Ghrelin is secreted primarily by the stomach. It circulates as acyl-(AG) and desacyl-(DG) forms. AG (but not DG) stimulates appetite. Ghrelin is elevated in Prader-Willi syndrome (PWS), suggesting it may cause the characteristic hyperphagia and overweight. Short-acting somatostatin analogue octreotide suppresses ghrelin in PWS. We hypothesized that long-acting octreotide (Sandostatin LAR®, L-Oct) would decrease AG and total (TG=AG+DG) ghrelin and compulsive behaviour towards food intake in adolescents with PWS. In this randomised, double blind, cross-over trial (24 wks washout period), we included 9 PWS subjects (age 15.6 [2.8] yrs, weight Z-score +2.0 [1.0], mean [SD]). They received either L-Oct (30 mg) or saline (Placebo [Plac]) IM every 4 wks for 16 wks. AG and TG (Linco®), glucose and insulin (during an OGTT, food-related behaviour as well as HbA1c and gallbladder ultrasound were evaluated at the beginning and end of each phase. Stat: ANOVA, repeated measures. Order of treatment (Plac vs L-Oct) did not affect the results. L-Oct caused a marked decrease in both AG and TG concentrations throughout the OGTT (Fig). BMI Z-scores remained similar before (+2.0 [1.0]) and after (+2.1 [1.0], P=0.5) L-Oct therapy. Appetite (Visual Analogue Scale), food seeking and obsessive/compulsive behaviours were not affected by L-Oct. Hba1c and 2-hour glucose were not significantly affected by L-Oct despite the expected decrease (-39%, P=0.02) in insulin area under the curve caused by L-Oct. L-Oct caused asymptomatic gallstones development in 4 subjects that improved/resolved after L-Oct discontinuation. Thus: 1. Prolonged L-Oct treatment caused a marked decrease in AG (-60%) and TG (-50%) that was sustained for 4 months. The absence of clinically relevant changes in BMI/behaviour towards food despite these major L-Oct-induced changes may suggest that ghrelin is not a primary cause of overweight in PWS or that effects of L-Oct on other appetite-regulating hormones mitigate those on ghrelin concentrations; 2. L-Oct is associated with a high incidence of gallstones in PWS.

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**FC7-104 Growth Hormone Secretion and Action**

A novel mutation in GH molecule (GH-E32A) affecting the correct GH mRNA splicing presented in a pedigree with IGHD type II

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An autosomal dominant form of GHD (IGHD II) characterized by short stature can result from heterozygous splice enhancer mutations that weaken recognition of exon 3, leading to increased production of the 17.5-kDa GH isoform relative to the 22-kDa isoform. We describe here a three-generation pedigree with all affected individuals carrying a single heterozygous base transition in exon 3 (E32A) coding for GH-E32A mutation of the GH-I gene causing familial IGHD II. The proband, a girl, presented with short stature (height -3.3 SDS) at the age of six years. GHD was diagnosed on standard GH provocation test. Recombinant human GH (r-hGH)-replacement therapy was initiated and resulted in an increase in growth velocity from 4-5 (-2.2 SDS) to 10.2 (+7.3 SDS) cm/year during the first year. Thereafter, the doses were continuously adapted on a regular basis according to the effect on growth velocity and IGF-I measurements.

Detailed functional characterization of GH-E32A mutation was first assessed through the splicing studies at the protein level. The splicing of wt-GH exclusively produced 22-kDa isoform with barely detectable amounts of 17.5-kDa GH isoform, while GH-E32A led to a significant increase (55% of total GH protein) in the production of the 17.5-kDa GH isoform. AtT-20 cells co-expressing both wt-GH and GH-E32A presented a significant reduction in cell proliferation and in GH-regulated secretion after forskolin stimulation when compared to the cells co-expressing wt-GH. These results were complemented with confocal microscopy analysis, which revealed a significant reduction of GH-E32A in co-localization with secretory granules compared to wt-GH.

Our clinical and experimental data indicate that the GH-E32A mutation found within ESE affects correct GH mRNA splicing causing IGHD II. Due to the increasing production of the 17.5-kDa isoform relative to the 22-kDa isoform, GH-E32A mutant exhibited a dominant-negative effect on both secretion of wt-GH and cell proliferation.

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**FC6-103 Adipose Tissue and Obesity**

Randomized placebo-controlled trial of metformin in pediatric patients with obesity

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Introduction: Metformin is a well-established oral hypoglycemic agent in the treatment of adults with type 2 diabetes mellitus and other conditions with insulin resistance. Unfortunately, currently exposable data regarding the role of metformin in insulin resistance associated with obesity in pediatric subjects are scarce. The aim of this randomized controlled trial was to compare the metformin therapy versus placebo in pediatric patients with obesity.

Methods: Sixty-eight participants (42 girls) aged 8-18 yr were randomized by computer-generated random number allocation to receive either metformin or placebo for 6 months (34 patients in each group).

Results: Metformin therapy over placebo significantly decreased both weight (+4.8 kg, p=0.03) and BMI (+2.3 kg/m², p=0.01). The usefulness of metformin over placebo was also found for VAAT (treatment effect = 8.1 cm²; p=0.02) as well as sc abdominal adipose tissue (treatment effect 47.1 cm²; p=0.01). Metformin therapy had a beneficial treatment effect on fasting insulin (-3.4 mU/liter; p=0.03) and insulin sensitivity (-2.1 mU/liter)-1min-1, p=0.03).

Conclusions: Metformin therapy has beneficial effects on body composition, fasting insulin and insulin sensitivity.

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**FC7-105 Growth Hormone Secretion and Action**

GH1 gene Phe25Tyr mutation: clinical, biochemical and molecular study of bioinactive GH syndrome in two families

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To establish the molecular basis for idiopathic isolated GH deficiency, GH1 gene was sequenced in a population of 683 index patients with chronic growth delay. Sequence changes other than the 25 SNPs present in the control population (Esteban 2007) were detected in 81. Among 53 different sequence changes, 7 were located in exons, predicted an aminoacid change (13.2% of changes) and were present in 9 index patients (1.3% of patients); 4 of these
changes have not been described to date (Arg19Hys, Phe25Tyr, Val173Leu, Ile179Leu).

The objective was to analyse the clinical and biochemical characteristics of two families carriers of a heterozygous Phe25Tyr mutation in GH1 gene and the in vitro functional characteristics of the mutated Phe25Tyr GH protein. Two families (one patient and the parents in family 1 and two brothers in family 2) were carriers of an identical allele for the 25 SNPs plus three additional changes (two single nucleotide mutations in exon 2, one of them predicting a Phe25Tyr protein, and a single nucleotide change in intron 4). The three patients were diagnosed around the age of 9, with height <-3 SDS, diminished GH secretion and IGF-1 levels and responded adequately to GH therapy. The two exonic changes could have originated from recombination with GH2 gene. The Phe25Tyr change could affect interaction of the GH molecule with the second GHR molecule. Recombinant Phe25Tyr protein presented a 30% reduction in immunoreactivity compared with the wild-type (WT). WT GH stimulated IGF-1 expression in cultured human fetal epiphyseal chondrocytes while the mutated Phe25Tyr failed to do so. GHK and IGF1 mRNA were over-stimulated.

In conclusion, two Spanish families with growth-retarded children, responders to GH therapy, were carriers of an identical GH allele with the mutation Phe25Tyr. In these children, the Phe25Tyr mutation was established as the cause of the bioinactive GH syndrome.

**FC7-106 Growth Hormone Secretion and Action**

**Anti-pituitary antibodies: a new feature of celiac disease at diagnosis with high prevalence in females**

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Patients with celiac disease (CD) are likely to develop organ and non-organ specific autoantibodies (Ab). Although short stature and increased risk of growth hormone (GH) deficiency (GHD) have been reported in CD, an autoimmune pituitary involvement was only hypothesised in the past years. Nowadays, the assay of anti-pituitary Ab (APA), currently clearly identified to autoimmune pituitary involvement was only hypothesised in the past years. Since no data are still available in newly diagnosed CD, we evaluated APA in CD patients before gluten free diet. We enrolled 72 (24 M and 48 F; mean age 5.2±3.7 yrs, mean height 0.2±1.0 SDS, mean BMI 0.1±1.8 SDS) biopsy-proven CD patients. One patient was on L-T4 because of autoimmune hypothyroidism and 2 had type 1 diabetes mellitus. PRL, TSH, FT4, IGF1, APA, anti-TPO and anti-TG Ab (ATA) were assayed. APA were detected in 27 pts (37.5%), more frequently (p=0.039) in females (22/48) than in males (5/24). We found no difference in ATA prevalence (overall 12.3%), means of age, height, BMI, FT4, TSH, and PRL according to the presence of APA. IGF1 was lower in patients with positive APA than in those without (-0.8±2.2 vs. 0.4±2.0 SDS, p=0.034). Six of 8 patients with positive APA underwent an insulin tolerance test and showed GH peak <8 ng/ml. Our study highlights that APA are frequently positive (37.5%) in newly diagnosed CD patients and prevalently associated with female gender. 75% of the tested patients with positive APA were low-responders for GH. We can not assess the exact role of these Ab in CD, but they have already been reported to identify short children prone to develop GHD. We are following our patients to evaluate whether APA could be harmless or indicate an early stage of pituitary impairment.

**FC7-107 Growth Hormone Secretion and Action**

**Studies for mitochondrial cytopathy (Kearns-Sayre syndrome) in children with growth hormone deficiency**

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Kearns-Sayre Syndrome (KSS) is a form of mitochondrial cytopathy leading to premature death in most cases. Multiple endocrine disorders are characteristic for KSS [diabetes mellitus, hypoparathyroidism, thyroiditis, hypogonadism and short stature (SS)]. Rearrangements and mutations of mtDNA occur in all cases and are the basic cause of a disease. The goal was to check the prevalence of KSS in children with SS and accompanying GH deficiency. Specific deletions of mtDNA molecule, characteristic for KSS, were studied. The special attention has drawn the group of children with short stature under rGH therapy, but without expected catch-up. We have selected the group of 17 children with severe short stature [mean hts SDS at diagnosis>-2.81] and some additional clinical features characteristic for KSS such as bilateral or unilateral ptosis, pigmented retinopathy and hypogonadism. Some patients were treated with rGH for years, but the success of treatment was limited. The remaining patients just initiated treatment or were not treated yet. DNA was isolated from patients and the set of primers for study protocol were designed so that the mutations could cover the whole mtDNA molecule, allowing detection of every possible deletion in mtDNA. Additional, smaller than normal, amplification product was the indication of a deletion. The exact size and breakpoint of the deletion was determined by direct sequencing of PCR product. As a result of this reaction we have identified three (3) patients with novel, sporadic deletions in mtDNA. Two of them were unsuccessfully treated with rGH (1) hts SDS from -6.0 to -6.6, (2) hts SDS from -3.8 to -2.9 and (3) hts SDS -2.9 has initiated the treatment. The prevalence of mtDNA deletions in this study group was 17.6%. These results also explained the poor clinical response in ordered treatment.

**FC7-108 Growth Hormone Secretion and Action**

**Enhanced protein degradation of the GHR/JAK2 complex through the proteasomes as a cause of abnormal GH signaling in GH transduction defect (GHTD)**

**Andrea Paola Rovio Gii**; Julia Karageorgou; Panos Ziros; Efthymia Basdra; John Varakis; Athanasios Papavasiliou; Bessie Spilioti

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Introduction: We have previously described 4 GHTD children with severe growth delay, a defect in the phosphorylation of STAT3, (pSTAT3), and impaired activation of the GH pathway due to negative regulation.

**Objective:** To examine the association of the negative regulator, CIS, with the impaired activation of the GH pathway and the possible involvement of the protein degradation mechanism in GHTD.

**Methods:** In fibroblasts from gingival biopsies of 2 controls and 2 GHTD patients with increased CIS expression, we performed activation and expression kinetics of Jak2, STAT3, STAT5 and CIS after induction by 200 ng/ml GH with or without 50ng/ml proteasome inhibitor MG132 in a time dependent manner using Western Immunoblotting.

**Results:** 1) In the GHTD fibroblasts, after hGH induction the expression kinetics of CIS protein showed increased expression of the total CIS protein including its ubiquitinated isoform in comparison with the controls at all studied time-points. The kinetics of Jak2 and STAT5 under the same conditions, showed retarded activation (max. phosphorylation at 30 min) in comparison to the controls (max. phosphorylation at 5-10 min). 2) After induction with hGH and MG132, decreased expression levels of total CIS were found in the GHTD fibroblasts, the ubiquitinated isoform was not detected at any time-point and physiological activation of all the components were found.
Homozgyosity of the d3-growth hormone receptor polymorphism is a major determinant of the total effect of rhGH on growth in girls with Turner syndrome

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The d3-GHR polymorphism was linked to a better first-year-growth response to GH in girls with Turner syndrome. Here, for the first time, we studied the long-term effect of GH therapy in Turner syndrome in relation to d3-GHR. We included all girls with Turner syndrome (n=48) who had been treated with GH during the last 18 years at our hospital and had reached final height. The mean GH dose was 38 µg/kg*d. The GH polymorphism was genotyped using a PCR multiplex assay which discriminates the fl (full length) allele from the d3 (minus exon 3) allele, and with a PCR assay specific for the fl allele. The fl/fl, d3/fl and d3/d3 genotypes were present in 24, 17 and 7 girls, respectively. At start mean height was -3.29 SDS, mean age was 9.1 yr. Height, age, target height, BMI and dose/duration of GH therapy were not different between the three groups. Mean weight SDS at start was lower in the d3/d3 group than in the fl/fl group (-1.15 versus -2.70) (P<0.02). Total gain in height was significantly higher in the d3/d3 group than in the fl/fl group of +1.92 SDS (0.25) than in the fl/fl group with +1.09 SDS (0.86) and in the d3/fl group with +1.13 SDS (0.82) (P<0.02). This outcome was based on a higher increase in height velocity during the first year of therapy (P<0.01) and especially on a longer duration of enhanced growth velocity in the d3/d3 group (P=0.03). The BMI of the d3/d3 group was remarkably low at the end of therapy (18.0 kg/m2) and different from the BMI of the others (22.9 and 23.4 kg/m2) (P<0.006).

Our data suggest that homozygosity for the d3-GHR polymorphism is associated with a unique long-time effectiveness of GH therapy on growth and a weight regulation towards lower BMIs in Turner syndrome.

IGFALS mutations define a new form of impaired postnatal growth associated to low circulating IGF-I, IGFBP-3, and acid-labile subunit (ALS) levels

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Up to 90% of circulating IGF-I and IGF-II are carried bound to either IGFBP-3 or IGFBP-5, and ALS in the form of tertiary complexes that extend their circulating half-life by preventing a quick plasma clearance. ALS synthesis is postnatally stimulated by growth hormone (GH), as are both IGF-I and IGFBP-3. Two different mutations in the ALS encoding gene (IGFALS, 16p13.3) have been recently reported in two patients with impaired postnatal growth presenting with very low IGF-I and IGFBP-3 levels in the absence of GH deficiency (GHD), thus supporting a role for ALS in the regulation of IGFs bioavailability during postnatal growth. We present the case of three unrelated male probands with idiopathic short stature whose main clinical characteristics at diagnosis are summarized in the enclosed table.

<table>
<thead>
<tr>
<th>Case Nr.</th>
<th>Chron. age (years)</th>
<th>Bone age (years)</th>
<th>Height (SDS)</th>
<th>IGF-I (ng/mL)</th>
<th>IGFBP-3 ALS (µg/mL)</th>
<th>Follow up (years)</th>
<th>Puberty onset (years)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4</td>
<td>3</td>
<td>-2.2</td>
<td>24±1</td>
<td>&lt;0.31</td>
<td>11</td>
<td>13</td>
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<tr>
<td>2</td>
<td>4</td>
<td>3</td>
<td>-3.8</td>
<td>30±1</td>
<td>0.41</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>13</td>
<td>-3.0</td>
<td>32±1</td>
<td>&lt;0.30</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Mutation screening of GHR, IGFBP3 and IGFALS genes was performed by dHPLC and DNA sequencing. Although GH stimulation tests were suggestive of partial GH insensitivity (GHI) in all cases, no mutations were found in GHR. IGFALS analysis revealed two novel homozygous mutations: a missense mutation at a conserved residue: N276S (probands 1 & 2), and a nonsense mutation: Q320X (proband 3). None of the mutations were detected in 100 healthy controls. No changes were found in IGFBP3.

The recessive N276S and Q320X IGFALS mutations dramatically decrease circulating ALS levels. As a consequence, serum IGF-I and IGFBP-3 are markedly low, indicating the lack of ternary complexes. IGFALS mutations should be considered as a possible cause of postnatal growth deficits in patients presenting with low IGF-I and IGFBP-3 levels in the absence of GHD and GH. Determination of serum ALS levels can be helpful in the diagnosis of patients with idiopathic IGF-I deficiency.
variants in the TCF7L2-gene have been associated with increased risk for type 2 diabetes in adults. To evaluate whether these risk variants confer a higher risk for obesity and early impairment of glucose metabolism in children, we genotyped five risk variants of the TCF7L2-gene in a representative cohort of 1029 Caucasian children and in an independent cohort of 283 obese children.

Applying a case control design, we observed a significantly lower prevalence of the rs11196265 and rs7893340 risk alleles in the obese compared to lean children (0.40 vs. 0.45, P=0.02). There was, however, no statistical significant difference between the genotypes and quantitative traits of obesity in neither the schoolchildren nor obesity cohort. Along with the marked elevation in BMI in obese children, they were significantly taller than lean children. This increase in height was independent associated with risk variants of the TCF7L2-gene, while in the normal representative cohort height appeared to be decreased in carriers of the minor alleles. This increase in height may be phenominal for the constitutional (growth) acceleration frequently seen in obese children that has been discussed as potentially accelerating diabetes manifestation along with autoimmune and insulin resistance in the accelerator hypothesis.

In the obese cohort, three risk alleles (rs7901695, rs7903146, rs1225572) were significantly associated with higher fasting and stimulated blood glucose levels independent of sex, age, pubertal stage, height, and BMI. Quantitative traits of insulin secretion appeared with a similar tendency but were not statistically significant.

Hence, our data indicate for the first time that TCF7L2-gene variants confer an increased risk for early impairment of glucose metabolism in obese children, which is consistent with adult studies identifying TCF7L2-gene variants to be decreased in carriers of the minor alleles. This increase in height to be decreased in carriers of the minor alleles. This increase in height may be phenominal for the constitutional (growth) acceleration frequently seen in obese children that has been discussed as potentially accelerating diabetes manifestation along with autoimmune and insulin resistance in the accelerator hypothesis.

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Hypochondroplasia and acanthosis nigricans.

New syndrome caused by the novel K650T mutation in the FGFR3 gene?
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Hypochondroplasia is a skeletal dysplasia characterized by short stature with disproportionately short arms and legs. Up to now, the FGFR3 is the only gene known to be associated with the disease and encodes for the FGFR3 tyrosine kinase receptor. Nevertheless, genetic heterogeneity is suspected. The N540K mutation appears in 72% of patients, and some rare mutations affecting codons 538, 540 and 650 accounts for a small number of cases.

Patient and family: The propositus is a 14 years old male with radiological finding of hypochondroplasia who showed acanthosis nigricans (AN). Adult members of the family with AN had been dermatologically studied and diagnosed as benign familial AN.

Genetic analysis. The FGFR3 exons 9, 10, 13, and 15 were PCR amplified and directly sequenced.

Results: The patient was heterozygous for the novel K650T (AAG→ACT) mutation. All the family members with AN and hypochondroplasia phenotype (height SDS -4.5 to -2) showed the same mutation whilst the members without the mutation had normal stature (SDS -0.26) and did not presented AN.

Discussion: The Lys-650 FGFR3 is located within a critical region of the tyrosine kinase domain of the receptor and different aminoacids substitutions at this site are related with phenotypes of diverse severity depending of the degree of the receptor activation: K650N and K650Q mild HC without AN, K650M SADDAN dysplasia (severe achondroplasia with AN) and K650E tanatophoric dysplasia. The new mutation seems to activate the FGFR3 receptor more than K650N and K650Q and less than K650M and K650E and is able to produce AN in addition to the hypochondroplasia.

Conclusion: A possible new syndrome which consists of hypochondroplasia plus acanthosis nigricans due to the novel K650T mutation in the FGFR3 gene is presented.

Intrauterine growth retardation (IUGR) has been linked to the development of type 2 diabetes in later life. The mechanisms underlying this phenomenon are unknown. Circulating free fatty acids (FFAs) represent an important nutrient and oxidative fuel for skeletal muscle. Growing evidence indicates that elevated plasma FFAs induce insulin resistance through inhibition of glucose transport activity. Circulating FFAs are converted into long-chain fatty acid CoA (LCFA-CoA) by the enzyme acyl-CoA synthase (ACS), thereafter the enzyme carnitine palmitoyltransferase-1 (CPT-1) regulates LCFA-CoA entry into mitochondria, where undergo beta-oxidation. Cellular oxidation of LCFA-CoA is regulated by the levels of malonyl-CoA, a potent inhibitor of CPT-1 activity. The enzyme malonyl-CoA decarboxylase (MCD) prevents the accumulation of LCFA-CoAs, by decreasing malonyl-CoA. We have recently described changes in the hypothalamic lipid sensing genes in IUGR rats at birth. The objective of this study was to investigate the effects of uterine artery ligation, a procedure which mimics uteroplacental insufficiency, on the skeletal muscle expression of key enzymes of LCFA metabolism. Bilateral uterine artery ligation was performed on day 19 of gestation in Sprague-Dawley pregnant rats. Sham-operated pregnant rats served as controls. Pregnant rats were allowed to deliver spontaneously. Muscle of the posterior limb was dissected at postnatal day 1 and processed by real-time PCR to analyze the expression of ACS, AMP-activated protein kinase (AMPK), CPT-1 beta isoform, and MCD. 14 sham and 8 IUGR pups were tested. Nonesterified fatty acids (NEFA) serum levels resulted significantly higher in IUGR pups (p<0.0001). A significant down regulation of ACS expression was observed in IUGR newborns (p<0.05). No changes in the expression of AMPK, CPT-1 and MCD were found. Our data suggest that an abnormal intrauterine milieu may alter IUGR skeletal muscle mitochondrial lipid metabolism.

Ghrelin is a peptide predominantly produced by the stomach, that exerts a lot of central and peripheral activities, among which endocrine actions and the regulation of energy balance. Data in newborns demonstrated that ghrelin is present starting from fetal life, but the majority of studies measured total ghrelin levels. In fact, this peptide circulates in blood in two distinct forms: acylated and des-acylated, that seems to have different roles in hormonal and metabolic functions. In order to understand the biological implications of these two forms, we measured plasma acylated (AG) and des-acylated ghrelin (DG) (ELISA kits, DRG Instruments GmbH, Marburg, Germany), leptin, adiponectin, insulin levels in 82 neonates (MF: 40/42) (72 AGA, 6 LGAs, 4 SGA) in cord blood. Acylated ghrelin levels were (median; 25th-75th centile)
FC9-119 Perinatal Endocrinology and Outcome
Antenatal glucocorticoid treatment for preterm birth is not associated with long-term metabolic risks at 19 years of age

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A single course of maternal glucocorticoid treatment is effective in reducing neonatal mortality after preterm birth. However, in animals, maternal glucocorticoid treatment is associated with lifelong hyperglycaemia and hypertension, and impaired nephrogenesis in offspring. Findings from studies in humans on this topic are highly contradictory due to a number of methodologic flaws and renal function after glucocorticoid exposure has never been assessed. Therefore, we assessed in a relatively large population whether antenatal glucocorticoid treatment for preterm birth is associated with long-term metabolic risks, including reduced renal function, at 19 years of age. We studied the effect of a single course of maternal betamethasone (24 mg) on body composition, insulin resistance, the serum lipid profile, blood pressure and estimated renal function in 19-year-olds born <32 gestational weeks and followed prospectively from birth participating in the Dutch Project On Preterm and Small-for-gestational-age infants (POPS) cohort. Neonatal survival in the betamethasone-exposed group (n=171) was 82% compared to 70% among unexposed (n=818) (log rank p =0.0016). We did not find any long-term adverse effects of antenatal betamethasone in participants at 19 years of age (n=365), with the exception of an effect on glomerular filtration rate (GFR) in women. In 19-year-old female survivors, GFR was lower after betamethasone: -7.4 (95% CI: -13.3 to -1.5) mL/min/1.73m2. We conclude that the reduction in neonatal mortality associated with a single course of maternal betamethasone is not accompanied by long-term metabolic risks in survivors of preterm birth. The only adverse effect found was lower GFR in women. Although this difference was not clinically relevant at 19 years, it might predict an increased risk of chronic renal failure in prematurely born women who were exposed antenatally to betamethasone.

FC9-120 Perinatal Endocrinology and Outcome
The ER22/23EK variant in the glucocorticoid receptor gene protects against postnatal growth failure and insulin resistance after preterm birth

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Preterm birth is associated with postnatal growth failure, abdominal fat accumulation, insulin resistance and hypertension, resembling increased glucocorticoid bioactivity. Indeed, there is some evidence from a small study that circulating cortisol levels are higher in adults born prematurely. It is unknown whether genetic variants in the glucocorticoid receptor gene could modulate the above phenotype. Therefore, we tested the effects of the ER22/23EK and N363S variants, associated with decreased and increased sensitivity to cortisol respectively, on linear growth and the adult metabolic profile in a birth cohort of men and women born <32 gestational weeks and followed prospectively from birth until 19 years of age. 249 Survivors of the Dutch Project On Preterm and Small-for-gestational-age infants (POPS) cohort underwent anthropometric assessment and blood pressure measurement, and venous blood was drawn for genotyping and determination of fasting glucose, insulin and cholesterol concentrations. The ER22/23EK variant (n =24) was associated with lower fasting insulin levels (mean difference after log-transformation: -0.09 [95% CI: -0.16; -0.01] mU/L) and HOMA-IR (mean difference after log-transformation: -0.09 [95% CI: -0.16; -0.01]), as well as with taller stature departing from the age of 1 year onwards. ER22/23EK carriers showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean, whereas stature in non-carriers was on average 0.5 SD below this mean. In contrast, the N363S variant (n =15) was not associated with any of the outcomes. We conclude that carriers of the ER22/23EK variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.
After age, sex, BMI and HOMA-IR, insulinogenic index was significantly lower in SGAgest compared to SGApop (p=0.001) and AGA (p=0.003) groups. In the SGAgest group, HOMA-IR was significantly shifted to the highest tertiles, while insulinogenic index – to the lowest tertiles of AGA distribution; SGApop subjects had both HOMA-IR and insulinogenic index predominantly in the highest tertiles.

In conclusion, subjects born AGA according to population references but failing to reach their genetic potential of intrauterine growth, according to individualized birth weight standards are at increased risk of metabolic disturbances, impaired insulin secretion and type 2 diabetes later in life.

**FC10-122 Sexual Differentiation and Tests**

**Genome-wide transcriptome analysis of peripheral blood mononuclear cells (PBMC)** reveals male, female and ambiguous gene transcription signatures

Paul-Martin Holterhus1; Jan-Hendrik Bebermeier1; Mahesh Appari1; Ralf Werner2; Annette Richter-Unruh2; Felix Reipe2; Olaf Hort2
1University Hospital Schleswig-Holstein, Kiel, Department of Pediatrics, Kiel, Germany; 2University Hospital Schleswig-Holstein, Lübeck, Department of Pediatrics, Lübeck, Germany; 3Endokrinologikum Wattenscheid, Private Practice of Pediatric Endocrinology, Wattenscheid, Germany

Sex-chromosomal gene transcription and androgen-programming are two mechanisms underlying sex dimorphic gene expression. PBMC are a potential model for sex-dimorphic gene expression as they express the androgen receptor (AR) — the key molecule in genital dimorphism. We compared gene expression patterns of PBMC of 10 normal XY males, 9 normal XX females and 17 XY- and XX-DSD patients. Total RNA was obtained from the normal males and females, labeled and hybridized against a common reference RNA to cDNA-microarrays (32,000 genes). Significance of differential expression was calculated comparing XY males and XX females by SAM (Significance Analysis of Microarrays). Identified genes served to classify the 17 DSD-individuals (CAH (n=5), SRD5A2-defect (n=2), Leydig cell hypoplasia (n=2), 17βHSDIII-defect (n=1), P450SCC-defect (n=1), Leydig cell hypoplasia (n=2), 17βHSDIII-defect (n=1), PANTHER (Protein ANalysis THrough Evolutionary Relationships) identified over-representation of programs related to cell structure, of genes like IGFR in phenotypic females and FZD6 in phenotypic males. Excessive differences in expression were caused by sex-chromosomal genes expression patterns resulting in male, female or even ambiguous genitalia. In contrast, after removing sex-chromosomal genes, clustering ordered normal- and DSD individuals mostly according to genital phenotype but independent of karyotype. This was due to over-expression of genes like IGFIR in phenotypic females and FZD6 in phenotypic males. PANTHER identified over-representation of programs related to cell structure, adhesion and cell cycle control (p=0.01-0.0001). In conclusion, PBMC contain both sex-chromosome-related and genital phenotype-related gene expression patterns resulting in male, female or even ambiguous gene transcription signatures. We assume that in addition to sex-chromosomes, AR-pathway must have influenced long term gene expression in PBMC, presumably by early actions at stem cell levels.

**FC10-123 Sexual Differentiation and Tests**

**Role of IGFs in human prepubertal testis:** differentiation of steroidogenic cells

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The role of IGFs has been demonstrated mainly in rodents in testicular growth and development, control of Leydig cell (LC) number and in the onset of steroidogenesis. In human immature testis the information available is scarce. In this study, the role of the GH/IGF system in post natal testicular activation was analyzed. Testes were collected from necropesies, following Hospital rules. For testicular immunochemistry, subjects of 3 age groups (Gr) were studied: Gr1 (n=11), neonatal (1- to 21-day-old newborns), Gr2 (n=13), postnatal testicular activation (1- to 7-month-old infants) and Gr3 (n=10), early puberty (12 to 16-month-old children). IGF1, IGF2, type 1 IGF receptor (IGFR), insulin receptor (IR) and GHR immunoperoxidase was analyzed. IGF1 was barely detectable in interstitial cells (IC) and LC in all age groups. Strong immunostaining of IGF2 was detected in LC of Gr1 and Gr2. Moderate staining of IGFR was observed. Strong staining of IR was found in LC of Gr2 and moderate staining in LC of the 3 Grs. Moderate staining of GHR was observed in LC of Gr2. In 6-day somatic testicular cell cultures of the 3 Grs, and in the absence of ICG or LH, IGF1 stimulated testosterone secretion in pmol/d . million cells (mean ±SEM 400 ± 58.9 % of basal condition, n=23, p=0.011, t test). P546sec expression (% positive cells) was also stimulated under IGF1 (1440 ± 405 % of basal, n=4, p=0.049). IGF1 stimulated cell proliferation index (203 ± 11.0 % of basal, n=4, p=0.007) and inhibited cell apoptotic index (43.8 ± 4.66 % of basal, n=15, p=0.021). These results provide the first evidence that the GH-IGFs system, mainly IGF2 via IR, might be one of the factors involved in the induction of infantile LC differentiation. GH might act only in Gr2. In vitro studies showed that IGFS regulate immature cell proliferation and apoptosis, as well as steroidogenesis. We propose that during the testicular activation of infancy, the GH-IGFs axis is involved differentiating immature LC pool and it prepares the infant testes for optimal response to stimulation by LH and testosterone secretion.

**FC10-124 Sexual Differentiation and Tests**

**Mutations in a novel exon of the LH receptor gene are associated with Leydig cell hypoplasia**

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Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals. 

Inactivating mutations of the human luteinizing hormone receptor (LHR) lead to decreased response of Leydig cells to LH. We have studied a family with a 46,XY girl suspected to have LCH and two 46,XY sisters with primary amenorrhoe.

At birth, the phenotypically girl (unrelated parents) presented with labial synchiae and palpable gonads. A karyotype 46,XY was found. Postnatal testosterone levels were low. The girl was gonadectomised. Histology showed fibrotic testis tissue. The obese Turkish sisters of consanguine parents were referred to departments of Gynecology because of primary amenorrhoe. Both presented with a lack of breast development and a blind ending vagina. Karyotyping revealed 46,XY. A second heterozygous mutation 559G was found in exon 6a, which is located in intron 6 of the LHR gene. In the two affected sisters we identified a homozygous mutation A557C in exon 6a. Exon 6a is a novel, primate-specific bona fide exon, within the LHCGR gene. It displays composite characteristics of an internal/terminal exon and possesses stop codons triggering nonsense-mediated LHCGR mRNA decay (NMD). Transcripts including exon 6a result in an intracellular, truncated LHCGR protein of 209 amino acids. Functional studies of the revealed a dramatic increase in the expression of the mutated internal exon 6a, thereby preventing the transcription of the normal LHR transcript. IGF1, IGF2, type 1 IGFR receptor (IGFR), insulin receptor (IR) and GHR immunoperoxidase was analyzed. IGF1 was barely detectable in interstitial cells (IC) and LC in all age groups. Strong immunostaining of IGF2 was detected in LC of Gr1 and Gr2. Moderate staining of IGFR was observed. Strong staining of IR was found in LC of Gr2 and moderate staining in LC of the 3 Grs. Moderate staining of GHR was observed in LC of Gr2. In 6-day somatic testicular cell cultures of the 3 Grs, and in the absence of ICG or LH, IGF1 stimulated testosterone secretion in pmol/d . million cells (mean ±SEM 400 ± 58.9 % of basal condition, n=23, p=0.011, t test). P546sec expression (% positive cells) was also stimulated under IGF1 (1440 ± 405 % of basal, n=4, p=0.049). IGF1 stimulated cell proliferation index (203 ± 11.0 % of basal, n=4, p=0.007) and inhibited cell apoptotic index (43.8 ± 4.66 % of basal, n=15, p=0.021). These results provide the first evidence that the GH-IGFs system, mainly IGF2 via IR, might be one of the factors involved in the induction of infantile LC differentiation. GH might act only in Gr2. In vitro studies showed that IGFS regulate immature cell proliferation and apoptosis, as well as steroidogenesis. We propose that during the testicular activation of infancy, the GH-IGFs axis is involved differentiating immature LC pool and it prepares the infant testes for optimal response to stimulation by LH and testosterone secretion.
**FC10-125 Sexual Differentiation and Testis**

**Involvement of a surface C-terminal ‘tail’ region of the androgen receptor in ligand binding may involve interaction with a novel coactivator**

Harriet Miles1; Trevor Bunch1; Raif Werner1; John Davies1; John Schwabe1; Ieuan Hughes2

1University of Cambridge, Paediatrics, Cambridge, United Kingdom; 2University of Cambridge, Department of Paediatrics, Addenbrookes Hospital, Hills Road, United Kingdom; UK-SH Campus Luebeck, Paediatrics and Adolescent Medicine, Luebeck, Germany; University of Cambridge, Paediatrics, Cambridge, United Kingdom.

**Introduction:** The extreme C-terminal 13 residues end or ‘tail’ of the androgen receptor (AR) shares a sub-family of nuclear receptors a surface location which is distant from the ligand binding hydrophobic pocket. In identifying a novel His917Arg (AR is 919 residues) substitution causing complete androgen insensitivity, we postulate a critical function of the tail for transcription not previously known in order for such a severe phenotype to occur.

**Aims:** Analyse the function of His917Arg following site directed mutagenesis. Based on structural modelling, analyse 4 adjacent residues through artificial mutation to alanine to understand the surface region of the AR.

**Methods:** Mutations His917Arg, His917Ala, Tyr915Ala, Lys861Ala, Gln858Ala and Tyr857Ala were created by site-directed mutagenesis and introduced into pSV AR vector for ligand binding and transactivation reporter gene assays in COS cells. Dissociation kinetic studies were undertaken using mibolerone. A two-hybrid assay was used to study N/C-terminal interaction.

**Results:** His917Arg, His917Ala and Tyr915Ala greatly reduced reporter gene activation with the remaining artificial mutants having WT activity. A 2-fold diminution in ligand binding and a slight increase in dissociation rate (½) His917Arg 101 min vs 184 min for WT) was insufficient to explain the AR dysfunction. Furthermore, there was only 30% reduction in N/C interaction. Also, the loss of function is not explained by any change side-chain size.

**Conclusions:** Functional analysis of both a novel, natural AR mutant that completely impairs androgen action and non-natural mutants predicted to be important from structural modelling does not fully explain the role of the AR ‘tail’ in ligand binding. Its surface location and distance from the ligand binding pocket suggests the mechanism may relate to interaction with a novel AR-specific coactivator.

**FC10-126 Sexual Differentiation and Testis**

**Sertoli cell function in boys with central precocious puberty (CPP)**

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Although rare in boys, CPP is an interesting model to evaluate the regulation of Sertoli cells. To date, only one study has partially characterized Sertoli cell function in CPP (Rey et al. 1993).

To further assess Sertoli cell function, five boys (4.3 ± 1.2 yr) with CPP were included in this retrospective, observational study. Measurements of LH, FSH, inhibin B [InhB], AMH and testosterone [T] were performed before, during, and after monthly GnRH treatment discontinuation (tripotrenin acetate 110-190 mcg/kg). Pre-treatment tests volume (TV) was 6.6 ± 1.0 ml, and LH 3.8 ± 1.4 IU/l, FSH 1.5 ± 0.4 IU/l, T 357 ± 59 ng/dl. Sertoli cell function was assessed by measuring serum InhB and AMH. Pre-treatment InhB was elevated (396 ± 57 pg/ml) and AMH low (87 ± 27 pmol/l) for chronological age. During treatment, LH, FSH and T returned to prepubertal values. TV persisted moderately increased (4.5 ± 0.8 ml), and InhB decreased to the upper normal prepubertal range (201 ± 37 pg/ml). AMH increased and remained at prepubertal levels (618 ± 78 pmol/l) throughout treatment. After treatment discontinuation, LH, FSH, T and InhB increased again to pubertal levels while AMH decreased, as expected.

In summary, like in normal puberty, CPP induces pubertal Sertoli cell maturation (increase in InhB and decrease in AMH). GnRH treatment curtails FSH, LH and T secretion, resulting in AMH normalization. However, InhB remains in the upper normal range, in correlation with the persistence of moderately elevated TV, probably reflecting an increased mass of Sertoli cells that is not restored to prepubertal values. In conclusion, this clinical model supports the hypothesis that peripheral InhB levels represent the result of two pools, one regulated by LH/FSH and the second by the mass effect of the prepubertal Sertoli cell population.

**FC10-127 Sexual Differentiation and Testis**

**Evaluation of the common marmoset as a model for investigating restoration of fertility in survivors of childhood cancer**

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1MRC Queens Medical Research Institute, Human Reproductive Sciences Unit, Edinburgh, Edinburgh, United Kingdom; 2Edinburgh Royal Hospital for Sick Children, Paediatric Oncology, Edinburgh, United Kingdom; 3Edinburgh Royal Hospital for Sick Children, Paediatric Endocrinology, Edinburgh, United Kingdom.

Infertility is a recognised late effect of treatment for childhood malignancy. Potential therapies include recovery of tissue/germ cells (GC) for transplantation and hormonal suppression of GC activity during treatment. Evaluation in a relevant animal model is required to test these strategies. We have previously compared expression of GC markers in human fetal testicular germ stem cells and cells committed to differentiation. We have also demonstrated that the Common marmoset is strikingly similar to the human with regard to organisation of spermatogenesis and phases of testicular development.

We investigated whether the testes of the Common marmoset contain different populations of GCs (stem/differentiating/proliferating) that parallel those in man and evaluated the effects of hormonal manipulation in an immature marmoset model on GC proliferation and/or maturation.

We used single/double immunohistochemistry on testis sections from newborn through to adult marmosets and compared cell populations in immature controls with those treated with GnRH antagonist. Oct4-positive GCs (putative stem cell spermatagonia) were found in clusters within the tubules of newborn animals; these cells were also positive for AP2γ. In older animals expression of Oct4 declined and the GCs were immunopositive for MAGE and VASA with co-localisation during transition. These results closely parallel the pattern of expression seen in human fetal testes. In contrast c-KIT was expressed in two phases, namely pre-pubertal and adulthood. Proliferation (Ki67 positive) of c-KIT positive cells was more frequent in the adult animals. GnRH antagonist treatment did not alter the pattern of GC marker expression or proliferation when compared to controls. These results have demonstrated that GC subpopulations in the immature marmoset parallel those in man. Functional maturation of GCs and cell proliferation were unaffected by hormonal suppression with GnRH antagonist.

Use of this model should aid in development of novel strategies to preserve fertility.
The PROP-1 gene is one of the several genes involved in pituitary organogenesis. Its inactivating mutations cause combined pituitary hormone deficiencies (CPHD).

The aim of this study was a long-term retrospective analysis of pituitary gland images and histological findings in resected pituitary tumors in patients with the PROP-1 gene mutation. In 63 CPHD patients aged 3-18 years, the radiological evaluation and the PROP-1 gene analysis were performed. Depending on the degree of abnormality in MRI or CT scan, the patients were operated or followed up by repeated MRI examinations. Tissue samples retrieved by the neurosurgeon, routinely stained with hematoxylin-eosin were reexamined. The PROP-1 gene mutation was found in 32 patients (17 girls, 15 boys). In 12 patients various degrees of pituitary enlargement were observed. In four patients, the size of the pituitary gland normalized at follow-up, whereas the pituitary in three patients waxed.

The pituitary gland enlargement in an 8-year-old girl was presumed to occur due to the coexistence of the sellar enlargement with its bottom destruction as seen on the radiograph and tumor absence on CT scan. In another two patients, pituitary tumors were removed - histology revealed epithelial cells, partially oxyphilic, forming tube-like or gland-like structures, surrounded by eosinophilic colloid; the findings corresponded to the intermediate lobe of the pituitary gland. Within 11 and 13-year follow-up, respectively, in these patients no tumor recurrence was observed. In patients with the PROP-1 gene mutation, the pituitary gland may be small in size, normal or enlarged, and it shows a tendency decreasing in subsequent years, with the exception of patients with pituitary tumors. We postulate that pituitary tumors in such patients originate and develop in the intermediate lobe. These tumors are slowly progressing, without any neurological impairment, or regress spontaneously and do not relapse after partial resection.
Growth hormone (GH) influences muscle function in short children born small for gestational age (SGA)

David D. Marta; Roland Schweizer; Ulrike Keim; Michael B. Ranke
University Children’s Hospital Tuebingen, Paediatric Endocrinology Section, Tuebingen, Germany

Objective: The anabolic effect of growth hormone involves an increase in muscle mass in GHD adults and children. In short children born SGA less is known about muscle changes under GH, e.g. whether the increase in muscle mass correlates with an increase in muscle function.

Aim: To investigate muscle function before and during GH treatment in short children born SGA.

Patients and methods: We studied 31 prepubertal short children born SGA (17 female). Age at start of GH was 6.0 [yrs]; height [SDS] at GH start, — 3.01; GH peak, 10.9 [µg/L]; median GH dose, 54 [µg/kg/d]. Time points of peak jump force (PFJ) [N] and peak jump power (PJP) [W] assessment using the Leonardo Jumping Platform (Novotec GmbH, Pforzheim, Germany) were at GH start, 12 months and, in a subgroup of 13 patients, at 24 months on GH treatment. For the calculation of age- and height-dependent SDS we applied the references published by Fricke et al. 2006.

<table>
<thead>
<tr>
<th>Time with GH</th>
<th>start</th>
<th>12 Months</th>
<th>24 Months</th>
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<tbody>
<tr>
<td>RIF [N]</td>
<td>350</td>
<td>430*</td>
<td>560*</td>
</tr>
<tr>
<td>RIF [SDS]CA</td>
<td>-0.79</td>
<td>-1.02*</td>
<td>-0.81</td>
</tr>
<tr>
<td>RIF [SDS]Height</td>
<td>-0.93</td>
<td>-0.90</td>
<td>-0.42</td>
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<tr>
<td>RIF [W]</td>
<td>330</td>
<td>537*</td>
<td>576*</td>
</tr>
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<td>RIF [SDS]Height</td>
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<td>-0.90*</td>
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Results: see table (*significantly different in comparison to values at GH start; CA = chronological age dependent SDS; Height = height dependent SDS).

At GH start, PJP and PFJ were low. PIF and PJP increased significantly during 12 and 24 months of GH treatment but not PIF [SDS]CA and Height. Interestingly, after 12 months of GH treatment, PIF [SDS]CA decreased significantly.

Conclusions: Our findings suggest that the increase in muscle mass during the first 2 years of GH treatment does not correlate with a concomitant increase in muscle force but with an increase in peak power.

ALTU-238 is being developed as a subcutaneously (SC) administered, once-per-week formulation of human growth hormone (hGH) that employs Altus’ proprietary protein crystallization technology. ALTU-238 was tested in a Phase I study in healthy adults and a Phase II study in growth hormone deficient (GHD) adults.

The objective was to compare the PK/PD dose-response between healthy and GHD adults. The hypothesis is that the PK/PD dose-response in healthy adults can predict the clinically relevant dose range for GHD adults. Healthy adults received single SC injections of ALTU-238 at 2.8 mg, 8.4 mg, 16.8 mg or 24.5 mg hGH (n = 6 per group). GHD adults received three weekly SC injections of 3.6 mg (n = 4) or 11.2 mg (n = 7). Doses were administered using a fine-gauge needle (29 or 30). PK and PD were measured for GH, insulin-like growth factor-1 (IGF-1), and IGF binding protein-3 (IGFBP-3) using serum samples following the first injection (Phase I) and first and third injections (Phase II).

GH and IGF-1 serum profiles in both healthy adults and GHD adults supported a once-per-week dosing regimen. The time lag between GH Cmax and IGF-1 Cmax was similar between the two studies (1.5 to 2 days). The slopes of the dose-response curves between the two studies were similar for GH AUC, IGF-1 Cmax and IGF-1 AUC, but the dose-response comparison for GH Cmax showed greater variability between the two studies. ALTU-238 was well-tolerated by healthy adults and patients with GHD and demonstrated GH, IGFBP-3, and IGF-1 serum profiles that support a once-per-week dosing regimen. Dose-response data from the Phase I study suggest that doses higher than 11.2 mg may be needed in some adult GHD patients to attain IGF-1 levels that approximate the mean ± 1 standard deviation (based on age and gender).

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group continued their respective treatment without change, up to 24 months. LB03002 in all dose groups was safe and well tolerated. Neither clinically relevant adverse events, nor abnormal laboratory parameters, nor obvious safety concerns, such as pubertal advancement or acceleration of bone age, were observed. Occasionally, injection site reactions were reported, mostly mild and these resolved within 2 - 3 days post-dose, without intervention.

Table 1: Mean HV (cm/year)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Month 0-12</th>
<th>Month 12-24</th>
<th>Month 0-24</th>
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<tr>
<td>LB03002 0.2</td>
<td>3.67</td>
<td>9.97</td>
<td>8.37</td>
<td>9.07</td>
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<tr>
<td>(N=11)</td>
<td></td>
<td></td>
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<tr>
<td>LB03002 0.5</td>
<td>3.84</td>
<td>11.63</td>
<td>7.98</td>
<td>9.80</td>
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<tr>
<td>(N=10)</td>
<td></td>
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<tr>
<td>LB03002 0.7</td>
<td>3.07</td>
<td>12.53</td>
<td>8.07</td>
<td>10.32</td>
</tr>
<tr>
<td>(N=12)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Genotropin</td>
<td>3.56</td>
<td>12.08</td>
<td>8.61</td>
<td>10.36</td>
</tr>
<tr>
<td>(N=10)</td>
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The expected attenuation of HVSDS in the 2nd year for LB03002 at 0.5mg/kg/week is closely comparable to that observed for Genotropin and is consistent with previously published data (Ranke et al, Acta Paediatr Scand Suppl 1991; 379; 109-113).

In GHD children, once-a-week administration of LB03002 (0.5mg/kg/week) over 48 months, was shown to be safe and well tolerated and delivered growth comparable to the daily control group.

*In cooperation with BioPartners and LG Life Sciences’ Study Group

**FC12-134** Endocrine Pancreas

Human pancreatic islet-derived precursor cells display mesenchymal stem cell features and differentiation capacity

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Strategies for cell-based therapy of type 1 diabetes mellitus are based on pancreatic islet replacement and islet regeneration. Human islet-derived precursor cells (hiPC) expressing nestin and c-met have been investigated as an additional source of beta-cells. These cells have been demonstrated to differentiate in vitro into insulin producing cells and are assumed to be of endodermal origin. In continuation of previous work we provide further evidence that hiPCs share a common phenotype with human bone marrow derived mesenchymal stem cells (hMSC) and, in addition, bear the potential to differentiate along mesenchymal maturation pathways. hiPC and hMSC phenotyping was performed by FACS and immunocytochemistry. Gene expression was examined by cDNA array analysis (Affymetrix U133 Plus Chip). hiPCs were expanded and subjected to osteogenic, chondrogenic and adipogenic differentiation media. Differentiation markers were analysed by RT-PCR and immunocytochemistry. Both cell types express nestin and c-met, hiPCs display a mesenchymal immunophenotype (SH3+, SH2+, CD29+, CD44+, CD45+) and a gene expression pattern similar to hMSC. Moreover, hiPCs could be induced to differentiate in vitro towards the osteogenic (osteocalcin, alkaline phosphatase, day 28), adipogenic (LPL, PPARgamma, day 14) and chondrogenic (collagen IX, X, day 21) lineages. Our results demonstrate that hiPCs and hMSCs share common phenotypes and similar mesenchymal differentiation capacities supporting the occurrence of endodermal to mesodermal transition in epithelial precursor cells. Understanding the molecular mechanisms that enable these cells to cross the traditional germ layer boundaries will help to develop effective strategies for in vitro generation and differentiation of specific phenotypes for the use in cell therapeutic approaches. Moreover, if hiPCs represent a population of pancreatic cells with stem cell capacity, these cells could be induced in vivo to differentiate into insulin secreting cells or to provide regeneration of beta-cells in diabetic pancreatic islet.

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Mosaic uniparental disomy for an ABCBC8 gene mutation in a patient with congenital hyperinsulinism (CHI)

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Congenital Hyperinsulinism (CHI) is a cause of persistent hypoglycaemia. Histologically there are two main subtypes of CHI, diffuse (D) and focal (F). The typical D form affects all β-cells and is most commonly due to recessive mutations in the genes (ABCC8 and KCNJ11) encoding the two subunits of the KATP channel. Despite the classification into F and D disease there are still some cases which represent a diagnostic challenge as they cannot be easily classified into either subgroup. Some of these “atypical” forms demonstrate pancreatic β-cell nuclear enlargement confined to single areas of the pancreas. The molecular pathophysiology of these “atypical” forms is unclear. We report the first CHI patient with mosaic uniparental disomy for an ABCBC8 gene mutation and “atypical” diffuse histology. This patient presented with severe hyperinsulinemic hypoglycaemia at 2 days after birth. The patient failed to respond to medical therapy and underwent a near total pancreatectomy. The ABCBC8 gene was sequenced in this patient. Histopathological examination of the patient’s resected pancreas identified “atypical” disease with β-cell nuclear enlargement present in only some pancreatic sections. A novel nonsense mutation (Q54X) in the ABCBC8 gene inherited from the father was identified. Microsatellite analysis showed mosaic segmental paternal uniparental disomy (UPD) encompassing the gene. The Q54X mutation was heterozygous in unaffected sections of the pancreas, but present at approximately 68% in diseased tissue. The expected attenuation of HVSDS in the 2nd year for LB03002 at 0.5mg/kg/week is closely comparable to that observed for Genotropin and is consistent with previously published data (Ranke et al, Acta Paediatr Scand Suppl 1991; 379; 109-113).

We report a novel case of CHI resulting from a nonsense mutation and mosaic UPD. Our findings suggest that “atypical” histological diffuse forms of CHI may be attributed to mosaicism for ABCC8 (or KCNJ11) gene mutations.
Congenital hyperinsulinaemic hypoglycaemia of infancy is defined by levels of C-peptide and insulin that are inappropriately raised for the level of plasma glucose, and may be transient (THHI) or persistent (PHHI). Whilst PHHI has been shown to be due to mutations in KCNJ11, SUR1, GLUD1, SCHAD or GCK, genes in 50% of cases, the aetiology of transient hyperinsulinaemia of infancy (THHI), which remits over the first few months or years of life, is usually unknown. HNF4A is a transcription factor expressed in liver, pancreas and kidney. Mutations in the HNF4A gene cause maturity onset diabetes of the young (MODY). It was recently shown that some individuals with HNF4A mutations and diabetes had transient hyperglycaemia as neonates [Pearson et al in press]. This is similar to the phenotype of the HNF4A beta cell specific knockout mouse. Our hypothesis was that mutations in HNF4A would be found in patients with THHI, not necessarily with a family history of diabetes.

We identified subjects with THHI; defined as evidence of inappropriate plasma insulin levels concurrent with hypoglycaemia, response to medical treatment, and remission of hyperglycaemia allowing cessation of medication. Informed consent was taken. Direct sequencing of HNF4A was carried out on 8 patients who were screened for HNF4A mutations. Two patients proved positive for separate heterozygous mutations in HNF4A; R76W, L263P. There were no significant differences between patients with and without HNF4A mutations. Mutations in HNF4A may be a significant cause of THHI. This is important as until now, the majority of THHI has been of unknown aetiology. Although THHI is relatively rare, finding HNF4A mutations is important as these children are likely to develop diabetes in adolescence which may respond better to oral sulphonylureas than insulin.

Neonatal diabetes mellitus, defined as insulin-requiring hyperglycaemia within the first 3 months of life, is a rare disorder which is usually associated with intrauterine growth retardation and low birth-weight. A baby boy of Turkish ancestry was born after 37 weeks gestation by CS due to poor fetal growth (birth weight, 1400 g). His parents were first cousins, and the mother had gestational diabetes. At two months of age, the boy was referred to our hospital with diagnosis of sepsis. Laboratory tests revealed hyperglycaemia (290 mg/dL). Serum C-peptide level was 0.45 ng/mL (N.1-1.4), and HbA1c was 7.9% (N.4.8-5.9). The patient was diagnosed as neonatal diabetes.

Molecular genetic studies revealed a novel homozygous missense mutation (T168A) in exon 5 in glucokinase gene. At age of 4 years, he was admitted to our clinic for a trial of glibenclamide therapy. Oral glibenclamide doses were gradually increased in 2 weeks up to a final dose of 0.85 mg/kg/day. Total daily sc insulin doses were tapered gradually. In order to assess beta-cell function, glucagon stimulation test (GST) was performed before and 1 month after the trial beginning. In the first GST, basal C-peptide level was 0.013 ng/mL, and the stimulated one was 0.020 ng/mL. Although there was a little rise in ratio of C-peptide response to hyperglycemia (6%), there was a striking increase (12-fold) in basal C-peptide secretion. In a 3-month period, HgA1c level dropped from 9.4% to 8.1%, and sc total insulin doses decreased %29, from 0.85 to 0.60 kg/day. Because sc insulin doses could not be tapered further, and of possible dangerous side-effects of this drug at very high doses, glibenclamide therapy was ceased at the end of 3rd month. In conclusion, we demonstrate for the first time that oral sulfonylurea therapy can also stimulate endogen insulin secretion, especially the basal secretion, and has a positive effect on glycemic control in patients with homozygous GCK gene mutation.

Objective: MODY 5 is caused by mutations in the TCF2 gene encoding the transcription factor HNF1β. Phenotype of patients includes adolescence-onset diabetes and malformation of kidney and genitalia. Recently, in patients presenting with MODY 5 phenotype, one-third of the cases was found to have large deletions of the whole TCF2.

Cases and methods: Patient 1 is a 17 year old boy who presented with extreme intrauterine and postnatal growth retardation (present height 132 cm, -6.45 SDS), congenital cholestasis with bile duct hypoplasia, unilateral cryptorchism and bilateral congenital kidney malformation (renal aplasia and hydrenephrosis). Hyperglycemia developed at age of 13 yrs without ketonemia and in presence of detectable C-Peptide levels. Patient 2 is a 14 year old girl with mild diabetes manifestation at age of 13 yrs. Associated abnormalities were congenital dysplasia of one kidney and uterus bicornis. Both patients were initially treated with insulin, patient two was successfully transferred to Glibenclamide (0.1 mg/kg/d), while patient one was continued on insulin. DNA of both patients showed no mutation of TCF2, but by quantitative Multiplex-PCR of Short Fluorescent Fragments (QMPSF) a monoallelic loss of the entire TCF2-gene was found in both patients. FISH analysis was applied to confirm a large gene deletion of approximately 1.4 Mb. Parents of both patients do not show this deletion (FISH analysis) and have a positive effect on glycemic control in patients with homozygous GCK gene mutation.
Cystic fibrosis-related diabetes: the role of peripheral insulin resistance and β-cell dysfunction

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in caucasians. Cystic fibrosis–related diabetes (CFRD) occurs in 30–40% of adults and is associated with increased morbidity and mortality, accelerated decline in lung function, decreased BMI, and increased likelihood of infection with Pseudomonas species. Insulin deficiency is an essential factor in the development of CFRD. An additional contribution of insulin resistance has been reported, but the role of IR in the pathogenesis of CFRD remains unclear. Our aim was to investigate the roles of peripheral insulin resistance (IR) and pancreatic β-cell dysfunction in the pathogenesis of cystic fibrosis-related diabetes (CFRD) in CF patients with no previous history of glycemic disturbances. Thirty-nine CF patients underwent oral glucose tolerance tests (OGTTs). Peripheral IR was measured using the homeostasis model assessment for insulin resistance (HOMA-IR). Pancreatic β-cell function was calculated by the HOMA2 computerized model as well as by the ratio between the 30-min increment in plasma insulin and the corresponding 30-min post-OGTT plasma glucose concentration. Of the 39 CF patients studied, 6, 26, and 7 were found to have normal, impaired and diabetic glucose tolerances, respectively. HOMA-IR was inversely correlated with fasting glucose (r=0.44, p=0.008). The mean HOMA-IR values of diabetic patients was significantly (p=0.03) increased compared with the patients with impaired or normal glucose tolerance (1.94±0.69, 1.03±1.1, and 0.99±0.4 mU/mmol, respectively). β-cell function was significantly (p=0.03) decreased in the diabetic group compared with the patients with impaired or normal glucose tolerance for both methods used: 30-min increment insulin/glucose ratio: 0.7±0.54, 1.44±0.91, and 2.16±1.05 mU/mmol, respectively, p=0.03; HOMA-%β: 36.6±12, 52.1±23.2, and 56.7±15.1 percent, respectively, p=0.003. Our results suggest that both peripheral IR and pancreatic ß-cell dysfunction with insulinopenia play a role in the pathogenesis of CFRD.