Our data suggest a role for Fgf8/FGF8 in forebrain and hypothalamic development in humans, suggesting an overlap in pathogenesis between KS and complex midline defects with hypopituitarism. Furthermore, this is the first report to implicate FGF8 mutations in Moebius syndrome.

**FC4-95 - Screening of a large cohort of pediatric patients with GH deficiency for mutations in genes involved in the regulation of pituitary development and GH secretion**

Werner F. Blum1; Elena P. Shavrikova2; Catherine Sampson3; Heike Stobbe4; Juergen Klamm4; Serge Amselem5; Roland W. Pfaeffle4

1Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany; 2PSI Company Ltd., Statistics, St. Petersburg, Russian Federation; 3Statprobe, Statistics, Cary, United States; 4University of Leipzig, Hospital for Children and Adolescents, Leipzig, Germany; 5University Pierre et Marie Curie, Hospital Armand Trousseau Inserm U933, Paris, France

GH deficiency (GHD) can be caused by mutations in genes involved in pituitary development and/or regulation of GH synthesis and secretion. To investigate the frequency of mutations in such genes we screened patients (pts) with severe GHD enrolled in the GeNeSIS observational program for mutations in GH1, GHRHR, GLI2, HESX1, LHX3, LHX4, POU1F1, PROP1, SHH and SOX3 using denaturing high-performance liquid chromatography (dHPLC) and DNA sequencing. A total of 709 pts (37% female) were included in this analysis (isolated GHD [IGHD] n=377; multiple pituitary hormone deficiencies [MPHD] n=315); 17 pts without mutations could not be assigned to either group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>IGHD (N=377)</th>
<th>MPHD (N=315)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH1</td>
<td>24 (6.4%)</td>
<td>3 (1.0%)</td>
</tr>
<tr>
<td>GHRHR</td>
<td>15 (4.0%)</td>
<td>NT</td>
</tr>
<tr>
<td>GLI2</td>
<td>NT</td>
<td>3 (1.0%)</td>
</tr>
<tr>
<td>HESX1</td>
<td>NT</td>
<td>5 (1.6%)</td>
</tr>
<tr>
<td>LHX3</td>
<td>NT</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>POU1F1</td>
<td>NT</td>
<td>2 (0.6%)</td>
</tr>
</tbody>
</table>
NT=not tested; no mutations were found in LHX4, SHH or SOX3. Pts with mutations differed from those without in the following ways (median [Q1/Q3] pval): younger age (5.2[1.9/8.2] vs. 6.3[3.1/10.8] yr, 0.022); lower bone age SDS (-3.6[-5.1/-2.2] vs. -2.8[-4.2/-1.6], 0.010); lower height SDS–target height SDS [-3.8,-5.3/-2.4] vs. -2.5[-3.6/-1.5], <0.001; lower peak stimulated GH (1.3[0.5/4.2] vs. 3.3[1.1/6.7] ng/mL, <0.001) indicating more severe GHD. There was no significant gender difference. Notably, 2 pts with IGHD had PROP1 mutations and 3 pts with GH1 mutations developed MPHD (1 histiocytosis with ADH deficiency, 2 TSH deficiency). In pts with MPHD there was a regional clustering of PROP1 mutations in Eastern Europe (Czechia, Lithuania, Russia): 29/78 (37.2%); in comparison Western Europe: 10/139 (7.2%); USA: 2/52 (3.8%); Asia-Pacific: 1/43 (2.3%); Japan: 0/3. We conclude that mutations in the investigated candidate genes occur at frequencies that justify genetic testing as standard medical care, taking into account the clinical implications associated with such mutations.

**FC4-96 - Further evidence that pituitary stalk and/or pituitary gland defects may be caused by mutations in holoprosencephaly (HPE) genes**

Christine Tatsi; Antony Voutetakis; Helen Valavani; Maria Alexandra Magiakou; Amalia Sertedaki; Catherine Dacou-Voutetakis

**Athens University, Medical School, First Department of Pediatrics, Athens, Greece**

**Background:** Previous data from our laboratory have shown one deletion and one point mutation in the TGIF gene in 2 patients with pituitary stalk interruption syndrome (PSIS). These two patients also presented single central incisor (SCI) without other characteristics of HPE.

**Objective and hypotheses:** Based on these findings, we hypothesized that PSIS and/or pituitary hypoplasia may constitute a form of HPE. Consequently we looked for mutations in two other HPE related genes: the Sonic Hedgehog (SHH) and SIX3, in patients with PSIS and/or pituitary hypoplasia. The SHH gene shows diencephalic expression and coordinates the anteroposterior and dorsoventral patterning in the hypothalamus and in the diencephalon. The SIX3 gene is also involved in the patterning of the vertebrate forebrain and it has been implicated in holoprosencephaly.

**Methods:** We screened 25 and 20 patients for mutations in the SHH and the SIX3 genes respectively, by PCR amplification of their coding regions and bidirectional sequencing.

**Results:** We found a novel heterozygous G to A nucleotide transition at position 1279 of exon 3 of the SHH gene in a female patient and her father. This molecular defect results in the substitution of glycine by arginine at codon 427, G427R. Codon 427 is found at the C-terminal domain of the SHH protein, which is secreted at the extracellular space and is conserved in pan troglodytes and rattus norvegicus. No mutation has thus far been detected in the SIX3 gene.

**Conclusions:** The present data as well as the previous findings of two molecular defects of the TGIF gene, suggest that PSIS and/or pituitary hypoplasia possibly represent mild phenotypes of holoprosencephaly in certain cases.

**FC4-97 - Diffusion tensor imaging reveals specific white matter abnormalities in children with Isolated Growth Hormone Deficiency**
Emma Alice Webb1; Michelle A O'Reilly2; Jon D Clayden3; KK Seunarine3; Naomi Dale2; Alison Salt2; Chris A Clark3; Mehul T Dattani1

1Institute of Child Health, Developmental Endocrinology Research Group, London, United Kingdom; 2Institute of Child Health, Neurosciences Unit, UCL Institute of Child Health and Developmental Vision Clinic, London, United Kingdom; 3Institute of Child Health, Radiology and Physics Unit, London, United Kingdom

Many studies have attempted to clarify the exact nature of the cognitive deficit found in individuals with an abnormal growth hormone (GH) axis. However, whilst some studies have drawn similar conclusions, there remains no consensus as to whether the GH-IGF-1 axis plays a significant role in neural development. No previous studies have used diffusion tensor imaging (DTI) to address this question. Thirteen children (mean age 8.5yrs) with isolated growth hormone deficiency (IGHD) (peak GH <6.7µg/l [mean 4µg/l]) plus a pathologically low IGF-I concentration for age (mean -2 standard deviations for age and sex), and eleven children (mean age 8.5yrs) with isolated short stature (ISS) (peak GH >10µg/l [mean 14.5µg/l], normal IGF-1 measurements and normal growth rate) were studied. All children were right-handed. All underwent a comprehensive neuropsychological assessment including the Weschler Intelligence Scale for Children (Fourth edition [WISC IV]) and the Movement ABC (mABC) test. DTI sequences were acquired on a 1.5T magnetic resonance imaging scanner. Corticospinal tract tractography was performed and fractional anisotropy (FA) (a measure of white matter coherence) and mean diffusivity (MD) (a measure of structural integrity) calculated. Children with IGHD performed significantly worse on the WISC IV (full-scale p<0.004, verbal p<0.002, performance p<0.002) [results controlled for maternal educational level and socioeconomic status] and the mABC test (p<0.005), and had significantly lower MD in the left corticospinal tract (p<0.03). Left Corticospinal tract FA correlated significantly with performance on the mABC (p<0.006, r=0.55). Children with IGHD have white matter abnormalities in specific regions of the brain, in association with deficits in cognitive function and motor performance. Currently the main aim of GH treatment in children is to optimise final height and maintain bone mass, if GH also has a significant impact on neural development and cognition this may have implications for clinical practice.

FC4-98 - Rationale approach to the diagnosis of severe growth hormone deficiency in the newborn

Gerhard Binder1; Melanie Weidenkeller1; Gunnar Blumenstock2; Markus Langkamp3; Karin Weber1; Axel R. Franz4

1University-Children's Hospital, Pediatric Endocrinology, Tuebingen, Germany; 2University of Tuebingen, Department of Medical Biometry, Tuebingen, Germany; 3Mediagnost Company, Assay Development, Tuebingen, Germany; 4University-Children's Hospital, Department of Neonatology, Tuebingen, Germany

Severe congenital growth hormone deficiency (GHD) of the newborn is a rare disease, which can cause life-threatening hypoglycemias beginning in the first week of life. Reviews and consensus papers on the diagnosis of GHD repeatedly state the lack of a practical evidence-based approach to the diagnosis of GHD in the newborn. Here, we provide for the first time sound reference values and a diagnostic cut-off for the growth hormone (GH) levels
in newborns at the age between the days 3 to 5. GH was measured in the eluate from 314 filter papers of the newborn screening test performed in our University Hospital by using a highly sensitive hGH-ELISA. Reference data are compared with measurements from 9 newborns with very high likelihood of having severe GHD, and cut-offs for the diagnostic work-up are defined. In the presence of clinical evidence, the diagnosis of neonatal GHD can be confirmed during the first week of life by a single randomly taken GH level < 7µg/L with 100 % sensitivity and 98 % specificity on the basis of our assay method. GH content in newborn screening cards stored for almost three years were not different to the content found in recently used screening cards indicating high immunological stability of GH over time. Therefore, the diagnostic approach can utilize stored screening cards. In addition, we observed a clear gender dichotomy in respect to GH with healthy female newborns having significantly higher GH levels than males (mean 17.5 versus 15.4 µg/L; P=0.014, linear regression analysis). Cigarette smoking during pregnancy was associated with higher (21.6 versus 16.2 µg/L; P=0.007)), transient tachypnea of the newborn with lower GH levels (13.1 versus 16.9 µg/L; P=0.004). These findings were confirmed by multiple linear regression analysis. We provide the first rational approach to the diagnosis of severe GHD in the newborn and evidence for gender dichotomy of the neonatal GH axis.

FC4-99 - A variable degree of alterations in glucose metabolism in a pedigree with an IGF-1 receptor defect
Angelika Mohn1; M. Loredana Marcovecchio1; Jürgen Klammt2; Tommaso de Giorgis1; Roland Pfaeffle2; Francesco Chiarelli1; Wieland Kiess2
1University of Chieti, Department of Paediatrics, Chieti, Italy; 2University of Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany
Growing evidence indicates a role of insulin-like growth factor-1 (IGF-1) in influencing β-cell function and insulin sensitivity. However, little is known on potential consequences of mutations in the IGF-1 receptor (IGF-1R) on carbohydrate homeostasis. We investigated the effect of a novel IGF-1R mutation in four family members: a 18-year old boy (index case), his sister and two paternal aunts. Glucose metabolism was assessed by oral glucose tolerance test (OGTT) and indexes of insulin sensitivity (HOMA-IR and WBISI) and secretion (insulinogenic index and HOMA-β). All family members carried a novel heterozygous C to A nonsense mutation (p.Tyr387X) within exon 5. The index case underwent an OGTT at the age of 18 years showing impaired glucose tolerance (IGT) (2h glucose: 155mg/dl) associated with reduced insulin sensitivity (HOMA-IR: 3.24). A second OGTT confirmed IGT associated with fasting hyperglycaemia (116 mg/dl), in the presence of deteriorated β-cell function, as indicated by reduced HOMA-β and insulinogenic index (table). His 47-year old obese aunt, similarly presenting fasting hyperglycaemia (132mg/dl) and IGT (155 mg/dl), showed a compromised β-cell function, with low basal and post-load insulin secretion, and insulin resistance. His 50 year-old overweight aunt had IGT (151mg/dl), with reduced stimulated insulin secretion. The obese sister (age: 26yr), who had had transient IGT during pregnancy, showed normal glucose metabolism in the presence of reduced insulin sensitivity (table).
<table>
<thead>
<tr>
<th>Index case</th>
<th>Sister</th>
<th>47-year-old aunt</th>
<th>50-year-old aunt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin resistance indexes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.74</td>
<td>4.17</td>
<td>4.54</td>
</tr>
<tr>
<td>WBISI</td>
<td>3.39</td>
<td>3.29</td>
<td>2.79</td>
</tr>
<tr>
<td><strong>b-cell function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (mU/ml)</td>
<td>6</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>2h Insulin (mU/ml)</td>
<td>96</td>
<td>82</td>
<td>57</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>42</td>
<td>140</td>
<td>74</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>23</td>
<td>16</td>
<td>2.1</td>
</tr>
</tbody>
</table>

This is the first study showing an association between a novel *IGF-1R* mutation and alterations in carbohydrate metabolism. The impaired glucose homeostasis, ranging from normal glucose tolerance in the presence of insulin resistance, to IGT and fasting hyperglycaemia in association with both insulin resistance and b-cell dysfunction, likely reflects disturbance of the IGF-1 modulation on β-cell function and insulin sensitivity. The different impairment in glucose homeostasis might be possibly related to an incomplete penetrance and expressivity of the same *IGF-1R* mutation.