Poster Presentations

P1-d1-163 Adrenals and HPA Axis 1

The role of S-palmitoylation of human glucocorticoid receptor in mediating the non-genomic actions of glucocorticoids
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Background: In humans, glucocorticoids (GCs) regulate a broad spectrum of physiologic functions and exert both genomic and non-genomic actions through their ubiquitously expressed glucocorticoid receptor (hGR). The rapid non-genomic actions of GCs are likely to be mediated by membrane hGRs that transduce the glucocorticoid signal via activation of kinases. S-palmitoylation plays an important role in plasma membrane (PM) localization and occurs through a highly conserved 9 amino acid motif in the ligand-binding domain (LBD) of steroid receptors. A highly homologous sequence is present in the LBD of the hGR protein, suggesting that the hGR might also undergo S-palmitoylation.

Objective and hypotheses: To determine the role of S-palmitoylation of hGR in mediating rapid glucocorticoid signaling following translocation and binding to the PM.

Methods: In vitro studies were performed to determine the specific residues within the 9 amino acid motif of the LBD of hGRs that are crucial for rapid glucocorticoid signaling. Specifically, we determined whether mutation of the amino acids at position -2, 0 and +5/6, relative to cysteine in the 9 amino acid motif within the 9 amino acid motif of the LBD of hGRα that are crucial for rapid glucocorticoid signaling. Specifically, we determined whether mutation of the amino acids at position -2, 0 and +5/6, relative to cysteine in the 9 amino acid motif in the LBD of hormone receptors. A highly homologous sequence is present to the PM localization of the receptor or colocalization with caveolin-1. Compared to wild-type hGR, hGR mutant receptors resulted in decreased activation of MAPK signaling from 60 min onwards. A similar reduction in wild-type GR-induced MAPK signaling at 60 min was observed after treatment with 2-bromopalmitate.

Conclusions: S-palmitoylation facilitates sustained activation of the MAPK pathway. Further studies are required to confirm that the hGR protein mediates this effect through the 9 amino acid motif in the LBD.

P1-d1-164 Adrenals and HPA Axis 1

Arterial hypertension in children: alterations in mineralocorticoid and glucocorticoid axis and their impact on pro-inflammatory, endothelial damage, and oxidative stress parameters
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Background and aims: The pathogenesis of arterial hypertension and its impact and determining factors with respect to cardiovascular damage in children is poorly understood. We evaluated the prevalence of alterations in the mineralocorticoid and glucocorticoid axes and their impact on pro-inflammatory, endothelial damage and oxidative stress parameters in hypertensive children.

Methods: 306 children (5-16 years old); Group 1: Hypertensives (n=111); Group 2: normotensives with hypertensive parents (n=101); Group 3: normotensives with normotensive parents (n=95).

Results: Fasting blood samples were drawn for hormone measurements (aldosterone, plasma renin activity (PRA), cortisol (F), cortisone (E)); inflammation variables (hsCRP, adiponectin, IL-6, IL-8, TNF-α); endothelial damage (PAI-1, MMP9 and MMP2 activities) and oxidative stress (malondialdehyde). Familial hyperaldosteronism type 1 (FH-1) was diagnosed when aldosterone/PRA ratio >10 was associated with the chimeric CYP11B1/CYP11B2 gene. The 11β-HSD2 activity was considered altered when the F/E ratio exceeded the mean + 2 SD with respect to group 3.

Comparison between the groups was done by Kruskal-Wallis Test.

Results: HF-1 was only detected in group 1, 4/115 children (3.4%). The F/E ratio was elevated (>4.3) in Group 1= 18/115 (15.6%); Group 2= 5/101 (4.9%) and Group 3= 5/95 (5.3%).

The comparison between groups 1, 2 and 3 showed differences in levels of F (ug/dl): 9.9[6.7-14.4]*, 8.5[6.2-11.1], 8.4[6.3-10.4]; E: 2.9[2.2-3.3]*, 2.6[2.0-3.2], 2.6[2.0-3.2]; hsCRP (mg/L): 1.2[0.4-2.3]*, **, 0.5[0.2-1.6], 0.5[0.2-1.3]; PAI-1 (ng/ml): 22.2[13.4-31.7]*, 18.8[9.8-27.3], 14.9[9.9-23.3] and MMP-9: 2.2[1.3-3.0]*, 1.8[1.2-2.5], 1.6[1.2-2.3]; *p<0.05 group 1 vs group 3, **p<0.05 group 1 vs group 2.

Conclusions: In hypertensive children, HF-1 and deficient 11β-HSD2 activity were detected in addition to increases in inflammation, subclinical and endothelial damage. These results highlight the importance of blood pressure measurement in the child population.
predicted height at baseline and after two years of treatment with anastrozole in these boys.

**Population and methods:** The study is conducted on twenty boys 8-12 years old diagnosed with prior premature adrenarche from years 2006 through 2011, who received anastrozole, 1 mg/day, for two years. These boys also have advanced bone age, delayed gonadarche in relation to the bone age and obesity (BMI +2 SDS). Predicted heights (determined from the Bayley-Pinneau tables) at baseline and two years after treatment were obtained and compared using the paired t-test.

**Results (Preliminary):** There is a significant increase in the mean predicted height of 6.8 cm two years after treatment with anastrozole compared to the predicted height at baseline in obese boys with premature adrenarche, delayed gonadarche and advanced bone age (p<0.02).

**Conclusions:** Anastrozole increases the predicted height in obese boys with premature adrenarche, delayed gonadarche and advanced bone age.

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**P1-d1-166 Adrenals and HPA Axis I**

**Hair cortisol as a novel monitoring tool for long-term hydrocortisone exposure in patients with congenital adrenal hyperplasia**

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**Background:** Congenital Adrenal Hyperplasia (CAH) is characterized by cortisol deficiency and androgen excess. Treatment titration is aimed at preventing androgen excess on the one hand, and iatrogenic Cushing’s syndrome on the other. CAH is associated with poor health at adult age, partly due to overtreatment. Measuring cortisol in scalp hair is a new technique providing the clinician with a value representing long-term systemic cortisol exposure.

**Objective:** We studied whether hair cortisol is a valuable monitoring-tool in the follow-up of children with CAH.

**Methods:** We collected hair samples from 23 CAH patients, 5-17yr/o, and 23 age and gender matched healthy controls. Cortisol was extracted from hair using methanol and cortisol levels were measured using an ELISA kit. Anthropometric characteristics were measured, hydrocortisone (HC) dose documented and androgen levels obtained from routine laboratory measurements.

**Results:** Hair cortisol levels were significantly higher in patients compared to controls (31.12 pg/mg vs. 14.85 pg/mg, p<0.001). Body mass index (BMI) standard deviation score (SD), weight-for-height and waist circumference were significantly higher in patients. In controls, hair cortisol was correlated with BMI SD (r=0.51, p=0.021), weight for height (r=0.57 p=0.009) and inversely with height SD (r=-0.45, p=0.045). No correlations were found between hair cortisol and anthropometric measurements, HC dose or androgen levels in patients. Cumulative HC dose was found to be positively correlated with weight for height and BMI SD when adjusted for salivary 17α-hydroxyprogesterone levels.

**Conclusions:** Hair cortisol measurement, provides us for the first time with the possibility of measuring long-term hydrocortisone exposure in CAH patients. Hair cortisol measurement could be a useful novel monitoring tool in addition to current CAH monitoring tools.

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**P1-d1-167 Adrenals and HPA Axis I**

**High prevalence of testicular adrenal rest tumours (TART) in adolescents with classic congenital adrenal hyperplasia (CAH)**

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**Background:** TART are one of the most important and frequently detected complications in adult male CAH patients. Because of their central localization of these benign tumours near the mediastinum testis, compression of the seminiferous tubules may lead to obstructive azoospermia and irreversible damage of the surrounding testicular tissue. Therefore, it may be important to detect and treat the tumours in an early stage. Ultrasound is a good method for detection and follow-up of TART, especially when they are not palpable by physical examination. TART is already detected in children, however the age of first detection is not known and screening of TART is not routinely performed.

**Objective:** Aim of our cross sectional study was to define the prevalence and age of first detection of TART in a group of 42 paediatric male CAH patients age 0 – 19 years old.

**Methods:** All male classic CAH children who are regularly followed at our outpatient pediatric endocrine clinic were included. Ultrasonographic evaluation was performed by an experienced radiologist (KK) with a high-frequency linear array transducer L 17 / 5 (17 MHz) in two directions (transversal and sagittal).

**Results:** Below the age of 10 years no TART were detected (16 patients). Above the age of 10 there was a clear increase in prevalence of TART: 10 – 12 years 28% (2 of 7 patients), 13 – 14 years 50% (4/8), 15 – 16 years 75% (3/4). Above the age of 16 TART were detected in 100% of the patients (7/7). The tumours were usually small (< 1 cm) mostly bilateral and generally not detectable by palpation.

**Conclusion:** In patients with classic CAH TART is already present in childhood with increasing prevalence in puberty. Based on our results we recommend regularly ultrasound from the age of 10 years in all boys with classic CAH.

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**P1-d1-168 Adrenals and HPA Axis I**

**Characterization of the novel missense mutation G250V in type II 3beta-hydroxysteroid dehydrogenase (3β-HSD2) gene found in a 46, XX (female) patient with congenital adrenal hyperplasia (CAH)**

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**Background:** 3βHSD2 deficiency is characterized by salt loss, incomplete masculinization in males and mild or absent virilization in females.

**Objective:** To characterize a novel missense mutation (G250V) in 3β-HSD2 gene.

**Methods:** We report a 7-month-old 46,XX girl referred because of precocious pubarche and postnatal eunogamy. Consanguinity was reported. She showed low serum cortisol, 4.8ug/dl, high ACTH, 2888 pg/ml, DHEAS, 53000 ng/ml and 17OHP 141 ng/ml, and plasma renin, 424 ng/ml. These data suggested 3βHSD2 deficiency. Enzymatic activity was analyzed by in vitro analysis of a mutant recombinant enzyme generated by site-directed mutagenesis after its transient expression in COS cells.

**Results:** A novel homozygous c.749G→T mutation in 3β-HSD2 gene resulting in a G250V change was found. This G is highly conserved in vertebrate 3β-HSD2 and is located in the substrate-binding domain of the enzyme. In silico PolyPhen and SIFT analysis predicted G250V to be a damaging substitution. Enzyme activity using 0.5 µM pregnenolone (P5) in the medium during 6h revealed relative conversion rates of P5 to P4 of 78±4% and 21±1% in WT and G250V-3β-HSD2 enzymes respectively. Using 0.5 µM DHEA, the relative conversion rate of DHEA to Δ4 during 6h was 87±8% and 23±7% in WT and G250V-3β-HSD2 enzymes, respectively. Immunofluorescence studies showed that both WT and mutant G250V-3β-HSD2 protein colocalized with endoplasmic reticulum.

**Conclusions:** We identified a novel G250V 3βHSD2 gene mutation which causes incomplete loss of enzymatic activity. Flux via the adrenal “backdoor” pathway, which converts 17OHP to DHT has been recently implicated in disorders of androgen excess. We hypothesized that this alternate pathway could not be activated in fetal 3β-HSD2 deficiency due to very low intracellular 17OHP substrate level explaining mild virilization or even normal sexual differentiation in females. Postnatal 3β-HSD1 activity might explain high serum 17OHP and clitoris stimulation.
Is the ACTH test useful in the diagnosis of late onset congenital adrenal hyperplasia?

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Background: Premature pubarche is the most common manifestation of non-classical congenital adrenal hyperplasia (NCAH); however, accelerated growth rate or bone age, hirsutism and clitoral hypertrophy in prepubertal children can be further forms of clinical presentation. The adrenal stimulation test with ACTH is the recommended method for diagnosing NCAH.

Objective and hypotheses: To define the specificity and sensitivity of basal plasma concentrations of 17-OH-progesterone (17OHPG), androstenedione, DHEA-S and testosterone as predictors of NCAH.

Patients and methods: Retrospective cohort study of patients undergoing ACTH for suspected NCAH (n = 280). NCAH was defined by a post- ACTH 17OHPG plasma level <10 ng/ml and confirmed by molecular genetic analysis. Univariate descriptive analysis and ROC curves were applied to determine the sensitivity and specificity of each of the parameters evaluated.

Results: Indications for ACTH test and their results are shown in the table I and Table II, respectively.

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>male</th>
<th>female</th>
<th>AGE</th>
<th>17-OHPG response to 60 min&lt;10 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>Mean (Range)</td>
<td>%</td>
</tr>
<tr>
<td>Pubarche</td>
<td>43</td>
<td>210</td>
<td>72 ± 13 (0.5-111)</td>
<td>27</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>0</td>
<td>11</td>
<td>6.0 ± 2.9 (0.9-9.2)</td>
<td>1</td>
</tr>
<tr>
<td>Accelerated bone age</td>
<td>0</td>
<td>1</td>
<td>8.8</td>
<td>0</td>
</tr>
<tr>
<td>Accelerated growth</td>
<td>2</td>
<td>2</td>
<td>6.5 ± 2.2 (3.4-8.8)</td>
<td>1</td>
</tr>
<tr>
<td>Clitoral hypertrophy</td>
<td>0</td>
<td>11</td>
<td>2.9 ± 2.8 (1.9-4.6)</td>
<td>0</td>
</tr>
</tbody>
</table>

Diagnosis 17OHPG BASAL 17OHPG 60 MIN Genetic Analysis

17OHP < 10 ng/ml
(n=251)
0.54 ± 0.37
32.4 ± 17.22
Not performed

NCAH (n=29)
8.73 ± 6.15 (2.0-25.2)
32.4 ± 17.22 (13.8-100)
Confirmed in all patients

Conclusions: 10.7% of patients were diagnosed of NCAH and confirmed by genetic studies. Basal plasma testosterone, androstenedione and DHEA-S concentrations were not helpful for identifying patients with NCAH. Basal plasma 17-OHPG 2 mg/ml concentrations had 100% specificity and 95% sensitivity for NCAH diagnosis, rendering the ACTH test unnecessary.

Plasma steroid profile in newborns and adolescents treated with Ritonavir-Lopinavir for HIV reveals profound adrenal impairment

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Background: Ritonavir-Lopinavir (r-LPV) is a human immunodeficiency virus 1 (HIV-1) protease inhibitor boosted by ritonavir, a cytochrome P450 inhibitor. It is prescribed in newborns as a post exposure prophylaxis in preterm infants and from the moment of diagnosis, rendering the ACTH test unnecessary.

Objective and hypotheses: To better describe the adrenal function impairment due to r-LPV and to obtain data in short term exposed newborns and long term exposed adolescents.

Methods: 2 newborns treated with r-LPV for 4 weeks and 3 HIV infected adolescents long term treated with r-LPV were analysed. Basal and ACTH stimulated plasmatic hormonal concentrations (cortisol, 17-hydroxyprogesterone, 17-hydroxyprogrenolone, DHEA, DHEA-S, androstenedione, testosterone) and ionogram were assayed.

Results: Adrenal function tests were abnormal in all patients. In newborns, high levels of 17-hydroxyproprgenolone (> 30 mmol/L at basal level and >100 mmol/L after ACTH stimulation) and of DHEA-S (> 6 000 ng/ml) were found. 17OHProgresterone was normal. DHEA-S levels normalized after treatment completion. In adolescents, all had elevated androstenedione levels (> 6.3 mmol/L, 2 had elevated 17 hydroxyproprgenolone and 1 had elevated DHEA-S (7176 ng/ml). All patients had normal basal and stimulated cortisol and mineralo-corticoic secretion.

Conclusions: The impact of r-LPV on adrenal function is confirmed. Short exposure on immature adrenal of newborns and long exposure on adolescents provoke adrenal anomalies with a preservation of cortisol secretion. These results suggest a 3JHSD2 block profile which needs to be further defined. *D,k and A,S contributed equally and should be considered as joint first author.

51st Annual Meeting of the ESPE

Horm Res 2012;78(suppl 1)
Background: Mutations in nicotinamide nucleotide transhydrogenase (NNT) and glutathione peroxidase 1 cause familial glucocorticoid deficiency in humans, characterized by a deficiency of glucocorticoids alone. Patients often present with hypoglycaemia which, if unrecognized and untreated, can lead to neurological impairments and may be life-threatening. NNT, a highly conserved gene, encodes an integral protein of the inner mitochondrial membrane. Under most physiological conditions, this enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of NADPH. Detoxification in mitochondria of reactive oxygen species (ROS) by glutathione peroxidases depends on this NADPH for regeneration of reduced glutathione (GSH) to oxidized glutathione (GSSG) to maintain a high GSH/GSSG ratio.

Objective: To study the effects of NNT ablation in an adrenocortical cell line. Methods: Generation of NNT knockdown (NNT-KD) and control (SCR) H295R cell lines by lentiviral delivery of shRNAs. Detection of superoxide production by Mitosox red staining. Western blotting of the apoptosis marker cleaved PARP. GSH/GSSG ratio measured by the luminescent GSH/GSSG-Glo Assay.

Results: Using shRNAs targeting NNT we achieved a 70% knockdown of NNT and >80% reduction in protein levels in H295R cells. NNT-KD cells showed not only increased levels of ROS and apoptosis but also a lower GSH/GSSG ratio when compared to SCR cells (18.82±8.75 vs 29.95±4.77; p<0.001) implying these cells also have an impaired redox potential.

Conclusion: Taken together with the fact that NNT mutations give rise to FGD, these results suggest that, in humans, NNT is of primary importance for ROS detoxification in adrenocortical cells, highlighting the susceptibility of the adrenal cortex to this type of pathological damage. Over time patients may develop other organ pathologies related to impaired anti-oxidant defence and will therefore need careful monitoring.

Background: Mutations in CYP21A2 are the most common cause of congenital adrenal hyperplasia (CAH). Rare, family or population specific mutations, account for around 5% of all CYP21A2 mutations. In order to enable genetic counseling in these families the disease phenotype associated with the rare mutations can be verified by in vitro functional analyses of the mutated P450c21 protein.

Objective: To verify the disease phenotype associated with five rare missense mutations identified in the CYP21A2 gene in patients investigated for non-classic CAH.

Methods: We have performed functional studies of five rare missense mutations (R233G, A265S, R341W, R366C, M473I) identified in the CYP21A2 gene in patients investigated for non-classic CAH. The mutant proteins were expressed in vitro in eukaryotic COS-1 cells and the enzyme activities toward the two natural substrates 17OHP and progesterone were determined in order to study the disease-causing state of the mutations. The results will be correlated with the patient phenotypes. The A265S mutation should most probably be considered as a polymorphism, while the M473I could represent a very mild mutation.

Conclusion: Genotype-phenotype relationships in CAH due to 21-hydroxylase deficiency can be improved by combining information gained from clinical, functional and structural studies.
P1-d1-175 Adrenals and HPA Axis 1

Genetic analysis of 16 patients with familial glucocorticoid deficiency from 1 centre
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Background: Familial glucocorticoid deficiency (FGD) is a rare inherited disorder characterized by isolated glucocorticoid deficiency and ACTH resistance. Approximately 50% of cases result from mutations in the ACTH receptor (melanocortin 2 receptor, MC2R), MC2R accessory protein (MRAP) and the steroidogenic acute regulatory protein (STAR). Recent mutations in three further genes have been reported; one encodes a DNA replication protein, mini-chromosome maintenance 4, and the other two, nicotinamide nucleotide transhydrogenase and glutathione peroxidase 1, are antioxidant genes.

Objective and hypotheses: We searched for underlying mutations in 16 patients with clinically diagnosed FGD from 11 families.

Methods: The clinical diagnosis of FGD was made in patients who presented with symptoms of glucocorticoid deficiency associated with low serum cortisol and high ACTH levels in the absence of mineralocorticoid deficiency. MC2R, MRAP and STAR genes were sequenced in all patients.

Results: We found 9 mutations in 16 patients. In MC2R we found a novel homozygous mutation, p.L225R, in one patient and the known mutation, p.D103N, was homozygous in three siblings. In MRAP we found a novel homozygous p.L53P mutation in two siblings, the previously seen IVS3ds+1 del G mutation was homozygous in two cousins, and a homozygous V26A mutation was found in one patient whose father has FGD due to the same mutation. STAR gene sequencing was normal in all patients. Seven of 16 patients had no mutation in MC2R, MRAP or STAR. The remaining patients may harbour mutations in one of the newly described FGD-causing genes.

P1-d1-176 Adrenals and HPA Axis 1

Longitudinal growth, adult height and hydrocortisone dose in 549 adult patients with congenital adrenal hyperplasia due to 21-Hydroxyase deficiency
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1AQUAP-AUSTudy group10
2OVG University Magdeburg, Department of Pediatrics, Magdeburg, Germany; 3University Erlangen, Department of Pediatrics, Erlangen, Germany; 4OVG University Magdeburg, Institute of Biometry and Med. Informatics, Magdeburg, Germany; 5University Essen, Department of Pediatrics, Essen, Germany; 6University Leipzig, Department of Pediatrics, Leipzig, Germany; 7University Tuebingen, Department of Pediatrics, Tuebingen, Germany; 8University Kiel, Department of Pediatrics, Kiel, Germany; 9University Vienne, Department of Pediatrics, Vienna, Austria; 10University Muenster, Department of Pediatrics, Muenster, Germany; 11Germany

Background: CAH due to 21-hydroxylase deficiency results in a mean reduction of adult height-SDS of -1.38 (systematic review by Muthusamy K, 2010).

Objective and hypotheses: To achieve normal growth, prevent overweight, infertility and testicular tumours it is recom-mended to treat with physiologic hydrocortisone doses and to avoid under- or overtreatment.

Methods: In this observational study, we correlated hydrocortisone doses (>15 mg/m2/d versus ≤15 mg/m2/d) with height-SDS at yearly intervals up to adulthood. 549 adult individuals (344 females) from 28 centres (79.8% born after 1980) with 10.865 examinations were analysed. 52% of patients were treated with daily hydrocortisone doses >15 mg/m2. ANOVA test was performed to check the influence of target height (TH), clinical form of CAH, onset of puberty.

Results: Corrected for TH, 31 out of 325 (9.54 %) had a reduced adult height < -2 SD. Length at birth was normal (+0.21, range -2.31 to 2.46 SDS), and slightly reduced (-0.34, -2.9 to +2.9) during infancy. From 3 - 4 years, mean H-SDS increased to a peak at 8 years (+0.72 SDS, range -2.7 to +6.3). In salt wasters, mean age at onset of puberty was not different from reference group. However, early puberty (m < 9y., f < 8y.) has been documented in 38 out of 208 children. H-SDS corrected for TH-SDS was significantly different with regard to the two HC dose groups has been found for different age groups: 1 year and 6 to 18 years (fig.).

Conclusions: Our data show that HC doses of <15 mg/m2/d during infancy and childhood result in normal height in adulthood. In salt wasters, mean age at onset of puberty was not different from reference group.
P1-d1-178 Adrenals and HPA Axis 1

Premature adrenarche may be an independent negative risk factor for atherosclerosis in girls
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Background: In girls, premature adrenarche (PA) refers to isolated pubic or axillary hair growth under 8 years. PA has been associated with metabolic complications in adulthood.

Objective and hypotheses: We aimed to evaluate whether the metabolic risk factors for girls with PA is increased when compared to body mass index (BMI) matched peers.

Methods: Forty-seven girls with PA and 45 BMI-matched prepubertal peers were compared with respect to anthropometric, hormonal measurements and insulin sensitivity (IS) screened by fasting glucose insulin ratio (FGIR) and homeostasis model assessment (HOMA), fasting lipids and atherogenic index of plasma (AIP). Correlations of these parameters within both groups were assessed.

Results: AIP was significantly lower in girls with PA than controls (p=0.001). In girls with PA, AIP significantly correlated with BMI standard deviation score (SDS) (r = 0.40, p=0.006), weight SDS (r = 0.4, p=0.005), FGIR (r = -0.39, p=0.022) and HOMA-IR (r = 0.37, p = 0.029).

Conclusions: PA may be an independent negative risk factor for atherogenesis. The risk of atherogenesis in girls with PA is increased in relation to decreasing IS and increasing BMI. The reduced risk of atherogenesis cannot be directly attributed to premature onset of DHEA-S secretion from the adrenals.

P1-d1-179 Adrenals and HPA Axis 1

Cyclical cushing syndrome in a 5-year-old girl with primary non-pigmented nodular adrenal hyperplasia in absence of PKA1R1A mutations
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Background: Cyclical Cushing Syndrome (Cyc CS) is an unusual form of hypercortisolism associated with ACTH-producing adenomas either with primary pigmented nodular adrenal disease (PPNAD) or with adrenal hyperplasia. PPNAD is a part of Carney Complex (CNC) or McCune-Albright Syndrome in most cases. Only few cases of CycCS due to micronodular adrenal hyperplasia in children have been reported to date.

Case report: A 5-year-old girl born to non-consanguineous parents was admitted to our clinic with clear “cushing” phenotype. She manifested at the age of two with the episode of bulimia, rapid weight gain and “moon” face. The symptoms regressed spontaneously within 1-2 months and recurred again in the next 2-3 months. More than five cycles appeared during three year period. Munchausen syndrome was excluded. Laboratory data confirmed ACTH-independent intermittent hypercortisolism (Table 1).

CT-scan revealed mild bilateral adrenal hyperplasia without adrenal mass. Liddle’s test was suggestive for the diagnosis of PPNAD. We did not find other components of CNC or McCune-Albright syndrome. Genetic analysis did not detect PKA1R1A mutations. The girl underwent laparoscopic bilateral adrenalectomy. Histological examination revealed non-pigmented micronodular cortex dysplasia. She has been doing well on hydrocortisone replacement during two year follow up, new manifestations of CNC were not seen.

Conclusions: We described a case of CycCS due to non-pigmented nodular adrenal hyperplasia in a child without addition signs of CNC. PKA1R1A mutations also were not found. Interestingly, the patient always had very low androgen levels. We suppose that this case possibly belongs to different group of adrenal hyperplasias in childhood distinct from PPNAD, and other genes than PKA1R1A probably underlies this adrenal abnormality.

P1-d1-180 Adrenals and HPA Axis 1

Comprehensive characterisation of two 17α-hydroxylase isoforms reveals differential enzymatic properties
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Background: Zebrafish is emerging as an effective system in endocrinology. Zebrafish synthesise steroids in the interrenal (counterpart of the mammalian adrenal), gonad and brain. Human 17α-hydroxylase (hCYP17A1) facilitates the 17α-hydroxylase and 17,20-lyase reactions in the adrenal and gonad. Two zCyp17a1 (zCyp17a1/zCyp17a2) enzymes exist in zebrafish.

Objective: To characterise the expression pattern and function of the two zCyp17a enzymes.

Methods: zCyp17a expression was determined by RT-PCR in embryos and adult tissues. Functional assays were performed in COS7 cells overexpressing zCyp17a or hCYP17A1. 17α-hydroxylase activity was assessed by the conversion of pregnenolone into 17α-hydroxyprogrenolone and progesterone into 17α-hydroxyprogesterone. The 17,20-lyase activity was measured by the conversion of 17α-hydroxyprogrenolone into DHEA and 17α-hydroxyprogesterone into androsterone.

Results: zCyp17a genes were expressed from fecondation and in the adult interrenal, gonad and brain. Both zCyp17a enzymes converted progesterone 6 times more efficiently than hCYP17A1. zCyp17a2 hydroxylated pregnenolone 2.5 and 20 times more efficiently than zCyp17a1 and hCYP17A1, respectively. Unlike hCYP17A1, zCyp17a1 efficiently synthesised DHEA and androsterone. zCyp17a2 lacked 17,20-lyase activity. In silico analysis using two newly developed three-dimensional zCyp17a models and the hCYP17A1 model suggest that our in vitro findings could be due to residue divergence within the substrate and redox interaction domains.

Conclusions: Herein, we prove that the zCyp17a enzymes are more efficient than hCYP17A1, showing a preference for the A5-pathway. Furthermore, we describe a zCyp17a enzyme naturally lacking 17,20-lyase activity. Expression data indicate a role of both zCyp17a genes during early embryogenesis. Importantly, our data provides novel insights into CYP17A1 structure-function relationships. Furthermore, it will help to establish zebrafish as a comprehensive model to further the understanding of human pathology.
Background: Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a rare AR disorder caused by mutations in AIRE, characterized by mucocutaneous candidiasis, multiple endocrinopathies and/or ectodermal dystrophy. Circulating tissue-specific autoantibodies are a hallmark of APECED, whose generation has been mainly related to the escape of autoreactive T cells from tolerance mechanisms. Recent data suggest a T cell-independent B cell-dependent BAFF mediated mechanism implicated in altered peripheral B cell selection and its possible contribution to the pathogenesis of autoimmune disease. However, it is not still clear how B lymphocytes and the autoantibodies they produce are related to immunodeficiency and to the autoimmune process. This question is of practical importance given the advances in immunotherapy targeting B cells in a growing number of autoimmune diseases. Objective and hypotheses: To characterize the B cell subsets distribution in peripheral blood in 12 APECED patients, and to evaluate the presence of B cell related cytokines, i.e. IL-21 and BAFF, in order to better understand whether an intrinsic B cell defect was present. Population and methods: Flow cytometric analysis of B cell subsets was performed in 12 Sardinian APECED patients (Image 1), compared to age-matched healthy donors. The following B cell subsets were analysed: transitional, naïve, IgM and switched memory, and plasmacells. ELISA assay was performed for cytokines determination in sera.

Results: A significant deregulation of B cells subsets was found in APECED patients, affecting principally two major subsets: 1) immature transitional B cells and 2) switch memory compartment. Concomitantly, we observed an increased in serum BAFF levels, which could be related to an exaggerated activation of IFN-γ pathway in DCs (Image 2).

Conclusions: Our hypothesis is that autoantibodies generation in APECED patients is mediated by an altered peripheral B cell selection not entirely dependent from T cells. We propose a BAFF dependent mechanisms acting on the immature transitional B cell compartment.

Image 2: Percentage of transitional-immature peripheral B cells CD19*CD24hiCD38hi in peripheral blood of APECED patients compared to age matched healthy controls. Shown dot plot of normal donor
**P1-d1-184** Autoimmune Endocrine Disease 1

**Prevalence and role of antibodies to ZnT8 in young patients with autoimmune thyroid disease and/or celiac disease**

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**Background:** Type 1 diabetes mellitus (T1D), autoimmune thyroid disease (ATD) and celiac disease (CD) are autoimmune conditions relatively common in paediatric age and frequently occur in association in the same subject. Autoantibodies to the islet-specific zinc transporter isofrom 8 (ZnT8Abs) are detected in the majority of T1D patients prior to and at clinical diagnosis. The presence of ZnT8Abs in other autoimmune diseases has not been investigated.

**Objective and hypotheses:** The aim of this study was to determine the prevalence and role of antibodies to ZnT8 in young patients with ATD and/or CD.

**Methods:** We analyzed ZnT8Abs, insulinoma-associated antigen-2 (IA-2) and glutamic acid decarboxylase 65 (GAD65) antibodies by radioimmunassay in 77 patients (mean age 19.0±7.2; 59 F/18M); 28 patients had ATD, 37 CD, and 12 CD and ATD in association. The presence of one or more diabetogenic DQ molecules was investigated in all patients.

**Results:** ZnT8 were positive in 3 patients (3.8%), GAD65 in 20 (25.9%) and IA-2 in 21 (27.2%) patients. In the table, autoantibodies positivity according to autoimmune diseases was reported.

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>ATD (n=28)</th>
<th>CD (n=37)</th>
<th>ATD+CD (n=12)</th>
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<tbody>
<tr>
<td>IA-2</td>
<td>6 (21.4%)</td>
<td>2 (5.4%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>GAD65</td>
<td>6 (21.4%)</td>
<td>1 (2.4%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>IA+GAD65</td>
<td>0 (0%)</td>
<td>4 (10.8%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>ZnT8+GAD65+IA</td>
<td>0 (0%)</td>
<td>2 (16.6%)</td>
<td></td>
</tr>
</tbody>
</table>

All patients with ZnT8Abs positivity, presented also very high titer of GAD65 and IA2 autoantibodies; 4 heterodimers and 2 heterodimers of susceptibility to TID were found in 2 and 1 patients, respectively.

**Conclusions:** Our results showed a low prevalence of ZnT8 autoantibodies, respect to the others, in patients with ATD and/or CD. No patients presented positivity only for ZnT8Abs. Determination of ZnT8Abs does not seem useful in the screening of the risk of TID in patients with ATD and/or CD.

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**P1-d1-185** Bone, Growth Plate and Mineral Metabolism 1

**Serum amino-terminal proC-type natriuretic peptide in girls with idiopathic central precocious puberty during GnRHa treatment**

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**Background:** The mechanism of linear growth reduction during GnRHa treatment in central precocious puberty has not been elucidated.

**Objective:** To investigate the pattern of serum amino-terminal proC-type natriuretic peptide (NT proCNP) in healthy girls throughout puberty, and the changes of serum NT proCNP in girls with idiopathic central precocious puberty (ICPP) before and during gonadotropin-releasing hormone analog (GnRHa) therapy.

**Methods:** Serum levels of E2, NT proCNP, insulin-like growth factor 1 (IGF-1), N-MID Osteocalcin (OC) and carboxy-terminal cross-linking telopeptide of type 1 collagen (β-CrossLaps) were measured in healthy 57 girls at different pubertal stages, and in 13 girls with ICPP at the beginning and the end of 6-month and 12th-month of GnRHa treatment. Height velocities of the 13 ICPP girls in each 6 months before and after GnRHa treatment was calculated.

**Results:** Serum NT proCNP level increases as the progress of pubertal development and peaks at the late puberty (P=0.01), paralleling with serum E2 and IGF-1 levels, like with the pattern of height velocity. All of serum NT proCNP, N-MID OC and β-CrossLaps level decrease significantly in ICPP girls at the end of 6-months of GnRHa therapy (P=0.01 or P<0.05), and remain the same low level at the end of 12th-month of GnRHa. Different from the above markers, serum IGF-1 level remains high before and during GnRHa treatment despite growth deceleration.

**Conclusions:** Linear growth reduction in girls with ICPP treated with GnRHa is due at least in part to decreased CNP-mediated long bone growth after estrogen inhibition. Serum proCNP can be used as a biological marker of long bone growth indicating the activity of epiphyseal growth plate.
Conclusions: In this cohort of patients mostly (84%) younger than 18 yr at HCT, BMD deficits were mild on average. However, age at HCT and time since HCT were significantly associated with BMD deficit. Younger age and shorter interval since HCT were associated with lower BMD Z-scores.

P1-d1-187 Bone, Growth Plate and Mineral Metabolism 1

Novel compound heterozygous mutations in SERPINF1 gene identified by whole exome sequencing in a korean patient with osteogenesis imperfecta

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Background: Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by bone fragility, frequent fractures and low bone mass.

Objective and hypotheses: Dominant COL1A1 or COL1A2 mutations appeared to be causative in the majority of OI types but rare recessive genes have also been reported including CRTAP, P3H1, LEPRE1, PLOD2, PPIB, SERPINH1, and SP7.

Methods: Given that many genes are involved in OI and COL1A1 and COL1A2 genes are difficult to be analyzed by conventional Sanger sequencing, an excellent tool for the identification of mutations in patients with OI is whole-exome sequencing. We applied whole exome sequencing to identify mutations in a Korean OI patient who showed an initially mild and then progressively worsening form of OI.

Conclusions: The present study shows that whole exome sequencing may be an excellent tool for the identification of mutations in patients with OI and also support the notion that SERPINF1 mutations are genetic causes of recessive OI regardless of ethnicities.

P1-d1-189 Bone, Growth Plate and Mineral Metabolism 1

Elite peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry

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Background: Intensive physical training in peripuberty may have a sport-dependent effect on bone mass acquisition.

Objective and hypotheses: The aim of this study was to compare the effects of sports that generate different mechanical loads, in terms of impact and intensity, on the bone mass acquisition in young girls over the peripuberty period.

Methods: Eighty peripubertal girls from 10.7 to 18.0 years old (mean 13.83±1.97) were recruited: 20 artistic gymnasts (AG; high-impact activity, mean hours training per week: 20.3±4.2), 20 rhythmic gymnasts (RG; medium-impact activity; 21.1±4.4 hr/wk), 20 swimmers (SW; no-impact activity; 14.5±5.9 hr/wk), and 20 age-matched controls (CON; leisure physical activity, mean hours training per week: 20.3±4.2), 20 age-matched controls (CON; leisure physical activity, mean hours training per week: 20.3±4.2), 20 age-matched controls (CON; leisure physical activity, mean hours training per week: 20.3±4.2). Given that many genes are involved in OI and COL1A1 and COL1A2 genes are difficult to be analyzed by conventional Sanger sequencing, an excellent tool for the identification of mutations in patients with OI is whole-exome sequencing. We applied whole exome sequencing to identify mutations in a Korean OI patient who showed an initially mild and then progressively worsening form of OI.

Conclusions: High-impact activity clearly had a favorable effect on bone mass acquisition and bone geometry, although the bone health benefits appeared more marked after menarche.

P1-d1-188 Bone, Growth Plate and Mineral Metabolism 1

17β-Estradiol Regulates C-type Natriuretic Peptide and Its Receptor NPR-B Expression in Rat Growth Plate Chondrocyte

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Background: Estrogen is a key regulator of growth plate development. It is found that c-type natriuretic peptide (CNP) regulated cartilage homeostasis and enchondral bone growth.

Objective and hypotheses: We observed the impression of different concentration estrogens on CNP and its receptor NPR-B levels and mRNA expression in rat growth plate chondrocyte.

Methods: Chondrocytes were isolated from growth plates in tibia of prepubertal rats and primarily cultured. After cell density was adjusted, 17β-estradiol (10^-4 mol/L, 10^-5 mol/L, 10^-6 mol/L, and 10^-7 mol/L) was added to the culture solution. And a control without 17β-estradiol in different levels.

Results: It was found that 17β-estradiol impacted on chondrocyte viability when concentration was within 10^-4 to 10^-6 mol/L, but had an inhibited effect at 10^-7 mol/L. Consistent to these findings, CNP level in culture solution was highest (0.49±0.02 ng/ml, P<0.05) when 17β-estradiol was at 10^-6 mol/L. Meanwhile, levels of CNP and NPR-B mRNA expression were significantly higher in 10^-5 mol/L group than any others. 2-mit values of CNP and NPR-B mRNA were 73.18±11.35 and 19.20±8.56 respectively (P<0.05). Chondrocytes which were cultured with 10^-6 mol/L expressed much more IGF-1 mRNA when compared to other groups. But level of IGF-1 mRNA expression declined significantly when 17β-estradiol was at 10^-7 mol/L, its level was even lower than at 10^-6 mol/L (P<0.05).

Conclusions: 17β-estradiol in different concentrations could modulate differentiation and proliferation of chondrocyte from growth plate of prepubertal rats through CNP/NPR-B pathway. But cell viability was not accordant with paracrine/autocrine activities of IGF-1 in chondrocyte when stimulated with 17β-estradiol in different levels.
P1-d1-190 Bone, Growth Plate and Mineral Metabolism 1

Hyperandrogenism in elite adolescent swimmers does not modify bone mass acquisition

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1CHU and UMI and Direction Régionale de la Jeunesse, des Sports et Cohésion Sociale, Hormonologie, Montpellier, France; 2Hopital La Peyronie, CHU Montpellier and UMI, Hormonologie, Montpellier, France; 3Hopital La Peyronie, CHU Montpellier and UMI, Medicine Nucléaire, Montpellier, France; 4Hopital St Elois, CHU Montpellier, CIC 1001, INSERM and Centre d’Investigation Clinique et Département d’Information Médicale, Montpellier, France; 5Hopital St Elois, CHU Montpellier, CIC 1001, INSERM and Centre d’Investigation Clinique et Département des Maladies Endocrinienes, Montpellier, France; 6Hopital La Peyronie, CHU Montpellier and UMI, Medicine Nucléaire, Montpellier, France; 7Unité d’Endocrinologie Pédiatrique, Montpellier, France

Background: Hyperandrogenism has been frequently reported in elite athletes. However, the effect of high testosterone levels on bone mass acquisition in this population remains largely unknown.

Objective and hypotheses: To investigate whether hyperandrogenism affects areal bone mineral density (aBMD), bone geometry and bone remodeling in young elite swimmers (SW).

Methods: Twenty-five SW (mean age: 14.9 ± 1.0 yr, training 15.2 ± 4.4 hours per week) and 21 controls (CON; mean age: 15.6 ± 1.6 yr). All participants had breast stage IV or V and a gynecological age of 18 months. The areal bone mineral density (aBMD) at whole body, total proximal femur, lumbar spine and mid-radius was determined using dual-X-ray absorptiometry. Hip structural analysis (HSAs software) was applied at the femur to evaluate bone geometry. Bone remodeling was evaluated by specific markers of bone formation and resorption.

Results: Two groups of SW were constituted on the basis of the total testosterone (T) level. Hyperandrogenic SW (HSW; n=15) presented higher T than SW with normal T (NSW; n=10) and CON (0.63±0.17; 0.36±0.07 and 0.38±0.14 ng/ml, respectively). The SHBG level (62.1±18.7 vs. 43.3±19.8 nmol/l) and the LH/FSH ratio (1.7±1.1 vs. 0.9±0.5) were higher and menstruation and so to PHP or to Carney complex (myxomas of the heart, lentiginosis and endocrine overactivity).

Conclusions: We recommend consideration of other infrequent genetic mutations of the GPCR-Gsa-cAMP-PKA pathway in patients with clinical signs of AHO / PHP who are negative for GNAS mutations.

P1-d1-192 Bone, Growth Plate and Mineral Metabolism 1

Bone Morphogenic Protein 1 (BMP1) causes Osteogenesis imperfecta with high bone mass in humans and zebrafish

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1Childrens Hospital, Endocrinology/Osteology, Cologne, Germany; 2Proteos, Institute of Molecular and Cell Biology, Singapore, Singapore; 3University Hospital Cologne, Institute of Human Genetics, Cologne, Germany; 4University Hospital Cologne, Cologne Center for Genomics, Cologne, Germany; 5University Hospital Cologne, CIC 1001, INSERM, Cologne, Germany; 6University Hospital Cologne, Institute of Developmental Biology, Cologne, Germany

Background: Osteogenesis imperfecta (OI) is a hereditary disease with high variability of clinical symptoms, formerly associated with mutations in CO-L1A1/2. Recently a widening range of causative genes has been discovered including BMP1.

Objective and hypotheses: In a consanguine family two sons showed symptoms of OI with increased fracture rate, high bone mass and high osteoclastic activity. They presented at the age of 5.0/1.9 years with normal height (+0.1/-0.7SD) and BMI (+1.8/-0.4SD). They showed no typical signs of a pathologic collagen production (dentinogenesis imperfecta, hypermobility of joints, hearing loss or discoloured sclera). First fractures occurred with 23/14 months. Serum calcium and alkaline phosphatase levels were normal. Deoxyxypyridinoline/creatinine were elevated.

Methods: Using whole-exome sequencing we identified a homozygous missense mutation in BMP1 in our patients. We implemented this mutation in zebrafish.

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Methods: Using whole-exome sequencing we identified a homozygous missense mutation in BMP1 in our patients. We implemented this mutation in zebrafish.

Results: Tab.: Clinical Features of patients and zebrafish

Findings

<table>
<thead>
<tr>
<th></th>
<th>patient 1</th>
<th>patient 2</th>
<th>zebrafish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth length and birth weight</td>
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<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Verbrale fractures</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Deformities of bones/fin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Deformities of extremities</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vertebral bone mineral density DEKA (z-Score)</td>
<td>+ (3.45D)</td>
<td>+ (2.15D)</td>
<td>+</td>
</tr>
</tbody>
</table>

Phenotypes of patients and zebrafish were comparable. Zebrafish showed a reduced secretion of procollagen from the ER and a reduced posttranslational glycolisatisation. A strong expression of bmp1a in larval zebrafish osteoblasts was observed.

Precise conclusions: We identified siblings with symptoms of OI with increased bone density despite increased osteoclastic activity with a mutation in the human transcript and zebrafish mutation into zebrafish. First time functional analysis demonstrated conservation of BMP1 function in osteogenesis imperfecta across species. 1 Attenuated BMP1 function compromises osteogenesis leading to bone fragility in human and zebrafish; Asharani et al (AJHG, in press).
Bone mineral status and prevalence of fractures in girls with Turner syndrome (TS); usefulness of phalangeal quantitative ultrasound (QUS) in addition to dual energy X-ray absorptiometry (DXA)

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Background: TS guidelines do not recommend performing a densitometric evaluation during paediatric age despite increased risk of osteoporosis later in life.

Objective and hypotheses: To assess bone mineral status and prevalence of fractures in girls with TS.

Methods: 24 girls with TS were assessed at 13.4±3.0 yrs (21 were treated with GH from 4.7±3.0 yrs and 13 received HRT from 2.2±1.3 yrs) and at 17.1±3.1 yrs (all treated with GH from 6.9±2.8 yrs, 21 received HRT from 4.6±1.9 yrs).

Results: We evaluated areal and volumetric bone mineral density (bBMD and vBMD) at lumbar-spine (L) and femoral neck (F) by DXA and amplitude-dependent speed of sound (AD-Sos) and bone transmission time (BTT) by phalangeal QUS. Prevalence of fractures was assessed in 50 girls as controls.

Conclusions: At baseline L-bBMD, F-bBMD and F-vBMD Z-score were reduced in TS (-1.1±0.7; p<0.0001; -1.3±0.7; p<0.0001; -0.8±0.6; p<0.0001 respectively), while L-vBMD Z-score was normal. AD-Sos and BTT Z-scores were reduced (-1.6±1.7; p=0.0001 and -1.9±1.6; p=0.0001, respectively). By QUS we identified a higher percentage of girls with reduced bone mineral status than by DXA (58.3% vs 8.4%; BTT vs L-DXA p=0.003, BTT vs F-DXA p=0.0013). At the end of follow-up L-bBMD, F-bBMD and vBMD Z-score persisted reduced while L-vBMD Z-score remained normal. AD-Sos and BTT Z-scores were reduced (-1.4±1.2; p<0.0001 and -1.7±1.0; p<0.0001, respectively). The prevalence of fractures was higher in TS (0.6±1.0 vs 0.2±0.5, p=0.0242). AD-Sos and BTT were lower in TS who reported almost a fracture than fracture-free patients (p=0.0211 and p=0.0385, respectively).Duration of GH therapy and HRT did not correlate with bone status.
Background: Hypophosphatemic rickets (HR) is a group of rare, inheritable disorders caused by excessive renal phosphate wasting. We have previously reported a significant difference in bone mineral apparent density (BMAD) between the hip and spine (Z-scores 1.0 and 2.1, respectively) in HR children.

Objective and hypotheses: To evaluate the discordance in BMAD by measuring the metacarpal medullary diameter and cortical thickness from hand X-rays.

Methods: A total of 17 children were recruited from a larger study on HR and had hand X-rays performed. Two patients were excluded due to poor image quality, thus X-rays from 15 patients and DXA of the spine and hip were available in 11 patients. All but one patient had a PHEX mutation or proton pump inhibitor (PPI)-linked disease (XLHR). The remaining patient had a DMP1 mutation.

BoneXpert (Visiana) was used to calculate the following parameters of the metacarpal bones: length (L), width (W), cortical thickness (T) and medullary diameter (M). HR patients were compared with age-matched healthy children from Switzerland.

Results: Since the measurements from the patient with a DMP1 mutation differed significantly from the group of XLHR, his values are reported separately in brackets. All values are reported as median [range]. XLHR patients had significantly broader metacarpal bones W=1.6 SD [0.4-3.9], p=0.001, (5.5 SD), due to a wider marrow diameter M=2.2 SD [0.5-3.6], p=0.001, (4.8 SD). Their cortical thickness was significantly reduced T=1.5 SD [-2.2 -0.8], p=0.001, (1.3 SD). The length was not different compared with controls L=0.1 SD [-1.7 – 0.9], p=0.5, (1.3 SD). Cortical thickness was negatively correlated with the paired differences between Z BMAD spine and Z BMAD hip (p=0.028).

Results: Children with HR have broader metacarpal bones with significantly thinner cortex compared to reference. Cortical thickness correlated with the discordant BMAD at the spine and the hip. HR caused by a DMP1 mutation may affect bone size more severely than XLHR.

P1-d1-198 Bone, Growth Plate and Mineral Metabolism 1

**Vitamin D intoxication associated with fish oil supplement in young children**

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Background: Vitamin D poisoning has been associated with contamination of cooking oil, adulteration of table sugar, over-fortification of milk, erroneous production of vitamin D supplements and incorrect use of vitamin D-containing preparations. However, there is no report on vitamin D intoxication associated with fish oil supplements.

Objective: We report here the unusual event of seven young children with vitamin D intoxication that appear to have been resulted from excessively vitamin D-fortified fish oil.

Subjects and methods: Three young children initially presented with unexplained vitamin D intoxication were meticulously inquired about dietary supplements. It was realized that they had been taking a fish oil supplement that was recently produced by a local manufacturer. Although the manufacturer immediately recalled the product, four additional patients taking same fish oil applied to our clinic during one-month period. After the patients were successfully treated with intravenous hydration, furosemide and pamidronate infusions, their medical records were reviewed.

Results: All seven patients whose ages ranged from 9 to 50 months had taken one or two bottles of same fish oil for 15 to 60 days. All had severe hypercalcemia (serum calcium, 16.2±1.8 mg/dl), hypercalciuria (urinary calcium/creatinine ratio, 1.6±1.0 mg/g) suppressed levels of parathyroid hormone (6.9±4.0 pg/ml), and elevated serum 25(OH)D3 concentrations [636±222 (340-962) ng/ml]. Two patients developed nephrocalcinosis. After three- to four-month follow-up period, serum 25(OH)D3 levels returned to normal limits (30-80 ng/ml) with a mean value of 63±15 ng/ml.

Conclusions: Vitamin D intoxication may be caused by errors in the manufacturing of fish oil supplements for children. Physicians should be aware that their patients may be taking such dietary supplements. To prevent the occurrence of such unintentional incidents, the manufacturers should rigorously monitor levels of ingredient of dietary supplements. Also, they should be tightly controlled by the authorities of government.
Asian, 32% Black). By 2009 nearly all CYP were treated with multiple daily injections or insulin pump therapy. Deprivation scores were derived from the UK 2010 Index of Multiple Deprivation. We used linear mixed level modeling to identify longitudinal differences between ethnic groups.

**Results:** At study end; Black CYP had higher HbA1c levels (9.4 (standard deviation 2.4) v South Asian 8.4 (1.9) v White 8.6 (1.7), P-value for ANCOVA =0.007) but South Asians had lower HDL cholesterol (1.4 (0.4) v White 2.0 (1.2) v Black 1.6 (0.4)mmol/L, P-value=0.03) and higher triglyceride levels (1.8 (1.1) v 0.9 (0.4) v 1.0 (0.5)mmol/L, P-value=0.001). In linear mixed models, after adjusting for socio-economic deprivation and other predictors; (a) Black ethnicity associated with poorer glycaemic control (P<0.001) and (b) South Asian ethnicity associated with higher triglyceride levels (P<0.001), independent of HbA1c.

**Conclusions:** The effect of insulin intensification on glycaemic control and markers of future cardiovascular disease risk in CYP with T1D differs in relation to ethnic group. Ethnic specific thresholds for intervention should be considered during childhood.

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**P1-d1-200 Diabetes and Insulin 1**

**The role of activation of PERK in activating glycogen synthase kinase 3 (GSK-3) by oleic acid (OA) in type 2 diabetes**

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**Background:** ER-stress induced apoptosis of beta cells is an important mechanism of type 2 DM. PERK can be activated by the overactivation of ER-stress which will induce beta cells apoptosis.

**Objective and hypotheses:** To reveal the role of ER-stress, GSK-3 and the potential signal pathway during beta cells apoptosis.

**Methods:** The alterations of ER-stress related signal factors and kinases induced by OA are assessed by western blot, and the changes of PERK and AMPK are analyzed by ELISA meanwhile. The phosphorylated GSK-3β and total GSK-3 are detected by western blot, while PERK are inhibited by the transfection of P58IPK plasmid and AMPK are inhibited by the inhibitor Compound C. Finally, the interaction between PERK and GSK-3 are identified by co-immunoprecipitation and direct immuno-fluorescence.

**Results:** 1. Activation of ER-stress related signal factors and kinases in INS-1 cells. The expression of GRP78, ATF6, XBP1, PERK and AMPK significant increased in the presence of 0.4 mM OA(P<0.01). 2. ELISA measurement of PERK and AMPK activity. Activity of PERK and AMPK significantly augmented in the presence of OA (P<0.01). 3. Detection of the alterations of GSK-3 after inhibiting the activity of PERK and AMPK. (1) GSK-3β was activated when treated with OA; (2) the phosphorylation of GSK-3β obviously increased after the inhibition of PERK by transfection of P58IPK plasmid(P<0.01). (3) the activity of GSK-3β had no change after the inhibition of AMPK by Compound C(P>0.05). 4. Identification of interaction between PERK and GSK-3 co-immunoprecipitation. Co-immunoprecipitation of AMPK by Compound C.(P>0.05). 4. Identification of interaction between PERK and GSK-3 co-immunoprecipitation. Co-immunoprecipitation of AMPK by Compound C. Finally, the interaction between PERK and GSK-3 are identified by co-immunoprecipitation and direct immuno-fluorescence.

**Conclusions:** The effect of insulin intensification on glycaemic control and markers of future cardiovascular disease risk in CYP with T1D differs in relation to ethnic group. Ethnic specific thresholds for intervention should be considered during childhood.

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**P1-d1-202 Diabetes and Insulin 1**

**Oppositely regulated transcription of lipogenic genes in adipose and liver tissues in obese adolescents with early type 2 diabetes**

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**Background:** Insulin resistance associated with altered fat partitioning in liver and adipose tissues is a prediabetic condition in obese adolescents. 

**Objective and hypotheses:** To examine the molecular mechanisms that link altered fat partitioning to insulin resistance, we measured the expression of key lipogenic genes in the abdominal subcutaneous adipose and liver tissues across the spectrum of glucose tolerance in equally obese adolescents. Imaging and clamp techniques were performed to best characterize their metabolic profiles.

**Methods:** Fifty-three obese adolescents underwent a subcutaneous periumbilical adipose tissue biopsy, OGTT, euglycemic-hyperinsulinemic clamp, MRI and DEXA scans. According to their 2h glucose, they were divided into three groups: #1 <120 mg/dl (n=27), #2 120-140 mg/dl (n=16), #3 >140 mg/dl (IGT/T2D; n=10). Liver biopsy was done in 8 subjects with persistent elevation in ALT.

**Results:** Insulin resistance, hepatic steatosis, abdominal VAT/SAT ratio, and subcutaneous adipocyte diameter significantly increased with increasing 2h glucose, while the fraction of large cells decreased. The expression of ChREBP, SREBP1c, FASN, ACC, LPL and GLUT4 was significantly lower in the subcutaneous adipose tissue of IGT/T2D vs NGT. In contrast, liver expression of ChREBP, SREBP1c, FASN and ACC was significantly higher in IGT/T2D. Adipose ChREBP expression and fraction had a significant negative correlation with 2h glucose. Repeated subcutaneous adipose tissue biopsy in 4 IGT patients after converting to NGT showed an increase in the expression of ChREBP, SREBP1c, FASN, ACC, LPL and GLUT4, as well as in the fractional. In vivo analysis of de novo lipogenesis in adipose tissue showed a reduced stimulation of lipogenesis in IGT/T2D.

**Conclusions:** Our data indicate that de novo lipogenesis is oppositely regulated in adipose tissue and liver from obese adolescents with early T2D. The resulting decreased ability to store fat in subcutaneous adipose tissue is likely to be an important contributor to the development of liver steatosis and insulin resistance.

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**P1-d1-201 Diabetes and Insulin 1**

**Silent diabetic cochleopathy in type 1 diabetes mellitus**

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**Objective:** To detect early asymptomatic hearing affection whether at the level of outer hair cells (OHC), inner hair cells (IHCs) and olivocochlear bundle and the relationship between these abnormalities and other variables such as diabetes duration, metabolic control, or presence of microvascular complications.

**Methods:** Seventy five adolescents with T1DM and 33 healthy controls participated in the study. Duration of DM, HbA1c levels, microvascular complications were analyzed All underwent basic audiological assessment to ensure normal middle ear function and hearing. Other tests comprised: transiently evoked otoacoustic emissions (TEOAEs) testing OHCs, TEOAEs with contralateral suppression testing the integrity of olivo-cochlear bundle and threshold equalizing noise (TEN) testing IHCs as evidenced by dead regions within the cochlea.

**Results:** Early asymptomatic OHCs affection as reflected by partial pass was detected in 33.75% of cases with diminished suppression compared to 9.1% in control . Eleven patients showed positive TEN Test reflecting resistance of IHCs to hyperglycemic injury . Patients had higher amplitude of TEOAE noise suppression when compared to controls (p<0.002). Mean difference in amplitude of TEOAE before and after suppression was significantly higher in diabetics with microvascular complications when compared to diabetics without complications at all frequencies ( p<0.001 for all). Duration of diabetes and microvascular complications (neuropathy, peripheral and autonomic neuropathy were not associated with the TEOAE suppression except for retinopathy(p<0.02). In contrast, poor metabolic control was associated with TEOAE noise suppression=(−0.443 P<0.001).

**Conclusions:** Cochleopathy can be detected in a relatively high proportion of subjects with Type 1 diabetes in spite of a normal audiometric hearing threshold. It should be considered as early central manifestations of diabetic neuropathy which is related to the degree of metabolic control and retinopathy independent of other microvascular complications.
P1-d1-203 Diabetes and Insulin 1

Birth weight influences the clinical phenotype and the metabolic control of patients with type 1 diabetes

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Background: High birth weight has been related to an increased risk of type diabetes (T1D), while low birth weight has been related to insulin resistance, metabolic syndrome and type 2 diabetes (T2D). Insulin resistance, related to poor metabolic control, has been described also in T1D patients.

Objective and hypotheses: Aims of the study were: 1) to analyze the distribution of birth size for gestational age in a large group of T1D patients 2) to investigate the impact of birth weight on clinical phenotype and metabolic control.

Methods: The clinical records of 602 Caucasian T1D patients were evaluated. Small for gestational age (SGA) and large of gestational age (LGA) were defined as birth weight <3rd percentile or >97th percentile for gestational age, respectively. Birth weights between 3rd and 97th were defined as appropriate for gestational age (AGA). The clinical characteristics of SGA, AGA and LGA were compared. Multivariable linear regression models were fitted in order to evaluate the independent effect of birth weight and the other covariates (age at T1D onset, gender, T1D duration) on different clinical outcomes (BMI, HbA1c, daily insulin requirement, HDL cholesterol, triglycerides).

Results: The proportion of SGA subjects was slightly decreased in comparison with the percentage theoretically expected in the general population (13 subjects - 2.15% versus 3%), while the percentage of LGA subjects was slightly increased (39 subjects - 6.47% versus 3%). Daily insulin requirement (U/kg/day) was significantly higher in SGA (SGA: 1.0±0.2, AGA: 0.8±0.2, LGA: 0.7±0.2 - p: 0.0009). In contrast, LGA showed higher BMI (SGA: 22.3±5.1, AGA: 22.2±3.7, LGA: 24.7±6.3 - p: 0.024) and increased HbA1c% (SGA: 8.4±1.8, AGA: 7.7±1.1, LGA: 8.4±1.8). Multivariable linear regression showed a significant negative impact of birth weight on daily insulin requirement (p <0.0001).

Conclusions: Suboptimal birth weight (both high and low) in T1D patients seems to be associated with clinical characteristics suggestive of insulin resistance.

P1-d1-204 Diabetes and Insulin 1

Immune-metabolic markers in children with type 1 diabetes: toward the possibility to predict progression of autoimmune diabetes

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Background: Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells. T1D development involves a complex interaction between pancreatic β-cells and both innate and adaptive immunity. In literature, there is a surprisingly lack of markers able to predict residual β-cell function and pancreas failure severity.

Objective and hypotheses: To elaborate a simple tool, based on immune/metabolic parameters measured at disease onset, able to predict residual β-cell function over time.

Methods: We studied 114 T1D patients (66 M, 48 F; age 2-17.5), at onset and after 12 months. We performed an immunophenotypic analysis of peripheral blood cells, including Myeloid (mDCs) and Plasmacytoid Dendritic Cells (pDCs), and we measured serum levels of several immune/metabolic/inflammatory molecules, including Leptin (Lep) and soluble Leptin Receptor (sLepR). Patients were dichotomized in 2 groups, severe or mild, according to C-Peptide (C-Pep) levels (≤ or > 0.5 ng/ml, respectively). A multivariate logistic regression analysis was performed to identify, among the parameters measured at onset, those able to discriminate patients with good or worse pancreatic function twelve months later.

Results: We identified two immune cell subsets, not previously associated to pancreatic function, whose number and percentage, measured at T1D onset, emerged as independent predictors of C-Pep secretion after 12 months. We defined a predictive model, based on the counting at disease onset of these two immune cell populations, able to predict pancreatic residual C-pep secretion, a surrogate measure of β-cell mass, one year after disease onset.

Conclusions: This study provides a simple decision rule that predicts residual β-cell function since disease onset. Our approach could be a valuable tool to evaluate disease severity and delineate the basis for selecting potential candidates for innovative immune-based therapeutic approaches able to prevent complete loss of β-cell mass.

P1-d1-205 Diabetes and Insulin 1

Pulse pressure in children and adolescents with type 1 diabetes mellitus in Germany and Austria

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Background: Impaired blood pressure regulation contributes to the development of diabetic cardiovascular complications. The influence of systolic (SBP) and diastolic blood pressure (DBP) is controversial. Peripheral pulse pressure (PP), the difference between SBP and DBP, is an indicator for arterial stiffness. T1DM causes increased arterial stiffening and advanced vascular aging in adult patients. However, little data are available for PP in children. Therefore, we studied PP regulation in type 1 diabetic children and adolescents.

Methods: Blood pressure measurements of 47153 patients with T1DM <20 years are documented in the DPK database. The average blood pressure of the most recent year was calculated and patients with antihypertensive medication were excluded. Blood pressure values of the diabetic patients were compared with the control populations of the “4th report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents (4th report)” and the German KIGGStudy.

Results: Pulse pressure levels are significantly elevated in diabetic children (PP T1DM 49.13±11.1 vs. 4th report 45.38±3 and KIGGS 44.58±4.6 mmHg (all p<0.0001, Wilcoxon test). PP is increased in 63% (4th report) or 67% (KIGGS) of the patients, respectively. Absolute PP is elevated independently of the control population and increases with age in both sexes. The rate of increased PP remains stable between 59 and 68%, irrespective of sex, age and the control population. Age, male sex, diabetes duration, insulin dose, BMI, and height are independent factors contributing to elevated PP levels and to a higher rate of increased PP. HbA1c is only related to increased PP levels (multiple linear regression).

Conclusions: Increased PP in type 1 diabetes is a marker for accelerated arterial stiffness and aging and should be considered as an additional risk factor in the treatment of diabetic children. The elevated PP values in children and adolescents with type 1 diabetes may contribute to their markedly high risk for early development of atherosclerosis.
Use of meglitinides (glinides) in adolescent patients with HNF1A-MODY (MODY3)

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Background: HNF1A-MODY (MODY3) is caused by a heterozygous gene defect of HNF1A and is characterized by a progressive malfunction of glucose-dependent insulin secretion. The current ISPAD guideline recommends sulphonylureas (su) as the first line therapy. Sulphonylureas however have the potential risk of hypoglycaemia. Objective and hypotheses: In adult HNF1A-MODY patients, nateglinide in contrast to glibenclamide displays lower prandial glucose levels and reduced risk of hypoglycaemic episodes. In paediatric patients this therapy has not yet been reviewed.

Methods: We report on follow up results of meglitinide treatment in three adolescent patients with the molecular diagnosis of HNF1A-MODY.

Results:

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation Nucleotide (protein)</th>
<th>Age at diabetes onset [years]</th>
<th>Gender</th>
<th>HbA1c at diabetes onset (%)</th>
<th>Mean HbA1c with insulin therapy (range)</th>
<th>Hypoglycaemic episodes (blood glucose &lt; 60 mg/dl)</th>
<th>BMI (SDS) before use of meglitinides</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.872dupC (p.Gly292ArgfsX25)</td>
<td>12</td>
<td>Female</td>
<td>7.4%</td>
<td>5.5% (5.2 – 6.0%)</td>
<td>None</td>
<td>23.8 kg/m²</td>
<td>Repaglinide 3 x 0.5 mg</td>
</tr>
<tr>
<td>2</td>
<td>c.162T&gt;C (p.Leu54Pro)</td>
<td>14</td>
<td>Male</td>
<td>7.0%</td>
<td>8.5% (7.3-8.8%)</td>
<td>&gt;5/week, predominantly at night</td>
<td>19.6 kg/m²</td>
<td>Nateglinide 4 x 180 mg</td>
</tr>
<tr>
<td>3</td>
<td>c.1192C&gt;T (p.Glu398Asp)</td>
<td>11</td>
<td>Male</td>
<td>10.1%</td>
<td>8.9% (7.8-9.8%)</td>
<td>&gt;5/week</td>
<td>19.2 kg/m²</td>
<td>Repaglinide 1.5 – 0.5 mg, NPH insulin 10 – 11 U</td>
</tr>
</tbody>
</table>

†Mean HbA1c one year before and one year after transferral to meglitinide therapy

Case 2 was initially transferred on glibenclamide after molecular HFN1A. Case 3 is an obese patient and we could not achieve a satisfying glucose control with repaglinide alone and so added NPH insulin (0.27 U/kg). Her compliance is fluctuant and attaining a good glucose control is difficult.

Objective: GATA6 mutations cause pancreatic agenesis and diabetes in human sporadic cases. We report two novel GATA6 mutations in a cohort of eight children with pancreas dysplasia and diabetes.

Methodology: We investigated GATA6 in eight children with inborn pancreas dysplasia and diabetes in whom other known candidate genes for monogenic diabetes and pancreas dysplasia had been excluded.

Results: We found two novel heterozygous GATA6 mutations (c.951_954del and c.754_904del) in two patients with sporadic pancreas dysplasia, diabetes, and severe cardiac defects (common arterial trunkus and tetralogy of Fallot), but not in the remaining six patients. GATA6 mutations in carriers exhibited dysplastic pancreas with absent head in one patient and a hypoplastic pancreas in another patient.

Conclusion: Our findings add two novel cases with GATA6 mutations, who suffer not only from pancreatic dysplasia but also from progressive, postnatal insocrine and exocrine pancreatic disease. Our findings contribute to the understanding of GATA6 function in human pancreatic and islet-cell (patho)physiological function. They could help promote regenerative treatment strategies for diabetes and expand the phenotype of patients reported with GATA6 mutations.

Longitudinal HbA1c values in children and young adults with type 1 diabetes over the last decade: results from the U.S. T1D Exchange clinic registry

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Background: The Diabetes Control and Complications Trial demonstrated that lower Hemoglobin A1c (HbA1c) was associated with lower risk of long-term complications. This resulted in recommendations for intensive therapy for patients of all ages with type 1 diabetes (T1D).

Objective and hypothesis: To assess glycemic control over time in a cohort of children and young adults with T1D. The authors hypothesized that with newer insulins and greater focus on intensified management, HbA1c would have declined steadily over the past decade.

Population and methods: The T1D Exchange Clinic Registry is a cohort of >20,000 individuals with T1D (mean age = 22.7 years) from 67 centers throughout the U.S. This analysis included 13,660 participants <25 years of age, with ≥ 1 year T1D duration. The cohort is 80% non-Hispanic white, 5% non-Hispanic black, 10% Hispanic, and 5% other. Data (including demographics, insulin regimens, and frequency of glucose testing) was collected at enrollment from medical records and participant surveys. HbA1c values were entered, for up to the past ten years. Mean HbA1c for each year was compared to age group, sex, and those on CSII were compared to other regimens.

Results: There was a statistically significant decline in HbA1c from 2000 to 2005 for all age groups (p values range from <0.001 to 0.02) except 18-24 years (p=0.55). There was a significant increase from 2005 to 2011 in all age groups (p<0.001) except 6-11 y/o (p=0.74) (Fig.). CSII therapy use has declined steadily over the past decade.
Increased over time, from 2.5% in 2000 to 15% in 2005 to 51% use in 2011.
In all age groups, those using CSII at the time of the HbA1c test had lower HbA1c than those not using CSII.

**Conclusions:** The expected decline in HbA1c seen from 2000-05 did not persist. The reason for HbA1c rise after 2005 cannot be explained by less intensive therapy.

**Figure. Changes in HbA1c Over Time.**

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**P1-d1-209 Diabetes and Insulin 1**

**Prestarium pharmacogenetic efficacy in predicting diabetic nephropathy in children and adolescents**

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**Aim:** The work was initiated to study efficacy of prestarium in normoalbuminuric patients with type 1 diabetes mellitus by ACE genotype upon primary diabetic nephropathy (DN) prevention.

**Materials and methods:** We examined normoalbuminuric 22 patients with type 1 diabetes mellitus aged from 12 to 17 years with DN duration ≥10 years divided into two groups by ACE polymorphism, that is, the one with II genotype (n=11) and the one with DD genotype (n=11). Activity of urinary neutral α-glucosidase was measured by rate of glucose production from maltose. PCR was performed by means of GenePackTM PCR Core reagent kit. All patients were prescribed with prestarium in the dose of 2.5 mg/day for 6 months.

**Results and discussion:** Systolic arterial pressure and diastolic arterial pressure were found reduced by 4.2% and 3.6%, respectively, heart rate decreasing by 6.4%. Pre- and post-therapy blood fibrinolytic activity was 15.3 ± 0.8% and 14.9 ± 0.6%, respectively, fibrinogen concentrations being respectively, 3.02 ± 0.2 g/l and 3.08 ± 0.2 g/l. Total cholesterol was found decreased by 4.4%, HbA1c being reduced by 16.7%. There was 7.1% and 6.8% reduction in proteinuria in the 1st and 2nd groups of patients, respectively. Glomerular filtration rate and creatinine were found decreased by 9.2% and 4.6% in proteinuria in the 1st and 2nd groups of patients, respectively. Glomerular filtration rate and creatinine were found decreased by 9.2% and 4.6% in proteinuria in the 1st and 2nd groups of patients, respectively.

**Conclusions:** Our data suggest significant relationships between bone, fat tissue and glucose metabolism in pediatric patients with T1DM. They could give a background to use leptin as an additional therapeutic agent in T1DM.

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**P1-d1-210 Diabetes and Insulin 1**

**Possible influence of bone and adipose tissue on glucose metabolism in children and adolescents with type 1 diabetes mellitus (T1DM) and associated factors**

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Jagiellonian University in Cracow, Medical College, Polish-American Pediatric Institute, Department of Pediatric and Adolescent Endocrinology, Cracow, Poland

**Background:** Recent studies have shown a new link between skeleton, fat tissue, and insulin action. However, clinical data are still limited, especially in children.

**Objective and hypotheses:** The aim of the presented study was to investigate the relationship between bone-derived osteocalcin (OC), osteoprotegerin (OPG), and Receptor Activator of Nuclear Factor NF-κB ligand (RANKL), and fat tissue-derived leptin and adiponectin, with results of treatment of type 1 diabetes mellitus (T1DM) in children and adolescents.

**Methods:** Seventy-eight patients, 43 girls and 35 boys, mean 11.5±4.3 year old with T1DM were included into the study. Blood samples were drawn at 8:00 a.m., after 8 hours fast. All above-mentioned parameters were measured by ELISA. HbA1c was measured by standardized ISCC method. Patients were divided into three groups according to HbA1c level, I - below 7%, II - between 7%-9% and III - above 9%. In statistical analysis ANOVA, and multiple regression analysis were used.

**Results:** The mean data ± SDS are presented in the Table 1.

<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>24</td>
<td>37.5±17.9</td>
<td>2.1±1.9</td>
<td>11.1±3.5</td>
<td>1.7±1.4</td>
<td>0.2±0.2</td>
<td>53±79</td>
<td>6.4±0.4</td>
<td>10.5±4.2</td>
<td>17.6±2.0</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>28</td>
<td>33.0±18.3</td>
<td>1.8±1.7</td>
<td>12.3±4.9</td>
<td>1.8±3.3</td>
<td>0.2±0.1</td>
<td>68±9</td>
<td>7.8±0.6</td>
<td>12.7±4.8</td>
<td>18.5±3.1</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>26</td>
<td>28.8±12.9</td>
<td>1.0±0.6</td>
<td>13.8±6.4</td>
<td>2.3±1.5</td>
<td>0.2±0.2</td>
<td>111±133</td>
<td>11.2±1.8</td>
<td>11.2±3.9</td>
<td>16.2±2.9</td>
<td></td>
</tr>
</tbody>
</table>

Multiple regression analysis adjusted for age showed that serum OC and leptin negatively correlated with HbA1c (r=-0.22, p=0.004 and r=-0.27, p=0.0001 respectively). In opposite, serum OPG correlated positively with HbA1c (r=0.26, p=0.02) as well as with adiponectin (r=0.26, p=0.02) and RANKL (r=-0.27, p=0.02). Moreover leptin correlated positively (r=0.47, p=0.002), and adiponectin (r=-0.29, p=0.0001) and RANKL (r=-0.24, p=0.001) negatively with BMI.

**Conclusions:** Our data suggest significant relationships between bone, fat tissue and glucose metabolism in pediatric patients with T1DM. They could give a background to use leptin as an additional therapeutic agent in T1DM.

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**P1-d1-211 Diabetes and Insulin 1**

**Reproducibility of vibration sensation threshold (VST) values in children and adolescents with type 1 diabetes mellitus (T1DM) and associated factors**

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**Background:** VST has been proven to accurately identify patients at risk to develop peripheral diabetic neuropathy (DN), including those with early neuropathic deficits. However in T1DM children and adolescents, there are no previous studies on the reproducibility of VST and associated factors.

**Objective and hypotheses:** To study in T1DM children and adolescents the reproducibility of VST and the factors affecting it.

**Methods:** 118 T1DM children and adolescents (mean±SD age: 13.5±3.4 years, diabetes duration: 5.7±3.5 years, HbA1c:7.9±1.4%) and 79 normal controls (aged 12.0±3.07 yrs), were evaluated by a single examiner. VST was measured twice on upper and lower limbs, using a biothesiometer. Concor-
dance between the two VST measurements was estimated using the Co-hen’s Weighted Kappa statistic (Kappa: 0.41-0.60: moderate concordance, Kappa=0.61-0.80: good concordance).

Results: Overall, there was a good concordance of VST values (Kappa=0.66-0.72), with high inter-rater agreement. However, in some cases, the concordance was lower, particularly in the groups with poor control or long duration of T1DM.

Conclusions: VST values were lower in the groups with poor control (HbA1c>9.5%) (Kappa=0.49-0.71). No association was observed between VST values and other variables such as age or sex.

Wolfram syndrome: new mutations, different phenotype

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Background: Wolfram Syndrome (WS) is an autosomal recessive neurodegenerative disorder characterized by non-autoimmune diabetes mellitus, optic atrophy, diabetes insipidus and deafness. The WS gene, WFS1, encodes a transmembrane protein called Wolframin which may serve as a novel endoplasmic reticulum calcium channel in pancreatic β-cells and neurons. WS is a rare disease, with a prevalence of 1/550,000 children, with a carrier frequency of 1/354.

Objective and hypotheses: We aimed to determine the genotype of WS patients in order to establish a genotype/phenotype correlation.

Methods: We clinically evaluated 12 children from 12 unrelated families (6 males, 6 females). Basic criteria for WS clinical diagnosis were coexistence of insulin-treated diabetes mellitus and optic atrophy occurring before 15 years of age. Genetic analysis for WFS1 was performed by direct sequencing. All of them were located in exon 8, except two in exon 4 and 5 respectively. Three new variants (c.2663 C>A; p.S888X, c.1381A>C; p.T461P e c.650A>G; p.E202G) were found. The male patient carrying the compound mutation [c.1066_1062delTTC] and the female patient carrying the compound mutation [c.409_424dup16] showed a severely different phenotype (diabetes mellitus and optic atrophy).

Background: Hybrid closed-loop insulin delivery (CL), coupling automated glucose-responsive between-meal insulin delivery with manual pre-meal boluses, may lead to post-prandial hypoglycaemia when meal boluses are over-estimated.

Objective and hypotheses: We evaluated CL with reduced meal insulin boluses in comparison with CL with standard meal insulin boluses in adolescents with type 1 diabetes (T1D).

Methods: Eight adolescents with T1D [M 3; age 15.9±1.5yrs; A1C 8.9±1.6%; mean±SD; total daily dose 0.9±0.7, 1.1±0.3U/kg/d; median (IQR)] studied at a research centre for 36h on two occasions in a cross-over design. Subjects were randomised to CL with either standard insulin boluses calculated using subjects’ pump bolus calculator or CL with boluses reduced by 25%. Boluses were given before main meals (50-80gCHO) but not with snacks (15-30gCHO). On both occasions, between-meal insulin pump delivery was manually adjusted every 15min as per advice of a model-predictive-control algorithm informed by a real-time continuous glucose monitor. Subjects undertook moderate-intensity exercise on a stationary bicycle at 140rpm heart-rate for 20min (morning and afternoon).

Results: Overall insulin delivery was lower with reduced prandial insulin boluses [61.9 (55.2, 75.0) vs 72.5 (63.6, 80.3) U/36h, p=0.01] and was confirmed by lower plasma insulin concentration [186 (171, 260) vs 252 (198, 336) pmol/l, p=0.002]. Plasma glucose was identical 8.4±0.9mmol/l on the two occasions (p = 0.97). Time spent in target glucose 3.9-10mmol/l was also comparable [74(66, 84)% vs 80(63, 96)%; p=0.87]. Time above 10mmol/l was 21.8±16.3, 33.5% vs 18.0±4.1, 34.2% (p=0.87) and time below 3.9mmol/l was 0(0, 1.5) vs 0(0, 1.8%) (p=0.88). Hypoglycaemia occurred once within 1.5h post-meal during CL with standard bolus. Conclusions: In conclusion, closed-loop insulin delivery combined with 25% reduction of meal insulin boluses may be beneficial as it decreases overall insulin exposure while maintaining comparable glucose control in adolescents with T1D.

Reducing meal insulin bolus during closed-loop to minimise risk of post-meal hypoglycaemia in adolescents with type 1 diabetes

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Background: Hybrid closed-loop insulin delivery (CL), coupling automated glucose-responsive between-meal insulin delivery with manual pre-meal boluses, may lead to post-prandial hypoglycaemia when meal boluses are over-estimated.
P1-d1-214 Diabetes and Insulin 1
Assessment of the relative contribution of insulin resistance (IR) and beta cell (beta-cell) dysfunction in the aetiology of impaired glucose tolerance (IGT) in young adult survivors of childhood leukaemia treated with bone marrow transplantation and total body irradiation (BMT/TBI)

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Background: IGT and Diabetes Mellitus (DM) are increasingly recognised in adult childhood leukaemia survivors treated with BMT/TBI. The mechanism is unclear.

Objective: To investigate IR and beta-cell function in BMT/TBI survivors.

Method: Leukaemia survivors treated with group 1 and without group 2 BMT/TBI were compared with obese subjects (group 3). IR was represented by composite-insulin-sensitivity index (ISIcomp) derived from Oral glucose tolerance tests (OGTT) and beta-cell function by acute-insulin-response after arginine (AIRarg) and glucose (AIRg) stimulation from Arginine intravenous glucose tolerance tests (aVGGTT). Body composition was assessed by Dual-emission X-ray absorptiometry (DEXA). Comparison was made by ANOVA with post hoc Scheffe at 5% significance.

Results: Groups 1(n=21), 2(n=31) and 3(n=30) were 21.1 (±16.1-26.2) and 21.5 (±16.1-26.9) and 17.8 (±16.1-24.8) years respectively. All BMT/TBI survivors received 10-14.4 Gy TBI aged 9.3 (±1.0-10.8) years. Abnormal OGTTs were reported in groups 1 (DM=2, IGT=7) and 3 (IGT=1). There were no group differences in beta-cell function but groups 1 & 3 were more IR than group 2. Groups 1 & 2 have lower fat and lean masses than group 3. Android/Gynoid fat-mass-ratios were higher in groups 1 & 3 than 2.

Conclusion: Despite exposure to TBI at a young age, BMT/TBI survivors showed no evidence of beta-cell dysfunction. BMT/TBI patients showed paradoxically increase in IR and high incidence of IGT despite a lower fat-mass index.

P1-d1-215 Diabetes and Insulin 1
Induction of ER stress by variants of the prohormone convertase 1 (PCSK1)-gene
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Background: Prohormone convertase 1 encoded by the PCSK1 gene processes multiple prohormones such as proinsulin. Some variants within this gene were associated with polygenic obesity and rare mutations cause childhood obesity and abnormal glucose homeostasis. The PCSK1 variants could lead to falsely folded proteins, accumulating in the ER causing the unfolded protein response (UPR), a protective mechanism, alleviating the load of misfolded proteins in the ER. However, a continuous activated UPR leads to apoptosis.

Objective and hypotheses: We hypothesized that variants of PCSK1 can lead to falsely folded proteins and subsequently harm beta-cells by inducing ER-stress and apoptosis. This could link PCSK1 variants to diabetes, the most common comorbidity of obesity.

Methods: We applied Hck293 cells and the insulinoma cells Ins1E and BTC3 to investigate the influence of the PCSK1 variants S24C, ΔEx8, R80Q, N221D and S307L on maturation and secretion by western blot. Enzyme activity of the newly identified variants S24C and ΔEx8 was determined by an enzyme assay. We evaluated ER stress and UPR activation by western blot and PCR of ER-stress mediators. Finally, we assessed the effect of overexpressed variants on proliferation versus apoptosis by FACS. Further, we explored the influence on insulin expression in the insulinoma cells by western blot.

Results: The S24C, R80Q and N221D variants were expressed and were secreted like the wild type; no secretion was detectable for ΔEx8. We found a ten-dentally reduced maturation and secretion of S307L. The enzyme activity of S24C was not impaired, whereas ΔEx8 is an unfunctional variant. Further effects on UPR, apoptosis and insulin expression are currently investigated.

Conclusions: Our preliminary results indicate that S24C, R80Q and N221D do not disturb the maturation and secretion of PC1; S307L seems to have a slightly decreased secretion. The disturbed expression and secretion of ΔEx8 indicate that this variant possibly induces ER-stress.

P1-d1-216 Diabetes and Insulin 1
Successful hepato-pancreatic transplant in a case of Martinez-Frias syndrome caused by the RFX6 mutation p.R181Q
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1Hospital Universitario La Paz, Pediatric Endocrinology, Madrid, Spain; 2IdiPAZ, UAM, Hospital Univ. La Paz & CIBERER, U753, ISCIII, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; 3IdiPAZ, UAM, Hospital Universitario La Paz, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; 4Hospital Universitario La Paz, Gastroenterology, Madrid, Spain; 5Hospital Universitario La Paz, Pediatric Surgery, Madrid, Spain

Background: The Martinez-Frias Syndrome (MFS) is a rare autosomal recessive characterized by intratrehe growth retardation, pancreatic hypoplasia associated with neonatal diabetes, duodenal atresia, gallbladder aplasia or hypoplasia, and neonatal hernomachrosis. Mutations in RFX6, a transcription factor involved in exocrine pancreas embryogenesis, have been recently described in six MFS patients who presented with a complete déficit of pancreatic endocrine hormones (Smith et al. 2010).

Clinical case: A female newborn, born from consanguineous parents, who presented intratrehe growth retardation (BW: 1.220 g, -3.27 SDS; BL: 41 cm, -2.56 SDS, CP: 31 cm, -0.76 SDS), duodenal atresia and malrotation, pancreatic hypoplasia, absence of gallbladder, cholestasis, liver failure, hermochromatism and hyperglycaemia (>200mg/dl). C-peptide and insulin were undetectable and autoimmunity tests were negative. Insulin treatment was started during the first 12 hours of life.

Methods and results: Mutation screening of RFX6 identified an homozygous missense mutation, c.541 C>T that alters a highly conserved residue, p.R181Q, located in the DNA binding domain. Both parents were non affected hetero-
zygous carriers. The same mutation had been previously described in a male MFS newborn who died at the age of three months (Smith et al 2010).

Follow-up: Over the following 20 months she had poor weight gain, refractory diarrhea, multiple sepsis events, malabsorption and a highly unstable glycemic control, in spite of parental nutrition, pancreatic enzyme supplementation and insulin therapy. Recently, at the age of 20 months, she successfully underwent a hepatopancreateic transplant, which resolved the diabetes (HbA1c anti 2%).

**Conclusions:** 1) Mutation screening of *RXR* should be considered in patients with neonatal diabetes and pancreatic hypoplasia. 2) Although hepatopancreateic transplant initially successfully resolved diabetes, it is too early to know whether it can fully compensate the pancreatic endocrine hormones deficit in the long term.

P1-d1-217 Diabetes and Insulin 1
**Variable phenotype in 5 patients with Wolcott-Rallison syndrome due to the same EIF2AK3 (c.1259delA) mutation**

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**Background:** Wolcott Rallison Syndrome (WRS) is a rare condition caused by mutations in EIF2AK3 gene. Patients typically have early onset diabetes, skeletal dysplasia and recurrent hepatic dysfunction. Other features including central hypothyroidism have been reported.

**Objective:** We aimed to compare the phenotype of two families WRS due to the same EIF2AK3 (c.1259delA) mutation

**Patients and methods:** Five patients from two unrelated consanguineous families with WRS who had regular assessment since diagnosis were studied. Direct sequencing of EIF2AK3 gene was performed in affected patients and their parents.

**Results:** All 5 patients presented with permanent neonatal diabetes (PND) and were homozygous for a frame shift mutation deletion (c.1259delA) of the EIF2AK3 gene which has been reported only in these families. Recurrent episodes of hepatic dysfunction encountered in 4 patients and 2 of them died with acute fulminant hepatic failure. Two children developed skeletal dysplasia and another 2 had transient central hypothyroidism, associated with viral illnesses. One family has a discordant phenotype with typical WRS features in the younger child compared to her elder sister with isolated PND. There was no evidence of neutropenia, developmental delay, renal dysfunction or exocrine pancreatic dysfunction with this genotype so far.

**Conclusions:** EIF2AK3 (c.1259delA) mutation has variable phenotype ranging from typical picture of WRS to isolated PNDM. In WRS thyroid dysfunction is a transient phenomenon reflecting euthyroid sickness status ranging from typical picture of WRS to isolated PNDM. There was no evidence of neutropenia, developmental delay, renal dysfunction or exocrine pancreatic dysfunction with this genotype so far.

P1-d2-218 Diabetes and Insulin 2
**Are GAD65 antibodies at delivery in mother and cord a risk or protection for the development of Type 1 diabetes (T1D) in the offspring**

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**Background:** Studies by our group suggested that TID can start “in utero” during a viral episode.

**Subjects and methods:** Sera titers to beta cell auto-antibodies (GAD65, Zn18, and IA2) were compared with anti-viral antibodies to Rota (SA11) and Enterovirus (coxB3) in maternal blood from 105 healthy women at birth and cord blood during 2 successive winter viral seasons. Auto-antibodies titers were determined by radio-ligand binding assays, anti-viral antibodies by ELISA, and newborn HLA DRB1 and DQB1 allele frequencies by PCR.

**Results:** As shown in the Table sera from 10% of chord and 8% of maternal serum samples were positive for GAD65. Of these, 20% and 25% had high Ab titers to rotavirus and 50% and 50% had high Ab titers to enterovirus, respectively. One mother and child had antibodies to Zn18, while no sample had antibodies to IA2. Class II HLA allele frequencies in individuals with high auto-antibodies and/or anti-viral Abs were similar to the frequencies in all enrolled newborns.

**Conclusion:** The presence of significant GAD65 titers in 10 newborns and 8 of their mothers may indicate autoimmune damage to their ß cells “in utero” caused by the transmission of a viral infection from the mother. Follow-up will determine whether this early trigger event will progress to development of TID or that GAD65 transmission from mother to fetus with induce tolerance.

*Number and % of paired maternal and cord blood samples for which maternal or cord had significant levels of autoantibodies or ≥1000 arbitrary units of anti-viral antibodies

<table>
<thead>
<tr>
<th>Cord Blood</th>
<th>Postpartum Maternal</th>
<th>Postpartum Maternal</th>
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<tr>
<td>Antibodies</td>
<td>Antibodies</td>
<td>Antibodies</td>
</tr>
<tr>
<td>GAD65</td>
<td>Rotarix</td>
<td>Enterovirus</td>
</tr>
<tr>
<td>10(9.1%)</td>
<td>2/10(20%)</td>
<td>5/10(50%)</td>
</tr>
<tr>
<td>Rotarix</td>
<td>2/2(100%)</td>
<td>1/2(50%)</td>
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<tr>
<td>Enterovirus</td>
<td>5/18(27.8%)</td>
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P1-d2-219 Diabetes and Insulin 2
**Prevalence of increased liver enzymes in children and adolescents with type 1 diabetes mellitus in the DPV cohort**

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**Background:** A persistent elevation of liver enzymes may be the first indicator of the underlying condition of Non alcoholic fatty liver disease (NAFLD). The prevalence of elevated liver enzymes in adult patients with type 1 diabetes (T1DM) ranges between 4.3 and 12%. Data about the prevalence of NAFLD in childhood T1DM is rare.

**Objective and hypotheses:** The aim of the study was to determine the prevalence of elevated aminotransferases and investigate possible relations to cardiovascular risk factors in a large cohort of children and adolescents with T1DM.

**Methods:** Data of 15232 patients (47.2% female) with T1DM from the German-Austrian DPV (Diabetes Patienten Verlaufsbeobachtung) were included into analyses. The observation period contains January 1995 until September 2011. Inclusion criteria were an age <20 years and at least one measurement of amino transferases (TA) in the most recent year of treatment. Elevated aminotransferases were defined as ALT and/or AST > 50 U/l. Patients with celiac disease were excluded from the analysis.

**Results:** Median age of the patients was 14.45 (10.8-17.2) years with a median T1DM duration of 14 years. 68.5% of the patients had T1DM for >10 years. 10% of the patients had 1x increased aminotransferases, 3.98% (n=606) 1x increased aminotransferases were defined as ALT and/or AST > 50 U/l. Patients with celiac disease were excluded from the analysis.

**Median age of the patients was 14.45 (10.8-17.2) years with a median HbA1c 7.94% (7.1-11.9) and median insulin dose of 0.84 (0.66-1.04) IE/kg. 95.39% (n=14530) of the patients had normal, 3.98% (n=606) 1x increased and 0.63% (n=96) ≥ 2x increased aminotransferases. In a linear regression model, increase of aminotransferases was positively related to age (p=0.001), HbA1c (p=0.001), insulin dose (p=0.01), dyslipidema (p=0.001) and arterial hypertension (p=0.048) and negatively related to height (<0.001). No
significant association was found between elevated aminotransferases and sex and weight.

Conclusions: In our cohort elevated liver enzymes were found in 5% of children and adolescents with type 1 diabetes. Patients with higher age, lower height, increased insulin dose, insufficient metabolic control as well as with cardiovascular risk factors like dyslipidemia and arterial hypertension have increased risk for NAFLD.

P1-d2-220 Diabetes and Insulin 2

Structural characterization of two novel mutations in the GCK gene cause maturity onset diabetes of the young, type2 (MODY2)

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Background: Glucokinase with GLUT2 acts as a glucose sensor and stimulates the release of insulin from pancreatic beta cells. Mutations of the glucokinase gene (GCK) can lead to different forms of diabetes, such as GCK-monogenic diabetes of youth type2 (MODY2), permanent neonatal diabetes and congenital hyperinsulinism.

Objective and hypotheses: The present study was designed to identify how glucokinase components that are required for glucose homeostasis are affected in patients with MODY2.

Methods: Two patients who fulfilled the clinical and biological criteria for MODY2 were genetically tested by DNA sequencing of the GCK gene. The identified mutations were further studied by in silico analysis using appropriate computational tools.

Results: Full mutation analysis of the patients’ GCK gene revealed the two novel mutations: the nonsense p.440stop and the missense p.R447P. In silico analysis demonstrated that p.R447P causes structural conformational changes and destabilize the functional properties of the protein leading to reduction in glucose and MgATP2- affinity.

The amino acid change at position 447, from arginine to proline, disrupts severely the helical protein structure due to distortion of the phi and psi torsion angles and as a result changes the inter-ionic interactions altering the orientation of helix a13 relative to the proximal helix a5. The novel p.440stop mutation inactivates the cytoplasmic enzymatic activity of the protein. p.E440stop is responsible for the loss of the C-terminal end of the GCK peptide that includes vital residues essential for the release of a13 helix during glucose binding.

Conclusions: This study presents two novel MODY2/GCK mutations and their conformational changes have been analyzed in silico. These mutations disturb the functional role of glucokinase, by understanding the role of such essential components during glucose homeostasis and the determination of a glucokinase allosteric site can serve as potential drug target sites that aim to treat type2 diabetes.

P1-d2-221 Diabetes and Insulin 2

Permanent neonatal diabetes and MODY-type diabetes caused by the homozygous or heterozygous glucokinase mutation D278E

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Background: Glucokinase (GCK) is the key enzyme in pancreatic beta cells regulating glucose-dependent insulin secretion. Heterozygous inactivating mutations of the GCK gene result in maturity onset diabetes of the young type 2 (GCK-MODY1), characterized by an autosomal dominant mode of inheritance and mild hyperglycaemia. Homozygous inactivating mutations are a rare cause of permanent neonatal diabetes mellitus (GCK-PND) with almost complete inability to secrete insulin from the first hours of life.

Objective and hypotheses: We report on a large family of polish origin with two members of consanguineous parents having PND and 4 other members with MODY type diabetes.

Methods: Samples from overall 11 family members, including all members with diabetes were collected as dried blood spot or EDTA blood. The 8 Exons and neighbouring intronic regions of human GCK were Saenger-sequenced in both directions, primer sequences and PCR conditions are available on request.

Results: Our index case with MODY type diabetes presented with increased fasting glucose (116 mg/dl) and HbA1c of 6.1% (N < 5.8%). Sequencing of GCK revealed a heterozygous D278E mutation, as it was finally also identified in 6 other family members. The 2 cases with PND were found to have the same mutation in the homozygous state. Heterozygous mutation carriers were characterized by MODY type diabetes (n=4) the female patients have been identified for the first time with gestational diabetes (n=3). The remaining 3 patients with the heterozygous mutation had no obvious symptoms of diabetes.

Conclusions: We report two cases with permanent, neonatal diabetes caused by the homozygous D278E mutation and 7 cases with a heterozygous D278E mutation and a mild phenotype with gestational diabetes or GCK-MODY. The homozygous D278E mutation has been reported earlier in one case with GCK-PND. This family demonstrates phenotype variability and needs for a systematic family screening preventing most importantly gestational diabetes in young women.

P1-d2-222 Diabetes and Insulin 2

Insulin sensitivity (S_i) in obese adolescents estimated by a novel implementation of the oral minimal model decreases with increasing serum alanine aminotransferase

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Background: Although mathematical models of glucose homeostasis like the oral minimal model are valuable research tools for the identification of early alterations in glucose metabolism, these models are often infrequently used due to high computational effort.

Objective and hypotheses: To prove diagnostic feasibility of a novel, readily accessible implementation of the oral minimal model.

Methods: The oral minimal model was paired with a “marginal likelihood” approach, which does not depend on parameters for the uptake except for one parameter ensuring automatic tuning of the uptake curve. The approach allows unsupervised evaluation of parameters of glucose homeostasis. Dynamic insulin sensitivity (S_i) and non-insulin dependent glucose disposal (SGI) were calculated in a sample of n=38 obese adolescents with suspected non-alcoholic fatty liver disease (7age 14.7 ±2.1 years, DBMI z-score 2.79 ±0.48, ØALT 40.1 ±41.2 U/l) from 3h oral glucose tolerance test data.

Results: Validation of the approach demonstrated a low bias <5% in the determination of S_i. Mean S_i in the study population was 6.6 ±4.4. Regrouping the population in quartiles for S_i revealed mean values for S_i ranging from 2.7 ±0.7 in the lowest quartile to 12.4 ±6.6 in the highest, most insulin-sensitive quartile. Mean S_i was 1.26 ±0.35 and ranged from 0.77 ±0.12 in the lowest quartile to 1.65 ±0.10 in the highest quartile. S_i decreased with increasing
ALT level (Spearman’s Rank r -0.4, p<0.05), with a mean Š of 10.8±7.1 in the lowest ALT-quartile to a mean Š of 4.2±2.5 in the highest ALT-quartile (p<0.05).

Conclusions: Our newly developed implementation of the oral minimal model with automatic adjustment of the uptake curve reliably identified a wide range of dynamic insulin sensitivity in obese adolescents. In our cohort, increasing ALT was associated with significantly reduced insulin-dependent glucose disposal and impaired inhibition of glucose production, demonstrating the feasibility of the approach in detecting early alterations in glucose metabolism in a clinical research setting.

P1-d2-223 Diabetes and Insulin 2

The evaluation of metabolic syndrome development risk and adiponectin, leptin, IGF-1, IGFBP-1 Levels in LGA born children during prepubertal period

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Background: In recent years, various studies showed increased risk of insulin resistance in large for gestational age (LGA) born children during prepubertal ages.

Objective and hypotheses: We aimed to evaluate metabolic syndrome risk and the relationship between insulin sensitivity and anthropometric and metabolic parameters in LGA born prepubertal children.

Methods: Forty (19 female,21 male) LGA born prepubertal children (mean age 6.1±2.5 years) were evaluated with respect to glucose, insulin, IGFBP-1, IGF-1, leptin, adiponectin levels. Their data were compared to that of prepubertal 49 (25 female, 24 male) appropriate for gestational age (AGA) children (mean age 5.4±1.8 years).

Results: LGA children were taller, heavier than AGA children but had similar BMI SDS, waist/hip circumference ratio as AGA born children. There were no significant differences in glucose, insulin, HOMA-IR and IGFBP-1 levels between children born LGA and AGA. Adiponectin levels (p<0.009) and IGF-1SDS (p=0.01) were significantly lower in children born LGA. Leptin levels were higher in LGA born children(p=0.000). Univariate variance analysis revealed that being born LGA and having a higher HOMA-IR(higher than 2.5) had significant interaction and was associated with a higher leptin level. The analysis revealed that birth weight SDS, BMI SDS, HOMA-IR were each independent predictors of leptin levels.

Conclusions: The finding of high leptin and low adiponectin levels in LGA born children in prepubertal ages in the absence of obesity show that metabolic derangements can start early in childhood. Adipocytokine levels can be used as early signs of insulin resistance.
As positive predictive values, age at diabetes diagnosis after 1 month and age at remission >12 months give 96% and 93% of chance to find a mutation in ABC28 or KCNJ11 genes.

Conclusions: Clinical characteristics help in the prediction of the genetic aetologies of NDM cases but overlap according to genotype. However, genetic studies are mandatory to elucidate each case and to define optimal treatment.

P1-d2-226 Diabetes and Insulin 2
Abnormal body composition and the progression to abnormal glucose homeostasis in young adult survivors of childhood acute lymphoblastic leukaemia (ALL) treated with bone marrow transplantation and total body irradiation (BMT/TBI)

Background: Insulin resistance (IR) and abnormal body composition (BC) are common in adult childhood leukaemia survivors treated with BMT/TBI, and glucose intolerance increasingly recognised.

Objective: To investigate the relationship between BC and IR in BMT/TBI subjects.

Method: Leukaemia survivors treated with (group1)(n=21) and without (group2)(n=31) BMT/TBI (TBI 10-14.4Gy) were compared with obese subjects (group3)(n=30). IR represented by composite-INSulin-sensitivity index (ISIComp) from oral glucose tolerance tests were compared with BC data from Dual-emission X-ray absorptiometry. Comparisons between groups were made by ANOVA with post hoc Scheffe and relationships between IR and BC explored by Pearson’s correlation with significance at 5%.

Results: Median(range) age was 21.1(16.1-26.2), 21.5(16.1-26.0) and 17.8(16.1-24.8) years respectively. IR was greater in groups 1&3 than 2. Total and trunk fat mass and fat-mass-index (FM-index) correlated with IR in groups 1&2 but not 3. Although group 3 has higher total and central fat masses and BMI than group 1, IR (ISIComp<2.5) became evident at lower degree of central adiposity/trunk fat=4.1±2.8kg) in group 1. There was no correlation between IR and lean mass in any groups.

Conclusion: Although BMT/TBI survivors have lower actual fat mass and FMI, they are more insulin resistant than those with “simple” obesity. Further investigations of mechanism of IR post TBI are required.

P1-d2-227 Diabetes and Insulin 2
Treg function as a marker to define the susceptibility to T1DM clinical onset in a healthy subject with antibodies positivity?

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Background: T regulatory cells are crucial elements in modulating immune responses. Their activity and/or number were shown to be increased in autoimmune diseases.

Objective and hypotheses: To investigate the phenotype and functional status of T regulatory cells in T1DM patients and 10 healthy controls.

Methods: We studied a 20-year-old T1 diabetic male (disease duration:14 years) and his healthy 14-year-old brother, who presented high-titer diabetes antibodies (IAA, ICA, IAA-2, GADA) for 3 years, though maintaining high first-phase insulin response (90th centile). CD4+CD25highCD127low Treg cells were measured in both subjects and in 10 healthy controls by FACScan, sorted and tested for functional analysis. Cells were isolated and cultured with autologous CD4+CD25-responder T cells (Teff) and stimulated with anti-CD3, anti-CD2 and anti-CD28 monoclonal antibodies-loaded beads. The suppressive capacity of Treg towards Teff cells in co-culture was expressed as the ratio between the percentages of cells proliferating in presence or in absence of Tregs according to the formula [100 x (% CFSE low CD4+CD25-T cells in co-culture / % CFSE low CD4+CD25-T cells alone)].

Results: The results of flow cytometric analysis showed similar Treg frequency in the diabetic patient and healthy controls. Treg function (see table) was significantly reduced in diabetic patient as compared to healthy brother and controls.

Poster Presentations
people prior to their clinic appointment.

Aims: 1) To analyse DNA rates when carers/patients were telephoned or texted prior to their clinic appointment over a 2-year period. 2) Did the change in attendance result in improved HbA1c’s?

Methods: Prospective 2 year study with the 1st 8 months serving as control (routine hospital appointments made by patient) followed by an 8 month period of calling carers/patients to remind them of their appointment and the third period of 8 months of text messaging reminders. Paired t testing was used to compare DNA rates in the control and intervention period. The overall control of the clinic as reflected by average HBA1c and HBA1c at end of each period (control/phone/text messaging) was also compared.

Results: Data for 104 patients available. The proportion of missed clinic appointments decreased over the time period, though not statistically significant. (p=0.45) Multi-level linear regression shows that children who attend more clinics have better control: for each additional clinic attended, HbA1c decreased by an estimated average of 0.3 (p=0.004). Those who were spoken to every time a call was made had significantly better attendance; Those who replied to all texts had better attendance (p<0.001), fewer DNA’s (p<0.001), and better control.

Conclusion: Clinic appointment reminders particularly text messaging can be used to increase attendance rates in a paediatric diabetes clinic and this is reflected in better glycaemic control.

P1-d2-229 Diabetes and Insulin 2

Future HbA1c is associated to the level of diabetic ketoacidosis at onset of T1DM

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Background: Since 1996 The Danish Childhood Diabetes Register has collected data from all Danish paediatric diabetes centres treating type 1 diabetic patients aged 0-15 years. All newly diagnosed type 1 diabetic patients < 15 years have been enrolled since 1996.

Objective and hypotheses: (i) To evaluate the frequency and severity of DKA at onset and (ii) its association to future metabolic control.

Methods: DKA status was defined as: (i) none: HCO3 > 15 or pH > 7.3; (ii) mild: HCO3 < 15 or pH < 7.3; (iii) moderate: HCO3 < 10 or pH < 7.2 and (iv) severe: HCO3 < 5 or pH < 7.1 from blood gas analyses. Central HbA1c determination from all participants were analysed by means of multiple regression using age, gender, ethnicity, diabetes duration and DKA status as explanatory variables in a compound symmetric repeated measurement model.

Results: A total of 2939 recordings (1414 girls (48.1%) and 1525 boys (51.9%)) in 3364 persons were included in the analysis as 425 individuals did not have complete DNA data sets. 2422 individuals (62.4%) presented without DKA, whereas 237 (8.1%), 233 (7.9%) and 47 (1.6%) presented in mild, moderate and severe DKA, respectively. The multiple regression analysis revealed association of higher HbA1c levels over time (average diabetes duration 4.49 yrs) of 0.10%, 0.21% and 0.47% (p=0.002) for mild, moderate and severe DKA presentations compared to no DKA at onset. The HbA1c levels increased significantly with age (p<0.001) and was higher in the immigrant population. There was no effect of gender.

Conclusion: Presentation of T1D in DKA associates to higher HbA1c years ahead. Possible explanatory factors e.g. residual beta-cell function or adherence to treatment needs further exploration.

P1-d1-230 Endocrine Oncology 1

Papillary carcinoma of the thyroglossal duct cyst: case report in a 12 year old girl

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Background: Thyroglossal duct cysts (TGDC) are the most common thyroid developmental anomalies accounting for 75% of midline neck tumors in children and in adults. Carcinoma of the TGDC has been reported in less than 1% in adults.

Objective and hypotheses: Describe the case of a pediatric papillary carcinoma of the TGDC.

Methods: Case report

Results: A 12 year old girl presented with an asymptomatic fast growing neck mass noticed 7 months previous to consultation. Her past medical history was unremarkable. US revealed a cystic-solid mass of 21 x13 mm with microcalcifications with a normal thyroid gland. With a presumptive diagnosis of TGDC a Sistrunk procedure was performed. Histological evaluation showed a papillary carcinoma of 12x6mm in the wall of a 35x25x25mm TGDC. The patient was referred to our Unit for follow up. Physical examination revealed an euthyroid pubertal girl with a non palpable thyroid gland without palpable cervical nodes. Neck US showed a normal eutopic thyroid gland without suspicious adenopathies. Neck and chest CT scan were normal. Histologic examination after total thyroidectomy revealed no tumor. Postoperatively, an ablative 131I dose of 50 mCi was administered. WBS performed on day 5 revealed focal radioidine uptake confined to the inferior cervical region. Cervical US revealed a small yugular adenopathy of 15x7mm with an heterogeneous vascularized rounded area. FNAB cytology was positive for papillary carcinoma with positive thyroglobulin in the needle wash-out .Surgical excision was performed with histologic diagnosis of papillary metastatic infiltration.

Conclusion: Although exceptional in pediatrics rapid growth of a thyroglossal duct cyst excluding infection and/or US signs suggestive of malignancy should alert of the possibility of TGDC carcinoma. The lack of thyroid involvement does not rule out the presence of metastasis and follow up should be the same as for differentiated thyroid cancer.

P1-d1-231 Endocrine Oncology 1

Chemotherapy not growth hormone (GH) implicated in second primary tumours in survivors of childhood brain tumours

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Background: Second Primary Tumours (SPT), such as surgically resectable meningiomas and thyroid tumors, are known late effects of Posterior fossa Brain Tumours (PFBT) attributed to high dose radiation scatter at the field’s edge. Concerns that their prevalence may be increased by GH therapy have been raised but adjuvant chemotherapy and genetic cancer predisposition may also contribute.

Methods and aims: In a long term (>10 years) evaluation of late outcomes, we performed surveillance MRI brain scans on 103 (65% Male) PFBT survivors (68 Primitive neuroectodermal tumours [PNET], 18 Astrocytomas, 17 Ependymomas) with a mean age of 21.8 (17-38) years transitioning to adult services. 54 (49%) had received childhood GH replacement, especially after PNET (80%). Non-CNS SPTs, prior oncological therapy and any known genetic cancer predisposition were noted.

Results: Over a median 14.4 (2.8-34.8) years, 20 SPTs occurred in 14 (29%)M neuraxially irradiated PFBT survivors, all but one (ependymoma), being PNET survivors. 11 had received prior GH therapy. Two patients each had 2 and 3 SPT’s respectively, one of whom was found to harbour a FAP gene mutation. 10 (50%) SPTs occurred in the 8 PNET patients given che-
motherly, these occurring earlier (12.3 vs 19 years) and tending to higher grade (sarcoma, leukemia) than in the 5 given chemotherapy. Three had never received GH.

Conclusion: PNET survivors are particularly susceptible to SPTs, 20% (1.5) developing at least one over an average time similar to that reported (13 vs 15 years), but at a significantly greater (4%) prevalence than (2-3%) expected. Adjuvant chemotherapy, not GH, doubled the risk of, and halved the time to, SPT after PNET. These novel and preliminary data suggest that in PNET, the 10-15% increase in survival brought by adjuvant chemotherapy is offset by its potentiation of the number and severity of SPT’s, in which GH replacement does not appear implicated.

P1-d1-232 Endocrine Oncology 1

Resveratrol induces cell death in human cancer cells and targets NAMPT

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Background: Nicotinamide phosphoribosyltransferase (NAMPT) is a key enzyme of NAD biosynthesis and regulates the activity of the NAD-dependent deacetylase sirtuin 1 (SIRT1). SIRT1 is implicated in multiple aging-related diseases including cancer. Resveratrol (Resv) is a potential SIRT1 activator and has been proven to be an effective chemopreventive agent.

Objective and hypotheses: We hypothesized that NAMPT acts as key player in the Resv-mediated apoptosis of cancer cells through a reduced metabolisation of nicotinamide and consequently inhibition of SIRT1.

Methods: HepG2 cells, Jurkat cells, primary hepatocytes and PBMC were cultured. Induction of apoptosis was measured by Annexin/PI staining and Western Blotting of phosphorylated p53. NAMPT protein level and its enzymatic activity were quantified using a NAMPT ELISA and a radioactive filter assay. NAD levels were measured by HPLC analysis.

Results: Resv reduced cell proliferation and caused an increase of cells in the S- and G2/M-phase of the cell cycle in HepG2 cells. Additionally, Resv induced apoptosis (68.7±10.7%), phosphorylation of p53 and an increased acetylation of p53. It also reduced the intracellular NAMPT protein by 48.1±15.4% and the NAMPT activity by 49.5±2.3%. In contrast, primary human hepatocytes did not show significant changes in NAMPT protein expression and cell viability. In human leukemia Jurkat cells we observed a reduction of cell viability after incubation with Resv. Treatment of leukemia cells with Resv and different chemotherapeutics induced cell death in an additive manner.

Conclusions: We demonstrated that Resv exerts opposite effects on cancer cells and normal, primary cells regarding cell viability, apoptosis, NAMPT protein amount and activity. In leukemia cells Resv might act as a chemo-sensitizer. The apoptotic effects of Resv on cancer cells might be mediated through a reduced NAMPT activity and consequent inhibition of SIRT1 which leads to an increased p53 mediated apoptosis.

P1-d1-233 Fat Metabolism and Obesity 1

Plasma brain-derived neurotrophic factor in prepuberlal obese children: results from a two-year lifestyle intervention programme

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Context: BDNF (brain-derived neurotrophic factor) is a neurotrophin potentially involved in the pathophysiology of obesity and metabolic syndrome in adults. In children, it has scarcely been studied.

Objective: To analyze plasma BDNF and its relationship with metabolic syndrome components before and after two years of a lifestyle intervention programme in a prepuberlal obese cohort.

Design and setting: Case-control study with a 2-year prospective follow-up in a referral paediatric endocrine outpatient centre. Patients and methods: Seventy-three prepuberlal obese children, 8.03±1.08 years old and 47 age and gender-matched lean controls were studied. Anthropometric parameters, blood pressure, platelet count, oral glucose tolerance test, homeostatic model assessment for insulin resistance (HOMA-IR), lipid profile, BDNF, diet and physical activity were evaluated. Weight loss was considered if z-score body mass index (BMI) decreased at least 0.5 SD.

Results: At baseline, BDNF tended to be lower in prepuberlal obese children compared to lean controls (p=0.076). BDNF did not correlate with any metabolic syndrome component. After two years, obese patients showed an increase in BDNF. Regression model analysis adjusted by age, sex, puberty, BMI, platelet count and HOMA-IR showed that BDNF increased in subjects who lost weight (p=0.036), practiced sports (p=0.008) and had an adequate carbohydrate intake (p=0.032).

Conclusions: Plasma BDNF tends to be lower in obese prepuberlal children than in lean controls, is not related to any other metabolic syndrome component and increases after a lifestyle intervention programme.

P1-d1-234 Fat Metabolism and Obesity 1

Age at adiposity rebound: a novel method to identify children at risk for overweight or obesity in the general population

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Background: BMI-for-age z-score (zBMI) is commonly used to screen children that are at risk for overweight or obesity. However, the sensitivity of zBMI in identifying these children is suboptimal. Adiposity rebound (AR) at young age correlates with excess weight gain, but has been insufficiently explored in screening of overweight and obesity in the general population.

Objective and hypotheses: To compare how the age at AR and zBMI in children aged 2-7 years predict development of overweight and obesity after 10 years of age.

Methods: Longitudinal growth data of full-term, appropriate-for-gestational age (AGA) healthy girls (n=2,606) and boys (n=2,628) with 25,837 and 25,629 height and weight measurements from birth to over 10 years of age were included. Age at AR was defined as the nadir of the fitted curve through the observed BMI-for-age data points. The screening accuracy of the age at AR and zBMI (at different ages) for the subsequent overweight and obesity were evaluated using Receiver Operating Curve-analyses after 10 years of age.

Results: Overall, zBMI showed good to excellent accuracy in predicting overweight or obesity in girls (AUCs 0.83 and 0.94) and fair to good accuracy in boys (AUCs 0.79 and 0.86) at 2-7 years (Figure 1). The accuracy of prediction increased with age. At 7 years overweight or obesity were detected already relatively well (AUCs 0.91 and 0.97 for girls, 0.89 and 0.94 for boys, respectively). However, the age at AR was superior to zBMI at 7 years for overweight and obesity, with AUCs 0.96 and 0.98 for girls and 0.96 and 0.98 for boys, respectively.

Conclusions: In full-term AGA children the risk for excess weight gain leading to overweight and obesity is identified more accurately by the age at AR than by zBMI at any age.
P1-d1-235 Fat Metabolism and Obesity 1
Association of urinary bisphenol a with insulin resistance in obese children and effects on adiponectin and resistin expression
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Background: Bisphenol A (BPA) is used in polymerization reaction to produce plastics for food and water containers, baby bottles, lining of food and beverage metal cans and medical tubing. Small amount of BPA can migrate from the polymers to food and water upon heating. High urinary BPA concentrations have been associated in adults with cardiovascular diseases and with diabetes and, recently, with insulin resistance in elderly subjects.

Objective and hypotheses: We wanted to determine i) whether BPA associates with insulin resistance in obese children ii) the effect of BPA on adipokine expression in human mature adipocytes.

Population and methods: We enrolled 98 obese children (age 10±2.3 years, BMI-z-score 3.7±1.5). Blood pressure, waist circumference, lipids, insulin able glucose were measured. Insulin resistance was evaluated by HOMA: Urinary BPA was measured by HPLC. Preadipocytes, obtained from subcutaneous abdominal adipose tissue, were differentiated to mature adipocytes and were treated for 24 h with increasing doses of BPA (1nM, 10 nM, 100 nM).

To evaluate the effect of BPA on adiponectin and resistin expressions, a Real-Time PCR was performed.

Results: Mean urinary BPA was 1.1mg/ml (0.47-1.3). No correlations were found between BPA and BMI-z-score, waist, lipids and blood pressure. A GLM showed a significant positive correlation between BPA and HOMA (p = 0.03), adjusting for waist circumference, age and sex. Adiponectin mRNA levels were significantly inhibited (nearly 4 fold) in the adipocytes treated with BPA. Resistin mRNA, on the contrary, was not detectable in not treated cells but was present, in dependant-dose manner, in BPA treated adipocytes.

Conclusions: Our results suggest that i) BPA may represent one of the factors able to modulate insulin resistance in childhood obesity and that ii) this action appears to be due to a combined effect of BPA on adipocyte expression and resistin expression stimulation.

P1-d1-236 Fat Metabolism and Obesity 1
Mechanography in childhood: new tests and new references
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Background: Mechanography offers tests that reflect everyday motor function, but solid reference values for young children were missing.

Objective and hypotheses: Mechanography offers The aim of this study was to establish reference values for body mass related peak force (RPF), peak power (RPP) and force efficiency (FE) in children for counter movement jumps, one legged hopping and chair rising tests.

Methods: A total of 868 subjects (432 male, 436 female) aged 3 to 19 years were studied. In addition to auxological measurements and maximal isometric grip force (MIFG), the following movements were performed and assessed using the Leonardo Mechanograph®: Single Two Legs Jump (s2LJ), Multiple One Legged Hopping (m1LH) and Chair Rising Test (CRT).

Results: We present weight-adjusted age-, gender-related normative values for each of these tests and discuss their dynamics through childhood. Body weight related results are reported as multiples of earth’s gravity (g). Maximal voluntary force (RPFmLH) during hopping on one foot was found to be constant and independent of age and gender in the age group between 5 and 19 at 3.33 g (SD 0.31 g). Peak jumping power in relation to body mass during counter movement jump for maximum height (RPPs2LJ) was found to be almost linearly increasing with age for males age 5 to 19 and female age 5 to 11 at a rate of 4.6 W/kg per year. RPPs2LJ for females age 12 to 19 increased only slightly by less than 10%. CRT time per repetition was constant and independent of age and gender. Peak power per body mass during the rise phase (RPPCRT) showed similar but smaller age and gender relations as peak power during s2LJ.

Conclusions: This data from a healthy Caucasian middle class population hardly touched by the obesity and media exposure epidemics provide ideal reference values for these tests; they have been incorporated into Leonardo Mechanograph®.

P1-d1-237 Fat Metabolism and Obesity 1
Metabolic and haemodynamic aspects of cardiovascular risk in obese adolescents
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Background: Epidemiologic data shows an increasing tendency of obesity in childhood. Furthermore the exact nature of cardiovascular risk in a paediatric population remains obscure.

Hypothesis: Detrimental cardiovascular changes in obese adolescents are a result of metabolic-hemodynamic relationships.

Methods: 117 obese and 32 overweight adolescents were studied using detailed anthropometry, tissue doppler echocardiography, 24-h blood pressure monitoring, carotid intima-media thickness (CIMT). Fasting lipids, carhs, leptin, adiponectin, TNF-α, uric acid concentrations were measured. Subjects were grouped according to BMI: +1-2SD (Gr.1); 2-3SD (Gr.2) and >3SD (Gr.3). Nonparametric statistic analysis and multiple regression technique were performed.

Results: There is a valid correlation between Left Ventricular Mass Indexed and increasing BMI, HOMA (p=0.004), FFA (p=0.004), waist to height ratio (p <0.003), TNF-α (p=0.032). Established eccentric LV remodelling is a result of both increased systolic BP (p=0.026) and hypercholesterolemia (p=0.001). Systolic function (by the EF,%) significantly correlated with the total cholesterol (p=0.037), HDL (p=0.005) and % body fat (p<0.017). Diastolic LV function was dependent on HOMA (p=0.001) and revealed reduced diastolic filling time. The 24hr BP profile is characterized by both increased systolic and diastolic BP. Levels of systolic BP demonstrate a direct relationship with BMI (p=0.003), uric acid (p=0.002), HOMA (p=0.002) and diastolic BP - with leptin (p=0.012), HOMA (p=0.016). Increasing levels of obesity are accompanied by increasing CIMT, vascular stiffness and maximum systolic flow velocity without significant changes in systolic-diastolic gradient. CIMT was associated with abdominal fat predisposition (p=0.038), diastolic BP (p=0.004), diastolic load (p=0.001), uric acid (p=0.03) and TNF-α (p=0.05).

Conclusions: Myocardial and vascular remodelling in paediatric obesity may be stepwise contributed by excess abdominal adipose mass, insulin resistance, chronic subclinical inflammation and hypertension.

P1-d1-238 Fat Metabolism and Obesity 1
Vitamin D status in obese children and its recovery after weight loss are influenced by the distribution of adipose tissue, adiponectin levels, ethnic background, pubertal stage and metabolic impairment
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Background: Socioeconomic background, sex and puberty can influence 25-OH-vitamin D (vD) levels in obese children. Its anthropometrical, metabolic and hormonal determinants remain to be characterized.

Objective and hypotheses: To evaluate the relationship between vD status, body fat, bone mineral density (BMD), metabolic impairment and adiponectin levels in obese children before and after weight loss.

Methods: Obese children (n=150; 11.8±2.9 years; 4±1.4 BMI-SDS; 50% sex; 75 Latinos and 75 Caucasians) were enrolled. Serum glucose, insulin, lipid profile, body composition (DXA), abdominal magnetic resonance imaging, liver ultrasonography, vD (Chromoluminiscence, Liaison®) and total (RIA) and HMW-adiponectin (ELISA) levels (Millipore®, USA), were studied at baseline and after BMI reduction of over 1.5 SDS (n= 33).

Horm Res 2012;78(suppl 1) 71
Results: Around 88% were vD insufficient (10-30ng/ml); 6.4% deficient (<10ng/ml), regardless of their sex, but with lower vD levels in adolescents (17.7±6.9 vs. 21.1±6.9, p<0.01) as in Latinos (15.9±6.0 vs. 21.9±6.8, p<0.001). vD negatively correlated with trunk fat (p<0.05), but not with visceral fat or BMD. Liver steatosis determined lower vD (13.1±5.8 vs. 16.1±4.4;p<0.05). vD positively correlated with total and HMA-aldosterone (both p<0.01), HDL (p<0.05) and negatively with VLDL, triglycerides and HOMA index (all p<0.01), with vD deficient children showing higher VLDL, triglycerides and HOMA (Table). Weight loss increased vD exclusively in prepubertal children (20.1±6.5 vs. 26.9±5.6; p<0.05).

Conclusions: Decreased vD levels in obese children are highly prevalent, independently associated with metabolic impairment and ectopic fat deposition and strongly influenced by puberty and ethnic background.

P1-d1-239 Fat Metabolism and Obesity 1

Can inflammatory marker levels help in detecting early complications of childhood obesity? 
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Background: Excess body weight may be associated with a state of chronic low-grade inflammation in childhood. There is increasing evidence that intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are biomarkers of endothelial dysfunction.

Objective and hypotheses: Aim of this study was to investigate the relationship of inflammation and endothelial activation in children with severe obesity and healthy controls. Data were also analyzed according to presence of insulin resistance (IR), metabolic syndrome (MS), and non-alcoholic fatty liver disease (NAFLD).

Methods: Thirty-seven (16 boys) obese children and adolescents (11.3±2.76 yr; BMI SDS 3.42±0.78) were examined and compared with 17 normal weight subjects matched for age and sex. Fasting levels of interleukin-6 (IL-6), ICAM-1, VCAM-1, and endothelin were determined. IR was assessed by the homeostasis method and MS was defined using Weiss criteria.

Results: Serum ICAM-1 concentrations resulted significantly higher in obese subjects compared to controls; IL-6, VCAM-1, and endothelin levels were non different. In obese patients VCAM-1 concentrations resulted positively correlated with both waist (r=0.33, p=0.048) and hip circumference (r=0.38, p=0.021). ICAM-1, despite high levels, was not correlated with measures of adiposity. Analyzing data according to presence/absence of IR, MS and NAFLD we did not find significant differences in inflammatory marker levels. Multivariate regression analysis did not identify each inflammatory marker as significant predictive factor for IR, MS, and NAFLD.

Conclusions: Considering the high levels of ICAM-1 and the correlation of VCAM-1 with measures of adiposity, our concern is on the correct approach in managing our obese subjects to precociously indentify the formation of the atherosclerotic plaque. Moreover, in our study inflammatory marker were not identified as predictor factors for IR, MS, and NAFLD so other studies are needed to better understand which other markers can help us for the precocious diagnosis of these complications.

P1-d1-240 Fat Metabolism and Obesity 1

Chronic lepton administration induces changes in circulating and adipose tissue inflammatory cytokines
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Background: Obesity is characterized by hyperleptinemia, reduction of insulin sensitivity and an augment in circulating inflammatory factors synthesized by adipose tissue, among other tissues.

Hypothesis: Leptin promotes synthesis of cytokines in subcutaneous and visceral adipose tissue, generating a proinflammatory profile related with insulin resistance.

Methods: We studied 18 male Wistar rats divided into three groups; rats receiving saline ivc (controls, C), treated ivc for 14 days with a daily dose of 12 µg of leptin (L) and a pair-fed group (PF) that received the same food amount consumed by L. Serum leptin and insulin were measured by ELISA, mRNA levels of interferon-γ (IFN-γ), interleukins (IL) 2, 4, 6 and 10 and tumor necrosis factor-α (TNF-α) by real-time PCR and serum and subcutaneous and visceral adipose tissue levels of these cytokines by multiplexed bead immunoassay. Activation of signal transducer and activator of transcription 3 (STAT3) and protein kinase B (Akt) and levels of suppressor of cytokine signaling 3 (SOCS3) and insulin receptor in both fat depots were performed by Western blot.

Results: Serum leptin, IL-2, IL-4, IFN-γ and homeostasis model assessment of insulin resistance (HOMA-IR) index were increased in L and TNF-α was decreased in PF and L. Serum leptin, IL-2 and IL-4 levels correlate positively with HOMA-IR index. In L group, an increase in mRNA levels of IL-2 was found in both adipose depots and IFN-γ only in visceral tissue. Activation of leptin signaling, measured by the phosphorylation of STAT3, is increased in subcutaneous fat of L, and insulin signaling, determined by phosphorylation of Akt, is decreased, in subcutaneous adipose tissue of L group.

Conclusion: Leptin mediates the production of inflammatory cytokines by white adipose tissue independently of food intake reduction, generating a state of peripheral insulin resistance.

P1-d1-241 Fat Metabolism and Obesity 1

Differential effects of increased bodyweight due to neonatal over-nutrition and a sucrose-enriched diet on hypothalamic inflammation and glial markers
Esther Fuente-Martínte; Cristina García-Cácereste; Miriam Granado; Miguel A. Sánchez-Garridote; Manuel Tena-Sempere; Jesus Argente; Julie A. Chowent
1Hospital Infantil Universitario Niño Jesús, Universidade Autónoma de Madrid, CIBERObn, Endocrinology, Madrid, Spain; 2University of Córdoba, Cell Biology, Physiology and Immunology, Córdoba, Spain

Background: Neonatal over-nutrition (NON) increases the propensity towards obesity in adulthood, with this being aggravated by a high fat diet. Recently hypothalamic inflammation and gliosis have been associated with obesity onset and exacerbation. However, current dietary habits also include increased carbohydrate consumption and little is known regarding its effects on hypothalamic inflammation.

Objective and hypotheses: Our aim was to determine how NON affects the response to a sucrose-enriched diet and if metabolic changes are associated with modifications in hypothalamic glial and inflammatory markers.

Methods: At birth, litters of Wistar rats were adjusted to 12 (control) or 4 (NON) pups. At weaning, half of each group received sucrose supplemented water (33% solution) ad libitum. Male rats were killed at 80 days of age (n=8-12/group) and serum and brains collected.

Results: NON rats weighed more than controls at weaning and sacrifice (ANOVA: p<0.0001). Sucrose intake decreased weight gain in spite of a higher Kcal intake, but increased fat mass and altered metabolic hormone levels (insulin: p<0.0001; leptin: p<0.0001; acylated ghrelin: p<0.01). Sucrose intake increased hypothalamic NPY mRNA levels in all rats (p<0.05), but increased AgRP and decreased LepR mRNA levels only in controls. NON increased the number of astrocytes in the arcuate nucleus (p<0.005), the num-

Poster Presentations
P1-d1-242 Fat Metabolism and Obesity 1  
**The metabolic response to a sucrose-enriched diet is differently affected by prenatal stress in males and females**  
Eva Baquedano1; Yolanda Diz-Chaves2; Luis Miguel García-Segura2; Julie A. Chown2; Jesús Argente1; Laura M. Frago1  
1Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, CIBERObn, Endocrinology, Madrid, Spain; 2Instituto Ramón y Cajal, CSIC, Functional and Systemic neurobiology, Madrid, Spain  

**Background:** Gestational stress affects fetal growth and has long-term effects on the response to later metabolic challenges. Increased sucrose consumption is suggested to be involved in the rise in obesity and some of the long-term effects of early stress differ between males and females.  

**Objective and hypotheses:** Our aim was to investigate whether prenatal stress affects metabolic parameters in response to a sucrose-enriched diet in adulthood and if this response is sexually dimorphic.  

**Methods:** Pregnant C57BL/6 mice were subjected to restraint stress from gestational day 12 to delivery. After weaning, mice were allowed to eat normal Chow ad libitum. At 2 months of age 8 mice of each group were given a solution of 33% sucrose instead of water for 2 weeks and then sacrificed. The experimental groups were: control+water (CtW), control+sucrose (CtS), prenatal stress+water (PSW) and prenatal stress+sucrose (PSS).  

**Results:** Serum leptin levels increased in PSS males and in CtS and PSS females. Both prenatal stress and sucrose intake decreased insulin and IGF-I levels. CtS and PSS males had increased POMC and CtS increased NPY mRNA levels. POMC and NPY mRNA levels were decreased in CtS, PSW and PSS females (Table 1).  

<table>
<thead>
<tr>
<th>MCTW</th>
<th>MCTS</th>
<th>MPSW</th>
<th>MPSS</th>
<th>FCTW</th>
<th>FCTS</th>
<th>FPSS</th>
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<tr>
<td>Leptin</td>
<td>±116</td>
<td>±137</td>
<td>±190</td>
<td>±238</td>
<td>±69</td>
<td>±143</td>
<td>±73</td>
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<tr>
<td>Insulin</td>
<td>±96</td>
<td>±30</td>
<td>±14</td>
<td>±45</td>
<td>±25</td>
<td>±50</td>
<td>±15</td>
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<tr>
<td>IGF-I</td>
<td>±68</td>
<td>±34</td>
<td>±29</td>
<td>±47</td>
<td>±47</td>
<td>±35</td>
<td>±53</td>
</tr>
<tr>
<td>NPY</td>
<td>±10</td>
<td>±23</td>
<td>±15</td>
<td>±15</td>
<td>±7</td>
<td>±10</td>
<td>±51</td>
</tr>
<tr>
<td>POMC</td>
<td>±10</td>
<td>±25</td>
<td>±52</td>
<td>±9</td>
<td>±10</td>
<td>±35</td>
<td>±21</td>
</tr>
</tbody>
</table>

Table 1. Leptin, insulin and IGF-I levels in serum. NPY and POMC mRNA levels measured by RT-PCR. M. males; F. females * p<0.05 vs CTW; # p<0.05 vs PSW; $ p<0.05 vs CTW.  

**Conclusions:** The metabolic response to a sucrose-enriched diet is affected by prenatal stress differently in males and females.

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P1-d1-243 Fat Metabolism and Obesity 1  
**Leptin-induced depletion of hippocampal somatostatinergic system promotes anorexigenic effects**  
Vicente Barrios1; Arancha Perianes-Cachero2; Emma Burgos-Ramos1; Lilian Puebla-Jiménez1; Sandra Canelles2; Jesús Argente1; Eduardo Arilla-Ferreiro2  
1Hospital Infantil Universitario Niño Jesús, Endocrinology, Madrid, Spain; 2Universidad de Alcalá, Biochemistry and Molecular Biology, Alcalá de Henares, Spain  

**Background:** Recent evidence indicates that the hippocampus, a brain area critical in learning and memory, is involved in the regulation of food intake and energy homeostasis. Leptin and somatostatin, as well as their receptors, are widely distributed in this area and have opposite functions in energy regulation in other brain structures. In addition, several actions of these hormones are mediated by opposite changes in intracellular cAMP levels.  

**Hypothesis:** Leptin-mediated suppression of food intake is related to changes in the hippocampal somatostatinergic system.  

**Methods:** We studied 18 male Wistar rats which were divided into three groups; controls (C), rats treated iv with 14 days with 170 µg of leptin (L) and a pair-fed group (PF) that received the same food amount consumed by L. Somatostatin content was measured by RIA, somatostatin receptors by a binding assay and activity of adenyl cyclase (AC) by a functional assay. The levels of the inhibitory G protein (Gi) subunits 1-3 and AC-1 and AC-VI isoforms, as well as activation of signal transducer and activator of transcription 3 (STAT3), protein kinase B (Akt) and cyclic AMP response element binding protein (CREB) were determined by Western blot.  

**Results:** The density of hippocampal somatostatin receptors is increased in PF and decreased in L, being due to changes in somatostatin receptor 2 protein levels. These changes in PF are concurrent with activation of hippocampal Akt and CREB. The inhibitory effect of somatostatin on AC activity, however, was lower in L group, coincident with lower G inhibitory ∼3 and higher AC-I isoform levels and STAT 3 activation.  

**Conclusions:** These results suggest that activation of somatostatinergic system after food restriction may be a mechanism to potentiate the hippocampal anorexigenic mechanisms in a situation of metabolic demand, whereas depletion of this inhibitory system after leptin infusion may represent a mechanism to potentiate anorexigenic effects of leptin.

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P1-d1-244 Fat Metabolism and Obesity 1  
**The Val/Met-polymorphism and serum levels of BDNF in German children and adolescents: impact on metabolic parameters associated with obesity**  
Agnes Kalenda1; Kathrin Landgraf2; Dennis Löfler3; Kathrin Dittrich4; Madlen Neef; Wieland Kieß; Antje Körner5  
1University of Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany  

**Background:** The neurotrophin BDNF plays an important role in the development of neuronal structures and is found altered in clinical entities such as depression, anxiety and eating disorders. In recent years, a direct interaction with metabolic pathways has been discussed. However, rare data exist on a possible connection between BDNF and early-onset obesity.  

**Objective and hypotheses:** The aim of our study was to investigate the association of both BDNF polymorphism rs6265 and BDNF serum levels with metabolic parameters in children.  

**Methods:** We genotyped the Val/Met-polymorphism rs6265 of BDNF in 1171 lean and 1002 obese children and adolescents. Furthermore, we measured BDNF serum levels by implementing an ELISA in a subset of 371 children. Statistical analyses were performed in order to investigate an influence of BDNF on parameters of glucose and lipid metabolism.  

**Results:** BMI-SDS was significantly lower in carriers of the minor Met-allele (r= -.071; p=0.001). Moreover, Met-allele carriers showed a reduced increase in levels of blood glucose following oral glucose challenge (AUC blood glucose: r= -.166; p=0.014) and reduced HbA1c levels (r= -.181; p=0.017), independent of BMI-SDS. This was only seen in the post-pubertal stage. An association of the polymorphism with fasting blood glucose or insulin parameters was not observed. BDNF serum levels were not changed in Met-allele carriers compared to wildtype. Furthermore, they were neither correlated with BMI-
Objective and hypotheses: The aim of this study is to investigate a potential regulatory role of the obesity-associated genes MTCH2, NERG1 and TMEM18 during adipose tissue development in vivo by applying the zebrafish (Danio rerio) as a model organism.

Methods: We identified orthologs of MTCH2, NERG1 and TMEM18 in the zebrafish. We analysed their expression profile in sequential developmental stages from fertilization to adult organs by quantitative real-time PCR and in situ hybridization. In addition, we assessed the effect of high-fat diet and starvation on candidate gene expression.

Results: In situ hybridization analyses of early developmental stages (4 hpf-96 hpf) revealed that mtch2 expression was mainly present in the developing brain, liver, and intestine, while nerg1 was restricted to neural tissues (eyes, brain, spinal cord). The expression of mtch2, nerg1 and tmem18 was significantly up-regulated at 9 dpf. At this stage, we could also detect first visceral adipocytes by Nile red staining. Moreover, mtch2, nerg1 and tmem18 were expressed in adipose tissue of adult zebrafish. Interestingly, both mtch2 and tmem18 mRNA levels were down-regulated in adipose tissue after 7 days of high-fat diet. In contrast, nerg1 expression was not affected by high-fat diet but up-regulated after starvation.

Conclusions: In zebrafish, mtch2, nerg1 and tmem18 are activated with the start of adipogenesis and regulated by nutrition indicating a potential role during adipogenesis in vivo.

P1-d1-246 Fat Metabolism and Obesity 1

Circadian clock gene expression in leukocytes of obese and lean subjects
Tobias Drechsler; Isabel Wagner; Daniela Friebe; Kathrin Dittrich; Dennis Löfler; Julia Gesing; Wieland Kiess; Kathrin Landgraf; Antje Körner
University of Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany

Background: Recent studies described associations of clock genes with glucose and fat metabolism. However, a potential association of clock gene expression with obesity in humans has not been assessed so far.

Objective and hypotheses: The aim of this study was to assess potential differences in clock gene expression levels in obese and lean subjects.

Methods: Based on previous publications, we selected three clock-genes (CLOCK, CR1, and PER2) and assessed their expression in leukocytes isolated of lean and obese subjects. We analysed the expression profile of each gene during a 29h period including standardized meals, oral glucose tolerance test, restig period and a 30 minute sport unit, and assessed the response to nutrition, sport and oral glucose load.

Results: Measuring CLOCK, CR1, and PER2, we could detect circadian rhythmity. Moreover, clock gene expression of Per2 was lowered to 78.4% in a subject with BMI 30.8 and to 13.7% in a subject with BMI 49.7 compared to a lean control, which points to a correlation between clock gene expression and BMI. Additionally, we observed an increased expression after the meals and oral glucose load, indicating an effect of metabolic factors on clock gene expression levels.

Conclusions: Our data support an association between clock-gene expression and BMI, indicating a bigger role of clock genes in obesity and obesity related diseases.

P1-d1-247 Fat Metabolism and Obesity 1

GHR exon 3 polymorphism and metabolic parameters in obese children
Felix Schreiner 1; Christian L. Roth 1; Bettina Gohike 1; Joachim Woelfler 1
1Children’s Hospital, University of Bonn, Pediatric Endocrinology, Bonn, Germany; 2Seattle Children’s Research Institute, Endocrinology, Seattle, WA, United States

Background: The growth hormone receptor exon 3 polymorphism (fl = full length / d3 = deletion of exon 3) has been associated with better growth response to rhGH in several study cohorts. We previously reported this variant to be associated with spontaneous catch-up growth and metabolic parameters such as HbA1c and fasting IGFBP1 levels in former extremely low birth weight preterm infants. Recently GHrD3 has also been linked to metabolic properties in obese children.

Objective: We analyzed auxological and metabolic parameters with respect to the GHR exon 3 polymorphism in a cohort of 97 obese children (mean age 10.8 yrs; range 9.7-17.2 yrs).

Results: Genotype frequencies (fl/fl 54%; fl/d3 38%; d3/d3 8%) were comparable with those reported from non-obese children. Auxological parameters including BMI-SDS (fl/fl 2.74±0.56; d3-carrier 2.71±0.50) did not differ between genotype groups. We also did not find significant associations with IG-I-IGF-I, IGFBP-3, DBP, or parameters of glucose metabolism such as fasting glucose, insulin, HOMA-IR, HbA1c, and IGFBP1. Ghrelin levels, which significantly correlated with age (R -0.30), BMI-SDS (R -0.21), IGF-I (R -0.47), IGFBP3 (R -0.30), IGFBP1 (R 0.53), fasting insulin (R -0.27), and leptin (R -0.43), appeared to increase with every GHR d3-allele inherited (fl/fl 1074.6 ±329.3; fl/d3 1199.6 ±486.6; d3/d3 1325.5 ±599.4 pg/ml; p=0.049 after correction for age; p=0.030 when analyzing only prepubertal children (n=41)). Leptin levels did not differ between genotype groups.

Conclusions: In summary, we did not find significant effects of the GHR exon 3 genotype on body size or parameters of glucose metabolism. However, Ghrelin levels known to be decreased in obese as compared to non-obese children seem to be lowest in fl/fl-homozygous individuals. Given the still incomplete understanding of the role of GHrD3 in growth and metabolism, this finding warrants further investigation.
**Results:** E/I ratio (p=0.007), ln(HF) (p=0.001), pupil diameter in darkness (p=0.007), and pupil re-dilation velocity (p=0.03) were negatively correlated with BMI-SDS. Gender, but not BMI-SDS, was significantly associated to SBR (lower parasympathetic: p=0.01; upper limbs: p=0.02). Latency of pupillary constriction showed a significant dependence on age (p=0.02).

**Conclusions:** These findings demonstrate widespread ANS dysfunction in overweight and obese children and adolescents, involving some organ systems. Both parasympathetic and sympathetic activity is reduced. The pattern of ANS dysfunction resembles that observed in normal-weight diabetic children and adolescents.

### P1-d1-249 Fat Metabolism and Obesity 1

**Improved oxidative stress and insulin sensitivity in obese prepubertal children with liver steatosis treated with vitamin E**

_Ebe D’Adamo; Loredana Marcovecchio; Tommaso de Giorgis; Chiara De Leonibus; Francesco Chiarelli; Angelika Mohr_  
University of Chieti, Department of Paediatrics, Chieti, Italy

**Background:** Liver steatosis is a frequent finding in obese children, and oxidative stress appears to be one of the main factors implicated in its pathogenesis. In obese adults, treatment with vitamin E has resulted in an improvement in liver histology, but there are no available data in children.

**Objective and hypotheses:** Our aim was to assess whether oral supplementation with Vitamin E might reduce oxidative stress in obese prepubertal children with steatosis.

**Methods:** 42 obese prepubertal children (16M/26F; BMI SDS: 3.2±0.5) affected by steatosis were randomised to treatment with vitamin E (n=21), at a dose of 600 mg/day, or placebo for 6 months. At baseline (T0) and after 6-month treatment (T6), BMI, oxidative stress (urinary isoprostanes (PGF-2α)), alanine aminotransferases (ALT), high sensitivity C-reactive protein (hsCRP), glucose and insulin were assessed. HOMA-IR was used as an index of insulin resistance.

**Results:** The obese and control groups were comparable for age (8.3±1.6 vs 8.4±1.3 y), sex (8M/13F) and BMI SDS (3.3±0.5 vs 3.1±0.4). In addition, at the beginning of the study, PGF-2α, HOMA-IR, ALT and hsCRP were similar between the two groups (all p>0.05) (Table). After 6-month treatment, levels of PGF-2α significantly decreased in children treated with vitamin E (p<0.001). A significant reduction was also found in ALT (p<0.001) and HOMA-IR (p<0.001). In contrast, no significant change in any of these markers was detected in the placebo group (Table).

### Table

<table>
<thead>
<tr>
<th></th>
<th>Vitamin E group (T0)</th>
<th>Vitamin E group (T6)</th>
<th>Placebo group (T0)</th>
<th>Placebo group (T6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI SDS</td>
<td>3.3±0.5</td>
<td>3.0±0.5</td>
<td>NS</td>
<td>3.1±0.4</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>PGF-2α (ng/ml)</td>
<td>1.6±0.3</td>
<td>0.5±0.2</td>
<td>&lt;0.001</td>
<td>1.2±0.6</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.5±1.5</td>
<td>2.1±1.2</td>
<td>&lt;0.001</td>
<td>2.7±1.6</td>
<td>2.4±1.5</td>
</tr>
<tr>
<td>ALT (μL)</td>
<td>39.9±11.6</td>
<td>32.2±10.6</td>
<td>0.001</td>
<td>35.4±8.8</td>
<td>36.5±10.8</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>4.0±0.3</td>
<td>0.4±0.3</td>
<td>0.05±0.4</td>
<td>0.4±0.2</td>
<td>0.3±0.2</td>
</tr>
</tbody>
</table>

**Conclusions:** In this study vitamin E supplementation was associated with a significant reduction in oxidative stress and with an improvement in insulin sensitivity. These data suggest that, as in adults, Vitamin E supplementation could represent a valuable treatment in obese children affected by steatosis.

### P1-d1-250 Fat Metabolism and Obesity 1

**Impact of obesity on cardiac geometry and function and on autonomic nervous function in children and adolescents**

_M. Loredana Marcovecchio; Ebe D’Adamo; Stefania De Marco; Chiara De Leonibus; Francesco Chiarelli; Angelika Mohr_  
University of Chieti, Department of Paediatrics, Chieti, Italy

**Background:** Subclinical cardiac abnormalities and impaired autonomic nervous function represent predisposing factors for cardiovascular disease in obese adults. However, there are scant data in obese youths.

**Objective and hypotheses:** To evaluate early cardiac abnormalities and autonomic nervous function in obese children and adolescents.

**Methods:** Doppler two-dimensional-echoangiographic studies and 24-hour ECG monitoring were performed in 30 obese (13M/17F; age: 11.3±2.2 years; BMI SDS: 2.1±0.6) and 13 age and gender matched normal-weight (9M/4F; age: 12.7±3.3 years; BMI SDS 0.3±1.1) children and adolescents. Left atrial (LA) and ventricular (LV) geometry were measured. LV diastolic function was assessed by mitral inflow velocities (peak early (E) and late (A) waves, the E/A ratio and E-wave deceleration time (DcT)). Myocardial flow velocities, including early and late diastolic mitral annular velocity (Em and Am) and their ratio (Em/Am) were also acquired. 24h ECG parameters included low- (LF) and high-frequency (HF) power, LF/HF ratio, and time-domain variables (SDNN, SDNNi, rMSSD, pNN50).

**Results:** In the obese group LA size was significantly increased compared to the control group, as indicated by higher maximal and minimal LA diameters and area (table). Obese children also showed diastolic filling abnormalities, as indicated by higher values of E, DcT, and Am and decreased Em/Am ratio.

<table>
<thead>
<tr>
<th></th>
<th>Obese group</th>
<th>Non-obese group</th>
<th>P</th>
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<tbody>
<tr>
<td>Maximal LA diameter (cm)</td>
<td>4.76±0.47</td>
<td>4.23±0.68</td>
<td>0.02</td>
</tr>
<tr>
<td>Minimal LA diameter (cm)</td>
<td>3.45±0.51</td>
<td>2.98±0.48</td>
<td>0.01</td>
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<tr>
<td>Left atrial area (cm²)</td>
<td>14.42±2.57</td>
<td>10.76±3.55</td>
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<tr>
<td>Left ventricular mass (g/m²)</td>
<td>69.77±13.58</td>
<td>61.48±11.82</td>
<td>NS</td>
</tr>
<tr>
<td>E (cm/sec)</td>
<td>96.8±14.05</td>
<td>85.83±12.4</td>
<td>0.02</td>
</tr>
<tr>
<td>A (cm/sec)</td>
<td>22.5±11.88</td>
<td>50.83±14.43</td>
<td>NS</td>
</tr>
<tr>
<td>E/A</td>
<td>1.92±0.48</td>
<td>1.8±0.54</td>
<td>NS</td>
</tr>
<tr>
<td>DcT (cm/sec)</td>
<td>193.15±36.57</td>
<td>162.3±25.96</td>
<td>0.02</td>
</tr>
<tr>
<td>Am (cm/sec)</td>
<td>9.18±2.83</td>
<td>6.92±1.83</td>
<td>0.01</td>
</tr>
<tr>
<td>Em (cm/sec)</td>
<td>20.14±4.3</td>
<td>19.75±2.09</td>
<td>NS</td>
</tr>
<tr>
<td>Am/Em</td>
<td>2.36±0.78</td>
<td>3.07±0.87</td>
<td>0.03</td>
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</tbody>
</table>

A significant and independent association with BMI SDS was found for all the above parameters (LA area: β=0.43; Am/Em: β=0.34; Em/Am ratio: β=0.43, all p<0.05). 24-h ECG monitoring showed a decreased LH/HF ratio in obese children compared to controls (1.39:0.9 vs 2.12:0.94, p=0.006), whereas the other ECG parameters were similar between the two groups.

**Conclusions:** Obese children showed increased atrial size and impaired LV diastolic function associated with an increased parasympathetic tone, suggesting an increased cardiovascular risk associated with childhood obesity.

**Background:** Data on serum apelin levels in obese children(OC) are few and controversial. No data are available concerning APJ gene mutations on childhood obesity.

**Objective and hypotheses:** To measure serum apelin levels in OC and adolescents(OA) and search for possible associations between them and the presence of the G212A APJ gene polymorphism.

**Population and methods:** Eighty-five obese individuals (43 children) and 45 lean matched for age/gender were enrolled in the study. All obese patients underwent oral glucose tolerance test (OGTT) and genomic DNA was extracted from peripheral blood amplified by PCR for genotyping G212A APJ gene polymorphism. Results are shown in Tables 1,2. Apelin levels were significantly lower in obese participants than in controls. The G212A polymorphism genotype frequency distribution did not differ between obese (GG=54.1%, GA=38.8%, AA=7.1%) and the HapMap polymorphism frequency distribution of the Caucasians (GG=46.9%, GA=39.8%, AA=13.3%, p=0.232). The G212A genotype was associated with significantly different apelin levels (One-WayANOVA test: p=0.016). The Bonferroni post-hoc test revealed that GG and GA group had significantly lower apelin levels.

### Table

<table>
<thead>
<tr>
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<tr>
<td>maximal LA diameter</td>
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<td>left atrial area</td>
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<td>left ventricular mass</td>
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<td>E</td>
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<tr>
<td>A</td>
<td>NS</td>
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<td>E/A</td>
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<td>DcT</td>
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<td>Am</td>
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<td>Am/Em</td>
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<table>
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<tr>
<td>apelin levels (mg/l)</td>
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<td>G212A polymorphism</td>
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</table>

**Results:** In the obese group LA size was significantly increased compared to the control group, as indicated by higher maximal and minimal LA diameters and area (table). Obese children also showed diastolic filling abnormalities, as indicated by higher values of E, DcT, and Am and decreased Em/Am ratio.
P1-d1-252 Fat Metabolism and Obesity 1

Asymmetric dimethylarginine, nitric oxide and oxidative stress in obese youth: relationship with 24-hour ambulatory blood pressure measurement

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1University of Edinburgh, Edinburgh, United Kingdom
2Department of Children’s Health, University of Chieti, Chieti, Italy

Background: Asymmetric dimethylarginine (ADMA), a competitive inhibitor of nitric oxide synthase (NOS), is a risk marker for cardiovascular disease, a relevant issue in obesity. The aim of the present study was to evaluate ADMA and Nitric Oxide (NO) concentrations as well as oxidative stress (PGF-2α), and define their association with twenty-four hour ambulatory blood pressure (ABP) in obese adolescents.

Methods: A group of 87 obese adolescents were recruited and compared with 51 healthy age and gender matched peers. In all subjects, fasting blood samples were obtained for the evaluation of ADMA, NO, insulin, blood glucose and lipid profile, and urine samples, for urinary PGF2α measurement. Blood pressure was evaluated by a 24-hour ABP monitoring in all subjects.

Results: ADMA (0.71±0.166 vs 0.54±0.101 μmol/L, P<0.001) and PGF-2α (21.61±14.25 vs 14.98±10.09 ng/ml, P=0.015) values were significantly increased while NO significantly reduced (48.97±15.67 vs 90.38±47.89 mmol/L, P<0.001) in obese adolescents compared to controls. 24-h systolic and diastolic blood pressure (SBP and DBP) as well as daytime and nighttime SBP and DBP were significantly higher in obese compared to lean subjects (all P<0.009). After dividing the obese group by tertiles of ADMA, SBP, DBP and the percent of non-dipper subjects progressively and significantly increased across tertiles. Multiple regression analysis showed that ADMA concentrations were significantly associated with oxidative stress and BMI-SDS.

Conclusion: Obese youth present increased ADMA and decreased NO levels compared to healthy peers and these markers are associated to impaired blood pressure regulation. Adiposity and oxidative stress represent the major factors influencing ADMA levels.

P1-d2-253 Fat Metabolism and Obesity 2

Evaluation of 9 gene variants in relation to obesity and anthropometric parameters in the Czech adolescent population

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Background: Genome-wide association studies have identified several gene variants associated with body mass index (BMI) and obesity so far. Objective and hypotheses: We performed a replication study of 9 previously reported variants in near genes PCSK1 (rs6232, rs6235), BDNF (rs92946, rs4923461), SEC16B (rs10913469), TMEM18 (rs7561317), SH2B1 (rs7498665), KCTD15 (rs29941) and FTO (rs9939609) in the Czech adolescent population. We investigated their association with BMI status (underweight, overweight and obesity) and related anthropometric parameters.

Methods: Genotyping was performed in 1443 adolescents, including 670 overweight/obese (BMI ≥90th percentile), 713 normal weight (BMI 10—90th percentile) and 60 underweight (BMI <10th percentile) adolescents aged 13—18 years. Anthropometric parameters were assessed in all individuals.

Results: FTO rs9939609 was associated with overweight and obesity (OR = 1.28, 95%CI 1.02—1.60, p = 0.04) and values of body weight, BMI, abdominal and hip circumference, body and trunk fat (p < 0.001). The minor allele of SEC16B rs10913469 increased risk of overweight/obesity (OR = 1.24, 95%CI 1.02—1.51, p = 0.04) and values of body weight, BMI, abdominal circumference and body fat (p < 0.05). The remaining variants had not significant odds ratios and only slight impact of the increasing number of risk alleles on body weight, BMI, abdominal and hip circumference, body and trunk fat was observed (p < 0.05). Interestingly, the risk G-allele of TMEM18 rs7561317 was negatively associated with underweight (OR = 0.56, 95%CI 0.36—0.88, p = 0.02).

Conclusions: In addition to widely replicated FTO gene variant we confirmed the association of the SEC16B variant with overweight/obesity and related traits in our population sample. The risk variant of TMEM18 was not associated with obesity, but it seems to be protective against underweight.

P1-d2-254 Fat Metabolism and Obesity 2

Morbid obesity in adolescence. Experience and preliminary mid-term results (18-24 months) with intragastric balloon

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Introduction: The intragastric balloon (IGB) is a non-invasive and reversible procedure and may constitute a useful tool together with behaviour and lifestyle changes in the treatment of morbidly obese adolescents prior to the decision to perform bariatric surgery.

Aims: Evaluation of results obtained at 18-24 months following IGB withdrawal in a group of morbidly obese adolescents.

Patients and methods: Eight candidates (5 girls, 3 boys; age range: 13.9 -17.9 years) were selected. All had BMI >40 with one or more severe co-morbidities and met the internationally-accepted criteria for bariatric surgery. IGB (B.I.B. Bioentecers®) were inserted endoscopically under general anaesthesia and left in place for six months. Patients followed a strict calorie-controlled diet.

Results: Patient clinical data, weight loss, BMI and BMI-z score at IGB removal at six months and at 18-24 months post-removal are shown in the table. Four patients maintained weight loss after IGB withdrawal (BMI loss: -11.0 ± 3.7). The remaining patients recovered or slightly gained their pre-IGB (+2.3 ± 3.7).
Background: In recent years, there has been an increasing attention to thyroid function in paediatric obese patients due to its possible role as metabolic and cardiovascular risk factor.

Objectives: a) to ascertain the association between thyroid function, lipid status and insulin resistance (IR) in nutritionally obese children and adolescents; b) to evaluate the frequency of hyperthyrotropinemia in our cohort.

Design: Cross-sectional study.

Methods: We examined 311 obese children and adolescents (163 females), mean age 9.2 ± 2.8 yrs (62.7% prepubertal) from a iodine sufficient region. Anthropometric, metabolic and hormonal variables were determined when patients were referred to our Outpatients Clinic during the period 2005-2010. Patients with thyroid autoimmune disease (TAD) and/or other chronic diseases were excluded.

Results: Hyperthyrotropinemia (TSH >4.5 mIU/L) was diagnosed in only 9 cases (2.9%, 2 females). In the remaining 302 euthyroid subjects (161 females) we did not find any correlation between TSH levels and BMI-SDS, lipid panel and IR. Conversely, FT4 levels were correlated with both BMI-SDS (r=0.139, p=0.016) and HDL cholesterol levels (r=-0.150, p=0.018). There were no differences of FT4 levels, lipid status and HOMA-IR between patients with TSH <2.5 mIU/L and those with TSH 2.5 - 4.5 mIU/L. Among the 187 prepubertal children, HOMA-IR was significantly higher in the 130 subjects with TSH between 2.5 and 4.5 mIU/L compared with those with TSH <2.5 mIU/L (3.41 ± 2.87 vs 2.60 ± 1.92, p = 0.026). No differences were found in pubertal adolescents.

Conclusions: In our paediatric obese population: 1) the prevalence of hyperthyrotropinemia resulted only slightly higher than that reported in general paediatric population (2.9 vs 1.7%), probably due to the preliminary exclusion of subjects with TAD; 2) TSH levels were not correlated with the severity of the obesity; 3) among the euthyroid prepubertal obese children IR was significantly higher in the subgroup with TSH between 2.5 and 4.5 mIU/L.

Method: We investigated whether leptin, a classic adipocytokine, receptor take a role for expression and production of proinflammatory cytokine, TNF-α through increased PLD activity in mouse alveolar macrophage (Raw 264.7).

Results: Dominant negative PLD1 decreased leptin-induced TNF-α expression and production. Treatment of Leptin activated the phospholipase Cγ (PLCγ)/Src/mTOR/JNK/p38 MAPK pathway. Leptin-induced PLD activation was attenuated by PLCγ inhibitors (PAO), Src inhibitor (PP2). These results indicate that PLCγ, Src act as upstream activators of PLD in leptin-treated Raw 264.7 cells. Furthermore, expression and production of TNF-α increased by leptin were also blocked by inhibition of PLCγ, Src, mTOR, JNK, p38 MAPK. Taken together, PLD1 acts as an important regulator in leptin-induced expression and production of TNF-α in Raw 264.7 cells.

Conclusions: Thus, we suggest leptin may contribute to development of asthma through increase expression and production of TNF-α in Raw 264.7 cells. Our result support obesity might contribute to pathogenesis of asthma in molecular level.
Background: Childhood obesity is associated with nonalcoholic fatty liver disease (NAFLD) and its more severe form, steatohepatitis (NASH). Recent studies have found associations between vitamin D deficiency (VDD), insulin resistance (IR) and NAFLD among overweight children.

Objective and hypotheses: To explore mechanisms and test whether VDD contributes to NASH progression.

Methods: We fed young (age 25d) Sprague-Dawley rats with a low-fat diet alone (LFD, group 1) or with vitamin D depletion (LFD+VDD, group 2). Additional rats were exposed to a Westernized diet (WD: high-fat/high-fructose corn syrup) that more typically consumed by overweight children, and was either replete (WD, group 3) or deficient in vitamin D (WD+VDD, group 4).

Liver histology was assessed using the NASH CRN scoring system and expression of genes involved in inflammatory pathways were measured in liver and visceral adipose tissue after 10 weeks.

Results: Weight gain, total caloric intake and Lee adiposity index were highest in the WD+VDD group. WD+VDD animals exhibited significantly greater hepatic steatosis compared to LFD groups. Lobular inflammation as well as NAFLD activity scores (NAS) were higher in WD+VDD vs. the WD group (NAS: WD+VDD 3.2±0.47 vs. WD 1.50±0.48, p<0.05). In both LFD and WD animals, VDD stimulated inflammatory gene expression more in the liver than in adipose tissue. Hepatic mRNA levels of toll-like receptors TLR2, TLR4 and TLR9, as well as resistin, interleukins IL-1beta, IL-4 and IL-6, were higher in WD+VDD vs. WD animals (p<0.05). Logistic regression analyses showed significant associations between NAS and liver mRNA levels of resistin, IL-1beta, IL-6 and IL-8 in WD+VDD animals.

Conclusions: VDD exacerbates NAFLD through TLR-activation possibly via endotoxin exposure and increased gut leakiness in a Westernized diet rat model. In addition it causes higher hepatic resistin gene expression, and up-regulation of hepatic inflammatory genes. These findings have implications for human NAFLD and also provide a novel model for experimental NASH.

P1-d2-256 Fat Metabolism and Obesity 2

Vitamin D deficiency activates hepatic toll-like receptors and exacerbates NAFLD in obese rats

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Background: AQP7 (41,37 and 34kDa) have been identified in rodents. Its involvement in childhood obesity.

Objective and hypotheses: To study AQP7 in lean and obese pre-pubertal children (Groups A:2mos-7yrs and B:8-12 yrs) and adolescents (Group C:10-15yrs). mRNA expression of AQP7 was studied with RT-PCR and ELISA. The correlation between RBP4 and IL-1 beta mRNA expression and secretion was significantly reduced upon incubation with MacCM in SGBS (by ~70 % using 10 % MacCM for 48 h) as well as in human primary adipocytes. IL-1beta was identified as a new marker of obesity

Poster Presentations
and potent cytokine regulating RBP4, as it down-regulated RBP4 mRNA and secretion in a time- and dose-dependent manner (inhibition by ~50% at 50 ng/ml after 48 h). Blocking IL-1beta signaling using a neutralizing IL-1R and a NFkB inhibitor (CAPE) abrogated the inhibitory effect of IL-1beta on RBP4 production. Most interestingly, RBP4 mRNA was negatively correlated with IL-1beta mRNA in subcutaneous adipose tissue obtained from 18 healthy female subjects (R = -0.55; p < 0.05).

**Conclusions:** RBP4 expression and secretion was inhibited in an in vitro model of inflamed adipose tissue. IL-1beta was identified as a new and potent inhibitor of RBP4 production. Adipose tissue inflammation and increased RBP4 levels are associated with the development of insulin resistance. We found that inflammatory conditions lead to a downregulation of RBP4 in adipocytes suggesting that adipose inflammation and the increase in circulating RBP4 are two unrelated processes.

**P1-d2-262 Fat Metabolism and Obesity 2**

**Serum di-ethylhexyl phthalate (DEHP) levels are associated with insulin resistance in girls**

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**Background:** Phthalates have documented biochemical activity as peroxisome proliferator-activated receptor activators and antiandrogens, which may contribute to the development of obesity and insulin resistance. Though a few studies have shown that concentrations of phthalates are associated with insulin resistance in adults, studies on association of phthalate concentrations with insulin resistance in children are limited.

**Objective and hypotheses:** We studied whether serum di-(2-ethylhexyl) phthalate (DEHP) levels are associated with obesity and insulin resistance in Korean girls.

**Methods:** A total of 155 girls (53 obese/31 overweight cases and 71 controls; aged 6 to 13yr) were enrolled. Anthropometry, physical activity and nutrient intake were analyzed, and serum DEHP levels were measured by gas chromatography/mass spectrometry method.

**Results:** Geometric mean serum DEHP levels were higher in obese (191.3±272.2 ng/mL) and overweight (189.6±226.1 ng/mL) subjects than in controls (50.1±164.9 ng/mL; P<0.0001). According to the increased DEHP quartile, prevalence of overweight and obesity increased (P<0.05). Subjects in the top DEHP quartile had higher HOMA-IR (4.2±6.6 in Quartile 4 vs. 2.1±1.1 in Quartile 1, P<0.05) and fasting blood glucose levels (P<0.05) compared with the subjects in the lowest DEHP quartile. Serum DEHP levels showed positive correlation with fasting blood glucose (r=0.30, P<0.001), fasting insulin (r=0.43, P<0.0001) and HOMA-IR levels (r=0.46, P<0.0001), whereas it did not had significant correlation with serum ALT or lipid profiles.

**Conclusions:** Serum DEHP levels showed significant positive correlations with insulin resistance. Prospective studies are needed to determine potential causal links between DEHP exposure and insulin resistance in children.

**P1-d2-263 Fat Metabolism and Obesity 2**

**Circulating IgG relates to insulin resistance and serum lipids in asymptomatic prepubertal children**

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**Background:** Chronic activation of the innate immunity is a key feature of the insulin resistance syndrome. Recent studies in mice suggest that B lymphocytes promote also insulin resistance by accumulating in adipose tissue and producing pathogenic IgG antibodies.

**Objective and hypotheses:** We aimed to study whether circulating IgG (seemingly including pathogenic antibodies produced by B cells) is associated with metabolic risk markers in asymptomatic prepubertal children.

**Methods:** Subjects were 177 school-age healthy prepubertal Caucasian children (93 girls and 84 boys) with normal height and weight distributions consecutively recruited among those seen within a setting of primary care. Circulating total IgG concentration, insulin resistance (HOMA-IR) and fasting lipids were assessed in all subjects. IgG levels were measured by nephelometry.

**Results:** Increasing IgG levels were associated with a less favorable metabolic phenotype, consisting of higher fasting insulin and HOMA-IR and lower HDL-cholesterol and HDL/TG ratio (all p<0.005 to p=0.0001). HOMA-IR increased by 60% from (0.43 ± 0.1 to 0.69 ± 0.1) and the HDL/TG ratio decreased by 25% (from 1.60 ± 0.1 to 1.20 ± 0.1) from the lowest to the highest tertile of serum IgG. These associations were attenuated but remained significant after adjusting for confounding variables such as gender, age and BMI (p<0.01 to p=0.001).

**Conclusions:** A less favorable metabolic profile is observed in healthy prepubertal children with higher circulating IgG. These results suggest an association of adaptive immunity with energy metabolism in children.

**P1-d2-264 Fat Metabolism and Obesity 2**

**The evaluation of dyslipidaemia, hypertension development risk and plasma atherogeneity index: triglyceride/HDL Ratio In LGA born children during prepubertal period**

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**Background:** Recent studies show that atherosclerotic process begins during childhood and clinical findings appear in adulthood. Being born large for gestational age (LGA) is a risk factor for metabolic disorders in advanced ages.

**Objective and hypotheses:** We aimed to investigate the risk of development of hypertension and dyslipidemia and to determine the plasma atherogeneity index (AIP) and triglyceride/HDL cholesterol (C) ratio in LGA born children in prepubertal ages.

**Methods:** Forty (19 female,21 male) LGA born prepubertal children (mean age 6.1±2.5 years) were evaluated with respect to total cholesterol, triglycerides, HDL-and LDL-C. Atherogenic index of plasma (AIP) was calculated as triglycerides/HDL-C ratio. To determine the cardiovascular risk, total cholesterol/HDL-C ratio and LDL-C/HDL-C ratio were calculated. Their data were compared to that of prepubertal 49 (25 female,24 male) appropriate for gestational age (AGA) children (mean age 5.4±1.8 year).

**Results:** LGA children were taller and heavier than AGA children but had similar BMI SDS, waist/hip circumference ratio, skinfold thickness as AGA born children. Systolic blood pressure and diastolic blood pressure (p=0.030, p<0.009, respectively) were higher in LGA children. There were no significant differences in triglycerides(TG) and VLDL-C levels between children born LGA and AGA. Total cholesterol and LDL-C (p=0.004, p=0.000, respectively) levels were higher in children born LGA than AGA. LDL-C levels were significantly lower in children born LGA than AGA(p=0.001). AIP (TG/HDL-C) was higher in children born LGA than AGA(p=0.022). Total Cholesterol/HDL-C and LDL-C/HDL-C ratios were higher in children born LGA than AGA.

**Conclusions:** Low HDL-C and high LDL-C levels and high AIP in LGA born prepubertal children with normal BMI SDS indicate that atherosclerotic changes start early in childhood ages even in the absence of obesity.
P1-d2-265 Fat Metabolism and Obesity 2

Association of FTO, TCF7L2 gene single nucleotide polymorphisms with obesity and metabolic parameters in Chinese Han children and adolescents
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Background: The prevalence of obesity in Chinese children and adolescents is ever increasing, but few studies focused on the genetic etiology.

Objective and hypotheses: To study the association of the SNP of FTO (rs9939609, rs1421085), TCF7L2(rs79031346) gene with the obese children and adolescents.

Methods: Subjects were divided into three groups: control, obese, and overweight group. Fasting Plasma Glucose (FPG), Triglyceride (TG), Total Cholesterol (TCH) and Fasting Insulin (Fins) were evaluated. Homeostasis Model of Assessment (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) were calculated to evaluate the insulin resistance. Taqman-MGB probe was used to detect genotypes.

Results: (1) The levels of FPG, Fins, TG and HOMA-IR were significantly higher in obesity/overweight group than that in normal control group. (2) FTO gene: The AA genotype frequency of rs9939609 was 2.7% in obesity group and 0.4% in overweight group and there existed significant difference compared with normal control group (genotype frequency 1.7%, P = 0.048, OR= 1.347). The CC genotype frequency of rs1421085 was 2.7% in obesity group and 0.9% in overweight group and there existed significant difference compared with normal control group (genotype frequency 1.7%, P = 0.076, OR = 1.388). There existed significant difference in BMI between the rs1421085 TC + CC, rs9939609 TA + AA genotypes when compared with their wild TT genotypes (P = 0.003; rs1421085: P = 0.0005). (3) TCF7L2 gene: There was no significant difference of allele C and T frequency of SNP rs7903146 between obese/overweight groups and control group (P>0.05). The level of HOMA-IR in CC genotype was significantly higher than that in CT genotype (P<0.046).

Conclusions: FTO gene rs9939609 and rs1421085 SNP are associated with obesity and BMI, and the SNP rs7903146 of TCF7L2 gene is associated with insulin resistance in Chinese children and adolescents. Both of the above SNPs are not correlated with metabolic parameters.

P1-d2-266 Fat Metabolism and Obesity 2

Effects of an outpatient obesity treatment program for adolescents on health-related quality of life
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Background: Interventions in obesity in adolescents aim at the prevention of medical comorbidities as well improving health related quality of life (HRQoL). Studies showed that HRQoL in obese adolescents is impaired and treatment programs can help to enhance positive determinants of HRQoL.

Objective and hypotheses: In an on-going evaluation of the standardised patient education program “Active Kids” we analysed HRQoL data of 65 adolescents (mean age 13.6 years, 49% females). They and their parents took part in a structured ten month outpatient obesity program composed of medical, nutritional and psychological trainings. Our analyses focused on the HRQoL before and after treatment.

Methods: At the beginning and after completion of the treatment program all participants answered the generic and the disease-specific obesity module of the KINDL HRQoL-questionnaire. Effects of the program on HRQoL were tested for significance using ANOVA to account for age and gender effects. We also compared the HRQoL scores with norms of the general population (KiGGS study).

Results: The participants had a mean standardized BMI (zBMI) of 2.5. After treatment the zBMI was significantly reduced by 0.2 (P<0.001). Compared to German norms most of the HRQoL mean scores at baseline were low, the obesity specific scores were comparable to other studies of obese adolescents. Participation in the program led to a significant increase from 52.7 to 59.6 in the self-esteem scale (p=0.028) and from 62.2 to 70.0 in the disease specific HRQoL scale (p=0.000) independently of age and gender. After treatment the self-esteem score reached the level of the general population.

Conclusions: The results confirm that the Active Kids program is effective in both reducing weight and enhancing self-esteem and obesity specific HRQoL. HRQoL should be included as an important outcome in clinical studies on the efficiency of intervention programs.

P1-d2-267 Fat Metabolism and Obesity 2

Physical activity in preschoolers: direct accelerometry in the course of the week and relation to weight status, TV consumption, and socioeconomic factors
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Background: Physical inactivity is a risk factor towards the development of obesity. Data on objectively measured physical activity (PA) in preschool children are scarce.

Objective and hypotheses: We measured PA in preschoolers (direct accelerometry) and evaluated differences in PA patterns over the course of the week. PA data were analyzed with regard to gender, anthropometrics, lifestyle, and socioeconomic parameters.

Methods: PA was measured in 119 children 3-6 years by direct accelerometry and analyzed in the 92 (40 girls) that wore it for at least 4 days including one day of the weekend (median/mean measuring time 23.5 h/21.8 h/d). PA questionnaires were completed by 103 parents and 87 caregivers to collect anthropometric, lifestyle, and socioeconomic data.

Results: Median daily PA (Metabolic equivalent: MET>3) was 4.3 hours (mean: 4.4 hours). Boys spent an estimated 52 min/week more being active (MET=6) than girls (95% confidence interval [6, 96] min/week, p=0.02). PA was lower during the weekend (3.7 h/d) compared to weekdays (4.5 h/d), p<0.01; 10.6), where a 95% confidence interval for the difference is [0.5, 1.0] h/d. There was not a significant difference in PA levels between overweight/obese children (median 4.7 h/d) and normal-weight peers (median 4.2 h/d). Daily media consumption increased with decreasing social class, both on weekdays (p=0.05) and during the weekend (p=0.01), but was not related to the amount of daily PA.

Conclusions: The negative impact of obesity-promoting factors known to be relevant in older children is rather low for preschoolers, but there is evidently a gradient in PA between weekdays and weekends already in this age group.

P1-d2-268 Fat Metabolism and Obesity 2

Inhibition of the phosphoinositide 3-kinase/mTOR pathway and influence on the viability of human PTEN-deficient lipoma cells
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Background: We identified a child with PTEN Hamartoma Tumor Syndrome (PHTS) and massive lipomatosis caused by a deletion in the phosphatase and tensin homolog (PTEN) gene. Treatment with rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), was only partially and transiently successful in this patient.

Objective and hypotheses: We tested in vitro whether pharmacological inhibition of AKT, PI3-kinase or mTOR leads to reduced viability or induction of apoptosis in lipoma cells of the above mentioned patient. Furthermore, we evaluated IGF-binding protein (IGFBP)-2 as potential marker for therapy success.

Methods: Cells from a lipoma of the patient with PHTS were maintained in
long term culture. Viability and apoptosis were assessed using WST-1 and Annexin/PI assay. IGFBP2 production was detected by ELISA and quantitative PCR.

Results: Lipoma cells had a lifespan of 91 population doublings with a doubling time of 25h. PTEN mRNA and protein levels were decreased and Akt phosphorylation was increased compared to human SGRS preadipocytes. The mTORC1 inhibitor rapamycin decreased viability by 43.4±2% and adipocyte differentiation by 72.7±5% (100nM), but did not induce apoptosis. The mTORC1/2 inhibitor WYE-354 decreased viability by 75.7±2% (5µM), whereas the PI3-Kinase inhibitor LY294002 (500µM) decreased viability by 98.09±1% and induced 65.0±3% apoptosis. The Akt inhibitor perifosine (100µM) reduced cell viability by 96.4±1% and induced 84.5±1% apoptosis. IGFBP2 serum levels were significantly elevated (1224-16ng/ml, reference 277-640ng/ml) in our patient, but did not show a consistent change during rapamycin therapy. Lipoma cells were found to secrete IGFBP2 in amounts comparable to other adipocytel cell lines.

Conclusions: mTORC1 inhibition by rapamycin reduced viability, but did not affect apoptosis of lipoma cells in vitro. In contrast, massive apoptosis was induced by Akt inhibitor perifosine. IGFBP2 was not useful as a biomarker for success of rapamycin therapy in our patient.

PI1-02-269 Fat Metabolism and Obesity 2

The assessment of segmental body fat composition with bioelectrical impedance analysis in childhood

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Background: Bioelectrical impedance analysis (BIA) is a simple and non-invasive technique with a high potential for the assessment of trunk and limb composition.

Objective and hypotheses: The aim of this study is to determine the growth pattern of body composition component according to whole body, trunk and limbs during childhood and adolescence.

Methods: A total of 4,151 (2,297 girls, 1,854 boys) children and adolescents aged 6–17 years were recruited for this study. Regional body fat percent (BF%) fat mass (FM) and fat free mass (FFM) distribution were evaluated using BIA. The measurements were made from total body, upper limbs, trunk, and lower limbs. We examined the growth patterns of these parameters according to gender and age.

Results: BF% and FM of the whole body were greater in girls than in boys, while FFM was greater in boys than in girls for all ages. This difference widened with age. The mean of BF%, FM, and FFM in each body part showed similar growth patterns especially in pubertal period. In the boys between 6 and 17 years old, BF% gradually decreased in the arms and legs but trunk BF% did not change much. This difference was less pronounced in girls than boys. FM and FFM gradually increased with age in each body part of both genders. The increase was more pronounced in the pubertal age (table 1, 2).

Conclusions: This data showed physiological changes at the whole body and limb composition in childhood and adolescence. The compositions of all body parts changed with age and gender. The accumulation of body composition according to body part is important for understanding childhood body composition and managing obesity.

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<th>Table 2: The composition of segmental body with BIA in boys</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>---</td>
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<tr>
<td>6</td>
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<td>7</td>
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<tbody>
<tr>
<td><strong>mean</strong></td>
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<td>±50</td>
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</tbody>
</table>
P1-d2-270 Fat Metabolism and Obesity 2

Respiratory function in obese children compared to lean children and the impact of body composition and maximum physical exercise

Kathrin Dittrich1; Isabel Wagner2; Matthias Raschpichtler1; Julia Gasing1; Maike vom Hove1; Freerk Prenzel2; Wieland Kissel3; Antje Körner1
1Centre for Pediatric Research, Hospital for Children and Adolescents, University of Leipzig, Department of Women and Child Health, Leipzig, Germany; 2University Hospital for Children and Adolescents, Leipzig, Germany, Department of Women and Child Health, Leipzig, Germany; 3Leipzig University Medical Center (IFB), AdiposityDiseases, Germany

Background: Respiratory function (RF) is supposed to be reduced in obese children, but findings are still heterogeneous. Some studies demonstrated negative effects of fat mass, particularly visceral fat, on RF in adults.

Objective and hypotheses: We aimed to evaluate, whether there is a difference in RF between lean and obese children in relation to body fat distribution and after maximal physical exercise.

Methods: A total of 77 children (40 girls, 37 boys) aged 14.6±2.7 years stratified into lean (n=37) and obese (n=40) children underwent spirometry before cycle ergometry and shortly after maximum effort was reached. Adipose tissue mass (AT) was estimated from Magnetic Resonance Imaging.

Results: For the z-score of the forced expiratory volume in 1 second (FEV1 z-score), the forced vital capacity (FVC z-score) and the maximum inspiratory flow (MMEF z-score), we did not detect any significant differences between lean and obese children. However, we identified a significantly increased respiratory resistance (Rocc) in the obese subjects (0.60±0.25 vs. 0.46±0.15; p=0.004), and a positive correlation with BMI z-score (r=0.41; p=0.001). Neither in lean nor obese children, FEV1 and FVC z-score changed significantly after cycle ergometry. After adjustment for age, sex, pubertal stage and BMI z-score, FEV1 z-score was significantly negatively correlated with the percentage of subcutaneous AT (SAT%) (r=-0.36; p=0.014). Similarly, FVC z-score was significantly negatively correlated with the percentage of total AT (TAT%) (r=-0.52; p=0.000), SAT% (r=-0.56; p=0.000) and visceral AT (VAT%) (r=-0.30; p=0.039). For the MMEF z-score we found analogical results for TAT% (r=-0.60; p=0.000), SAT% (r=-0.50; p=0.000) and VAT% (r=-0.29; p=0.049).

Conclusions: We did not find major differences in RF between lean and obese children, except of an elevated Rocc in obese children. In addition, maximum physical exercise did not cause deterioration in RF. Nevertheless, the amount of adipose tissue seems to have negative implications on RF.

P1-d2-271 Fat Metabolism and Obesity 2

Dietary salt intake and metabolic profile of children and adolescents with severe obesity

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Background: The consumption of highly processed foods (HPF) is one of the markers of dietary habits leading to obesity. The total excretion of urinary Na (U(Na)) is a useful biomarker of the total intake of calories belonging to HPF, as more than 80% of total salt intake derives from this kind of foods. Furthermore, high salt intake has been associated with significantly increased risk of total cardiovascular disease.

Objective and hypotheses: To evaluate the salt consumption in a group of severe obese (i.e. z-score BMI≥3) children and adolescent and its correlation with dietary habits and metabolic risk factors associated with obesity (i.e. insulin-resistance).

Methods: We considered dietary and urinary data from a total of 92 obese normotensive children and adolescents. Mean age was 12.55 ± 3.03 (9.1 – 17.9), BMI 30.35 ± 6.74, z-score of BMI 3.87 ± 0.97. 30 age-matched non obese children served as control group for urinary Na values. Dietary data were calculated on three-day dietary records before urine collection and insulin-line-resistance was evaluated by HOMA-IR.

Results: Mean U(Na) in obese patients was 135.25 ± 38.48 mmol/d, corresponding to 14.7 ± 4.2 g of salt intake, versus 44.6 ± 10.7 in controls (p<0.001). In 80 % of subjects the Na excretion exceeded the recommended upper limit of 100 mmol/d established for adults. There was a positive trend between age, BMI (absolute and z-score) and values of Na excretion, without reaching statistical significance. HOMA-IR was directly correlated with complex carbohydrate intake calculated on dietary data (p = 0.29) and inversely correlated with U(Na) (p = 0.33).

Conclusions: Salt consumption appears to be strictly related to dietary habits leading to development and persistence of obesity, even in children and young adolescents. A reduction in the consumption of salty food could therefore have a beneficial effect on body weight status.

P1-d2-272 GH and IGF Physiology 1

Characterization of a novel combined heterozygous IGF1R and SHOX mutation of a patient with short stature

Eva-Maria Radermacher1; Jürgen Klammt2; Anja Barnikol-Oettler1; Janina Caliebe1; Monique Losskoclo3; Michael Ranke4; Gudrun Rappolt3; Marina Schlöke1; Heike Stobbe1; Jan M Wif5; Wieland Kissel2; Roland Pfülf6
1University Hospital Leipzig, Hospital for Children and Adolescents, Leipzig, Germany; 2SKL Hospitals, Children’s Hospital, Heilbronn, Germany; 3Leiden University Medical Center, Laboratory for Diagnostic Genome Analysis, Center for Human and Clinical Genetics, Leiden, Netherlands; 4University Hospital Tübingen, University Children’s Hospital, Tübingen, Germany; 5Ruprecht-Karls-University, Department of Molecular Human Genetics, Heidelberg, Germany; 6Leiden University Medical Center, Department of Pediatrics, Leiden, Netherlands

Background: Longitudinal growth is a trait regulated by a plethora of genes. Especially, the insulin-like growth factor 1 receptor (IGF1R) plays an essential role in growth regulation. For bone development the transcription factor short statured homeobox gene (SHOX) has been proposed to be of fundamental importance.

Objective: To investigate the underlying pathomechanism of growth failure of a patient, who carries a heterozygous exon 6 deletion in the IGF1R and a heterozygous point mutation in the SHOX gene (p.Met240Ile).

Methods: MLPA, long range PCR and DNA sequencing were used to identify the mutations and to define the deletion breakpoints. IGF1R mRNA stability was investigated by RT-PCR. We performed immunoblot to study IGF1R expression and IGF1 dependent receptor phosphorylation in patient’s fibroblasts.

Results: The AGA-born patient showed growth retardation at the age of six years with a height of 102.5 cm (-3.3 SDS) and weight of 15.9 kg (-2.6 SDS). The mother bearing the IGF1R deletion had an adult height SDS of 1.1. Final height of the father harboring the SHOX mutation was normal (-0.9 SDS). Furthermore, patient’s IGF1 levels were in low-normal range and the boy presented no additional abnormalities. In patient’s fibroblasts we revealed that the 5.2 kb spanning IGF1R deletion results in nonsense-mediated mRNA decay leading to IGF1R haploinsufficiency. Accordingly, IGF1 stimulated phosphorylation studies showed a decrease in IGF1R autophosphorylation but, unexpectedly, AKT/PKB activation was not impaired.

Conclusions: In summary, we have identified the genetic cause of IGF1 resistance in a growth retarded boy and disclosed the underlying pathomechanism. Due to the unexpected activation pattern of the AKT/PKB signaling branch and a possible additive effect of the parental IGF1R and SHOX mutations, investigations to explore the interplay between both signaling pathways in cell models are in progress.
P1-d2-273 GH and IGF Physiology 1

Establishment of the standardized centile curves and reference intervals of serum insulin-like growth factor-I (IGF-I) levels in Japanese population using LMS method

Tsuwoshi Isomori1; Akira Shimatsu2; Susumu Yokoya3; Toshiaki Tanaka4; Kazuo Chihara5; Naoki Hizuka6; Akira Teramoto7; Keita Tatsunami8; Katsumi Ichihara9; Noriyuki Katsumata10; Kenji Fujieda11
1Graduate school of medicine, The University of Tokyo, Department of pediatrics, Tokyo, Japan; 2The Foundation for Growth Science in Japan, GH and its Related Factors Study Committee and GH Treatment Study Committee, Tokyo, Japan; 3The Ministry of Health, Labour and Welfare, The Research Study Group on the Hypothalamic-Pituitary Dysfunction, Tokyo, Japan

Background: Measurements of insulin-like growth factor-I (IGF-I) are useful not only for diagnosis and management of patients with growth hormone (GH) related disorders but also for assessing nutritional status. We have previously reported the population based references of serum IGF-I in 1996. However, they did not entirely reflect data in the transition period.

Objective and hypotheses: The aim of the present study was to re-establish age and gender specific national standard of normative data for IGF-I in Japanese population.

Methods: The study included 1,685 healthy Japanese subjects (845 males, 840 females) aged from 0 year to 83 years. The subjects suffering from diseases which may affect serum IGF-I levels were excluded. Obese or thin adult subjects were also excluded. Serum samples were obtained and IGF-I concentrations were determined by two kinds of commercially available immunoradiometric assays. The reference intervals were calculated using the LMS method. It is important to analyze the data covering from infants and elders by the LMS method since IGF-1 values had sharp peak around the later puberty.

Results: Median IGF-I levels were increased up to 310 ng/ml in boys at the age of 13-14 years and 349 ng/ml in girls at age of 13 years, respectively, which rapidly declined and reached 124 ng/ml and 163 ng/ml in males and females, respectively, at the age of 70 years. Pretreatment IGF-I measurements in patients with severe GH deficiency were obtained from the database of the Foundation for Growth Science, Japan (523 boys and 224 girls, age range: 4 months to 18 years) and from the clinical study for adult GH deficiency in patients with severe GH deficiency were obtained from the database of the Foundation for Growth Science, Japan (523 boys and 224 girls, age range: 4 months to 18 years) and from the clinical study for adult GH deficiency in patients with severe GH deficiency were obtained from the database of the Foundation for Growth Science, Japan. GH and its Related Factors Study Committee, Tokyo, Japan; The Foundation for Growth Science in Japan, GH and its Related Factors Study Committee and GH Treatment Study Committee, Tokyo, Japan; The Ministry of Health, Labour and Welfare, The Research Study Group on the Hypothalamic-Pituitary Dysfunction, Tokyo, Japan.

Conclusions: As shown by the present study, the association of severe IGF-I and IGFBP-3 deficiencies to mild growth retardation is suggestive of ALS deficiency, particularly when associated to pubertal delay. The increasing number of ALS deficient reported patients indicates that this condition may be more prevalent that previously suspected.

P1-d2-275 GH and IGF Physiology 1

Three novel IGF1R gene heterozygous mutations in unrelated children with pre and postnatal growth retardation and microcephaly

Gabriela Guercio1; Diana Monica Warman1; Marta Ciaccio1; Mariana Azz2; Carmen Riu3; Milagros Aguilar3; Matías Juárez3; Roxana Marino1; Esperanza Benenzstein1; Eduardo Chaler1; Mario Aurelio Rivarola1; Alicia Belgorosky2
1Hospital de Pediatría Garrahan, Endocrine Service, Buenos Aires, Argentina

Background: Several IGF1R gene mutations have been described as a cause of growth retardation due to IGF1 insensitivity.

Objective: To analyze the IGF1R gene for mutations in three children suspected to have IGF1 insensitivity. Population: Three children (2 boys, 1 girl) were evaluated. All patients were born small for gestational age (SGA) and presented microcephaly. The two boys were evaluated at 18 months (P1) and 2.8 years of age (P2). They showed a mild dysmorphic phenotype. External genitalia and scrotal testes were normal. A 3.2-year-old girl (P3) also presented a Klippel Feil malformation. P2 and P3 showed no postnatal catch-up growth, while P1 reached a normal Heigh SDS at 2 years of age without changes in head circumference. P1 and P3 also presented developmental delay.

Results: Basal and stimulated serum GH and basal serum IGF-1 and IGFBP3 levels were quite variable among them. No chromosome 15 anomalies were detected. Three novel heterozygous mutations, de novo R1256S (P1), N359Y (P2), and R1337C (P3) were detected in the IGF1R gene. The aminoacid substitutions were located at highly conserved aminoacid residues in the protein (location, P1 and P3 exon 21, P2 exon 20). These mutations were predicted to affect protein function using the sequence homology based SIFT tool, the structure-based PolyPhen approach and the Mutation Taster. The father and sister of P3, carrying the R1337C mutation, were phenotypically normal. P1 and P2 started high-dose rGH treatment. After 5 months of therapy, P1 maintained his growth velocity (6.8 cm/years). P2 gained 0.73 Height SDS after 1 year of treatment.

Conclusions: Even thought IGF1R molecular studies should be considered in children with an undiagnosed history of SGA without postnatal catch-up growth and microcephaly, the clinical and biochemical picture and the response to rGH, among subjects carrying IGF1R haploinsufficiencies, are quite variable.
Data on serum ALS levels in short children born SGA are unknown and associations with onset of puberty have not been established.

**Objective and hypotheses:** To determine serum ALS levels in short SGA children compared with those in controls and to assess the association between serum ALS levels and age at start of puberty.

**Methods:** Serum ALS levels were measured in 319 short SGA children (mean age 8.1 yr) at start of growth hormone treatment and in 516 subjects with normal stature (159 children, 357 adults). Age at start of puberty was available in 239 short SGA children.

**Results:** ALS SDS values of the SGA group were lower compared with those of controls (P<0.001). Mean (SD) ALS levels in short SGA subjects was -0.4 (1.6) SDS. In 40 children (13%), ALS levels were ≤2 SDS, which occurred more in girls than in boys (P<0.001). ALS levels ≥2 SDS were more common in boys (P<0.001). ALS SDS levels were significantly correlated with height SDS, weight SDS and BMI SDS. Also, strong positive correlations were observed with serum IGF-I and serum IGFBP-3 levels. There was no significant correlation between ALS levels and age at start puberty.

**Conclusions:** Short SGA children have lower serum ALS levels compared to controls, albeit less reduced than the IGF-I and IGFBP-3 levels. There is no correlation between serum ALS levels and age at start puberty.

**Table 1. Clinical and laboratory characteristics**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls</td>
<td>319</td>
<td>165±154</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>319</td>
<td>38.4±3.8</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>209</td>
<td>-3.0 (1.6)</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>314</td>
<td>-2.2 (1.1)</td>
</tr>
<tr>
<td>Target height SDS</td>
<td>307</td>
<td>-0.6 (0.8)</td>
</tr>
</tbody>
</table>

At start of GH treatment

| CA (yr) | 319 | 8.1 (3.1) |
| Height SDS | 319 | -2.9 (0.6) |
| Weight SDS | 319 | -2.8 (1.0) |
| BMI SDS | 319 | -1.3 (1.1) |
| IGF-I SDS | 319 | -1.2 (1.3) |
| IGFBP-3 SDS | 319 | -1.1 (1.0) |
| ALS SDS | 319 | -0.4 (1.6) |

**Table 2. Correlations between ALS SDS and clinical parameters**

<table>
<thead>
<tr>
<th>R</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Height SDS</td>
<td>0.22 (&lt;0.01)</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.28 (&lt;0.01)</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.19 (0.001)</td>
</tr>
<tr>
<td>IGF-I SDS</td>
<td>0.55 (&lt;0.001)</td>
</tr>
<tr>
<td>IGFBP-3 SDS</td>
<td>0.68 (&lt;0.001)</td>
</tr>
<tr>
<td>CA boys at start of puberty</td>
<td>0.06 0.50</td>
</tr>
<tr>
<td>CA girls at start of puberty</td>
<td>0.10 0.32</td>
</tr>
</tbody>
</table>

**P1-d2-277 GH and IGF Physiology I**

**Impaired growth hormone signaling pathway in skin fibroblasts of normal newborn boys compared with prepubertal boys**

Paula Ocaarange; Fernanda Morales; Alvaro Matamala; Ximena Gaete; Germán Itiguez; Fernando Cassorla

University of Chile, Faculty of Medicine, Institute of Maternal and Child Research, Santiago, Chile

**Background:** Growth hormone (GH) is required for normal postnatal growth, but it is unclear whether linear growth is dependent on GH during the newborn period, when GH circulating levels are relatively high, and serum IGF-I are relatively low. This suggests that GH sensitivity may be low during the newborn period.

**Objective and hypotheses:** To determine the intracellular activation of JAK2 and STAT5 after GH stimulation and the expression of ALS in skin fibroblasts obtained from normal newborn boys and prepubertal boys undergoing elective circumcision or surgery.

**Methods:** We studied the intracellular activation of JAK2 and STAT5 and the ALS expression in 8 newborn boys and 9 prepubertal boys with normal stature. The activation of these proteins was studied by Western Blot in the non-nuclear fractions of fibroblast cultures obtained from a skin biopsy, both under basal conditions and following stimulation with rhGH (200 ng/mL) for 15-60 minutes. The expression of ALS was determined by RT-PCR after 16 h of stimulation in presence or absence of rhGH.

**Results:** Western blot analyses in the newborns revealed a lack of activation of JAK2 and STAT5 and in the expression of ALS after stimulation with rhGH when compared to the respective basal levels. Similar experiments conducted in prepubertal boys showed a rapid activation of JAK2 and STAT5, and in the expression of ALS after rhGH stimulation. The values are given as mean ± SEM.*P<0.05.

**Molecular features of the subjects enrolled in the study**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>JAK2 activation</th>
<th>STAT5 activation</th>
<th>ALS expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>1.06 ± 0.02</td>
<td>1.05 ± 0.03</td>
<td>1.36 ± 0.19</td>
</tr>
<tr>
<td>Prepubertal</td>
<td>0.97 ± 0.09</td>
<td>1.19 ± 0.08</td>
<td>1.43 ± 0.17</td>
</tr>
</tbody>
</table>

**Conclusions:** These results suggest that the GH signaling pathway is attenuated in fibroblasts from newborn boys. Our data provide support for the concept that normal newborns show evidence of decreased GH sensitivity compared to normal prepubertal boys. (Supported by FONDECYT 1095118)

**P1-d2-278 GH and IGF Physiology I**

**Phenotypic features of 17 patients with a defect of the IGF-1 receptor and the growth response to growth hormone therapy**

Marie J.E. Watenkamp*; Dennis Cramer; Bouwdewijn Bakker*; Edgar van MI; Dick Mul; Roelof J. Dink; Hermine A. van Duyvenvoorde; Stina A. Scheltinga; Sarina G. Kant; Willie Bakker-van Waarde; Annemarie Verrijn Stuart; Wilma Oostdijk*; Jan M. Wf; Monique Losekoof

VU University Medical Center, Pediatrics, Amsterdam, Netherlands; *Reinier de Graaf Gasthuis, Pediatrics, Delft, Netherlands; Jeroen Bosch Hospital, Pediatrics, Den Bosch, Netherlands; Haga Ziekenhuis/Juliana’s Children Hospital, Pediatrics, Den Haag, Netherlands; Catharina Hospital, Pediatrics, Eindhoven, Netherlands; Leiden University Medical Center, LGDA, Clinical Genetics, Leiden, Netherlands; Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands; University Medical Center Groningen, Pediatrics, Groningen, Netherlands; University Medical Center Utrecht, Pediatrics, Utrecht, Netherlands; Leiden University Medical Center, Pediatrics, Leiden, Netherlands

**Background:** The first reported patients with a heterozygous defect of the IGF-1 receptor (IGF1R) showed a small birth size for gestational age (SGA), postnatal growth failure, low head circumference (HC) and IGF-I levels above the mean for age. However, later publications report a more variable phenotype. The effect of growth hormone (GH) therapy has only been described in individual cases.

**Objective and hypotheses:** To describe the spectrum of phenotypic characteristics and the effect of GH treatment in a large group of patients with a defect of IGF1R.

**Methods:** DNA of patients from 8 hospitals with a phenotype suggestive for an IGF1R defect was analyzed. Patients were classified in three subgroups: (a) terminal 15q deletion, including IGF1R defect of the IGF-I receptor and the growth hormone (GH) therapy has only been described in individual cases.

**Poster Presentations**
patients with an isolated IGF1R defect. 14 out of 17 patients had feeding difficulties in the first year. Table 1 shows the response to GH treatment in patients with a terminal 15q deletion (a) or a pathogenic mutation of IGF1R (b) expressed as SDS (mean ± SD).

<table>
<thead>
<tr>
<th>terminal 15q deletion (n=7)</th>
<th>pathogenic IGF1R mutation (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height at start GH</td>
<td>±0.6</td>
</tr>
<tr>
<td>BMI at start GH</td>
<td>±0.9</td>
</tr>
<tr>
<td>GH at start GH</td>
<td>±0.8</td>
</tr>
<tr>
<td>Δ height after 1 year GH</td>
<td>±0.3</td>
</tr>
<tr>
<td>Δ height, last measurement</td>
<td>±1.1</td>
</tr>
<tr>
<td>Δ HC, last measurement</td>
<td>±1.2 ± 1.3</td>
</tr>
<tr>
<td>Max IGF-1 under GH</td>
<td>±4.2 ± 1.1</td>
</tr>
</tbody>
</table>

Conclusions: In patients with SGA, short stature, small HC and IGF-I levels > 0 SD, or unexpected high IGF-I levels under GH treatment, IGF1R analysis is indicated. There is a modest growth response to GH treatment on height and head circumference in patients with a pathogenic IGF1R defect.

P1-d2-279 GH and IGF Physiology 1

**Evidence of both growth hormone (GH) deficiency and neurosecretory dysfunction 10 years after childhood traumatic brain injury**

**Background:** Traumatic brain injury (TBI) in childhood can cause hypopituitarism. The reported prevalence of hypopituitarism varies and may be influenced by age, severity of trauma but also by timing and method of hormonal evaluation.

**Objective and hypotheses:** Aim was to assess the long-term impact of childhood TBI on the GH-IGF-I axis.

**Methods:** 18 participants with moderate (admission Glasgow Coma Score 9-12) or severe (GCS 3-8) TBI (time from injury 7-11 years) and 7 controls matched for age, BMI, sex and pubertal stage. All participants were clinically assessed and underwent an overnight 12 hour GH venous profile (15-20 min sampling) followed by an insulin tolerance test (ITT). GH was assayed with C obst ECLA and deconvolution software (pulse_XP) was used to assess both total GH secretion and pulsatile secretion (pulse frequency, duration, amplitude). Statistical analysis: Mann-Whitney U test.

**Results:** All participants were euthyroid and most were postpubertal (17/18 TBI and 6/7 controls). Four TBI subjects had abnormal GH responses in the ITT (GH peak <3mcg/l). Deconvolution analysis showed that total GH secretion, number of secretion events, mean secretion pulse mass/amplitude and inter-pulse intervals were statistically different between groups. IGF-I levels - although within the normal range - were significantly lower in the TBI group.

**Conclusions:** TBI in childhood is associated with significant GH-IGF-I axis abnormalities 10 years post injury with evidence of both GH deficiency and neurosecretory dysfunction.

<table>
<thead>
<tr>
<th>TBI group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
</tr>
<tr>
<td>Age (years), m (SD)</td>
<td>20.0 (4.2) vs 17.9 (2.2)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>15 (85%) vs 6 (86%)</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>22.4 (3.1) vs 21.7 (3.3)</td>
</tr>
<tr>
<td>GH-Area under curve (SD)</td>
<td>1492 (726) vs 2515 (1360)</td>
</tr>
<tr>
<td>GH-Secretion events (SD)</td>
<td>7.18 (1.67) vs 5.14 (0.69)**</td>
</tr>
<tr>
<td>Mean secretion amplitude (SD)</td>
<td>0.30 (0.13) vs 0.56 (0.363)*</td>
</tr>
<tr>
<td>Mean secretion inter-pulse interval (minutes) (SD)</td>
<td>78.7 (26.6) vs 102.8 (14.9)***</td>
</tr>
<tr>
<td>Mean secretion pulse mass (SD)</td>
<td>5.9 (2.8) vs 14.1 (9.1)**</td>
</tr>
<tr>
<td>IGF-I [nmol/l (SD)]</td>
<td>39.2 (9.1) vs 51.4 (15.4)*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01

P1-d2-280 GH and IGF Physiology 1

**The effect of homozygosity versus heterozygosity for IGFALS gene mutations on insulin resistance and bone strength**

Wolfgang Hogle1; David Martin1; Nicola Crabtree2; Timothy Barrett2; Jan Frystyk2; Jeremy Tomlinson1; Lou Metherell3; Ron Roselfeld3; Vivian Hwa2; Stephen Rose4; Joanna Walker2; Nicholas Shaw2; Birmingham Children’s Hospital, Dept of Endocrinology & Diabetes, Birmingham, United Kingdom; University Children’s Hospital, Paediatric Endocrinology & Diabetology, Tuebingen, Germany; Birmingham Children’s Hospital, Dept of Nuclear Medicine, Birmingham, United Kingdom; University Hospital Aarhus, Medical Research Laboratories, Aarhus, Denmark; University of Birmingham, Centre of Biomedical Research, Birmingham, United Kingdom; Barts and the London School of Medicine, William Harvey Research Institute, London, United Kingdom; Oregon Health Sciences University, Dept of Paediatrics, Portland, United States; Heartlands Hospital, Dept of Paediatrics, Birmingham, United Kingdom; Portsmouth Hospital, Dept of Paediatrics, Portsmouth, United Kingdom

**Background:** Acid-labile subunit (ALS) deficiency inhibits ternary complex formation leading to primary IGF-I deficiency and short stature. Potential metabolic consequences such as insulin resistance and low bone mass have not been in great detail.

**Objective:** This study aimed to measure insulin sensitivity, lipid profile and bone strength in members of 4 affected families, and explore possible gene-dose effects.

**Methods:** 4 patients (7-21 years) with homozygous mutations in the IGFALS gene and 12 heterozygous carriers had intravenous glucose tolerance tests performed. Bone imaging included dual-energy x-ray absorptiometry (DXA) of spine, hip and total body, peripheral quantitative computed tomography of the radius as well as automated radiogrammetry of the hand.

**Results:** Height z-scores in patients (median -3.75 [range -4.25 to -2.62]) were significantly lower compared to carriers (-1.77 [-2.21 to +0.26], p=0.001). Glucose disappearance rate (k), HOMA-IR, fasting insulin, glucose and lipid levels were similar between the two groups. 3 carriers (age 29, 53 and 55y) had k rates below 1%, and elevated fasting glucose levels, indicating diabetes mellitus. Bone density measured by DXA was lower in patients (lumbar spine Z-score -1.6 [-2.6 to -0.9]) compared to carriers (-0.7 [-2.5 to 0.9], p=0.04), likely influenced by their short stature. One postmenopausal carrier fulfilled the criteria for osteoporosis. Radius total and trabecular densities were similar between the two groups yet metacarpal bone width was significantly lower in patients (Z-score -2.67[-3.5 to -2.56]) than in carriers (-1.28 [-1.92 to -0.54], p=0.001). Percent body fat was normal and no subject had a history of low impact fractures.

**Conclusions:** 3/12 IGFALS gene carriers had type 2 diabetes and there was some evidence of insulin resistance in this cohort. ALS deficiency causes impairment in bone lengthening and widening but is insufficient evidence for a reduction in bone density, with some evidence for a gene-dose effect.

P1-d2-281 GH and IGF Physiology 1

**Both fasting ghrelin and leptin concentrations are higher in prepubertal SGA children than AGA children**

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**Background:** Ghrelin, a 28-amino-acid octanoylated peptide, predominantly produced by X/A cells in the lacrimal oxyntic mucosa, is natural ligand of the type 1a growth hormone receptor. However, it is mainly an orexigenic hormone. In healthy children, ghrelin concentration negatively correlates with age, body mass index (BMI), as well as leptin and insulin-like growth factor type I (IGF-I) concentrations. Recently, increased ghrelin levels were ob-
served in children born small for gestational age (SGA), as well as in children with growth hormone deficiency and neurosecretory dysfunction.

**Objective and hypotheses:** The aim of the study was to compare the fasting ghrelin, leptin and IGF-I concentrations in healthy prepubertal SGA children to children with idiopathic short stature born appropriate for gestational age (AGA).

**Methods:** Sixty five SGA children (birth weight below -2.0 SD), aged 4.85-9.75 years (mean±SD: 6.81±1.32 years) and 33 AGA children with idiopathic short stature, aged 3.6-10.75 years (mean±SD: 7.66±2.2 years) were qualified into the study. The control group consisted of 8 healthy, prepubertal AGA children, aged from 5.53 to 10.2 years (mean ± SD: 8.39±2.26 years) with normal body height and normal body weight. In each child, fasting ghrelin, leptin and IGF-I concentrations were measured.

**Results:** BMI SDS values were not different among groups. Ghrelin concentrations in SGA (2537.63±1598.36 pg/mL) were significantly higher than in AGA ISS children (1500.98±796.47 pg/mL) and than in Controls (1188.84±351.83 pg/mL). Also leptin concentrations were higher in SGA children than in AGA ISS children (4.97±5.01 ng/ml vs 2.8±3.02 ng/mL, p<0.05). Moreover, IGF-I SDS were higher in SGA children than in AGA ISS children.

**Conclusions:** It is possible that increased secretions of ghrelin, leptin and IGF-I are adaptive mechanisms to achieve normal growth and weight gain in prepubertal SGA children.

### P1-d3-282 GH and IGF Treatment 1

**Radiological features in patients (pts) with SHOX deficiency (SHOX-D) and Turner syndrome (TS) before and after 2 years of GH treatment**

**Background:** Pts with SHOX-D have variable degrees of skeletal anomaly, from none to pronounced mesomelic dysplasia.

**Objective:** To examine baseline differences & the effect of 2-yr GH treatment on radiological features in pts with SHOX-D & TS.

**Methods:** A 2-yr controlled clinical trial, 51 pts with SHOX-D were randomized to GH (NOGHTx-N: 27; 0.35 mg/kg/wk) or non-treatment (SHOX-noGH, N=24). 26 GH-treated pts with TS were included for comparison. Endocrine & genetically confirmed SHOX-D or TS, age ≥2yr; bone age (BA) <8yr (F), <10yr (M), <9yr (TS); prepubertal; height (ht) <3rd %ile & ht velocity <25th %ile.

**Results:** At baseline, SHOX-noGH, SHOX-GH & TS pts had similar age, BA & ht SDS (Table). Tibial tuberosities & Kosowicz sign (hypertrophic internal femoral condyle) were less common in SHOX-GH vs TS. Prevalence of radial bowing & moderate/severe carpal wedging appeared higher in SHOX-D vs TS (but p<0.05). Baseline radial & ulnar lengths were not significantly different between SHOX-D & TS. After 2-yr of study, no significant difference was observed between SHOX-GH & SHOX-noGH for prevalence or severity of any assessed anomaly. However, there were differences at 2-yr between SHOX-GH & TS (Table). Prevalence of radial bowing was significantly greater in SHOX-GH than TS, & despite no difference in ht gain, radial & ulnar lengths were significantly shorter in SHOX-GH vs TS. Carpal wedging was more severe in SHOX-GH vs TS.

### P1-d3-283 GH and IGF Treatment 1

**Mortality rates for GH-treated (GHTx) patients (pts) in paediatric and adult observational studies**

**Background:** Long-term follow up of the French SAGHe cohort (n=6928) adult pts with history of GHTx for childhood isolated idiopathic GH deficiency [IsiIGHD], idiopathic short stature [ISS] or small for gestational age birth (SGA) demonstrated a standardized mortality ratio (SMR) of 1.3 (95% CI, 1.08-1.64) vs French adult general population (Carel JC et al 2012;JCEM 97:941).

**Objective:** To assess mortality rates for GHTx pts in 2 prospective observational studies: n=11076 IGHD, ISS & SGA children in GeNeSIS, n=11428 adults with childhood-onset (CO) GHD in HypoCOCs.

**Methods:** Death rates were calculated per 100000 person-years (PY) for IsiIGHD, ISS, SGA in GeNeSIS (mean 3yr GHTx) & per 1000 PY for COGHD in HypoCOCs (mean 4yr GHTx). SMRs were calculated using country, age, & sex-specific general population mortality data from CDC & WHO.

**Results:** Five deaths (age 4-20y) were reported among IsiIGHD, ISS & SGA pts in GeNeSIS, for an overall death rate of 19.5/100000 PY; SMR 0.32 (95% CI, 0.16-0.75): IsiIGHD, 3 (diabetic ketoacidosis; respiratory failure; road accident); ISS, 1 (septic meningitis); SGA, 1 (disconnected V-P shunt). 14 deaths (age 19-53y) were reported in COGHD pts followed in HypoCOCs, for an age/sex-standardized observed death rate of 3.2/1000 PY (vs 707 CDC age/sex-adjusted US rate of 700/1000). All deaths were in pts with non-idiopathic GHD (due to craniopharyngioma,7; other brain tumor,2; empty sella,2; pituitary hemorrhage,1; unspecified,2). Causes of death were recurrent/2nd intracranial tumor,2; suicide,2; injury/accident,2; carotid artery stenosis, cerebral hemorrhage, pancreatitis, respiratory failure, sepsis 1 each; unspecified,3.
### P1-d3-284 GH and IGF Treatment 1

#### Comparative proteomic analysis in children with idiopathic short stature (ISS) before and after short-term recombinant human growth hormone (rhGH) therapy


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**Background:** Recombinant human growth hormone (rhGH) treatment is recommended for children with idiopathic short stature (ISS). Discovery of biomarkers predicting the responsiveness to rhGH has tremendous clinical implications. Two-dimensional difference gel electrophoresis (2-D DIGE) that separate proteins from gels using different fluorescent dyes between specimens, is used to discover new biomarkers.

**Objective and hypotheses:** This study was undertaken to identify new serum biomarkers differentially expressed by short-term rhGH therapy in patients with ISS.

**Methods:** The study included 11 children (nine males and two females) with ISS. They were treated with rhGH at a dose of 0.31±0.078 mg/kg/week for three months. To examine the differences in serum levels of specific proteins associated with growth response, immunodepletion of six highly-abundant serum proteins followed by 2-D DIGE analysis, and subsequent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, was carried out in order to generate a panel of proteins differentially expressed after short-term rhGH therapy.

**Results:** Fourteen spots were differentially expressed after rhGH treatment. Among them, apo E and apo L-1 expression were consistently enhanced, while SAA was reduced after rhGH therapy. The differential expressions of these proteins were subsequently verified by western blot analysis using sera of the before and after rhGH treatment. This study found that only the serum apo L-1 levels were significantly elevated after rhGH therapy (P<0.01).

**Conclusions:** 2-D DIGE is a powerful strategy for the discovery of predictive biomarkers. This study suggests that rhGH therapy influences lipid metabolism and that apo L-1 protein can be used as a predictive marker of the short-term effects of rhGH therapy in ISS patients.

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### P1-d3-285 GH and IGF Treatment 1

#### Cut-off limit of serum growth hormone (GH) in pharmacological test of GH secretion for ICMA immunoassay with the IRP IS 98/574

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4Alicia Belgorosky**

**Background:** Different cut-off values, from 3 to 10 ng/ml, depending on the assays, have been defined for pharmacological test (Pit) of growth hormone (GH) secretion, based on biochemical and clinical studies. Recently, a 22K recombinant GH isoform IRP IS 98/574 have been commercialized. We had previously defined a 6.1 ng/ml value, in terms of ICMA IRP IS 80/505 as serum GH cut-off limit in Pit of GH secretion.

**Objective and hypotheses:** Our aim was to assess which is the GH deficiency (GHD) diagnostic cut-off limit of serum GH in Pit of GH secretion for ICMA immunoassay with the IRP IS 98/574.

**Methods:** We analyzed serum GH concentration in 138 serum samples, using (ICMA calibrated with both IS 80/505 and IS 98/574. Blood samples (n=138), from 92 different individuals, (103 males and 35 female; age range, 2.5 15.0 years) were used. All samples were selected from arginine and clonidine Pit. **Results:** We found high significant linearity (y = 0.7307x + 0.2768, r²<0.001) between ICMA IS 98/574 (y) and ICMA IS 80/505 (x). The y value for fixed x value of 6.1 ng/ml in the ICMA IS 80/505 was calculated, and a diagnostic cut-off limit for ICMA IS 98/574 was estimated at 4.7 ng/ml. ICMA IS 98/574 / ICMA IS 80/505 ratio plot and 95% prediction interval was 0.79±0.17. Linearity and Bias were reconfirmed by Passing Bablock analyses and the Wilcoxon test respectively.

**Conclusions:** High significant linearity and differences among the assay with different IS were found. To suspect the diagnosis of GHD, we propose 4.7 ng/ml as the serum GH cut-off limit value in Pit for ICMA immunoassay with the IRP IS 98/574. Finally for each GH assay, an appropriate cut-off value for serum GH maximum peak response to Pit should be strongly recommended.

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### P1-d3-286 GH and IGF Treatment 1

#### Leptin correlates positively with anabolic GH effects in prepubertal short children growing SDS channel-parallel on GH treatment

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**Background:** Few studies have evaluated the metabolic outcomes of growth hormone (GH) treatment in prepubertal short children during different growth phases. We have studied individualized GH treatment in the maintenance growth phase and have previously found that IGF-1 lost its importance as one of the main growth factors after reaching near mid-parental height (MPH). This was shown by lacking correlations between IGF-1 and anabolic GH effects, i.e. A height, A lean soft tissue and A bone mineral content. We found high significant linearity (y = 0.7307x + 0.2768, r²<0.001) between ICMA IS 98/574 (y) and ICMA IS 80/505 (x). The y value for fixed x value of 6.1 ng/ml in the ICMA IS 80/505 was calculated, and a diagnostic cut-off limit for ICMA IS 98/574 was estimated at 4.7 ng/ml. ICMA IS 98/574 / ICMA IS 80/505 ratio plot and 95% prediction interval was 0.79±0.17. Linearity and Bias were reconfirmed by Passing Bablock analyses and the Wilcoxon test respectively.

**Objective and hypotheses:** This study was to construct two groups - one with half the original dose, reduced individualized dose (RID, 17–50 µg/kg/ day) and the other to remain at the original dose, unchanged individualized dose (UID, 17–100 µg/kg/day). A third group remained on fixed standard dose (FIX) constituted the control group (43 µg/kg/day). All three groups were indistinguishable at the randomization. In total, 98 prepubertal children, who were initially short due to either isolated GH and/or IGF deficiency participated in this 2-year randomized trial.

**Methods:** We focused on the metabolic outcome of children growing channel-parallel within ± 0.3 heightSDS after two years of maintenance growth phase. We have studied individualized GH treatment in the maintenance growth phase and have previously found that IGF-1 lost its importance as one of the main growth factors after reaching near mid-parental height (MPH). This was shown by lacking correlations between IGF-1 and anabolic GH effects, i.e. A height, A lean soft tissue and A bone mineral content.

**Objective and hypotheses:** This study was to construct two groups - one with half the original dose, reduced individualized dose (RID, 17–50 µg/kg/ day) and the other to remain at the original dose, unchanged individualized dose (UID, 17–100 µg/kg/day). A third group remained on fixed standard dose (FIX) constituted the control group (43 µg/kg/day). All three groups were indistinguishable at the randomization. In total, 98 prepubertal children, who were initially short due to either isolated GH and/or IGF deficiency participated in this 2-year randomized trial.

**Results:** We observed a leptin to be highly correlated with the anabolic GH effects in 18 children growing closest to the height SDS mean (Figure 1). They included 10 children receiving RID, 4 receiving the UID, and 4 in the fixed dose group (FIX).
We performed a retrospective review of SGA patients and its relationship with growth hormone treatment

**Background:** Short children born SGA are at increased risk for diabetes and cardiovascular disease. Growth Hormone (GH) treatment can also increase this metabolic risk.

**Objective:** Analyze the metabolic risk parameters in SGA patients at baseline and during treatment with GH in our region.

**Population and methods:** We performed a retrospective review of SGA children in treatment with GH in our region, using as data sources the GH Committee Register. We established 2 groups according to HOMA-RI (≤or > 3.16). Other metabolic risk parameters were evaluated: HDL-C, triglycerides (TG), systolic (SBP) and diastolic (DBP) blood pressures and GH dosage. Description of categorical (%) and continuous (mean±SD) variables. Comparative study of different parameters with Kruskal-Wallis non-parametric test.

**Results:** 76 patients (36 M, 40 F). Birth height 42.9±4.3cm (-2.8±1.2SD) and weight 2067.9±680g (-2.1±0.7SD). 31% were premature. HOMA-IR >3.16). Other metabolic risk parameters were evaluated: HDL-C, triglycerides (TG), systolic (SBP) and diastolic (DBP) blood pressures and GH dosage. Description of categorical (%) and continuous (mean±SD) variables. Comparative study of different parameters with Kruskal-Wallis non-parametric test.

**HOMA-IR:**

- **% Initial n=74**
  - ≤3.16: 89.2%
  - >3.16: 10.8%
- **% First year n=76**
  - ≤3.16: 75%
  - >3.16: 25%
- **% Fourth year n=16**
  - ≤3.16: 75%
  - >3.16: 25%

HOMA-IR ≤3.16 group had statistically significant minor IGF-1 mean at first and fourth year with treatment and independent of pubertal status.

**HOMA-IR**

- **IGF-1 first year (ng/ml)**
  - ≤3.16: 240±91
  - >3.16: 375±123
- **IGF-1 fourth year (ng/ml)**
  - ≤3.16: 351±177
  - >3.16: 541±59

p=0.022* p=0.040**

The percentage of SGA who started puberty grew 5.7%, 14.8% and 58.8% at baseline, 1 year and 4 years of GH treatment. There were no statistically significant differences between HOMA-IR at 1st year and 4th year in relation with other parameters.

**Conclusions:** HOMA-IR >3.16 group doubled during first year with GH treatment. This is the only metabolic risk parameter which has changed. This group also showed higher IGF-1 values than HOMA-IR ≤ 3.16 group.
P1-d3-289 GH and IGF Treatment 1

**Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study**

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**Background:** Knowledge about the effects of GH-treatment on cognitive functioning in children with PWS is limited.

**Objective and hypotheses:** To evaluate the effect of GH-treatment on cognitive functioning in children with PWS is limited.

**Methods:** Fifty pre-pubertal children, aged 3.5 to 14 years were studied in a randomized controlled GH trial during 2 years, followed by a longitudinal study during 4 years of GH-treatment. Cognitive functioning was measured biennially by short forms of the WPPSI-R or WISC-R, depending on age. Total IQ (TIQ) score was estimated based on 2 subtest scores.

**Results:** During the RCT, mean SD-scores of all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, while in untreated controls mean subtest SD-scores and mean TIQ score decreased and became lower compared to baseline. This decline was significant for the Similarities (p=0.04) and Vocabulary (p=0.03) subtests. After 4 years of GH-treatment, mean SD-scores on the Similarities and Block design sub-tests were significantly higher than at baseline (p=0.01 and p=0.03, respectively) and scores on Vocabulary and TIQ scores remained similar compared to baseline. At baseline, children with a maternal uniparental disomy had a significantly lower score on the Block design subtest (p=0.01), but a larger increment on this subtest during 4 years of GH-treatment than children with a deletion. Lower baseline scores correlated significantly with higher increase in Similarities (p=0.04) and Block design (p=0.0001) SD-scores.

**Conclusions:** Our study shows that GH treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract reasoning and visuospatial skills, during four years of GH-treatment. Furthermore, children with a greater deficit had more benefit from GH-treatment.

P1-d3-290 GH and IGF Treatment 1

**Long-term growth hormone therapy is associated with a dose-dependent increase in height SDS and insulin-like growth factor I SDS in short Japanese children born small for gestational age**

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**Background:** In children with short stature born small for gestational age (SGA), growth hormone (GH) treatment is associated with an acceleration of growth allowing most to achieve an adult height adequate for their target height. The pathophysiology of growth failure may arise from anomalies in the GH-IGF axis, including deficiencies in GH/IGF-1 concentrations and/or GH/IGF-1 sensitivity.

**Objective and hypotheses:** There is limited data on long-term GH therapy in Japanese children with SGA. This report investigates the relationship between GH dose, Height SDS (HSDS) and IGF-I SDS.

**Methods:** Data were analysed from a 156-week extension of a 104-week multicentre, randomised, double-blind, parallel group trial investigating the efficacy and safety of GH. Sixty-five children with SGA (age 3–8 years) received GH at 0.033mg/kg/day (n=31, 64.5% male, mean age 5.34 years) or 0.067mg/kg/day (n=34, 58.8% male, mean age 5.27 years). Change from baseline in HSDS, IGF-I SDS, and bone age (BA) were recorded.

**Results:** After 260 weeks, HSDS for chronological age (CA) was significantly positively correlated with ΔIGF-I SDS (n=57, r=0.664; p<0.0001). A greater increase in ΔHSDS and ΔIGF-I SDS was observed in the 0.067 than 0.033mg/kg/day group; correlation between ΔHSDS and ΔIGF-I SDS was shown in both (0.067mg/kg/day; r=0.579; p=0.0010; 0.033mg/kg/day: r=0.715; p<0.0001). ΔHSDS was positively correlated with ΔIGF-I SDS in male (n=36; r=0.707; p<0.0001) and female (n=21; r=0.685; p=0.0006) patients. Mean bone age (BA) was behind CA at baseline (BA/CA ratio <1) but increased during GH treatment, reaching 1.01 in the 0.033mg/kg/day group and 1.09 in the 0.067mg/kg/day group at 260 weeks.

**Conclusions:** In short Japanese children born SGA long-term GH therapy was associated with a dose-dependent increase in IGF-I that positively correlates with changes in HSDS. No acceleration of BA was noted in the low dose group but a minor acceleration was seen in the high dose group.

P1-d3-291 GH and IGF Treatment 1

**Increlex®-treated children enrolled in the increlex growth forum database (IGFD) in Europe: 2 years interim results on safety and effectiveness**

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**Background:** The post-registration authority, European Increlex® (Mecasermin [rDNA Origin] Injection) Growth Forum Database (EU-IGFD) was initiated in Dec 2008 to monitor the safety and effectiveness of Increlex® in children with growth failure.

**Objective:** Report 2-yr data from the registry, particularly for naïve pre-pubertal patients (pts).

**Methods:** Multicenter, open-label observational study.

**Results:** As of Oct 2011, 128 pts were enrolled in 9 countries: 34% female, 81% pre-pubertal, 60% growth treatment-naïve, 81% with severe primary IGF-1 deficiency. Mean (SD) age at first injection was 10.2 (3.9) yrs. Median treatment duration was 480 days (i.e. 183 pt-yrs). Median dose was 40, 110 and 113 µg/kg BID at start of treatment, yr 1 and yr 2 respectively. A total of 88 targeted adverse events (AEs) were reported for 43 pts (37%), the most frequent being hypoglycaemia (18 pts, 16%) serious for 4 pts (3%). New treatment-related serious AEs since last report were papilloedema, thyroid nodule, splenic infarction, toxoplasmosis (1 pt each). Effectiveness data are summarised by mean (SD) of height SDS, Height Velocity (HV).

<table>
<thead>
<tr>
<th>N*</th>
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<th>N*</th>
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<td></td>
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<td></td>
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<td></td>
<td>Yr 2 11</td>
<td>-2.6 (1.4)</td>
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* N data available at the timepoint

**Conclusions:** These 2-yr interim results from EU-IGFD registry did not show any new safety signals. However, we recommend special care for patients with complex syndrome, particularly if related to proliferative conditions. On average, HV increased after 1 yr of treatment and results of 2nd yr HV should be interpreted with caution due to the limited number of data and the large inter-individual variability observed in response to treatment.
Different growth pattern in response to GH therapy in SGA and AGA GH-deficient prepubertal children

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Background: Birth weight may influence the first year response to growth hormone (GH) therapy in GH-deficient (GHD) children, while its role during the following years of treatment is not fully elucidated.

Objective: We examined the growth of small for gestational age GHD children (SGA-GHD) and appropriate for gestational age GHD (AGA-GHD) children during the first five years of GH therapy.

Methods: Twenty-three SGA-GHD (birth weight and/or length ≤ -2 SDS for sex and gestational age; 11 F and 12 M; age: 1.3-12.3 years) and 26 AGA-GHD (11 F and 15 M; age: 3-12 years) were randomly enrolled and followed for five years after the start of GH treatment, yearly collecting height, weight height velocity and body mass index (BMI). Both SGA-GHD and AGA-GHD patients received rhGH at the recommended weekly dose of 0.25 mg/kg subcutaneously, in six daily doses.

Results: Although SGA-GHD subjects had significantly reduced weight and length at birth, at time of GH diagnosis the height-SDS and BMI-SDS were comparable between the two groups. During the first years of treatment height-SDS was still not different between SGA-GHD and AGA-GHD, although the values were always slightly lower in SGA-GHD; only from the fourth year of treatment AGA-GHD showed significantly increased height-SDS (figure). Pre-treatment height velocity SDS was similar between SGA-GHD and AGA-GHD (SGA-GHD: -1.82±0.30 SDS, AGA-GHD: -2.33±0.21 SDS), but it became significantly higher (SGA-GHD: 1.72±0.30 SDS, AGA-GHD: 2.67±0.21 SDS; p=0.039) in AGA-GHD during the first year of treatment. However, in the following years height velocity SDS was still comparable between the two groups.

Conclusions: In conclusion, height-SDS is comparable between SGA-GHD and AGA-GHD in the first years of GH treatment, but it becomes significantly reduced in SGA-GHD children from the fourth year on. Further studies are needed for confirming if this “waning effect” persists also in the following years of treatment.

Metabolic effects of LB03002, a sustained release formulation of rhGH, in children with GH Deficiency

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Background: Growth hormone has profound effects on body composition and lipid metabolism in children as well and adults. We previously demonstrated in a randomized phase III multicentre study that once weekly LB03002 is comparable to daily rhGH regarding safety and efficacy and now present data on metabolic parameters.

Objective and hypotheses: The effect of LB03002 treatment on glucose metabolism and lipid parameters was assessed.

Methods: 167 previously untreated children with growth failure (HTSDS ≤ -2 unless organic GHD, HVSDS ≤ -1) due to idiopathic or organic GH deficiency (GH peak ≤ 7 ng/mL in two tests) were randomized to receive either once weekly LB03002 (0.5 mg/kg) or once daily rhGH (0.03 mg/kg) for 12 month. Patients treated with daily rhGH were switched to weekly LB03002 in the second year.

Results: Selected growth and metabolic parameters (mean±SD):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First year/second year treatment</th>
<th>Weekly/weekly (N=87)</th>
<th>Daily/weekly (N=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV (cm/yr)</td>
<td>Baseline: 2.64±1.11</td>
<td>2.87±1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st year: 11.72±2.58</td>
<td>12.16±3.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd year: 8.33±1.92</td>
<td>7.28±2.34</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>Baseline: 4.70±1.60</td>
<td>4.51±0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 12: 4.58±1.49</td>
<td>4.43±0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 24: 4.43±1.33</td>
<td>4.22±0.94</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>Baseline: 4.01±1.08</td>
<td>4.09±0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 12: 4.40±0.68</td>
<td>4.66±0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 24: 4.56±0.48</td>
<td>4.47±0.68</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>Baseline: 5.07±0.38</td>
<td>5.04±0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 12: 5.13±0.29</td>
<td>5.14±0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 24: 5.35±0.36</td>
<td>5.33±0.38</td>
<td></td>
</tr>
</tbody>
</table>

Growth parameters were comparable for both groups. There were no relevant differences between treatment groups in metabolic parameters (glucose, insulin, haemoglobin A1c, cholesterol, and triglycerides). Cholesterol slightly decreased, while fasting glucose, insulin, and HbA1c increased over time but remained within the normal range. Changes from baseline of the assessed parameters were not clinically relevant. No patients developed diabetes.

Conclusions: The data show that weekly treatment with LB03002 has a comparable metabolic effect as treatment with daily rhGH.

*In cooperation with Biopartners’ and LG Life Sciences’ GH Study Group
The relationship between baseline IGF-I levels, first year growth and metabolic outcomes in children born small for gestational age during high dose growth hormone therapy - North European SGA Study (NESGAS)

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Background: Children born small for gestational age (SGA) exhibit wide variations in GH/insulin-like growth factor-I (IGF-I) axis activity and this heterogeneity may identify individuals with poor growth responses or adverse metabolic effects. We explored the variations in growth and glucose metabolism in response to a fixed GH dose over 1 year in SGA children with respect to IGF-I levels at baseline.

Methods: The North European Small for Gestational Age Study (NESGAS) is a multicenter study (n=110, 69 males) of GH therapy in prepubertal short SGA children. Patients received GH therapy at 0.75 µg/kg/day for 1 year. Glucose metabolism was assessed by a short intravenous glucose tolerance test. Insulin sensitivity (IS) and insulin secretion were estimated from HOMA and acute insulin response (AIR), respectively. Disposition index (DI) provided a measure of insulin secretion adjusted for IS.

Results: One year of GH therapy led to marked increases in height SDS and IGF-I-SDS, and was associated with a significant decrease in IS (p<0.0001). An increase in AIR was observed (p<0.0001), however, this compensation was partial resulting in a decrease in DI (p=0.032). Children in the highest IGF-I-SDS tertile at baseline were the least insulin sensitive both at baseline (p<0.02) and at one year (p=0.007), and had reduced ΔHSDS (p=0.006) and ΔIGF-I-SDS (p<0.0001) responses compared with other tertiles. ΔIGF-I-SDS was related to AIR (r=0.30, p=0.007) and DI (r=0.29, p=0.005) at one year.

Conclusion: The finding of reduced height gains and changes in IGF-I during GH therapy in children with higher IGF-I-SDS at baseline are indicative of GH and IGF-I resistance. Defining heterogeneity by baseline IGF-I-SDS is useful in terms of predicted growth response, but may also be relevant to metabolic outcomes.

GH treatment in children with Prader Willi syndrome: 3 years longitudinal data in prepubertal children from KIGS database

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Background: Prader Willi Syndrome is a rare disorder due to lack of expression of the paternally derived chromosome 15q11-13, in most children due to a deletion or uniparental maternal disomy. Children with PWS have impaired growth and growth hormone (GH) treatment has shown to improve growth. However, longer term data of a large group of GH-treated prepubertal children with PWS have not yet been reported.

Objective and hypotheses: To evaluate growth during 3 years of GH treatment in a large group of prepubertal children with PWS.

Methods: 415 prepubertal children (224 boys) with PWS who were treated with GH for 3 years. Their longitudinal data were registered in KIGS database (Pfizer International Growth Database).

Results: Mean(SD) birth weight and birth length SD score (SDS) were -1.25 (1.1) and -0.23 (1.4), resp. Mean (SD) mid parental height SDS was -0.11 (1.1). Prior to start of GH, 128 children had a GH stimulation test with a mean (SD) GH peak of 8.1 (9.3) µg/l and mean (SD) serum IGF-I-SDS -1.11 (1.5). All children remained prepubertal during the 3 years of GH treatment. Mean (SD) GH dose was 0.23 (0.06) mg/kg/wk. Table 1. Growth data during 3 years of GH treatment in 415 prepubertal children.

Conclusions: These data from a very large group of prepubertal children with PWS demonstrates that growth hormone treatment significantly improves growth of these children, resulting in a complete normalization of their stature within a few years.

Once-weekly, CTP-modified hGH (MOD-4023) effectively maintains IGF-I levels within the normal range in growth hormone deficient adults, supporting initiation of clinical development in children

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Background: Growth Hormone (GH) replacement therapy currently requires daily injections, which may cause poor compliance and distress for patients. CTP-modified hGH (MOD-4023) is being developed for once-weekly administration in GH Deficient adults (GHDA) and children.

Objective and hypotheses: The phase II study in adult GHDA patients evaluated the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of MOD-4023 in GHDA.

Methods: 39 GHDA currently treated with daily GH were randomized and switched to 3 dose levels of once-weekly MOD-4023 to evaluate safety and PK/PD profile (30%, 45% or 100% of each patient’s cumulative weekly hGH dose). The study was comprised of two stages, Stage I included an optimization period and 4 weeks of once-weekly MOD-4023. Stage II is an optional 16 week extension period of once weekly MOD-4023 to collect further safety information and confirm the results obtained in Stage I. Here we present the results of Stage I.

Results: MOD-4023 was well-tolerated and a dose dependent response of IGF-1 concentration was demonstrated. In most patients, IGF-I levels were maintained within ±2 SDS during the 4 weeks, without exceeding >2 SDS at peak levels. In two cohorts (45% and 100%), the mean IGF-I values were comparable to those obtained with daily hGH at steady state. No drug-related Serious Adverse Events were reported during the study. The adverse effects reported (mainly headaches) were consistent with known hGH related side-effects, and were mostly mild. MOD-4023 was not immunogenic.

Conclusions: Once-weekly, repeated doses of long-acting MOD-4023 were shown to be safe and well tolerated in GHDA. IGF-I levels were maintained...
within the normal range in most MOD-4023 treated patients for the entire 4 weeks. Based on the positive results of Stage I, the pediatric clinical development program was initiated and a phase II study in naive, pre-pubertal GH Deficient children is currently ongoing.

P1-d3-297 GH and IGF Treatment 1
Response to growth hormone (GH) treatment in GH deficient survivors of bone marrow transplantation with total body irradiation (BMT/TBI) in childhood
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1University Hospitals Bristol NHS Foundation Trust, Paediatric Endocrinology and Diabetes, Bristol, United Kingdom; 2University of Bristol, Institute of Child Health, Bristol, United Kingdom

Background: Childhood BMT/TBI survivors are often short adults, despite GH treatment for growth hormone deficiency(GHD). Final height is adversely affected by reduced spinal and pubertal growth but there are no data examining the GH response after TBI.

Objective and hypotheses: To assess the response to GH treatment and GH sensitivity using IGF-1 generation tests in GHD BMT/TBI survivors compared to controls with isolated GHD.

Methods: 41 subjects were investigated.13/22 BMT/TBI survivors were GHD(Group 1) and 6/19 controls had isolated GHD(Group2) in insulin tolerance tests. All GHD subjects had pre-treatment IGF-1 generation tests(Igf-1 day 1 and day 5 after 4 days of GH (33mcg/kg/day)). Auxology and whole body DEXA for body composition were assessed before and after 12 months GH treatment. Fat free mass index (fat free mass / height2) was calculated. GH dose was the same in both groups (5mcg/m2/week).

Results: Group1:6 post-pubertal, 7 pre-pubertal. Group 2: All 6 pre-pubertal. Height velocity increased from 3.4 to 9.0cm/yr (p=0.001,N=7) and from 4.0 to 10.7cm/yr (p=0.001,N=6) and height SDS improved from -1.24(1.60) to -0.75(1.80) (p=0.004) and -2.17(1.05) to -1.84 (0.89) (p=0.001), in groups 1 and 2 respectively. IGF-1 in generation tests increased from 23.0(13.0-64.1) to 55.0(31.7-81.6)mcg/l (p=0.001,N=13,group 1) and 24.9(8.5-53.8) to 47.0(16.1-82.3)mcg/l (p=0.002,N=6,group 2). Body fat decreased in both groups: from 11.89(2.09) to 13.06(1.60)(p=0.002,N=13,group 1) and 27.8(14.4%) to 23.5(16.1) to 27.8(14.4%) (p=0.034,N=6,group 2). Fat mass index increased in both groups: from 11.89(2.09) to 13.06(1.60)(p=0.002,N=13,group 1) and 12.45(1.04) to 15.16(1.78)kg/m2 (p=0.005,N=6,group 2). The magnitudes of all these changes did not differ between the groups.

Conclusions: Paired data comparing growth and IGF-1 responses, and body composition changes before and after 12 months GH treatment in BMT/TBI survivors with GHD and non-BMT controls with isolated GHD showed similar responses to GH treatment. We found no evidence of GH resistance in BMT/TBI survivors.

P1-d3-298 GH and IGF Treatment 1
IGF-I in isolated GHD (IGHD) and non-GHD boys; results from two randomized clinical trials with GH treatment to adult height
Elena Lundberg1; Kerstin Albertsson-Wikland2; Björn Jonsson3; Bert Kristad1
1Umeå University, Institute of Clinical Science, Paediatrics, Umeå, Sweden; 2Institute of Clinical Science, The Sahlgrenska Academy at University of Gothenburg, Department of Pediatrics, Göteborg Pediatric Growth Research Center (IGP-GRC), Gothenburg, Sweden; 3Uppsala University, Department of Women’s and Children’s Health, Uppsala, Sweden

Background: GH children are more responsive to GH than non-GHD children regarding growth. Knowledge regarding IGF-I responsiveness is scarce. Objective and hypotheses: To compare changes and mean levels of IGF-I in GHD vs non-GHD boys from two clinical trials on GH treatment to adult height (AH). The same GH dose would give IGF-I levels higher in a GHD vs a non-GHD group.

Methods: The IGHD boys (n=85) had ≥2 prepubertal yr on GH 33µg/kg/d (GH+) and were at onset of puberty randomized to 67 µg/kg/d (GH(GH+), n=50) or GH0 (n=35). The non-GHD boys (n=58), were randomized to GH

(IGH) (n=35) or GH(GH) (n=23) at GH start and 36 boys had one prepuberal yr. IGF-I was measured at baseline, 3m and yearly and converted to SDS. Study levels of IGF-I were defined as individual mean levels from 12m to study stop for the non-GHD group and for the GHD group from 12m from randomization close after onset of puberty to AH and the ΔIGF-I as change from start to mean level. Heights were converted to SDS vs the childhood reference and at AH vs the reference at 18 yrs.

Results: The prepubertal ΔIGF-I was significantly lower in the non-GHD GH group vs the GH0 (1.6±1.2 vs 2.3±0.9, p=0.046) and vs the GHD GH+ (2.7±1.3, p=0.020) and GH0 (2.5±1.9, NS vs GH+). The non-GHD prepubertal IGF-I study levels differed in GH+ vs GH0 (0.6±0.8 vs 3.1±1.1, p=0.008) but not in relation to and within the other groups. As a single variable, baseline IGF-I explained 13-14% of the variance in total & prepubertal height SDS gain. Using multivariate regression prepubertal and pubertal ΔIGF-I were significant predictors of total, prepubertal and pubertal height SDS gain.

Conclusions: The ΔIGF-I, and pubertal level was found to be lower for the non-GHD GH group when IGHD and non-GHD boys randomized in clinical trials to GH+ or GH0 were compared. Explaining total height gain for the merged group, ΔIGF-I but not GH dose was informative; thus it is not the dose per se that is important, but the responsiveness.

P1-d3-299 GH and IGF Treatment 1
Final height after long-term growth hormone therapy in SHOC2 mutation-positive patients
Federica Tamburro1; Emanuela Scarano2; Annamaria Pern3; Benedetta Vestrucci1; Michele Torella1; Monica Guidetti1; Laura Mazzanti1; Rare Disease and Aulogy Unit, Department of Pediatrics, S Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Background: Abnormalities of the RAS-MAPK signaling pathway has been identified as the cause of Noonan syndrome and related disorders (RASopathies). A single missense mutation in SHOC2 gene determine a specific condition previously termed Noonan-like syndrome with loose anagen hair (NS/LAH) [1,2]. Typical features are NS facial phenotype, growth retardation frequently associated with proven growth hormone (GH) deficiency, developmental delay with ADHD disorder, hair anomalies, darkly pigmented skin with eczema or ichthyosis, hypernasal voice, cardiac defects.

Methods: In our group of NS/LAH patients, 3 of them (1 male and 2 female) reached the final height (FH) with GH (dose 7 mg/m2/week) for a mean period of 12.7±2.2 yrs. 2 patients presented SHOC2 mutation and a female showed SHOC2 and SOS1 mutation. All the patients had a severe GH deficiency (GH peak < 4 ng/ml after arginine and L-dopa test or clonidine test) and bone age and puberty were delayed. A female was treated with L-tyrosine for hypothryroidism. No patient showed pubertal growth spurt.

Results: FH was 161.3 cm in the male (<3rd centile for H and age - Target H (TH) <25th centile) with height gain of 0 SD after GH therapy; in females, 147.1 cm (<3rd centile - TH <25th centile) with height gain of +0.4 SD and 148.5 cm (<3rd centile and TH > 50th centile) with height gain of +1.79 SD.

Conclusions: Differently to PTPN11+ patients, two-thirds of SHOC2+ subjects show severe GH deficiency and short stature. This is the first study reporting FH data: the height gain after long-term GH-therapy was variable and no pubertal growth spurt was observed. This behaviour seems to confirm the hypothesis of an interconnection between SHOC2 and GH signal pathway that remain to be elucidated in NS/LAH subjects. Other studies with FH data in SHOC2+ patients will be mandatory to collect statistically significant groups of subjects.

2. Cordeddu et al., Nat Genet 2009; 41(9):1022-1026
GH and IGF Treatment 1

A proposal of a decision-making score for GH treatment modulation in Prader-Willi syndrome

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1Università degli Studi dell’Insubria, Pediatrics, Varese, Italy; 2Università Vita e Salute San Raffaele, Pediatrics, Milan, Italy; 3Ospedale Pediatrico Bambino Gesù, IRCCS, Endocrinology and Diabetology Dpt, Pfeldor (Rome), Italy; 4Azienda Ospedaliera Triviglio, Pediatrics, Treviglio (BG), Italy; 5IRCCS Istituto Auxologico Italiano, Auxology, Verbania, Italy; 6Università degli Studi di Modena, Pediatrics, Modena, Italy; 7Ospedale di Rho, Pediatrics and neonatology, Rho (MI), Italy

Background: Following few cases of death reported in Prader-Willi syndrome (PWS) children during GH treatment, several international committees recommend serum IGF-1 monitoring, otorhynolaryngologist examination and polysomnography before and during GH treatment in PWS. However there is no general consensus on how to interpretate these evaluations for a safe GH therapy management.

Objective: To develop a practical decisional tool, based on current clinical evidence and international recommendations that provides useful and reliable criteria to modulate GH therapy in children with PWS.

Methods: We developed a 15-point score, which measures the respiratory risk on the basis of polysomnography parameters (respiratory disturbance index, and mean SpO2), otolaryngologist examination by flexible fibroendoscopy (Brodyk and Wang criteria for assessment of tonsils and adenoid hypertrophy) and levels of IGF-1. This score was named “POI” (Polysomnography, Otolaryngologist, IGF1). We used it retrospectively, as preliminary validation, in 7 patients in order to compare the therapy modulations established by empirical criteria and those resulting by using the score.

Results: In five patients the modulation based on empirical criteria was the same of that suggested by our score, in one case POI resulted slightly more restrictive and in another case slightly more tolerant.

Conclusions: Our proposal of a score to be used in the modulation of GH therapy in Prader-Willi syndrome, before becoming a tool for standardization of this treatment, requires first to be tested on a wider sample of patients in order to obtain general consensus.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values (points)</th>
<th>Values (points)</th>
<th>Values (points)</th>
<th>Values (points)</th>
<th>Score</th>
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<tbody>
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<td>&gt;95% (2)</td>
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<td>50-75% (2)</td>
<td>&gt;75% (3)</td>
<td>&gt;6 (4)</td>
</tr>
</tbody>
</table>

**TOTAL:**

Score: ∑ of 15

0-3: Start at 0.03 mg/kg/day maintain or increase the dosage
4-6: Start at 0.015 mg/kg/day maintain the dosage
7-9: Start at 0.01 mg/kg/day 50% dosage reduction
10: Do not start treatment discontinuation of the treatment

GH and IGF Treatment 1

ADD and stimulant use in children receiving growth hormone therapy: an analysis of the KIGS (Pfizer International Growth Study)

Bradley S. Miller1; Ferah Aydin2; Frida Lundgren3; Mitchell Gaffney4
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Background: Stimulant use for the treatment of Attention Deficit Disorder (ADD) has been associated with growth failure. Prevalence of ADD has been reported to be 3-8% worldwide in school-aged children.

Objective and hypotheses: Identify the frequency of ADD and stimulant use in children treated with Genotropin.

Methods: Children enrolled in KIGS with idiopathic GH deficiency (IGHD), idiopathic short stature (ISS), and Turner Syndrome (TS) were evaluated for the associated diagnosis of ADD prior to initiation of Genotropin. Concomitant medication entries for stimulant medications were captured. Baseline auxologic information was also extracted.

Results: Out of 75,261 children enrolled in KIGS between 1990 and 2011, 1,748 children (2.3%) with ADD stimulant therapy were identified. When analyzed by country of origin, a significantly greater number of children was from the U.S. (1,092/14,794=7.4%) than from the rest of the world (66/60,467=1.1%) (p<0.001). In the U.S., the frequency of ADD by treatment group was: IGHD: 8.7%, ISS: 8.2%, and TS: 3.8%. ISS, GHD, ISS and TS children with ADD treated with stimulants from whole cohort were taller (p<0.0001) and thinner (p<0.0001) than those in all 4 groups without ADD at the time of initiation of Genotropin therapy.

Conclusions: There is significant difference in the frequency of ADD stimulant use in U.S. vs non-U.S. children in KIGS. These differences in prevalence may reflect regional differences in diagnosis and treatment of ADD. The overall prevalence of ADD in children with IGHD, ISS, and SGA in KIGS was similar to worldwide prevalence estimates of ADD in the general population. The overall prevalence of TS, however, was less severe and BMI lower than in non-ADD counterparts. We speculate that low BMI diagnosis of ADD stimulant use may lead to referral at heights that are less severely affected.
SDS) and 643 µg/ml (+2.71 SDS), respectively, even under a relatively lower rhGH dosage of 0.022 mg/kg/d. Due to the marked IGF-I increase and the concomitant incidence of an epitheломa of Malherbe, rhGH treatment was interrupted. During the first 6 months after rhGH withdrawal, GI remained at 7.2 cm/yr (IGF-I 367 ng/ml +2 SDS; IGFBP3 634µg/mL +1.8 SDS), decreasing to 3.2 cm/yr during the following year. At her last check up (height 121.5 cm; -2.46 SDS) she showed a striking increase of body fat and BMI (22.73).

Conclusions: IGF1R haplinsufficiency due to heterozygous IGF1R deletion produces a clinical phenotype characteristic of IGF1RS. In our patient, low dose rhGH treatment quickly normalized the growth rate but dramatically increased circulating IGF-I. Hence, pro and cons of rhGH treatment in these patients should be carefully considered.

P1-d3-303 GH and IGF Treatment 1
Long-term efficacy of GH therapy in 37 short children with Silver-Russell syndrome
Gerhard Binder1; Melanie Lief2; Joachim Woelfle3; Roland Schweizer1
1University Children’s Hospital, Pediatric Endocrinology, Tuebingen, Germany; 2University Children’s Hospital, Pediatric Endocrinology, Bonn, Germany

Background: Long-term efficacy of GH in patients with Silver-Russell syndrome (SRS) is unknown.
Objective: We aimed to compare final heights of GH-treated and GH-untreated.
Individuals: 37 GH-treated patients (16 females) were diagnosed according to strict clinical criteria and partipated in this retrospective study with a matched control group conducted at our hospital. Molecular analysis revealed IGF2-H19 epimutations in 12 and mat/PD7 in 5 patients (4 not tested). At start of GH therapy mean age was 7.54 /6.73 y (males/females) and mean height was -3.02/-3.96 SDS (Prader). Mean target height was -0.13/-0.05 SDS. Mean duration of GH therapy was 5.53/5.65 y. Mean GH dose was 54+/–14 µg/kg/d. In 16 patients (9 males) puberty was blocked by GNRHa analogs for 2.4 y (mean). The untreated cohort comprised 13 individuals (5 females) with a mean height of -3.19 SDS, a mean age of 7.4 y and a mean target height of -0.53 SDS.
Methods: The primary endpoints were adult height SDS at a growth velocity <1 cm/yr or at an age >18 y and the gain in adult height (cm) in comparison to untreated matched by an outcome-blinded statistician according to gender, height, target height and age at puberty start.
Results: GH treated (males/females) reached a mean final height of -1.84 SDS (+4.00 –2.49 SDS (+0.83) gaining +1.18 SDS/+1.47 SDS in comparison to the height SDS at GH start. Mean height SDS of the untreated cohort stayed unchanged (-3.12 SDS). The matched treated males were in mean 11.1 cm (+6.6) taller while the effect size in females was +4.0 cm (+12.7).
Positive predictors of final height after GH were height at start and duration of GH therapy. Height gain was highest in the shortest.
Conclusion: GH therapy improves final height in SRS to the same extent as in non-syndromic SGA children.

P1-d2-305 Gonads and Gynaecology 1
Serum levels of anti-Müllerian hormone in girls with central precocious puberty before, during and after gonadal suppression with GnRHa agonist
Caspar P. Hagen; Sørensen Kaspar; Juul Anders
University of Copenhagen, Rigshospitalet, Dep. of Growth and Reproduction, Copenhagen, Denmark

Background: Serum levels of Anti-Müllerian hormone (AMH) reflect the ovarian follicle reserve.
Objective and hypotheses: To evaluate if treatment with long-acting GnRHa agonist (GnRHa) affects serum AMH levels in girls with central precocious puberty (CPP).
Methods: Tertiary pediatric centre. Prospective clinical study of 15 patients with CPP who were treated with subcutaneous injections of leuprolide acetate (Procren 3.75 mg every 28th day). Evaluations were done before, 3 and 12 months after initiation, as well as 6 months after discontinuation of GnRHa treatment. Healthy age-matched girls (n=129) served as controls. Main outcome measures were basal serum levels of AMH, estradiol, inhibin B, FSH and LH, as well as GnRHa-stimulated levels of FSH and LH.
Results: At baseline, the median (range) of AMH levels in the patients was 20.3 (2.0 – 30.0) pmol/L, not significantly different from levels in age-matched controls (p = 0.058). After three months of GnRHa treatment, AMH levels declined to 10.4 (2.0 – 27.0) pmol/L, p = 0.007. From 3 to 12 months of treatment, AMH levels did not change significantly, levels at 12 months: 14.4 (2.0-29.6) pmol/L, p = 0.582. After discontinuation of GnRHa treatment, AMH recovered to levels similar to what was found prior to start of treatment; levels after treatment: 18.8 (5.8 – 46.9) pmol/L, p = 0.508.

Figure 1

P1-d2-304 Gonads and Gynaecology 1
Deficient expression of genes involved in the endogenous defense system against transposons in cryptorchid boys
Panuk Hadziselimovic1; Nils Omar Hadziselimovic2; Philippe Demougin3; Gunthild Krey4; Eduard Oakeley5
1Kindertagesklinik Liestal, Pediatrics, Liestal, Switzerland; 2Biocentre Basel, Molecular Genetics, Basel, Switzerland; 3Institute for Andrology, Histology, Liestal, Switzerland; 4Novartis Institutes for Biomedical Research, Molecular Biology, Basel, Switzerland

Background: Mini-puberty is the period between 30 and 80 days after birth when testosterone and gonadotropin surges occur in male infants to induce the transformation of gonocytes into Ad spermatogonia. Cryptorchid boys with impaired mini-puberty develop infertility despite successful surgical treatment.
Objective and hypotheses: Uncontrolled retrotransposon activity results in genomic instability and germ cell death. In response to the danger posed by transposons, organisms have evolved an endogenous defense system that employs a particular class of small RNAs known as piwi-interacting RNAs (piRNAs) to identify and selectively silence transposons The decreased germ cell count found in high azoospermia risk group of cryptorchid boys could be the result of uncontrolled transposon activity inducing genomic instability and germ cell death.
Methods: A genome-wide analysis of 18 cryptorchid was performed with Affymetrix chips.
Results: We found that 6 of 8 genes that are important for transposon silencing were not expressed in the high azoospermia risk group of cryptorchid boys but were expressed in the low azoospermia risk group. Two genes, CBX3 and DNMT1, were equally expressed in all groups. Impaired expression of the DDX4, MAEL, MOV10L1, PIWIL2, PIWIL4, and TDRD9 genes in the group of cryptorchid boys at high risk of infertility indicates that gene instability induced by impaired expression of transposon silencing genes contributes to the development of azoospermia. Identical expression of the CBX3 and DNMT1 genes in all groups studied indicates gonadotropin and testosterone epigenetic independence in contrast to 6 other genes.
Conclusions: We observed that the majority of genes responsible for transposon silencing were not expressed in the high azoospermia risk group of cryptorchid boys, indicating that this altered expression may be responsible for the massive germ cell loss in these patients. Intact mini-puberty appears to be essential for the development of the endogenous defense system mediated by transposon silencing.
Conclusions: Circulating AMH declined significantly following gonadal suppression with long-acting GnRHa, but recovered to pre-treatment levels after discontinuation of GnRHa. Thus, GnRHa treatment did not seem to affect the ovarian follicle pool permanently in pubertal girls.

Patients: Sixty-one girls participating in the Dutch PWS Cohort study. Serum AMH, gonadotrophins, E2 and inhibin B and A levels were compared with reference values.

Results: AMH levels in girls and female adolescents with PWS were comparable to reference levels between 6 months and 22 years of age. From 10 years of age, FSH and LH levels increased to above the 5th percentile compared to reference levels. E2 and inhibin B levels were in the low normal range in the majority, and inhibit A levels were low, but detectable in almost half the female adolescents with PWS. The median age at puberty onset was comparable, but the median ages at attaining Tanner M3 (p=0.05) and M4 (p=0.0001) were significantly higher in girls with PWS than in healthy references.

Conclusions: Our study shows that the primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. We found no classical hypogonadotropic hypogonadism. However, maturation of follicles and progression of pubertal development are impaired. As these impairments are not absolute, ovulation and thus conception cannot be ruled out in individual adolescent girls with PWS.

Conclusions: The investigation shows that healthy and pregnant adolescent girls had kisspeptin median levels 0.03 ng/ml. Among the girls with amenorrhea kisspeptin levels was lower 0.01 ng/ml (p=0.014). Girls with mastopathy (dyshormonal dysplasia) had kisspeptin levels 0.05 ng/ml (p=0.033). Serum kisspeptin levels were significantly higher in adolescent girls with polycystic ovary syndrome than in control group (0.3 vs 0.03 ng/ml, p<0.01).

Conclusions: Kisspeptin levels is various in healthy adolescent girls, girls with menstrual disorders, mastopathy, with polycystic ovary syndrome. In this study, we demonstrated that serum kisspeptin level was significantly lower in girls with amenorrhea and higher in girls with mastopathy and polycystic ovary syndrome. Serum kisspeptin may be used as a marker of sexual disorders, mammary diseases and polycystic ovary syndrome in girls.

Background: The etiology of hypogonadism in girls with Prader-Willi Syndrome (dyshormonal dysplasia) have kisspeptin levels 0.05 ng/ml (p=0.033). Serum kisspeptin levels were significantly higher in adolescent girls with polycystic ovary syndrome than in control group (0.3 vs 0.03 ng/ml, p<0.01).

Conclusions: Kisspeptin levels is various in healthy adolescent girls, girls with menstrual disorders, mastopathy, with polycystic ovary syndrome. In this study, we demonstrated that serum kisspeptin level was significantly lower in girls with amenorrhea and higher in girls with mastopathy and polycystic ovary syndrome. Serum kisspeptin may be used as a marker of sexual disorders, mammary diseases and polycystic ovary syndrome in girls.

Objective and hypotheses: To evaluate gonadal function longitudinally in girls and female adolescents with PWS.

Methods: Longitudinal assessment of AMH, gonadotrophins, estradiol (E2), inhibin B and A and pubertal development in girls and female adolescents with PWS.

Patients: Sixty-one girls participating in the Dutch PWS Cohort study. Serum AMH, gonadotrophins, E2 and inhibin B and A levels were compared with reference values.
Anogenital distance and the masculinisation programming window: a clear association in isolated hypospadias

Ajay Tharankaroyo; Ken Ong; David Dunger; Daniel Carroll; Martyn Williams; Carlo Acerini; Ieuan Hughes

University of Cambridge, University Department of Paediatrics, Cambridge, United Kingdom; Institute of Metabolic Science, MRC Epidemiology Unit, Cambridge, United Kingdom; Cambridge University Hospitals NHS Trust, Department of Paediatric Surgery, Cambridge, United Kingdom

Background: The concept of a masculinisation programming window (MPW) in the male fetus is established from rodent studies. Anogenital distance (AGD) is a reliable readout of factors acting during the MPW in animals.

Objective and hypotheses: To explore AGD in boys with hypospadias, a condition whose origin coincides with the MPW.

Methods: Boys with isolated hypospadias were recruited (n= 53, age 12.1±6.5 months) for a cross-sectional assessment of AGD, measured from the centre of the anus to the perineo-scrotal junction. AGD and penile length were compared to age-appropriate data from 485 normal full-term boys from the Cambridge Baby Growth Study (CBGS) with repeated measurements between 0-2 years. Standard deviation scores for AGD and penile length were calculated using the LMS method.

Results: Of the 41 boys with no prior surgery, the type of hypospadias was glanular, penile and perineal in 19, 17 and 5 patients respectively. Boys with hypospadias had lower weight (-0.33±1.54 vs 0.01±0.96 SDS, p=0.027) and length (-0.22±1.30 vs 0.29±0.93 SDS, p<0.0001) compared with normal boys; they also had markedly shorter AGD (-0.88±0.90 vs 0.03±0.77 SDS, p<0.0001) and penile length (-1.41±1.29 vs -0.02±0.82 SDS, p<0.0001), and these differences persisted after adjusting for weight, height and age.

In boys with hypospadias, AGD was positively correlated with penile length (r=0.29, p=0.039), but did not differ by the type of hypospadias.

Conclusions: The aetiology of hypospadias is usually unknown. Nevertheless, an association of AGD with hypospadias points to the developmental stage in humans when disruption of organogenesis occurs. This observation and the reported links between AGD and sperm count, and testosterone in adults indicates that AGD is programmed long term. Our findings provide additional supporting evidence for the use of AGD as a biomarker of in utero exposure to endocrine disrupting agents which may affect the development of tests and male reproductive tract.
P1-d2-312 Gonads and Gynaecology 1

**LH-RH peripheral response and AMH level in adolescent girls with hyperandrogenism**

Polina Bogdanova\(^1\); Maria Kareva\(^1\); Irina Yarovsky\(^1\); Alexander Ilyin\(^1\);
Olga Zlotnikova\(^1\)

\(^1\)Endocrinology Research Centre, Pediatric Endocrinology, moscow, Russian Federation; \(^2\)Endocrinology Research Centre, Gynecological Department, moscow, Russian Federation; \(^3\)Endocrinology Research Centre, Pediatric Endocrinology, Buenos Aires, Argentina; \(^4\)Endocrinology Research Centre, Gynecological Department, moscow, Russian Federation

**Background:** There are two main items that may be used to assess the dysfunction of ovarian tissue in hyperandrogenism (HA) – steriodogenesis and folliculogenesis. Women presenting with so-called “typical” polycystic ovarian syndrome (PCOS) seem to have higher stimulated 17-hydroxyprogesterone (17-OHP) level after LH-RH stimulation, suggesting impairment of steriodogenic pathways. Anti-Mullerian hormone (AMH) precisely reflects the number of small follicles in the ovary. Taken together LH-RH test and AMH might serve as a good way to define hyperandrogenic ovarian dysfunction.

**Objective and hypotheses:** Adolescent girls with early pubarche in anamnesis (earlier than 10 yrs) are more likely to have adrenal HA, those who present with HA but underwent all the stages of puberty timely (telarche prior to pubarche) are more likely to have ovarian HA.

**Methods:** <25 girls matching PCOS Rotterdam criteria, all of them 2 yrs past menarche. 14 with early pubarche in case history (gr1) and 11 without (gr2). LH-RH test was performed during first 5-7 days of menstrual cycle with 17-OHP measurement 12 hours after LH-RH administration. AMH was measured by ELISA. All patients underwent pelvic ultrasound and hirsutism assessment (Ferriman-Gallway score).

**Results:** Adolescent girls with normal puberty were significantly different from those with early pubarche having bigger ovarian volume, less number of vessels per year, less level of hirsutism. Stimulated 17-OHP in gr1 was 3.7 [2.3:4.1]nmol/l vs. 6.2 [3.6:7.0]nmol/l in gr2 (p=0.01). Same differences were found in the levels of AMH: gr1 – 3.2 [1.5:4.5]μg/ml vs. gr2 – 8.9 [5.3:11.9]μg/ml (p=0.006).

**Conclusions:** Adolescent girls with HA and normal puberty seem to have ovarian dysfunction as a leading feature of disease by clinical and laboratory findings. LH-RH test and AMH measurement are useful to assess ovarian dysfunction in first years after puberty. Weather the girls with HA and normal puberty are more prone to develop “typical” PCOS in their mean age is left to be determined.

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### Table 1: Ovarian Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Gr1 (n=14)</th>
<th>Gr2 (n=11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian Volume, ml</td>
<td>8.4 [7.7:14.1]</td>
<td>14.0 [11.0:18.0]</td>
<td>0.05</td>
</tr>
<tr>
<td>Number of menstrual cycles per year</td>
<td>11.0 [10.0:12.0]</td>
<td>5.0 [3.0:11.0]</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hirsutism (F-Gallway score)</td>
<td>17.5 [13.0:25.0]</td>
<td>6.2 [5.5:9.0]</td>
<td>0.0002</td>
</tr>
<tr>
<td>AMH, ng/ml</td>
<td>3.2 [1.5:4.5]</td>
<td>8.9 [5.3:11.9]</td>
<td>0.008</td>
</tr>
<tr>
<td>17-OHP basal, nmol/l</td>
<td>2.8 [2.5:3.7]</td>
<td>2.4 [1.9:3.2]</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>17-OHP after LH-RH, nmol/l</td>
<td>3.7 [1.9:3.1]</td>
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<td>0.01</td>
</tr>
</tbody>
</table>

**P1-d2-313 Gonads and Gynaecology 1**

**Primary ovarian insufficiency in adolescent patients**

Andrea Arcari\(^1\); Maria Eugenia Escobar\(^1\); Alejandra Ginaca\(^2\);
Pamela Garabada\(^3\); Violeta Chiauzzi\(^3\); Graciela Del Rey\(^1\); Rodolfo Rey\(^1\);
Mirta Grygarden\(^1\)

\(^1\)Hospital de Niños Ricardo Güiterz, Endocrinology, Buenos Aires, Argentina; \(^2\)Hospital de Niños Ricardo Güiterz, Immunology, Buenos Aires, Argentina; \(^3\)Instituto de biología y medicina experimental, Immunology, Buenos Aires, Argentina

**Background:** Primary ovarian insufficiency (POI) is diagnosed when a woman younger than 40 years old has amenorrhea and serum FSH levels in the menopausal range. In the absence of oophorectomy, chemotherapy, irradiation or chromosomalopathies, POI is a heterogeneous condition whose etiology remains unknown in 90% of the cases. Scanty information exists about POI in adolescence.

**Objective and hypotheses:** To describe the phenotype and the prevalence of autoimmunity in an adolescent cohort with POI.

**Methods:** We performed a cross-sectional study of clinical, endocrine and ovarian ultrasound characteristics of 35 consecutive POI adolescents. We analyzed clinical records of girls with pubertal delay, primary or secondary amenorrhea and two FSH levels >30 mIU/ml. Patients with Turner syndrome, oophorectomy, chemotherapy and radiotherapy were excluded. FSH and LH were measured by IFMA, serum AMH by ELISA and estradiol by DELFIA. Karyotype was performed in all patients. Screening for organ and non organ specific autoantibodies by immunofluorescence, antiovarian antibodies by immunoblotting, antibodies against FSH-receptor by a radiometric assay and pelvic ultrasound were performed.

**Results:** Median age at diagnosis was 15.8 years. Familiar history of POI and of autoimmune disease were present in 31% and 37% respectively. Seventy one percent presented with pubertal delay or primary amenorrhea, 29% had secondary amenorrhea. Pelvic ultrasound showed follicles in 37% of patients. In 15/32 girls various organ and non organ specific antibodies were found. Three of 5 patients (9%) with autoimmune diseases had antiovarian antibodies. AMH levels, evaluated in 13 girls, were low or undetectable. All patients had 46, XX karyotype.

**Conclusions:** In our cohort of adolescents 9% had autoimmune diseases plus antiovarian antibodies. Besides, 31% had different autoantibodies suggesting that an autoimmune mechanism could be involved. However the etiology of POI remains unknown in a high number of patients. More specific studies are needed to clarify this point.

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### Table 2: Laboratory Results

<table>
<thead>
<tr>
<th></th>
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</tr>
</tbody>
</table>

**P1-d2-314 Gonads and Gynaecology 1**

**The effect of short course treatment with raloxifene on pubertal gynecomastia**

Iuliana Gherla\(^1\); Cristina Patricio Dumitrescu\(^1\);
Andreea Cristiana Breha\(^2\); Andra Carageorgheopol\(^2\);
Camelia Procopciuc\(^2\)

\(^1\)“C.I.Parhon” National Institute of Endocrinology, Pediatric Endocrinology, Bucharest, Romania; \(^2\)“C.I.Parhon” National Institute of Endocrinology, Research Laborator, Bucharest, Romania

**Background:** Pubertal gynecomastia, affecting 50% of Tanner 3-4 pubertal boys may necessitate a surgical approach due to its dimensions/benign structural alterations; selective estrogen receptor modulators might be a convenient medical alternative in these cases.

**Objective and hypotheses:** To assess the efficacy of SERM - raloxifene in the medical management of persistent/important/cystic pubertal gynecomastia.

**Methods:** Prospective study over 15 pubertal boys with important/complicated pubertal gynecomastia (maximum diameter of breast tissue on ultrasound assessment over 3 cm, tenderness and/or cystic lesions/ductal dilatations); the patients received a 3 to 9 months course treatment with raloxifene 60 mg/day, clinical and US assessment were done every 3 months until the completion of treatment.

**Results:** Symptomatic amelioration was accomplished in all patients - reduced tenderness and breast swelling. All patients with cystic lesions/ductal dilatations had a minimum 50% reduction of these lesions at 3 months with their complete disappearance at 6 months of treatment. Excepting one patient that had a fibrous organization of the glandular tissue with only a slight reduction of its dimensions on US, all other patients had a reduction of breast tissue dimensions of minimum 50% after 3 months of treatment. The pubertal progression was normal; there was a slight increase of plasmatic estradiol values in 30% of cases after 3 months of treatment; all patients had normal statial growth and bone maturation. There were no side effects.

**Conclusions:** Raloxifene - a selective estrogen receptor modulator with blocking action on breast estrogen receptors seems to be an efficient and safe therapeutic option in important/complex pubertal gynecomastia.
**P1-d2-315 Growth 1**

**Predicting the growth response to growth hormone in patients with SHOX deficiency or Turner syndrome by the Cologne prediction model**

*Heike Hoyer-Kuhn¹, Daniel Kowalski¹, Werner F. Blum¹, Eckhard Schoenau¹*

¹University of Cologne, Cologne, Germany

Background: Growth hormone (GH) treatment in children with SHOX deficiency or Turner syndrome is approved and widely used to increase height (HV) and adult height. Prediction of the growth response continues to be a challenge. A comparatively accurate method is the “Cologne Prediction Model” developed in GH-treated children with GH deficiency.

Objective and hypotheses: The aim of this analysis was to investigate whether this model can also be applied in patients with SHOX deficiency or Turner syndrome to predict HV in the first year of GH treatment.

Methods: This analysis of a multinational trial included prepubertal patients with SHOX deficiency (N=49, 26 with Leri-Weill syndrome, 21 with idopathic short stature, 2 with unspecified phenotype) or Turner syndrome (n=26), confirmed by chromosome/DNA analysis, who received somatropin treatment at a dose of 0.05 mg/kg/day. First year HV prediction by the Cologne Model uses the following variables: relative bone age retardation and IGF-I at baseline, urinary deoxypyridinoline cross-links at 4 weeks and HV at 3 months.

Results: HV and height SDS increased significantly during the first year of GH treatment in both SHOX deficiency and Turner syndrome groups. The Pearson correlation coefficient between Cologne predicted vs. observed first year HV was 0.63 (adjusted r² = 0.39) in patients with SHOX deficiency and 0.79 (adjusted r² = 0.61) in patients with Turner syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHOX deficiency (N=49)</th>
<th>Turner syndrome (N=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females/males (n)</td>
<td>26/23</td>
<td>26/20</td>
</tr>
<tr>
<td>Age (yr) at start</td>
<td>8.3±2.7</td>
<td>7.5±1.9</td>
</tr>
<tr>
<td>Bone age SDS at start</td>
<td>-0.9±0.9</td>
<td>-1.0±1.1</td>
</tr>
<tr>
<td>IGF-I SDS at start</td>
<td>-0.8±1.2</td>
<td>-1.1±1.2</td>
</tr>
<tr>
<td>Height SDS at 1 yr</td>
<td>-3.2±0.8</td>
<td>-3.7±0.9</td>
</tr>
<tr>
<td>Height SDS at 1 yr</td>
<td>-2.6±0.9</td>
<td>-2.9±1.0</td>
</tr>
<tr>
<td>Pre-treatment HV (cm/yr)</td>
<td>5.3±1.5</td>
<td>4.0±1.3</td>
</tr>
<tr>
<td>Observed first year HV (cm/yr)</td>
<td>8.4±2.7</td>
<td>8.9±2.0</td>
</tr>
<tr>
<td>Predicted first year HV (cm/yr)</td>
<td>8.1±1.1</td>
<td>9.3±1.6</td>
</tr>
<tr>
<td>Predicted first year HV – observed first year HV (cm/yr)</td>
<td>-0.3±2.1</td>
<td>0.4±2.25</td>
</tr>
</tbody>
</table>

Table: Characteristics of study populations at start and after 1 yr of GH treatment (mean±SD)

Conclusions: The results of this analysis demonstrate that the Cologne Model can be used to predict the growth response in patients with SHOX deficiency and Turner syndrome with reasonable precision in the first year of GH treatment. The results are comparable to those in other indications such as GHD. This suggests that the Cologne Model may be useful in clinical practice for individual dose adjustment in patients with SHOX deficiency.

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**P1-d2-316 Growth 1**

**High frequency of submicroscopic chromosomal deletions and duplications in dysmorphic patients born small for gestational age**

*Ana Canton¹, Tatiane Rodrigues², Ana Krepsch³, Ivo Arnhold³, Berenice Mendonca³, Carla Rosenberg³, Alexander Jorge³*

¹Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia Genetica LIM25, Sao Paulo, Brazil; ²Instituto de Biotecnologia da Universidade de Sao Paulo, Departamento de Genetica e Biologia Evolutiva, Sao Paulo, Brazil; ³Hospital do Cancer AC Camargo, Centro Internacional de Pesquisa e Ensino Oncologico, Sao Paulo, Brazil; *Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular LIM42, Sao Paulo, Brazil

Background: The etiology of prenatal onset growth retardation with postnatal persistence is heterogeneous, often encompassing complex genetic disorders of difficult diagnosis.

Objective: To analyze the frequency of submicroscopic chromosomal deletions and duplications in a group of patients born small for gestational age (SGA) without a known cause.

Methods: We evaluated 32 patients with pre and postnatal growth retardation associated with other physical or developmental defects (dysmorphic features or intellectual disability), but without criteria for the diagnosis of known syndromes. Array-based comparative genomic hybridization (aCGH) was performed with DNA from all patients. The array comprised 60,000 oligonucleotides, with a probe spacing of 50kb over the whole genome. The results were compared with copy number variations (CNVs) already described in normal controls databases and 18 relatives were evaluated for familial segregation.

Results: We identified 15 CNVs (7 duplications and 8 deletions) in 13 of the 32 patients (40%). None of these imbalances has been reported in healthy individuals. Considering their sizes, their gene contents and their familial segregations, at least 8 CNVs, found in 6 patients (I to VI), were categorized as probably pathogenic. These imbalances and their sizes were as follows: I) a 1.6Mb dup(10)(q26.2;26.3) and a 4.5Mb del(10)(q26.3); II) a 2.5Mb del(22)(q11.21); III) a 2.5Mb dup(5)(q21.3); IV) a 0.4Mb del(20)(p13); V) a 25Mb dup(8)(q24.2;24.3) and a 1.3Mb del(15)(q26.3); VI) a 3.5Mb del(3)(q27.1;q27.3). All patients had normal G-banded karyotyping, except for the patient V whose result was reported as 46,XY,15p-.

Conclusions: The frequency of pathogenic CNVs in patients born SGA associated with dysmorphic features or intellectual disability was high (at least 18%), showing the importance of aCGH as a clinical genetic test to clarify the diagnosis of these patients and to identify new chromosomal regions implicated in this condition.

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**P1-d2-317 Growth 1**

**Clinical, hormonal and immunological phenotype in individuals heterozygous for STAT5B mutations**

*Renata Scalzi¹; Carlos Tonelli¹; Patricia Pugliese-Pires¹*

¹Facultad de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia Genetica - LIM25 - Disciplina de Endocrinologia Pediatrica, Criciuma - SC, Brazil; ²Hospital das Clinicas da Universidade do Extremo Sul de Santa Catarina, Ambulatorio de Endocrinologia Pediatrica, Criciuma - SC, Brazil; ³Laboratorio Pasteur, Geral, Curitiba - SC, Brazil; 4Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular - LIM42, Disciplina Endocrinologia, Sao Paulo - SP, Brazil

Background: STAT5b is a protein involved in the signaling pathway of growth hormone (GH) and interleukins. Homozygous inactivating mutations in STAT5B gene cause GH insensitivity with chronic pulmonary disease and/or other immune dysfunctions. The effect of heterozygous STAT5B mutations on phenotype is still poorly characterized.

Objective and hypotheses: To test the hypothesis that STAT5b haploinsufficiency could cause an intermediate phenotype between patients with homozygous mutations and controls.
Methods: Twenty-seven direct relatives of two patients homozygous for the p.L1426X161 (c.424_427del) STAT3B mutation were evaluated. We compared clinical and laboratory characteristics between relatives heterozygous for this STAT3B mutation and non-carriers. In this way the differences related to different genetic backgrounds could be minimized.

Results:

<table>
<thead>
<tr>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=7)</td>
<td>(n=20)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>Height SDS (SD)</td>
<td>-0.3 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Basal GH (µg/L)</td>
<td>34.0 ± 4.5</td>
<td>34.0 ± 4.5</td>
</tr>
<tr>
<td>IGFBP-3 SDS (ng/mL)</td>
<td>5.2 ± 1.8</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>14.0 ± 6.0</td>
<td>11.0 ± 5.0</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>13.7 ± 10.7</td>
<td>11.0 ± 14</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>5.6 ± 2.8</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>Respiratory allergy</td>
<td>100%</td>
<td>57%</td>
</tr>
<tr>
<td>Lymphocytes (cell/mm³)</td>
<td>1000 / 1000</td>
<td>1656 ± 408</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>759 / 1013</td>
<td>966 ± 143</td>
</tr>
<tr>
<td>IgA (mg/dL)</td>
<td>221 / 252</td>
<td>313 ± 191</td>
</tr>
<tr>
<td>IgE (UI/mL)</td>
<td>NA / NA</td>
<td>385 ± 291</td>
</tr>
</tbody>
</table>

* Statistical difference between individuals homozygous for the STAT3B mutation and non-carriers. NA – not available.

Conclusions: Individuals homozygous for the studied STAT3B mutation are shorter than their non-carriers relatives, although in the normal height range. In the same direction, IGFB-1 and IGFBP-3 SDS were lower in heterozygous individuals, although without reaching significance. A tendency for more respiratory allergy and a significant increase in the IgE levels were also observed in carriers. These results suggest a mild phenotype in individuals homozygous for STAT3B mutations, but it is necessary to expand the number of studied relatives in this and other families to confirm the present findings.

P1-d2-318 Growth 1

**Effectiveness and cost effectiveness of automated growth monitoring in children in primary care: population based cohort study**

**Ulla Sankilampi**, Antti Saari, Laura Valpio, Tiina Laine

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**Background:** The assessment of linear growth in children is a well-established part of preventive health care. However, no evidence exists on how growth monitoring should be optimally organised. We developed a conceptually novel automated growth monitoring (AGM) strategy and implemented it into an electronic health record system in primary care.

**Objective and hypotheses:** We tested whether AGM, in comparison to standard growth monitoring (SGM), would improve referral rates, the appropriateness of referrals and the diagnostic yield of disorders that affect growth in a cost effective way.

**Population and methods:** A prospective, population-based cohort study with one-year AGM intervention including computerised screening algorithms and automated online consultation prior to referral was conducted in the child population (0.01-12 years) of a large municipality in Finland. Main outcome measures were the referral rate to secondary care for abnormal growth, diagnostic yield of growth disorders, diagnostic delay, and cost effectiveness of growth monitoring, all in comparison to the preceding year with SGM.

**Results:**

- The yield increased from one per 4,090 children (eight new growth diagnoses in SGM year) to one per 675 children (48 in AGM year) among the 32,404 and 32,718 children screened, respectively. 24 of 48 children diagnosed during AGM had abnormal screening results during SGM, but had not been referred leading to a median diagnostic delay of 2.0 years (0.1-10.3 years).
- AGM strategy was cost saving: price of one new growth diagnosis was 10,292€ versus 55,961€ in SGM.

P1-d2-320 Growth 1

**Determinants of early growth response and final height gain to GH treatment in patients with SHOX deficiency: results of a multi-centre trial**

**Werner F Blum**, Judith L Ross, Christopher J Child, Alan G Zimmermann, Daniel Kowalski, Eckhard Schoenau, Guadrun A Rappold

1 Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany; 2 Thomas Jefferson University, Department of Pediatrics, Philadelphia, United States; 3 Eli Lilly and Company, Lilly Research Laboratories, Windlesham, United Kingdom; 4 Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, United States; 5 PSI Company Ltd., Biometric, Warsaw, Poland; 6 University of Cologne, Children’s Hospital, Pediatric Endocrinology, Cologne, Germany; 7 Heidelberg University, Department of Molecular Human Genetics, Heidelberg, Germany

**Background:** Patients with mutations of the Short Stature Homeobox-containing (SHOX) gene have impaired growth, with or without a spectrum of skeletal anomalies consistent with mesomelic skeletal dysplasia. Growth hormone (GH) has been shown to improve the growth rate and final height.
Objective and hypotheses: The aim of this analysis was to identify determinants of the growth response to GH. 

Methods: Prepubertal children (27/22 females/male [F/M]; 21 with a phenotype of idiopathic short stature (ISS), 26 with Leri-Weill syndrome (LWS), 2 with unspecified phenotype) with genetically confirmed SHOX deficiency received GH treatment (tx) at 0.05 mg/kg/d. Study entry criteria were: age ≥3yr; bone age <8yr (F), <10yr (M); height (Ht) >3rd %ile (or Ht <10th %ile and height velocity [HV] <25th %ile). At study closure, 28 patients had attained final height (FH; last Ht <2cm/yr or last bone age ≥14yr [16yr] in F [M] or judgment by investigator). Multiple regression analyses were performed with 1-yr HV, 1-yr delta HtSDS, 4-yr delta HtSDS and FH SDS gain (FH SDS - HtSDS at start of GH tx) as response variables and various auxological and biochemical explanatory variables. 

Results: At start of GH tx mean±SD age was 8.3±2.7 yr, bone age SDS -0.8±0.9, and HtSDS -3.2±0.8. Duration of GH tx at FH was 6.2±2.0 yr. The response variables increased significantly during GH tx.

Table. Parameter estimates (P-values) of stepwise multiple regression models for various response variables.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>1st-yr HV</th>
<th>1-yr delta HtSDS</th>
<th>4-yr delta HtSDS</th>
<th>FH SDS gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>46</td>
<td>46</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>Gender (M:G,F:1)</td>
<td></td>
<td></td>
<td></td>
<td>0.76 (0.003)</td>
</tr>
<tr>
<td>ISS(0)/LWS(1)</td>
<td></td>
<td>-0.51 (0.013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at start (yr)</td>
<td>-0.28 (0.003)</td>
<td>-0.10 (≤0.001)</td>
<td>-0.11 (0.007)</td>
<td></td>
</tr>
<tr>
<td>Bone age SDS at start</td>
<td></td>
<td></td>
<td></td>
<td>-0.34 (0.022)</td>
</tr>
<tr>
<td>IGFBP-3 SDS</td>
<td>0.10 (0.039)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of GH tx (yr)</td>
<td></td>
<td></td>
<td></td>
<td>0.13 (0.003)</td>
</tr>
</tbody>
</table>

While young age at start predicted a better GH response during the first 4 yr, it was not a significant predictor of FH SDS gain. A good FH SDS gain was predicted by female sex and more pronounced bone age retardation. The following variables did not remain in any model: HtSDS at start, target HtSDS, IGF-I SDS.

Conclusions: The early growth response to GH tx in children with SHOX deficiency is significantly determined by the age at start of tx: the younger the better. In contrast, a good height gain to adult height can be expected, if bone age at start of GH tx is substantially retarded.

Body composition and circulating high-molecular-weight adiponectin and IGF-I in infants born small for gestational age: breast- versus formula-feeding

Giovanna Sebastiani; Marta Díaz; David Sanchez-Infantes; Abel López-Bermejo; Francis De Zegher; Luciani Carvalho; Marcela M. Franca; Aline P. Otto; Evelaryn F. Costalonga; Vinicius N. Brito; Ivo J. P. Arnholt; Berenice B. Mendonça

Background: The incidence of Isolated Growth Hormone Deficiency (IGHD) is estimated to be 1:4000-1:10000. Mutations in GH1 and GHRHR are known causes of IGHD but a large number of patients remain without molecular diagnosis. The study of Neurod4 knockout mice showed that this gene is critical for maturation and expansion of somatotropes by regulating the expression of GHRHR. We hypothesized that Neurod4 loss-of-function mutations could underlie some cases of IGHD.

Objective: To screen NEUROD4 for mutations in patients with IGHD.

Methods: Mutations in GH1 and GHRHR were ruled out in all patients. All patients met the diagnostic criteria of the Brazilian centre: the patients had short stature (heights SD score < –2) and peak GH levels > 5μg/ml by immunoradiometric assays in two GH stimulation tests. The entire coding region of NEUROD4 was evaluated in 30 patients (17 males) by Sanger Method using automatic sequencing.

Results: Ten patients presented a heterozygous allelic variant HGVS NM_021191.2: c.31 C>T previously described as polymorphism rs2656804. This variant is a 3′PRIME-UTR type and its position in the transcript is 1405. It is not conserved among species. The other 20 patients presented the most common allele C/C. Clinical and MRI findings are described in the table below: Table 1 – Clinical features and MRI findings of 30 patients with IGHD.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Consanguinity</th>
<th>Familial cases</th>
<th>Anterior pituitary hypoplasia</th>
<th>*Abnormal posterior pituitary</th>
<th>**Abnormal stalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>10</td>
<td>23</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>4</td>
<td>15</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

*Includes ectopic or non visualized posterior pituitary. **Includes transection or absent stalk. 

Conclusions: Despite the role that Neurod4 has in somatotrope development in mice, we found no loss-of-function mutations implicated in the aetiology of IGHD in a selected group of Brazilian patients.

P1-d2-322 Growth 1

Absence of NEUROD4 mutations in patients with congenital isolated growth hormone deficiency

Fernanda A. Correa; Luciani R. Carvalho; Marcela M. Franca; Aline P. Otto; Evelaryn F. Costalonga; Vinicius N. Brito; Ivo J. P. Arnholt; Berenice B. Mendonça

Background: The incidence of Isolated Growth Hormone Deficiency (IGHD) is estimated to be 1:4000-1:10000. Mutations in GH1 and GHRHR are known causes of IGHD but a large number of patients remain without molecular diagnosis. The study of Neurod4 knockout mice showed that this gene is critical for maturation and expansion of somatotropes by regulating the expression of GHRHR. We hypothesized that Neurod4 loss-of-function mutations could underlie some cases of IGHD.

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**P1-d2-323** Growth 1

**Copy number variations in patients with idiopathic short stature**

Hermine van Duvenvoorde1; Sarina Kant2; Wilma Oostdijk2; Antoinet Gijsbers3; Marcel Karperien4; Marie-José Valenkamp4; Cees Noordam5; Paul Voorhoeve6; Sandy van Goor6; Monique Losekoot3; Claudia Ruivenkamp3; Sanne Meeuwsen2; Jan-Maarten Wit1

1Leiden University Medical Center, Pediatrics, Endocrinology and Metabolic Diseases, and Center for Human and Clinical Genetics, Leiden, Netherlands; 2Leiden University Medical Center, Center for Human and Clinical Genetics, Leiden, Netherlands; 3Leiden University Medical Center, Pediatrics, Leiden, Netherlands; 4University of Twente, Developmental BioEngineering, Enschede, Netherlands; 5VU University Medical Center, Pediatrics, Amsterdam, Netherlands; 6Radboud University Nijmegen Medical Center, Pediatrics, Nijmegen, Netherlands; 7Catholic University Nijmegen Hospital, Pediatrics, Nijmegen, Netherlands; 8Haga Hospital/Juliana Children's Hospital, Pediatrics, Den Haag, Netherlands

**Background:** Height is a highly heritable and classic polygenic trait. Recent Genome-wide Association Studies have revealed that at least 180 genetic variants influence adult height. However, newly described variants explain only about 10% of the phenotypic variation in height. It is estimated that in approximately 80% of the short children presenting to a pediatrician the underlying cause cannot be identified, which classifies them as having idiopathic short stature (ISS).

**Objective and hypotheses:** To identify rare copy number variants (CNVs) that may influence height, using whole genome SNP array analysis in patients with ISS.

**Methods:** 180 patients (96 males) with ISS were included. To detect CNVs in these patients, whole genome SNP array analysis was performed, using either Affymetrix GeneChip Human Mapping 250K or Illumina Human-Hap300 BeadChip arrays and their corresponding analysis software. The detected CNVs were classified into four different groups: I, known pathogenic; II, potentially pathogenic, not described in the Database of Genomic Variants (DGV); III, not described in the DGV, but not containing any protein-coding genes; and IV, polymorphic variant described in the DGV or observed in our in-house reference set. All type II CNVs were assessed with Ensembl and DECIPHER for gene content and familial cases, respectively. If parents were available, segregation analysis was performed by SNP array.

**Results:** In 4 cases (2%) type I CNVs were found and in 45 cases (25%) type II CNVs. The type I CNVs were located in the chromosomal regions containing SHOX and the IGFR1. The four most interesting type II CNVs were further analyzed, which led to 3 novel candidate genes.

**Conclusions:** Whole genome SNP array analysis identified known pathogenic CNVs in 2% and potentially pathogenic CNVs, which may influence the regulation of longitudinal growth, in 25% of 180 patients with ISS.

**P1-d2-324** Growth 1

**A quest for copy number variations causing extreme tall stature**

Hermine van Duvenvoorde1; Claudia Ruivenkamp3; Dick Mul4; Wilma Oostdijk1; Marcel Karperien1; Monique Losekoot1; Alberto Pereira1; Sarina Kant5; Jan-Maarten Wit1

1Leiden University Medical Center, Pediatrics, Endocrinology and Metabolic Diseases, and Center for Human and Clinical Genetics, Leiden, Netherlands; 2Leiden University Medical Center, Center for Human and Clinical Genetics, Leiden, Netherlands; 3Leiden Children's Hospital, Pediatrics, Den Haag, Netherlands; 4University of Twente, Developmental BioEngineering, Enschede, Netherlands; 5Leiden University Medical Center, Endocrinology and Metabolic Diseases, Leiden, Netherlands

**Background:** Height is a highly heritable and classic polygenic trait. In the majority of individuals with extreme tall stature (ETS) no genetic cause is known.

**Objective and hypotheses:** To identify new loci that may influence height by identifying rare copy number variants (CNVs) in people with ETS.

**Methods:** 18 adults with ETS (from +2.4 to +4.4 SDS) were studied. Clinical and family history was obtained. Whole genome SNP array analysis using Affymetrix 262K NspI arrays was performed to detect CNVs. All potentially pathogenic CNVs were assessed with Ensembl and DECIPHER for gene content and similar cases, respectively. If family members were available, segregation analysis was performed.

**Results:** In 4 (3 males) of the 18 cases potentially pathogenic CNVs were identified and further analysis was performed. Additional segregation studies suggested that the identified CNV was familial in 2 of the cases and probably is not an explanation for ETS. In a 204.2 cm (+2.9 SDS) tall male, a 1p34.1 duplication containing part of MAST2 and PIKK3R3 (that binds the intracellular part of the IGFR1) was observed. His son and daughter with tall stature (+1.9 and +1.8 SDS) both carried the duplication. We recently identified a case with a similar duplication, but without tall stature, in our in-house reference set. In the fourth case, a male with a height of 201.9 cm (+2.5 SDS) 3 aberrations were detected; a duplication of 12q21.1 (also identified in his sister with a normal height), a 13q33.1 deletion (no genes) and an extra copy of the Y chromosome (47,XY).

**Conclusions:** Whole genome SNP array analysis in 18 cases with ETS identified potentially pathogenic CNVs in 2 cases (11%). Additional research showed that the 1p34.1 duplication, although of interest because it contains part of PIKK3R3, is probably not the cause of ETS. The observed extra Y chromosome in the other case is known to contribute to increased height, but the role of the 13q33.1 deletion still remains unclear.

**P1-d2-325** Growth 1

**Growth retardation, delayed mental development and mild dysmorphic features in a boy with a novel interstitial deletion of chromosome 8p21.1-p12**

Gian Luigi Scialdone1; Annalisa Deodati2; Giuseppe Scirè3; Anna Maria Nardone1; Diana Postorivo1; Marta Bertoli4; Marco Cappa2; Stefano Cianfarrani2; Sarina Kant1; Jan-Maarten Wit1; Celine Costentin5; Bambino Gesù Children's Hospital-University “Tor Vergata”, Endocrinology Unit, Rome, Italy; 2Bambino Gesù Children's Hospital-University “Tor Vergata”, Molecular Endocrinology Unit, Rome, Italy; 3Ospedale San Pietro FBF, UOSD di Genetica Medica, Rome, Italy; 4Bambino Gesù Children's Hospital, Endocrinology Unit, Rome, Italy; 5University “Tor Vergata”, Molecular Endocrinology Unit, Rome, Italy

**Background:** Few cases of interstitial 8p deletions have been described in the literature with a rather heterogeneous phenotype. We report on a boy with in whom an array-CGH disclosed a novel interstitial 8p deletion.

**Clinical report:** At birth he weighted 2730g; he showed a delay in psychomotor development associated with strabismus and nistagmus. At 8 yrs he was referred to us for short stature. Screening for celiac disease and other chronic diseases resulted negative; peak GH responses to two stimulation tests normal as were IGF1 levels. Since mild dysmorphic features were noted (epicanthus and ptosis, strabismus, turricefalwy, downward mouth angles) a karyotype was performed which resulted normal. A complete skeletal X-ray assessment and brain MRI scan revealed no anomalies. Height was 138.5cm (-4.1 SDS), testicles were 6ml, PH2, G2. LH-RH test showed a peak FSH value of 18.92 and a peak LH value of 54.14 mUI/ml; testosterone was 266.5 ng/dl. Bone age was constantly delayed. Mother’s and sister’s age at menarche was 15 yrs.

**Methods:** Array-CGH analysis of lymphocytes was performed according to the manufacturer’s protocol on a whole genome oligo-array 105K (BlueGene, UK).

**Results:** Array-CGH analysis detected a microdeletion in the region 8p21.1-p12, spanning 7.6 Mb, which was confirmed by FISH. Rearrangement is de novo.

**Conclusions:** The deletion harbouring in our patient is slightly smaller than other previously described in the same region. The case confirms the heterogeneous phenotype of 8p interstitial deletions, showing some clinical features in common with previously described patients (growth retardation, psychomotor development delay, mild facial dysmorphic features) but not others expected according to the genes of the deleted portion: a hypogonadotropic hypogonadism, which could have been predicted in accordance with the presence of GNRH1 gene in the deleted region, is not present, up to now; the mild delay in puberty might be interpreted taking in account the familial data.

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P1-d2-325 Growth 1

**A quest for copy number variations causing extreme tall stature**

Hermine van Duvenvoorde1; Claudia Ruivenkamp3; Dick Mul4; Wilma Oostdijk1; Marcel Karperien1; Monique Losekoot1; Alberto Pereira1; Sarina Kant5; Jan-Maarten Wit1

1Leiden University Medical Center, Pediatrics, Endocrinology and Metabolic Diseases, and Center for Human and Clinical Genetics, Leiden, Netherlands; 2Leiden University Medical Center, Center for Human and Clinical Genetics, Leiden, Netherlands; 3Leiden Children’s Hospital, Pediatrics, Den Haag, Netherlands; 4University of Twente, Developmental BioEngineering, Enschede, Netherlands; 5University of Twente, Developmental BioEngineering, Enschede, Netherlands; 6University of Twente, Developmental BioEngineering, Enschede, Netherlands; 7Catholic University Nijmegen Hospital, Pediatrics, Nijmegen, Netherlands; 8Haga Hospital/Juliana Children’s Hospital, Pediatrics, Den Haag, Netherlands

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**Results:** In 4 cases (2%) type I CNVs were found and in 45 cases (25%) type II CNVs. The type I CNVs were located in the chromosomal regions containing SHOX and the IGFR1. The four most interesting type II CNVs were further analyzed, which led to 3 novel candidate genes.

**Conclusions:** Whole genome SNP array analysis identified known pathogenic CNVs in 2% and potentially pathogenic CNVs, which may influence the regulation of longitudinal growth, in 25% of 180 patients with ISS.
Background: In girls with central precocious puberty(CPP) treated with gonadotropin-releasing hormone analog (GnRHa) for improvement of final adult height (FAH), the expected effects on FAH is not achieved if the linear growth decrease apparently because of the over-accelerated bone maturation. 

Objective and hypotheses: The study was designed to evaluate the effect of combined use of stanzanolol on the FAH in girls with idiopathic CPP and apparent decrease linear growth during GnRHa therapy.

Methods: 60 girls with idiopathic CPP and decreased growth velocity (GV) were separated into 3 groups (20 girls in each group) based on the following types of interventions: group1, GnRHa+stanzanolol (25-30ug/kg/d, every 3-months followed by 3-months discontinuation, total duration: 12±3.6 months), group2, GnRHa+growth hormone (1-1.1u/kg.w for 11.4±5.7 months), group3, GnRHa alone.

Results: 1. GV increased significantly both in group2 (2.8±0.6 vs 6.4±2.1cm/yr, p<0.01) and in group3 (2.8±0.6 vs 6.3±2.2cm/yr, p<0.01) during combined therapy, but maintained at low levels in group1 (3.9±1.0 vs 3.3±0.9cm/yr, p>0.05). 2. FAH was significantly higher than predicted AH before combined therapy, as well as than target height both in group1 (156.3±2.9 vs 150.8±3.7cm, p<0.01, 156.3±2.9 vs 153.9±2.6cm, p<0.01), and in group2 (157.3±4.7 vs 152.6±3.9cm, p<0.01, 157.3±4.7 vs 154.4±4.7cm, p<0.01), but was similar to predicted AH and target height in group3(154.1±5.5 vs 153.1±6.2cm, p=0.06, 154.1±5.5 vs 155.6±6.3cm, p=0.26). 3. Menarche occurred 13.2±1.4, 15.8±10.7, 10.2±4.8 months after discontinuation of therapy in group1, group2 and group3 respectively. No hirsutism, clitorism or irregular menstruation was recorded in girls treated with stanzanolol.

Conclusions: Combine use of stanzanolol can improve FAH in girls with CPP and apparent decreased linear growth during GnRHa therapy.

Results: AMDM was genetically confirmed in all the patients. Five different mutations were found and all of them were novel. Four patients were homozygous and one was a compound heterozygous, see table. The parents of all the patients and some relatives were mutation carriers.

Background: The PREDICT long-term follow-up study investigates relationships between conventional biomarkers, genetic polymorphisms, gene expression and long-term auxological changes in GH treatment-naive prepubertal children with GHD or TS during GH therapy.

Objective: To study the association between baseline (BL) gene expression and Year 1 growth response to GH therapy in children with GHD and TS.

Methods: Height velocity (HV) after 1 year of GH therapy was grouped into quartiles and correlated with BL gene expression measured using Affymetrix Human Genome U133 Plus 2.0 Arrays. Gene ontology of associated changes and biological network inference were assessed using Ingenuity Pathway Analysis (IPA) software. Predictive modelling was performed using K-near-est neighbour classification, with 1-level cross validation.

Results: In BL GHD (n=71) and TS (n=41) samples, differences in expression of 526 and 90 probe sets, respectively, correlated with the lowest quartile of HV (analysis of variance, p<0.01). There was no overlap between probe sets. Associated gene ontology was enriched for growth-related processes (false discovery rate corrected p-value, q<0.05) and protein ubiquitination (q<0.05) in GHD, along with nitrogen metabolic pathways (q<0.1) and regulation of transcription (q<0.1) in TS. IPA identified multiple inferred pathways in both conditions associated with metabolic functions, cell cycle and pathways related to apoptosis and growth (q<0.05). Predictive modelling identified a group comprising 26 probe sets in GHD and 71 probe sets in TS associated with poor response to GH treatment, which had positive predictive values of 0.48 and 0.62, and negative predictive values of 0.86 and 0.89, respectively.

Conclusions: Using BL gene expression, candidate biological pathways associated with low response over the first year of GH therapy have been identified in prepubertal children with GHD and TS. BL gene-expression profiles may contribute to a predictive model for individualized treatment.
P1-d2-329 Growth 1

Single nucleotide polymorphisms (SNPs) associated with growth response over 3 years on growth hormone (GH) therapy and changes from baseline through the third year of GH therapy in children with GH deficiency (GHD) and Turner syndrome (TS)

Pierre Chatelain1; Roland Pfäffli; Klaus Kapelari2; Jean-Pierre Salles3; Armand Valiesa4; Benoit Desteneuze5; Peter Clayton6
1Université Claude Bernard, Hôpital Mère Enfant de Lyon, Department Pédiatrie, Bron, France; 2University of Leipzig, Department of Pediatrics, Div. of Pediatric Endocrinology, Leipzig, Germany; 3Brûl’s Universitätsklinik für Kinder- und Jugendheilkunde, Department Pädiatrie 1, Innsbruck, Austria; 4Hôpital d’Enfants, Service d’Endocrinologie pédiatrique, Toulouse, France; 5Merck Serono SA, Biomarker Technologies - Bioinformatics, Geneva, Switzerland; 6Merck Serono S.A. - Geneva, Endocrinology, Geneva, Switzerland; 7University of Manchester, Manchester Academic Health Sciences Centre, Manchester, United Kingdom

Background: The PREDICT long-term follow-up study investigates the link between SNPs in growth- and metabolism-associated genes and long-term growth in children with GHD or TS receiving GH therapy.

Objective: Evaluate the association of SNPs with growth response (cm/yr) in years (Y) 1-3 of GH therapy in children with GHD or TS.

Methods: Treatment-naive children with classic idiopathic GHD or TS were prepubertal at start of GH therapy and Tanner stage 1-2 at Y3. Median GH doses were 35, 33 and 32µg/kg/day for GH and 50, 49 and 50µg/kg/day for TS at Y1, Y2 and Y3, respectively. 1451 SNPs from 98 candidate genes were successfully genotyped. Growth (Y1: n=110, Y2, n=93, Y3, n=73; TS: Y1: n=60, Y2, n=43, Y3, n=56). Associations of SNPs with growth (cm) over Y3 and changes from baseline (BL) through Y3 were assessed in continuous analyses by Kruskal–Wallis non-parametric tests. Adjusted p-values less than 0.05 were considered significant.

Results: 30 of 45 SNPs in 5 genes were associated with growth response in Y3 (AKT2, GAB1, PROP1, SLC2A4 and -4A) and 7 from BL to Y3 (FGF3, GRB2, JAK2, PI3KR1 and -2, SHC1, SLC2A4). SNPs in both FOS and GRB2 were associated with growth from both BL to Y2 and BL to Y3. None of these genes had been identified previously as associated with Y1 growth, although the genes associated with Y1 growth did include GRB10. For TS, SNPs in 4 genes were associated with growth response in Y3 (FGF3, CYR61, PI3KCB, PI3KR2) and 4 from BL to Y3 (ARRB1, FGF3, PTPN1, SLC2A1). SNPs in both FGF3 and PTPN1 were associated with growth from both BL to Y2 and BL to Y3. None of these genes were associated with Y1 growth. Y2 and Y3.

Conclusion: Results suggest that in children with GHD or TS receiving GH therapy, genes associated with first year (catch-up) growth differ from those that associate with longer term response (over the first 2 or 3 years of treatment). Growth factor receptor-bound proteins are, however, involved in both phases in GHD. These data point to the diversity of genetic modulation of growth response to GH treatment.

P1-d2-330 Growth 1

Magnetic resonance imaging (MRI) of the cranial and functional findings in 15043 children with non-acquired growth hormone deficiency (GHD) from KIGS

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Background: Neuro-imaging of the CNS by MRI has become an essential part of the diagnostic work-up in children with GHD. Aims: To document the frequency and diversity of neuro-anatomical anomalies in a very large cohort with GHD (max GH to provocation tests < 10 µg/L) and to associate the findings with patients characteristics.

Methods: In 15043 of 43725 children documented 1987-2011 within KIGS (Pfizer International Growth Database) with non-acquired GHD (idiopathic IGHD, Neurosecretary Dysfunction [NSD] or GHD of known congenital cause) results of cranial MRI (85% before GH treatment) were reported by the investigators. In cohorts of patients with normal MRI and abnormal pituitary (pituitary hypoplasia, empty sella [ES], Hypoplastic anterior pituitary) and in 3/12 (25%) mutation negative. The final height reached from 5 patients SOS1+ was normal, while it was low in 2 cases PTPN11+.

Results: In 4032 [26.8%] children various abnormal MRI findings were documented within (N=2968) and outside the pituitary region. The most frequent were: pituitary hypoplasia (N=1178 [7.9%]), ES (N=449 [3.0 %]) and HME (N=1019 [6.8%]). In 2361 children IGHD or NSD were diagnosed before MRI was done, but MRI abnormalities were observed in subsequent MRIs (e.g. pituitary hypoplasia: N= 974; HME: N=459). The characteristics in patients with GHD were found related to the neuro-anatomical abnormality (see Table 1).

Table 1: Patients Characteristics

<table>
<thead>
<tr>
<th>MRI Diagnosis</th>
<th>Normal</th>
<th>Pit. Hypoplasia</th>
<th>Empty Sella</th>
<th>HME</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1031</td>
<td>178</td>
<td>449</td>
<td>1018</td>
</tr>
<tr>
<td>maxGH µg/L</td>
<td>6.6</td>
<td>3.9</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>MPHD %</td>
<td>11.1</td>
<td>22.1</td>
<td>52.1</td>
<td>49.8</td>
</tr>
<tr>
<td>Birth delivery</td>
<td>2.2</td>
<td>2.4</td>
<td>3.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Height - MPHD</td>
<td>1.57</td>
<td>2.12</td>
<td>2.34</td>
<td>2.71</td>
</tr>
<tr>
<td>Age - GH start yrs</td>
<td>11.0</td>
<td>9.7</td>
<td>8.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Median values; * = p< 0.01 vs. Normal MRI. Group comparison by Wilcoxon.

Conclusions: In children with non-acquired GHD neuro-anatomical abnormalities are present in high frequency and great variety. There is an association between anatomical and functional abnormalities of the pituitary, which may have implications for the outcomes of treatment and the prognosis. Standardisation for the interpretation of MRI findings (e.g. pituitary size) is needed.

P1-d2-331 Growth 1

Abnormal growth in Noonan syndrome: correlation between growth parameters and genotype

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Background: Noonan Syndrome (NS) is an autosomal dominant disorder characterized by short stature, dysmorphic features, congenital heart defects and other anomalies. Familial or de novo mutations in PTPN11, RAF1, SOS1, KRAS, and NRAS are detected in 60-75% of cases.

Objective and hypotheses: The aim of this study was to find out a possible correlation between the linear growth, the GH secretion and the genotype in NS patients.

Methods: A cohort of 34 patients affected by NS diagnosed by Van der Burgt criteria was studied: 13 had a PTPN11 mutation, 9 SOS1 (in one case associated with RAF1) and 12 were mutation negative. All of these patients underwent a clinical and aulogical evaluation, GH secretion was evaluated in 29 patients.

Results: Short stature was detected in 9/13 (69%) PTPN11+ patients, in 8/12 (67%) mutation negative and only in 2/9 (22%) of SOS1+ patients. The average H-SD was significantly higher in the SOS1+ group compared to PTPN11+ and to mutation negative groups, while no significant difference was found between the latter two groups. The average H-SD of PTPN11+ and of mutation negative groups was significantly lower (p<0.002) than their respective target height. The average H-SD was significantly higher in the SOS1+ group compared to PTPN11+ -R2, SHC1, SLC2A4 and to mutation negative groups, while no significant difference was found between the latter two groups. The average H-SD of PTPN11+ and of mutation negative groups, while no significant difference was found between the latter two groups. The average H-SD was significantly higher in the SOS1+ group compared to PTPN11+.
Acid-Labil Subunit (ALS) haploinsufficiency and phenotypic variability of IGFALS p.N276S mutation carriers
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Background: Recent reports have indicated that ALS haploinsufficiency due to heterozygous inactivating IGFALS (16p13.3) mutations, may be a factor involved in the aetiology of moderate postnatal growth deficit associated with IGF-I deficiency. p.N276S is a founder IGFALS mutation in the Spanish population, which has been reported in 5 unrelated families.


Clinical case: The index case is a male with postnatal growth deficit since the age 1.4 yrs, born at term from non consanguineous parents. Father height: 171.5 cm (-0.91 SDS); mother height: 149 cm (-2.51SDS). His main auxological characteristics at diagnosis and during the follow up period are summarized in table 1. His clinical history noted peculiar facial features, mild hypertelorism, cranio-facial dysmorphism, clumsiness of the 5th finger, gastro-esophageal reflux and persistent cosinophilia since age 2.5 yrs. At age 10 yrs he was diagnosed with esophagitis.

Follow-up: During the follow up period his height remained between -1.79 and -2.47 SDS. Hormonal tests revealed consistently decreased IGF-I and IGFBP3 levels and a peak GH of 18.8 ng/ml (Propranolol). Serum ALS concentration was 55% of age-matched healthy controls. At his last control, age 14.9 yrs, his height was 149.5 cm (-1.95 SDS, Tanner III).

Methods and results: Genetic analyses: Mutation screening of IGFALS identified the mutation c.827A>G, p.N276S, in heterozygosis in the index case, mother and in 2/3 siblings, whose main characteristics are summarized in Table 2.

Conclusions: Although serum ALS levels were significantly decreased in all mutation carriers (45.58% of controls), the consequences of ALS haploinsufficiency on growth and final stature differs among the carriers, suggesting that ALS haploinsufficiency is a contributing risk factor but not the single determinant of the postnatal growth deficit observed in the index case.

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Development of new Danish growth charts
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Background: Current Danish growth charts are based on measurements of children born in 1954-1973. A recent Danish study of parentally reported heights from children aged 0-5 year suggested secular increases in height, weight and BMI compared to the current national references. Therefore, new Danish height curves based on contemporary children are needed.

Objective and hypotheses: To develop new Danish growth charts for children aged 0-20 years, and to compare them with historic Danish references and the WHO standards.

Methods: A combined cross sectional and longitudinal Danish population-based study on 4099 children born in 1983-2002 was conducted. We applied the following inclusion criteria: singleton births, GA ≥37 and GA<42, breastfeeding ≥3 months, no smoking during pregnancy, white Caucasian, BMI ≥ -2 SD and BMI ≤ +2 SD (based on old references). This resulted in 2057 and 2650 height measurements from 624 girls and 652 boys, respectively. Growth charts were made from the Generalized Additive Models for Location, Scale and Shape (GAMLSS). Medians and centiles were compared with national references and WHO standards.

Results: Overall, contemporary height curves differed significantly from historic charts. In boys, median height at 3-8 years was approximately 2 cm higher and this difference increased to 6-10 cm in 11-15 year olds. In boys, 1.1-1.5 % of measurements were below the 3rd percentile of historic height charts. Compared to WHO standards, approximately 1 % of height measurements were below the -2 SD line and 7-8 % were above the +2 SD line at all ages. In girls, similar patterns were observed.

Conclusions: New Danish growth standards for children aged 0-20 years differ from WHO growth standards with Danish children being significantly taller. A secular trend in height is observed compared to historic national references.

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Analysis of data from the ANSWER Program® from pediatric patients treated with growth hormone who were prescribed aromatase inhibitor therapy
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Background: The American Norditropin Studies: Web-enabled Research (ANSWER) Program®, a US-based registry, has collected data on patients treated with Norditropin® (somatropin rDNA origin, Novo Nordisk A/S).

Objective and hypotheses: To analyze baseline characteristics and longitudinal data in growth hormone (GH)-treated patients who were prescribed aromatase inhibitor therapy (AIT: anastrozole or letrozole) at the discretion of the physician.

Methods: As of October 2011, 57 male GH-naïve patients with GH deficiency (n=34), idiopathic short stature (n=17), or other growth disorders (n=6) were included in this analysis.

Results: For the overall population (n=57), mean ±SD) chronologic age (CA) at GH start and AIT start, respectively, were 12.2±3.3 y and 15.1±1.9 y. At start of AIT, the number of patients classified as Tanner stage II, III, IV, or V was 6, 15, 8, and 25, respectively. Mean height standard deviation score (HSDS) increased from baseline (−2.1±0.8) to start of AIT (−1.1±0.8). In a longitudinal population of 13 patients with data before and after AIT (Table 1), the mean duration of GH before AIT was 3.8±2.6 y and mean duration of AIT was 0.9±0.4 y. HSDS increased from −2.3±0.4 at baseline, to −1.2±0.6 at start of AIT, and to −0.8±0.7 after AIT. Bone age (BA) increased from 9.4±3.9 y at baseline, to 13.1±0.9 y at start of AIT, to 14.1±1.5 y.
after AIT. Mean BA/CA ratio increased from 0.81 at baseline to 0.96 at start of AIT, and remained at 0.96 after AIT.

Conclusions: AIT was initiated during GH treatment coincident with advanced age and Tanner stage, suggesting that physicians were concerned about a diminishing treatment window for optimizing growth due to skeletal maturation and impending epiphyseal fusion. In the longitudinal population, BA/CA ratio showed accelerated increase before AIT and remained unchanged after AIT, coincident with a continued increase in HSDD. These results are consistent with a potential effect of AIT in slowing bone maturation and prolonging the period during which GHT may increase growth potential.

Table 1. Patient characteristics before and after AIT

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Start of AIT</th>
<th>After AIT</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N Mean (SD)</td>
<td>N Mean (SD)</td>
<td>N Mean (SD)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>13 10.18 (4.23)</td>
<td>13 13.95 (2.06)</td>
<td>13 14.88 (2.17)</td>
</tr>
<tr>
<td>HSDD</td>
<td>13 –2.29 (0.44)</td>
<td>13 –1.17 (0.64)</td>
<td>13 –0.83 (0.65)</td>
</tr>
<tr>
<td>BA (y)</td>
<td>9 9.44 (3.92)</td>
<td>13 13.12 (0.94)</td>
<td>13 14.10 (1.47)</td>
</tr>
<tr>
<td>BA/CA ratio</td>
<td>9 0.81 (0.17)</td>
<td>13 0.96 (0.14)</td>
<td>13 0.96 (0.13)</td>
</tr>
</tbody>
</table>

P1-d2-335 Growth 1

Auxiological characteristics of patients with constitutional delay of growth and puberty (CDGP)

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Background: CDGP is a common cause of idiopathic growth delay in childhood. It is not due to endocrine or any other known disease. Several auxiological characteristics have been reported to aid in the diagnosis before delay of puberty becomes evident.

Objective and hypotheses: Up to now, after endocrinological work-up, repeated clinical visits for auxiological re-evaluation are required for reliable diagnosis of CDGP. The present study aims at defining model parameters which might allow prediction of future growth before pubertal age is reached.

Methods: We studied a group of Caucasian children (Index 229, Siblings 126) with a positive family history of CDGP. We built a subgroup of 43 children (11 female, 32 male, age 5.4-18.7 years), well characterized with regard to CDGP. All of them had retarded bone-age and no evidence of other known causes of small stature (including familiar short stature). We analyzed height and weight from birth through early childhood (data of well-child visits) to find evidence of growth deceleration. These data as well as height, weight, sitting-height, subischial-leg-length and some ratios at first presentation were calculated as mean standard deviation scores (SDS).

Results: At birth, length and weight were –0.75 SDS. Growth deceleration became evident between age 2 and 4, with SDS at age 4 being -1.45 for height. This difference remained stable until presentation (mean age 12.2). At this time, normal results were obtained for BMI (-0.37). A SDS of -1.51 was found for sitting-height and -1.02 for subischial-leg-length. SDS-Difference of sitting-height and subischial-leg-length was significantly (p<0.05) different before (-0.96) and at the beginning of puberty (-0.33).

Conclusions: Our study indicates that the data of the well-child visits, together with endocrinological and radiological work-up before puberty can be used to predict the diagnosis CDGP. Early and reliable diagnosis of this norm-variant of growth would save medical resources.

P1-d2-336 Growth 1

Establishment of hGH cut-off concentrations for stimulation tests in children and adolescents by the IDS-iSYS hGH assay

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Objective: Growth hormone deficiency (GHD) is confirmed if hGH peaks of two stimulation tests fail to exceed a cut-off in the range between 5-10 ng/mL. This analytical procedure requires a low variability, since otherwise the diagnosis GHD is severely biased and depends more on the quality of the laboratory method than on the presentation of the patient. We, therefore, compared hGH concentrations in serum samples applying commercially available hGH assays and established a new cut-off for interpretation of GH stimulation tests.

Methods: Samples for method comparison were obtained from 312 children with short stature suspected for GHD (8.00-3.44 years of age, 168 males, 144 females). Seven assays were used for GH measurement (iSYS, IDS, IMMULITE 2000, Siemens; Liaison, DiSorin; UniCel Dxl 800 Access, Beckman Coulter; Auto DELFIA, PerkinElmer; BC-IRMA, Beckman Coulter; ELISA, Medagnost). Samples for cut-off determination were retrieved from stimulation tests of 46 paediatric patients who failed the suspected diagnosis GHD by normal growth data in late life.

Results: The IDS-iSYS method was used as the reference assay. The regression equation (correlation coefficient) between the methods was: iSYS = 0.902 x Immulite-0.040 (r = 0.964), iSYS = 1.025 x Liaison + 0.401 (r = 0.919), iSYS = 1.124 x Dxl - 0.889 (r = 0.880), iSYS = 0.846 x AutoDELFIA + 0.523 (r = 0.922), iSYS = 1.381 x BC-IRMA + 0.873 (r = 0.927), and iSYS = 1.114 x Medagnost-ELISA + 0.924 (r = 0.869). The preliminary data deducted from stimulation tests of paediatric patients without GHD suggested a cut-off of 7.18 ng/mL with a diagnostic specificity of 97.5%.

Conclusions: There was a good or acceptable correlation between different hGH assays. Considering conversion factors of recombinant hGH preparations the established cut-off concentration was in agreement with results from last published data approximately 50 years ago.

P1-d2-337 Growth 1

De novo IGF1R gene deletion in an IUGR-SGA boy with high IGF1 levels and without catch-up growth

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Background: Impaired foetal growth has been related to quantitative or functional IGF1 deficiency. Hemizygosity or mutations of IGF1 receptor gene (IGF1R), located in 15q26.3, has been reported in 2% of short stature IUGR subjects with or without mental retardation and dysmorphic feature.

Objective: To report a case of short stature investigated for alterations in IGF1R gene.

Patient and methods: A 2.3-year-old boy with mild psychomotor retardation, born SGA (bw 1.7 Kg; ga 36 w) has been evaluated for severe short stature (75.5 cm; -3.22 SD and -1.7 SD below his target height). Coeliac disease and hypothyroidism were excluded. Bone age was delayed of about 1 year. IGF1 levels were >97° centile for age (206 ng/ml). No significant dysmorphic signs were observed at a careful clinical examination. The clinical picture suggested an IGF1 resistance requiring genetic confirmation.

Results: CGH-Array was performed showing a de novo 15q26.2-26.3 deletion of 4.07 Mb. The deleted region encompasses 12 OMIM genes, including IGF1R.

Conclusions: In our patient the genetic results support the hypothesis that short stature and high IGF1 levels are due to IGF1 peripheral resistance. However the correlation between genotype and phenotype requires further investigations. Since some patients are described compound heterozygotes for mutations in IGF1R, we could consider to study the sequence of the entire gene, in order to discover a possible point mutation in the other allele. Furthermore, functional analysis could be useful to study the expression of the protein in...
fibroblasts of the patient. In our case the IGF1 levels are particularly high suggesting severe resistance, while other cases of IGF1R deletion reported in the literature, responsive to GH treatment, have low or high-normal IGF1 levels. We wonder if this case, with high levels of IGF1, may or may not favourably respond to GH treatment.

P1-d2-339 Growth 1

Height and health-related quality of life: a nationwide population-based study
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Background: Short stature is increasingly viewed as an unfavorable health status and treatment for short stature in childhood is commonly recommended with the purpose of improving adult health-related quality of life (HRQoL). However, there is only limited data available concerning the consequences of body height for HRQoL in adulthood.

Objective and hypotheses: To investigate the relationship between body height and HRQoL in a national representative, cross-sectional household survey.

Methods: 8857 men and 9248 women, aged 18-50 years in 2003 from the national general non-institutionalized population were randomly sampled. Scores on the eight subscales of the Medical Outcomes Study 36-item Short Form (SF-36) were the primary outcomes. Univariate and multivariate linear regression analyses were used to evaluate the effect of height on HRQoL while controlling for age and various socio-economic variables and pathological conditions.

Results: Height was found to be a very weak predictor of HRQoL both for men and women. Only heights lower than 149.2 cm and 136.0 cm, and higher than 203.6 cm and 188.7 cm, in men and women respectively, were associated with a clinically significant reduction in physical functioning. The effects of body height on other (mental, social) dimensions of HRQoL were negligible or undetectable.

Conclusions: Height appears to have minimal consequences for physical functioning, and negligible effects on other dimensions of HRQoL. These results do not support the treatment of childhood short stature to increase adult HRQoL.

P1-d2-340 Hypoglycaemia 1

Activating mutations in the AKT2 gene cause a novel syndrome of hypoinsulinaemic hypoglycaemia and hemi-hypertrophy
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Background: Hypoglycaemia is a common problem in the newborn, infancy and childhood periods and can be due to many different causes. Hyperinsulinaemic hypoglycaemia is the most common cause of severe hypoglycaemia and is due to unregulated insulin secretion from pancreatic β-cells.

Objective and hypotheses: To understand the molecular basis of a unique phenotype in three patients characterised by recurrent severe hypoinsulinaemic hypoglycaemia associated with macrosomia and left-sided hemi-hypertrophy.

Methods: Exome-wide sequencing was undertaken and resulting variants were filtered to exclude those that were common or not predicted to alter amino acid sequences.

Results: 326 rare mutations were found including the heterozygous c.49G>A mutation in the AKT2 gene, leading to substitution of glutamate 17 in the pleckstrin homology (PH) domain for lysine. Sanger sequencing confirmed the heterozygous mutation in lymphocytes and left and right sided dermal fibroblasts from all patients but not from parents consistent with a de novo germline mutation. Overexpression of AKT2 p.Glu17Lys in 3T3-L1 adipocytes produced non insulin dependent membrane localisation of the GLUT4 glucose transporter. Consistent with this the mutant AKT2 exhibited plasma membrane localization even in serum-starved HeLa cells and to produce tonic nuclear exclusion of FoxO1 in 3T3-L1 cells.

Conclusions: This is the first study to describe the molecular basis of a novel disorder which leads to autonomous activation of the insulin signalling pathway and hemi-hypertrophy. AKT2 is an upstream positive regulator of the Mammalian Target of Rapamycin (mTOR) which is an atypical protein kinase that controls growth and metabolism in response to nutrients, growth factors and cellular energy levels. This study illustrates the key role played by AKT2 in regulating insulin action and has opened up new treatment options for these patients.
P1-d2-341 Hypoglycaemia 1

Assessment of pancreatic exocrine function in children following near-total pancreatectomy for diffuse congenital hyperinsulinism

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Background: Diffuse congenital hyperinsulinism (CHI) is a clinically heterogeneous disease, which if unresponsive to medical therapy (diazoxide, octreotide and continuous feeding) may require near-total pancreatectomy. There are very few studies describing long term pancreatic exocrine function in a large cohort of patients with diffuse CHI following near-total pancreatectomy.

Objective: To describe the clinical characteristics, genetic aetiology and pancreatic exocrine function in a large cohort of medically unresponsive diffuse CHI patients following near-total pancreatectomy.

Methods: Retrospective review of case notes of children treated with near-total pancreatectomy for diffuse CHI over a 10-year period.

Results: Twenty-three children (mean age 7.5±4.1 yr) had near-total pancreatectomy for diffuse CHI. Of these, 21(91%) had mutations (homozygous/compound heterozygous) in ABCC8/KCNJ11 genes. All had faecal elastase measured within 3 months of pancreatectomy. Of these, 17(74%) had faecal elastase either undetectable (<15μg/g) or suggestive of severe pancreatic exocrine insufficiency (<15-100μg/g). Of these, 7(41%) did not show clinical evidence of pancreatic exocrine insufficiency (pale stools, abdominal discomfort, flatulence, weight loss) over a mean follow up period of 6.1±3.5yr and are currently not on pancreatic enzyme supplementation. Mean weight and height SDS of these ten children (mean age 7.6±5.0yr) is 0.59±1.27 and 0.48±1.43 respectively. The remaining 10(59%) with undetectable faecal elastase, developed clinical pancreatic exocrine insufficiency and needed pancreatic enzyme supplements. Mean weight and height SDS of these ten children (mean age 7.7±3.5yr) is 0.35±0.77 and 0.19±1.08 respectively.

Conclusions: In our large cohort of 23 children with near-total pancreatectomy for diffuse CHI, faecal elastase proved to be a poor marker for pancreatic exocrine function. No correlation was found between faecal elastase and clinical pancreatic exocrine insufficiency. Faecal elastase should be used with other markers to assess pancreatic exocrine function.

P1-d2-342 Hypoglycaemia 1

Medical treatment of congenital hyperinsulinism: side effects and response to treatment depending on the underlying mutation

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Background: Congenital hyperinsulinism (CHI) is a heterogeneous genetic disorder leading to an unregulated secretion of insulin from pancreatic beta cells. Medical treatment of CHI still remains challenging and keenly depends on the clinicians experience. Knowledge about efficacy, dosage and safety of the medication in current use is limited. An optimisation of pharmacological treatment is particularly important for diffuse CHI as it may avoid subtotal pancreatectomy.

Objectives: Aim of this study was to review the literature on long-term conservative treatment in CHI. In addition, we evaluated the underlying disease-causing mutation (ABCC8, KCNJ11, GLUD1, GCK, HADI, HNF4A, UCP2) with respect to the clinical phenotype and response to pharmacological treatment.

Methods: We searched MEDLINE (from 1947) and EMBASE (from 1988) using the OVID interface. The last search was run in March 2011. In addition, we searched reference lists of retrieved articles and reviews. No language restrictions were applied.

Results: Data for more than 1000 patients with CHI were evaluated. 600 patients had received long-term conservative treatment. Common side-effects of treatment with diazoxide (DZX) were hypotension, fluid retention and dyspeptic syndrome. Severe side effects such as encephalopathy and heart failure were rare. Typical side effects of octreotide included tachyphylaxis and abdominal discomfort. Gallstones, necrotizing enterocolitis and reduced growth velocity were less frequent. No side effects were described for treatment with calcium channel blockers. However, half of the patients treated with these agents required extra medication. About 70% of patients with a mutation in ABCC8 or KCNJ11 and about 14% of patients with a mutation in GCK were resistant to treatment with DZX. The majority of patients with a mutation in GLUD1, HADI, HNF4A or UCP2 were responsive to DZX.

Conclusions: This review provides the basis for a structured discussion with regard to optimal treatment strategies in children with the heterogeneous disorder CHI.

P1-d2-343 Hypoglycaemia 1

Surgery in non-focal congenital hyperinsulinism: less may be more

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Background: In children with non-focal congenital hyperinsulinism (CHI) who are resistant to medical therapy extensive pancreatic resections (95-98%) have been recommended. However, the incidence of diabetes until puberty has been reported in up to 90% after subtotal pancreatectomy.

Objective: We evaluated a less extensive, more selective minimal invasive surgical approach.

Patients and methods: 7 children (mean age 19 months) and one adult with severe non-focal congenital hyperinsulinism which was not responsive to medical therapy underwent laparoscopic biopsies under frozen section control. A laparoscopic pancreatic tail resection removing approx. 30-40% of the gland was performed in all patients. In one child additionally an open resection of the uncinate process was carried out. Today the follow-up time is 5.4 months (2 - 11 months).

Results: In six patients no genetic mutation, in one a heterozygous mutation in ABCC8 and homozygous KCNJ11 mutations in another have been detected. Three children revealed the recently described segmental mosaic form of CHI, four children and the adult a classical diffuse CHI. After surgery, one child with segmental mosaic CHI can be estimated as cured. Five children are off any medication today and manage their glucose level by starch-enriched meals. The adult is euglycemic without further treatment. There were no complications except one hematoma.

Conclusions: In 8 patients with non-focal congenital hyperinsulinism, resistant to medical therapy (diazoxide or octreotide) a restrictive, frozen-section guided laparoscopic pancreatic tail resection led to considerable improvement in quality of life for children treated in the past by mutilating pancreatectomy. Further work is necessary to identify this subgroup within the molecular and clinical heterogeneous diagnosis of CHI.

P1-d2-344 Hypoglycaemia 1

Genotype phenotype correlations in congenital hyperinsulinism

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Background: Congenital hyperinsulinism (CHI) is characterized by clinical and genetic heterogeneity. Mutations in the K-ATP channel are reported in nearly 50%, with null or reduced functional activity.

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Conclusions: GIP levels were increased by approximately 3-fold more than the other patients and were increased by approximately 15-fold higher than the other patients (one-way ANOVA, p > 0.05). However, in the atypical patient postprandial plasma GLP-1 levels were significantly higher than those in the other patients (one-way ANOVA, p < 0.05). Seven patients had transient hypoglycaemia and no identified genetic abnormalities. In the family of patients classified as diffuse, focal and transient CHI patients (one-way ANOVA, p < 0.05), no gene defects were found to be associated with the atypical patient. Seven families had transient hypoglycaemia and no identified genetic abnormalities. In the family of patients classified as diffuse, focal and transient CHI patients (one-way ANOVA, p < 0.05), no gene defects were found to be associated with the atypical patient.

Results: We detected 48 mutations in ABCC8 and 5 different mutations in KCNJ11 in 36 and 6 unrelated patients, respectively, including 28 novel mutations. In ABCC8, we identified 20 missense mutations, 7 nonsense mutations, 7 frame-shift mutations, 2 in-frame deletions, and 1 splice-site/splicing mutations. In KCNJ11, 1 different missense mutations were found and one in-frame deletion. Six mutations of ABCC8 and one KCNJ11 mutation were recurrent in more than one family. Molecular results suggested focal type CHI in 9 children (6 ABCC8 and 3 KCNJ11) and could be proven by Dopa-PET/CT and histology. 8 patients carried a heterozygous ABCC8 mutation affecting one of the nucleotide binding domains. In 5 of them family history or published data suggested that the respective mutations act in a dominant manner. Furthermore in 26 children a diffuse type CHI was confirmed by compound-heterozygosity or homozygosity for K-ATP channel mutations (23 ABCC8 and 3 KCNJ11).

Conclusions: In 36 of affected children mutations of ABCC8 (with 48 different alleles) and in 6 of KCNJ11 (with 5 different alleles) could be identified. In all 9 histological confirmed focal type CHI a paternally inherited mutation of ABCC8 or KCNJ11 was detected. In the majority of cases molecular findings in the family are good predictors of the phenotype.

Objective and hypotheses: In order to use mutational data as biomarker for diagnostic and treatment decisions it is crucial to correlate them with valid clinical data.

Methods: 113 patients (incl. 7 families) referred between 2004 and 2011 with clinical diagnosis of CHI were screened for mutations in the genes ABCC8 and KCNJ11 by bidirectional sequencing of all coding exons and adjacent intronic regions. Segregation was tested for all families where parental samples were available.

Results: We detected 48 mutations in ABCC8 and 5 different mutations in KCNJ11 in 36 and 6 unrelated patients, respectively, including 28 novel mutations. In ABCC8, we identified 20 missense mutations, 7 nonsense mutations, 7 frame-shift mutations, 2 in-frame deletions, and 1 splice-site/splicing mutations. In KCNJ11, 1 different missense mutations were found and one in-frame deletion. Six mutations of ABCC8 and one KCNJ11 mutation were recurrent in more than one family. Molecular results suggested focal type CHI in 9 children (6 ABCC8 and 3 KCNJ11) and could be proven by Dopa-PET/CT and histology. 8 patients carried a heterozygous ABCC8 mutation affecting one of the nucleotide binding domains. In 5 of them family history or published data suggested that the respective mutations act in a dominant manner. Furthermore in 26 children a diffuse type CHI was confirmed by compound-heterozygosity or homozygosity for K-ATP channel mutations (23 ABCC8 and 3 KCNJ11).

Conclusions: In 36 of affected children mutations of ABCC8 (with 48 different alleles) and in 6 of KCNJ11 (with 5 different alleles) could be identified. In all 9 histological confirmed focal type CHI a paternally inherited mutation of ABCC8 or KCNJ11 was detected. In the majority of cases molecular findings in the family are good predictors of the phenotype.
Background: Adverse experiences in utero might result in persistent modulating consequences on metabolism and cardiovascular system ("fetal programming"). This sensitive period has been extended as "developmental programming" to the early extrauterine life of premature children. The hypothalamic-pituitary-adrenal (HPA) axis is susceptible to programming and may be linked to risk of disease later in life.

Objective and hypotheses: In our study we hypothesized that premature infants born with extremely low birth weight (ELBW, birth weight < 1000g) have higher levels of urinary steroid metabolites than healthy controls.

Patients: 27 ELBW-infants (17 girls, 10 boys, all prepubertal, age 8-11 yrs) were included. Control children were age and sex matched. There was no significant difference of height, weight or BMI between the groups. All results were adjusted according to body surface area.

Methods: Urinary steroids were extracted, enzymatically hydrolysed, derivatized and profiled by gas chromatography-mass spectrometry using selected ion monitoring. 36 different steroid metabolites were quantified.

Results: All 36 measured steroid metabolites were higher in the ELBW boys (six significantly) in comparison to controls. In girls 33/36 steroid metabolites were higher (19 significantly). Sums for mineralocorticoid precursors, cortisol production, 11-β-HSD-activity, and the adrenal androgen production are shown in the table:

<table>
<thead>
<tr>
<th>Sum of [Mean (µg/l)]</th>
<th>Female ELBW (n=17)</th>
<th>Female Control (n=17)</th>
<th>P-value</th>
<th>Male ELBW (n=10)</th>
<th>Male Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralcorticoid precursors (THB + THA + Allo THB + 18α-THB)</td>
<td>1016</td>
<td>467</td>
<td>0.002</td>
<td>1725</td>
<td>472</td>
<td>0.02</td>
</tr>
<tr>
<td>Cortisol production (Tetrahydrocortisol + Allo-TetraHydrocortisol + Cortisol + β-Cortolon + Tetrahydrocortisol + Allo-TetraHydrocortisol + Cortisol)</td>
<td>10,717</td>
<td>6200</td>
<td>0.007</td>
<td>5849</td>
<td>4311</td>
<td>0.2</td>
</tr>
<tr>
<td>11-β-HSD activity (Tetrahydrocortisol + Allo-TetraHydrocortisol + Tetrahydrocortisol)</td>
<td>1.94</td>
<td>1.46</td>
<td>ns</td>
<td>1.48</td>
<td>0.23</td>
<td>ns</td>
</tr>
<tr>
<td>adrenal Androgens (Androstene-17,17-diol=5-Androstene-3,17,17-diol=5-Androstene-3,17,17-diol=5-Androstene-3,17,17-diol=5-Androstene-3,17,17-diol=5-Androstene-3,17,17-diol=5-Androstene)</td>
<td>1087</td>
<td>550</td>
<td>0.03</td>
<td>1145</td>
<td>502</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusions: These findings suggest that the premature stressful extra-uterine environment has long-lasting effects on the function of the hypothalamic-pituitary-adrenal-axis. We found significantly increased urinary androgen excretion, and a significant higher cortisol production rate and higher mineralocorticoid precursors in the ELBW infants pointing to an overall increased activation of the adrenals in ELBW. Global 11-β-HSD-activity was not significantly different and does not seem to account for the changes in cortisol metabolism.

Background: Fibroblast growth factor 21 (FGF21) is considered a key regulator in adaptation to fasting. Recently, a causative role of increased FGF21 in inhibition of skeletal growth during prolonged undernutrition was suggested in mice, mediated by antagonistic effect on GH action and reduced IGF1 synthesis (Kubicky et al., Endocrinology 2012). However, there are no studies on its role in chronic growth failure in man.

Objectives and hypotheses: We hypothesised that FGF21 is a mediator of extrauterine growth retardation in very preterm (PT) infants. We assessed FGF21 at birth and at 1, 3, and 5 weeks of age (w1-w5), and growth from birth to term in 32 very PT infants (56% boys, median gestational age 28.2 weeks, range 23.4-31.9; median birth weight (BW) 990g, range 480-1910; median birth length (BL) 36.8cm, range 28.0-44.0).

Methods: Plasma FGF21 was measured with an ELISA kit. BW, BL, weight, and length at term (median postmenstrual age 40.0 weeks, range 37.0-41.6) were converted into SDS with population-based birth size reference. LG-transformation was used for normality. Mixed model and linear regression were used for analyses.

Results: FGF21 was unmeasurably low at birth in 85% of infants. In mixed model adjusted for gestational age, sex, BW-SD and BL-SD, a significant increase from w1 to w5 was observed (p<.021). Increasing BW-SD had a significant negative effect on lgFGF21 (p=.003). Only 6.2 and 3.1% of infants had BW or BL <-2SD, whereas at term, 34.4% had both weight and length <-2.0SD. In linear regression model, w1-w3 FGF21 had a significant negative correlation (beta=-.455, p=.001) with length-SD at term, and BL-SD a positive correlation (beta=.499, p=.001).

Conclusions: These results indicate that elevated FGF21 is independent associated with extrauterine growth failure in very PT infants. This supports the hypothesis that FGF21 inhibits skeletal growth in humans as well.
Subjects and methods: Eight hundred and nine babies from mothers registered on the Cohort Study for Children and Mothers at NCCHD were involved in this study. One hundred eleven subjects were born small for gestational age (SGA). The economical background of participants was relatively homogenous. Cord blood samples were obtained at delivery, IGF-I, insulin, leptin, and adiponectin were measured by commercially available kits.

Results: Subjects' birth weight was 2903.9±493.3 g (mean±SD), birth length was 48.49±1.94 cm. Birth weight showed significant correlation with IGF-I (R^2=0.093, P<0.0001), insulin (R^2=0.023, P<0.0001), leptin (R^2=0.122, P<0.0001), and adiponectin (R^2=0.08359, P<0.0001) in cord blood. Birth weight among subjects with full-term, AGA delivery significantly correlated with maternal weight before pregnancy but not with maternal age. In SGA children (n=111) among our subjects, birth weight strongly correlates with IGF-I and adiponectin (R^2=0.249, P<0.0041 and R=0.254; P<0.008 respectively) and weakly correlates with leptin (R^2=0.157, P=0.048), but not with insulin levels. IGF-I, leptin and adiponectin levels in cord blood were significantly higher in AGA than in SGA children (P<0.0001), SGA children showed significantly higher BMI increment than AGA children during one month after birth.

Conclusion: These results indicate the contribution of IGF-I on fetal growth. Leptin and adiponectin might contribute for fetal growth, however, it is more likely that the levels of these factors show only the results of fetal growth. The significant correlation of maternal weight before pregnancy and child's birth weight suggests the involvement of genetic factors. The factors that have influence on early postnatal growth are under investigation.

P1-d3-350 Perinatal and Neonatal Endocrinology 1
Serum levels of receptors for advanced glycation end products in normal-weight and obese children born small and large for gestational age
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Background: Children born small (SGA) and large (LGA) for gestational age are at increased risk of cardio-metabolic complications later in life. Interestingly, in recent years the soluble (sRAGE) and the endogenous secretory (esRAGE) receptors for advanced glycation end products have been proven to be involved in the pathogenesis of cardiovascular diseases.

Objective and hypotheses: Our aims were to evaluate sRAGE and esRAGE in normal-weight (NW) and obese (Ob) pre-pubertal SGA and LGA children compared to subjects born appropriate (AGA) for gestational age, and to explore the potential association of these markers with birth weight (BW), insulin resistance (IR) and obesity.

Methods: We categorized 130 pre-pubertal children into 6 groups according to BW and obesity. sRAGE, esRAGE and homeostasis model assessment of IR were evaluated.

Results: sRAGE and esRAGE were lower in Ob SGA and LGA children than Ob AGA subjects (all P<0.05), and in NW SGA and LGA children than NW AGA subjects (all P<0.05). In a multiple linear regression analysis with sRAGE as the dependent variable, SGA (β=−0.237, P=0.008) and LGA (β=−0.414, P<0.0001) BW categories as well as HOMA-IR (β=−0.275, P=0.01) were significantly related to sRAGE, independently of BMI-SDS, age and gender. In a second model, using esRAGE as the dependent variable, SGA (β=−0.307, P=0.001) and LGA (β=−0.400, P<0.0006) BW categories as well as HOMA-IR (β=−0.246, P=0.04) were significantly related to esRAGE, independently of BMI-SDS, age and gender.

Conclusions: sRAGE and esRAGE are decreased in pre-pubertal SGA and LGA subjects, particularly in those showing excess body weight during childhood. Furthermore, IR emerged as an independent determinant of reduced RAGE levels. Further longitudinal studies are needed to verify the effect relationship between IR and RAGE in these children.

P1-d3-351 Pitutary 1
Timed-12h urinary gonadotropin during gonadotropin-releasing hormone analog stimulation testing for diagnosing the onset of hypothalamic-pituitary-gonadal axis in girls
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Background: GnRH stimulation testing requires venipuncture and multiple blood sampling at short intervals, an accurate but at times not always comfortable method. Furthermore, GnRH is commercially limited.

Objective: To estimate usefulness of timed-12h urinary gonadotropin during GnRH analog (GnRHa) stimulation testing for diagnosing the onset of hypothalamic-pituitary-gonadal (HPG) axis.

Methods: Following injecting tripotren (08:30 am, Decaptyl, 0.1 mg, s.c.), consecutive double timed-12h urine samples were respectively collected in 42 girls who suffered from disorders of growth or pubertal development. Gonadotropin was assayed by immunochemiluminescent assay (ICMA).

Results: There were 34 girls with the onset of HPG axis and the remaining 8 girls in the situation of pre-puberty. The correlation coefficient between the serum peak LH (PLH) and the content (concentration × volume) of the first urinary LH (FULH) was 0.759, and for PLH and the second urinary LH (SULH) content 0.746. The means of FULH content and SULH content in girls with the onset of HPG axis were all more than girls who were in pre-puberty (P<0.05). The areas under the receiver operator characteristic curves for diagnosing the onset of HPG axis for PLH, FULH content and SULH content were 0.982, 0.952 and 0.982, respectively. When PLH, FULH content and SULH content were respectively no less than 4.12 IU/L, 1.130 IU and 0.433 IU, the sensitivities were respectively 97.1%, 88.2% and 97.1%, and all three indexes were 100% specific.

Conclusions: It is concluded that timed-12h urinary LH content assayed by ICMA during GnRHa stimulation testing provides an effective, noninvasive methods for the diagnosis of onset of HPG axis in girls, and SULH content may be better than FULH content.

P1-d3-352 Pitutary 1
Initial hypothalamic involvement is the major risk factor for impaired prognosis and quality of life in childhood craniopharyngioma regardless of chosen treatment strategies – results of kraniofonyrmegom 2000
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Background: Hypothalamic obesity has major impact on prognosis and quality of life (QoL) in childhood craniopharyngioma (CP). The pathogenic relevance of initial hypothalamic involvement versus treatment-related hypothalamic lesions is a matter of controversy.

Methods: 120 patients were recruited prospectively (2001-2007) and evaluated after 3 yrs of follow-up. BMI and QoL at diagnosis (dgx) and 36 mo after dxg were analyzed based on reference assessment of tumour localization and post-surgical hypothalamic lesions using a novel grading system (no, anterior,
posterior involvement/lesion). Treatment was analyzed regarding neurosurgical strategy of 50 participating neurosurgical centres and the centre sizes. Based on patient load during the 6-year recruitment, centres were categorized as small (1/6 yrs), middle (2-5 yrs) or large-sized centres (>5 yrs). Results: BMI SDS at diagnosis was similar in patients with or w/o hypothalamic involvement. Surgical lesions of anterior and posterior hypothalamic areas were associated with higher increases in BMI SDS during 36 mo post-diagnosis compared to patients without or only anterior lesion (+1.8 BMISD, p=0.033; +2.1 BMISD, p=0.011), negatively impacting QoL in patients with posterior lesions. Surgical strategies varied between the 50 neurosurgical centres. Patients treated in small centres presented with a higher rate of hypothalamic involvement compared to middle and large-sized centres. Treatment in large centres was less radical, the rates of complete resection and hypothalamic surgical lesions lower than those of middle-sized and small centres. However, multivariable analysis showed that pre-operative hypothalamic involvement was the only independent risk factor for severe obesity (p=0.002). Conclusions: Radical strategies leading to posterior hypothalamic lesions are not recommended. Because our results show that initial hypothalamic involvement has an apriori effect on the clinical course, our recommendations are based on recognizing CP as a chronic disease.

P1-d3-354 Pituitary 1
Novel KAL1 sequence variants associated with septo-optic dysplasia (SOD) in three female patients
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Background and aims: KAL1 is essential for GnRH neuronal migration and olfactory bulb development with mutations implicated in Kallmann syndrome (KS; hypogonadotropic hypogonadism with anosmia). KAL1 is located in the X-chromosome pseudoautosomal region, which may account for the recent report of female KS patients exhibiting KAL1 variations. There is increasing evidence of overlapping genotypes/phenotypes between KS and congenital hypopituitarism including septo-optic dysplasia (SOD). Therefore, we aimed to screen 421 patients with the latter for mutations in KAL1.

Methods: The coding region of KAL1 was assessed by direct sequencing. In vitro functional analyses of identified variants included immunocytochemistry and western blot analyses for protein secretion from Cos7 cells as well as a novel quantitative luciferase-reporter assay.

Results: Two variants [p.K185N (n=1) and novel p.P291T (n=2; sisters)], occurring at highly conserved residues and absent from 480 controls, were identified in three female patients with SOD. Each had optic nerve hypoplasia and GH deficiency, with the p.K185N variant also being associated with TSH deficiency and an ectopic posterior pituitary. A qualitative decrease in secretion of mutant p.P291T protein was shown by its retention in Cos7 cells and a 40% decrease in transcriptional activity (p<0.001). Secretion of p.K185N was unaffected but the variant was associated with a 21% decrease in transcriptional activity (p=0.01). This variant is located between protein-protein interaction domains and may affect the binding of the protein to FGFR1 and heparan sulfate.

Both variants were inherited from the unaffected mothers and are suggestive of variable penetrance or digenicity in the affected individuals, none of whom exhibit variations in any of the other known KS genes.

Conclusions: We implicate KAL1 in females with hypopituitarism/SOD for the first time to our knowledge, reflecting an overlap between KS and SOD that has also been observed with FGFR8, FGFR1 and PROKR2 variants.

P1-d3-355 Pituitary 1
Screening of LHX2 in patients presenting growth retardation with posterior pituitary and ocular abnormalities
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Background: In humans, pituitary hormone deficiency may be part of a syndrome including extra-pituitary defects like ocular abnormalities. Very few genes have been linked to this particular phenotype. In the mouse, Lhx2, which encodes a member of the LIM class of homeodomain proteins, was shown to be expressed during early development in the posterior pituitary, eye and liver, and its expression persists in adulthood in the central nervous system. Lhx2-/- mice display absence of posterior pituitary and intermediate lobes, malformation of the anterior lobe, anophthalmia and they die from anemia.

Objectives and hypotheses: We tested the implication of the LHX2 gene in patients presenting pituitary hormone deficiency associated with ectopic or non visible posterior pituitary and ocular defects.

Methods: A cohort of 76 patients, including two familial cases, was studied. Direct sequencing of the LHX2 coding sequence and intron/exon boundaries was performed. LHX2 transcriptional activity on pituitary promers was tested in vitro.
Results: Seven heterozygous sequence variations were identified, among which two are novel missense (p. Ala203Thr and p. Val333Met). In vitro, LH2X activates transcription of TSHβ, PRL, and POU1F1 promoters in HEK293 cell line. A synergistic action of POU1F1 and LH2X was also shown on these promoters. The two variations were tested and no significant difference was observed leading to the conclusion that there are not deleterious.

Conclusions: These results suggest that, if LH2X is involved in pituitary hormone deficiency associated with posterior pituitary and ocular defects, it would be a rare cause of this disease condition.

P1-d3-357 Pituitary 1

Genetic screening in a cohort of 2030 patients with congenital hypopituitarism: current knowledge and future directions

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Background: Congenital hypopituitarism (CH) encompasses a spectrum of disorders: isolated or multiple pituitary hormone deficiencies with/without associated midline or ocular defects and/or structural pituitary abnormalities. Known genetic factors have explained a relatively small percentage with varying results depending on the cohort. We analysed the results of genetic screening in a large cohort of patients with CH performed in our centre. Patients and methods: Over 15 years we screened 2366 individuals including 2032 patients and 334 unaffected family members. 540 had combined pituitary hormone deficiencies (CPHD), 522 had variable hypopituitarism, 540 septo-optic dysplasia (SOD) and its variants (26%), 368 isolated GH deficiency (IGHD) (18%) and 62 had midline clefts including holoprosencephaly (5%). 17.4% (n=362) had a positive family history. According to phenotype patients were screened for mutations in HESX1, SOX2, SOX3, OTX2, LHX3, LHX4, SIX3, SHH, GLI2, CHD7, FGF8/FGFR1, KAL-1, PROK2/PROK2, PROP1, POU1F1, GHI, GHRHR.

Results: In patients with SOD HESX1 mutations were identified in 1.3% (7/540); mutations in SOX3(n=13) and OTX2(n=4) were rare but accounted for almost 28% and 4.5% respectively of cases with severe eye phenotypes (13/45 and 4/45). Changes in SOX3 dosage were uncommon (n=6). Variations in FGF8 were identified in <1% whilst PROK2 variations were the commonest (2%) but their contribution to phenotype is yet to be established. In patients with CPHD PROP1 mutations were identified in 3.3% (n=18) and POU1F1 in 2.2% (n=12) with higher percentages in familial cases. Genetic changes (GH1, GHRHR) were identified in 34 patients (9%) with IGHD.

Conclusions: Known genetic factors account for 5% of CH (n=106); this encompasses isolated or multiple pituitary hormone deficiencies with/without associated midline or ocular defects and/or structural pituitary abnormalities. Known genetic factors have explained a relatively small percentage with varying results depending on the cohort. We analysed the results of genetic screening in a cohort of 2030 patients with congenital hypopituitarism; current knowledge and future directions would be a rare cause of this disease condition.

P1-d3-358 Pituitary 1

Pituitary and ocular features of a novel OTX2 mutation in a family

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Background: The orthodenticle homologue 2(OTX2) homeobox gene encodes a transcription factor important in ocular and pituitary development. More than 30 OTX2 heterozygous mutations have been described with varied phenotype expression.

Objective: We describe a novel OTX2 mutation with marked phenotypic variability within a family.

Methods: Review of clinical features of novel OTX2 mutation in family.

Results: Phenyotype: Case1 (father) had 4 children with the same partner: 3 affected (cases 2-4), 1 unaffected. One child from a previous relationship had hypermetropia and squint with no other information available. Table 1 provides a summary of the clinical features seen in this family case series. Table 1: Summary of ocular abnormalities, pituitary dysfunction, additional co-morbidities and MRI findings for each case.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Ocular abnormalities</th>
<th>Pituitary dysfunction</th>
<th>Additional co-morbidities</th>
<th>MRI findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (male)</td>
<td>Hypermetropia, L-sided amblyopia, normal optic discs &amp; macula</td>
<td>No</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>Case 2 (male)</td>
<td>Rotary nystagmus, L convergent squint &amp; amblyopia, reduced vision in R eye, pale optic discs, mild bilateral ptosis</td>
<td>No</td>
<td>Transposition of the great arteries, developmental delay, autism, hearing loss</td>
<td>Thick corpus callosum, cerebellar hypoplasia</td>
</tr>
<tr>
<td>Case 3 (female)</td>
<td>See-saw nystagmus, hypermetropia, pale optic discs, corrected vision reduced in both eyes, no squint</td>
<td>Growth hormone deficiency</td>
<td>Developmental delay, high frequency hearing loss, poor coordination</td>
<td>Small pituitary fossa, ectopic posterior pituitary</td>
</tr>
<tr>
<td>Case 4 (male)</td>
<td>B/L microphthalmos, atrophic irides, retinal &amp; optic nerve aplasia, squint, horizontal nystagmus, choroidal coloboma, ptosis</td>
<td>Growth hormone deficiency</td>
<td>Severe developmental delay, R-sided congenital nasal cleft</td>
<td>Abnormal sella, ectopic posterior pituitary</td>
</tr>
</tbody>
</table>

Genotype: Direct DNA sequencing found a previously unreported heterozygous mutation c.249G>A in exon 2 of OTX2. Although this mutation is synonymous for p.Gln83, it is likely to be pathogenic as it alters the last nu...
P1-d3-359 Pituitary 1

**PROKR2 Variants in multiple hypophyseal with pituitary stalk interruption**

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**Background:** Pituitary stalk interruption represents a frequent feature of congenital hypopituitarism but only rare cases have been assigned to a known genetic cause.

**Objective and hypotheses:** Using a candidate gene approach we tested several genes of potential roles in hypopituitarism with pituitary stalk interruption. We hypothesized that ectopic posterior pituitary may be a consequence of defective neuronal axon projections along the pituitary stalk or defective angiogenesis of hypophysial portal circulation. Considering the role of the prokineticin 2 pathway in angiogenesis and neuronal migration, we screened PROKR2 and PROKR2 genes. Methods: PROKR2 and PROKR2, and all genes previously known to be involved in hypopituitarism with pituitary stalk interruption (LHX4, HESX1, OTX2, SOX3) were screened in 72 index cases with PSIS from the GENHYPOPIT database. In vitro studies were performed to assess the functional consequences of allelic variants. Results: We identified two heterozygous PROKR2 mutations (p.Leu173Arg and p.Arg85His) previously reported in isolated hypogonadotrophic hypogonadism, and a novel PROKR2 variant (p.Ala51Thr) that, in contrast with both other mutations, did not impair receptor signaling activity. Three allelic variants of HESX1 were identified: the heterozygous p.Phe156Ser and the nonsense of OTX2 mutations, and the extreme variability of their clinical manifestations. Conclusions: We report PROKR2 variants in congenital hypopituitarism with pituitary stalk interruption suggesting a potential role of the prokineticin pathway in pituitary development.

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P1-d3-360 Pituitary 1

**A balanced translocation disrupting a copy of the hominoid-specific TBC1D3 gene embedded in a CNV region in syndromic CPHD**

Kalotina Machini; Bénédicte Duriez; Vardenia Rakover; Philippe Duquesnoy; Florence Dartot-Le-Moal; Nathalie Collot; Serge Arsenault

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**Background:** The etiology of CPHD has been resolved only in a small proportion of patients. Over the last few years, accumulating sequencing data has illustrated the major role of copy number variation (CNV) regions in genome architecture and evolution. Such regions can be associated with sporadic, Mendelian or complex diseases. However, no example of chromosomal translocation interrupting these sequences has so far been reported.

**Objective and hypotheses:** The aim of this study was to identify the molecular basis of a syndromic form of CPHD in a young girl with de novo translocation involving chromosomes 17 and 14. The patient had deficits in GH, TSH and ACTH, and anterior pituitary hypoplasia. She was also diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD).

**Methods:** Genomic DNA of the patient and her parents was studied by means of array-based SNP/CGH analysis. The patient’s DNA was subjected to FISH analyses, followed by targeted sequencing. Transcripts were analyzed in lymphoblastoid cell lines.

**Results:** The array CGH data were in favor of a balanced translocation. We mapped the breakpoints of the translocation in a region of segmental duplication of chromosome 17, which has been shown to confer risk of developing autism spectrum disorders. The translocation disrupts one copy of the hominid-specific gene TBC1D3, which is absent in lower species and present in multiple copies in the human genome and has been shown to regulate the highly conserved EGF pathway in humans. This chromosomal rearrangement does not affect the gene copy number of TBC1D3 and no chimeric transcripts were detected in the patient’s lymphoblastoid cell line, raising the possibility that the translocation affects gene expression only in the pituitary and possibly in a specific developmental window.

**Conclusions:** Overall, these data, which unveil the pathogenic role of a translocation disrupting a CNV region, strongly suggest that the hominoid-specific gene TBC1D3 plays a key role in pituitary development.
whether this discrepancy in the incidence of UPD arises from under-diagnosis or because of ethnic differences. Subtle phenotypic differences between the deletion and UPD genotypes may be related with the age of diagnosis and their clinical importance keeps unknown, a question worthy of further study.

P1-d3-362 Programming/Epigenetics 1

Metabolic syndrome and endothelial dysfunction in a population born small for gestational age

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Background: Being born SGA is associated with metabolic syndrome (MS) and cardiovascular disease (CD).

Objective: To evaluate the presence of markers predictive of MS and CD in a population born SGA and to assess its relationship with spontaneous catch-up growth and rhGH treatment.

Population and method: Cross-sectional study: 181children born SGA (95 SGA with rhGH, 86 SGA with spontaneous catch-up growth) and a control population (n=88) born appropriate for gestational age. They were classified as prepubertal and pubertal according to Tanner stage. Parameters analyzed: perinatal auxology, physical examination, blood pressure (BP), carotid intima-media thickness (IMT-c) and analytic markers of MS. Longitudinal study of carbohydrate metabolism in 89 SGA rhGH treated.

Results: In the prepubertal group, patients born SGA with spontaneous catch-up growth showed values of systolic BP, diastolic BP, IMT-c, insulin, insulin/glucose ratio and HOMA index, statistically significantly higher compared to patients born SGA with rhGH and control group. In the pubertal group similar glucose ratio and HOMA index, statistically significantly higher compared to prepubertal and SGA and the control group. The IMT-c correlated negatively with birth weight and length and positively with age, weight, BMI, systolic BP, diastolic BP, triglycerides, LDL-cholesterol, insulin and HOMA index. Longitudinal carbohydrate metabolism evaluated by HOMA index did not show significant changes.

Conclusions: Children born SGA with spontaneous catch-up growth show lower insulin sensitivity determined by insulin/glucose ratio and HOMA index than children born SGA with rhGH.Prepubertal children born SGA with spontaneous catch-up growth show signs of endothelial dysfunction, indicative of an increased cardiovascular risk that persists during puberty.In children born SGA without spontaneous catch-up growth, rhGH treatment did not induce modifications in any of the studied parameters.

P1-d3-363 Programming/Epigenetics 1

A high birth weight is associated with increased risk for type 2 diabetes and obesity

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Background: In parallel to increasing maternal weights the proportion of infants born large for gestational age has increased during recent decades. The association between low birth weight (BW) and adult disease is well known. Much interest is now focused on the effects of a high BW.

Objective and hypothesis: To investigate the hypothesis that a high BW increases the risk of adult metabolic disease.

Population and methods: This is a cohort study of term, single births in Sweden 1973-1982, n=759 999 (subjects born small for gestational age are excluded). The registers used are the Swedish Medical Birth Register, the Inpatient Care Register, the Drug Register and the Causes of Death Register. Hazard ratio (HR) was calculated in relation to BW for components of the metabolic syndrome. Follow-up time is January 1998 to July 2010.

Results: Males with a BW above 2 SDS had a 1.9 fold increased risk (HR 1.57, 95% CI 1.33-1.87) for obesity, whereas the risk for females was 1.3 fold increased (HR 1.34, 95% CI 1.21-1.47). For males and females with BWs above 3 SDS the risks for adult obesity were higher, 2.5 fold (HR 2.48, 95% CI 1.65-3.74) and 1.8 fold (HR 1.81, 95% CI 1.41-2.32) increased, respectively. Males with a BW above 2 SDS had a 2.3 fold increased risk (HR 2.27, 95% CI 1.57-3.27) for type 2 diabetes, whereas those with a BW above 3 SDS had a 5.4 fold increased risk (HR 5.42, 95% CI 2.68-10.94). No increased risk for type 2 diabetes in relation to BW was seen for females.

Conclusions: Being born with a high BW, particularly very high BW, increases the risk for type 2 diabetes in young adult males, but not in females. The risk for obesity increases with increasing BW for both genders.

P1-d3-364 Programming/Epigenetics 1

Childhood adiposity is associated with maternal long chain polyunsaturated fatty acid status in late pregnancy

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Background: Studies in adults have shown an association between greater n-3 long chain polyunsaturated fatty acid (PUFA) status and lower risk of obesity. Maternal diet during pregnancy has been linked to offspring body composition. However, the specific nutrients and underlying mechanisms require characterisation and it is unclear whether maternal PUFA status during pregnancy influences offspring body composition.

Objective: To investigate the associations between maternal PUFA status during pregnancy and offspring body composition in childhood.

Methods: We evaluated the relationships between maternal plasma PUFA status (n-3 and n-6) in late pregnancy(34 weeks gestation) and proportionate body composition assessed by whole body DXA(Hologic Discovery) in their children at 4 and 6 years of age in a population-based prospective mother-offspring cohort. Linear regression methods were used to investigate these associations (yielding standardised regression coefficients).

Poster Presentations
Results: Complete data were available in 287 mother-child pairs. Whole body percentage fat mass (FM) and lean mass (LM) in children were differentially associated with maternal PUFA status. Maternal n-6 concentration was positively related to offspring percentage FM but negatively to percentage LM at 4 years (ß=0.13, p=0.025 & ß=0.13,p=0.023 respectively), with similar results at 6 years of age. There were less robust relationships between maternal total n-3 PUFA and offspring body composition. However, n-3 PUFA as a percentage of the total fatty acid pool was negatively related to percentage age FM and positively to percentage LM at 4 years (ß=0.17,p=0.003 & ß=0.17,p=0.003); associations at 6 years were in the same direction, but were not statistically significant.

Conclusions: Our results are consistent with adult studies demonstrating an association between PUFA status and obesity and suggest that maternal PUFA levels during pregnancy might influence offspring body composition. These findings may help inform nutritional strategies in pregnancy aimed at optimising offspring health.

P1-d3-365 Programming/Epigenetics 1
Early origins of the metabolic syndrome: role of small size at birth, early postnatal weight gain and adult IGF-I
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Background: The relationship between low birth weight and increased risk for Metabolic Syndrome (MetS) in later life has been frequently described. Factors involved in that association might be accelerated early weight gain and decreased IGF-I levels in adulthood as these factors have been associated with both small size at birth and components of the MetS.

Objective: To unravel mechanisms involved in the association between small size at birth and components of MetS in early adulthood.

Methods: In 280 young adults of the PROGRAM study, aged 18-24 yr, we investigated associations of birth weight and gain in weight for length during early life, and adult IGF-I SDS, with number of MetS components (ordinal regression analyses), prevalence of MetS components and MetS (logistic regression analyses), and other metabolic parameters (linear regression analyses). Revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of MetS. The other metabolic parameters were C-reactive protein (CRP), insulin sensitivity, trunk fat mass, total cholesterol, and LDL cholesterol.

Results: More gain in weight than length SDS in the first three months of life was significantly associated with an increased number of MetS components (Odds ratio: 1.34), prevalence of MetS (Adjusted odds ratio: 2.51, Table 1) prevalence of low HDLc (Odds ratio: 1.49), higher CRP levels (p-value=0.009) and lower insulin sensitivity (p-value=0.007) at the age of 21 years. Low birth weight SDS was associated with lower insulin sensitivity (p-value=0.036), but low birth weight SDS and adult IGF-I SDS were not significantly associated with any of the MetS components, or MetS prevalence at 21 years.

<table>
<thead>
<tr>
<th>Metabolic Syndrome</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>Birth weight SDS*</td>
<td>0.492</td>
<td>0.224-1.080</td>
</tr>
<tr>
<td>Delta weight SDS 0-3 mo#</td>
<td>2.509</td>
<td>1.200-5.247</td>
</tr>
<tr>
<td>Adult IGF-I SDS~</td>
<td>0.867</td>
<td>0.409-1.795</td>
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* Adjusted for sex, age, SES, gestational age.
# Birth length SDS, #Delta length SDS 0-3 mo, ~IGF-BP3 SDS

Conclusions: Our study demonstrates that higher gain in weight for length in the first three months of life is associated with a higher prevalence of MetS at 21 years, whereas low birth weight and low adult IGF-I are not.

P1-d3-366 Programming/Epigenetics 1
Elevated insulin concentrations at birth and at prepubescent age are associated with an altered BMI course during childhood – results of the Ulm birth cohort study (UBCS)
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Background: The natural course of BMI-values of a child shows an increase after birth and reaches a maximum at the age of one. Thereafter BMI-values decrease, reach a minimum at pre-school age and increase until young adulthood. The reversal point is called adiposity rebound.

Objective and hypotheses: The identification of factors which are associated with an altered BMI course is in the focus of current interest.

Methods: The Ulm Birth Cohort study is a prospective study with a longitudinal design. At baseline examination n=1066 mothers and their newborns were recruited for the study. The weight and height development of the children was documented from birth until the latest follow-up at age of 8 yrs. At baseline an umbilical cord blood sample was taken. At the 8-year follow-up a fasting blood sample of the children was taken. The concentrations of insulin in the umbilical cord blood sample and in the fasting blood sample of the children at the age of 8 yrs were measured (ELISA). Insulin concentrations were categorised in quartiles. Three groups of insulin concentrations were defined: elevated insulin concentration at birth and at the age of 8 (both within upper quartile); decreased insulin concentrations at birth and at the age of 8 (both within lower quartile), normal insulin concentrations (all the others).

Results: The BMI courses of the children of the three groups differ significantly (figure 1). Children with elevated insulin concentrations show a BMI increase after birth and reach a maximum at the age of one. Thereafter BMI-values decrease, reach a minimum at pre-school age and increase until young adulthood. The reversal point is called adiposity rebound.

Conclusions: The present data show that higher insulin concentrations in children from birth to 8 yrs of age are associated with an altered BMI course during childhood. Higher insulin levels might be programmed during the fetal period and may stimulate postnatal weight gain.
P1-d3-367 Puberty and Neuroendocrinology 1

24 months treatment experience of two leuprolide acetate 3 month depot formulations for children with central precocious puberty

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Background: We have recently demonstrated short term efficacy and safety of leuprolide acetate (LA) 3 month (M) depot 11.25 or 30 mg in children with central precocious puberty (CPP) treated for 6M (Lee PA, et al. JCEM, May 2012, 97(5).

Objective and hypotheses: To assess long term hypothalamic-pituitary-gonadal axis suppression and the safety profile of LA 3M depot 11.25 or 30 mg in children with CPP treated for 24M.

Methods: 72 children (baseline mean age 8.5±1.6 yrs, 65 females) with CPP treated with 11.25 (N=34) or 30 mg (N=38) 3M depot LA with continued LH suppression at 6M were followed for up to 24M of additional treatment. Peak stimulated LH <4 mU/mL was considered suppressed. Adverse events (AEs) data were collected.

Results: Peak stimulated LH was suppressed in 90.3% and 96.9% of subjects at 12M and in 87.5% and 100% of subjects at 24M in the 11.25 and 30 mg groups, respectively. In the 11.25 mg group, 5 subjects escaped during the 24M treatment; 3 of 5 subjects were suppressed at their subsequent visit; 2 of 5 were considered failures at the subsequent visits but had no evidence of progression in Tanner Staging. In the 30 mg group, 2 subjects escaped LH suppression, but were suppressed at the subsequent visit. Continued positive effects in suppressing pubertal progression and sex steroid levels and slowing bone age maturation were observed. The majority of subjects who discontinued treatment (n=43) were considered ready to progress through physiologic puberty. AEs were comparable between groups with injection site pain being the most common AE (26.5% in 11.25 mg and 23.7% in 30 mg).

Conclusions: The 2 doses of LA 3M depot are safe and provide persistent maintenance of LH suppression in children with CPP over 24 months of additional treatment.

P1-d3-368 Puberty and Neuroendocrinology 1

Utility of basal luteinizing hormone levels for detecting central precocious puberty in girls

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Objective: The hallmark of puberty is the progressive increase in gonadotropin-releasing hormone (GnRH) activity, reflected by an increase in the circulating concentration of luteinizing hormone (LH). The GnRH stimulation test has become widely used in the evaluation of precocious puberty. The aim of our study was to investigate whether the GnRH stimulation test could be simplified with fewer hormonal measurements.

Methods: A total of 1015 girls were referred to Ajou University Hospital for evaluation of precocious puberty between 2008 and 2011. All subjects underwent GnRH-stimulation tests as part of their evaluation. Serum LH and follicle stimulating hormone (FSH) were measured by immunoradiometric assay before and after the GnRH injection.

Results: Of the 1015 subjects, 663 (65.3) were included in the pubertal response group and 352 (34.7%) were in the prepubertal response group. Basal LH level was identified as a significant predictor for central precocious puberty (CPP). Based on the ROC curve, the optimal cut-off point of basal LH related to “pubertal responses” was 1.1 IU/L, which was associated with 72.1% sensitivity and 51.6% specificity, with an area under the ROC curve of 0.649 (95% confidence interval 0.614-0.683)

Conclusion: A single basal LH measurement provides adequate information to discriminate girls with CPP from prepubertal girls and is useful for initial laboratory screening test in most of the girls who were evaluated for early pubertal signs.

P1-d3-369 Puberty and Neuroendocrinology 1

Estrogen receptor alpha polymorphisms and idiopathic central precocious puberty in girls

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Objective: Estrogen plays a crucial role in the development and function of the reproductive physiology. Estrogens regulate cellular activity through binding to estrogen receptor α (ERα) and β (ERβ). ERα polymorphisms have been associated with age at menarche, menopause onset, and fertility. The aim of this study is to investigate the relationship of ERα gene polymorphisms with central precocious puberty (CPP) in girls.

Methods: Two hundred and four (201) Korean girls with idiopathic CPP were included in this study along with 100 healthy Korean female adults with pubertal maturation within normal age, who served controls. A uxoroidal and endocrine parameters were measured, and both patients and controls were genotyped for PvuII (397T→C) and XbaI (351A→G) polymorphisms of ERα gene.

Results: There was significantly lower incidence of the CC genotype of PvuII polymorphism among CPP girls than controls (11.9% vs. 22%, P=0.021). Pubertal onset was also later for carriers of CC genotype of PvuII polymorphism compared with carriers of TT genotype (7.28 ± 1.35 years vs. 7.57 ± 1.40 years, P=0.30). In addition, there was no significant difference in XbaI polymorphism between patients and controls.

Conclusion: The present study indicated that the PvuII polymorphism of ERα gene might function as a modulating factor in the onset and progression of puberty. However, no solid conclusion can be made and further studies are necessary to validate the function of these polymorphisms.

P1-d3-370 Puberty and Neuroendocrinology 1

Timing of puberty: reversal of congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations

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Objective: To assess long term hypothalamic-pituitary-gonadal axis suppression and the safety profile of LA 3M depot 11.25 or 30 mg in children with CPP treated for 24M.
Vitamin D and calcium levels are associated with glucose metabolism and pubertal timing in Danish children

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Background: Vitamin D (VD) is a key regulator of calcium homeostasis. Low VD levels have been associated with adverse effects on glucose metabolism, as well as with early pubertal timing. Whether or not low VD influences glucose metabolism and pubertal timing directly or through effects on calcium homeostasis is unresolved.

Objective and hypothesis: The objective was to evaluate associations of VD, parathyroid hormone (PTH) and albumin corrected calcium (Ca) levels with glucose metabolism and pubertal timing in healthy children and girls with central precocious puberty (CPP).

Methods: One-hundred and eighty healthy children (girls = 115) from the COPENHAGEN Puberty study, and 11 girls with CPP were evaluated cross-sectionally by whole-body DEXA-scan, oral glucose tolerance test (insulin sensitivity, ISI), peak insulin (PI) levels) and pubertal staging. Fasting blood samples were analyzed for VD, PTH, calcium as well as IGF-1 and reproductive hormone levels.

Results: Ca, but not VD, levels were positively associated with PI (p = 0.001), and negatively associated with ISI (p = 0.013) in healthy children. In healthy girls, logistic regression analyses revealed that earlier age at onset of breast development was associated with higher Ca (p = 0.037) and lower VD levels (p = 0.039), respectively. In healthy girls ≥ 12 years, significant higher levels of LH, FSH, IGF-1 and PI were found in the highest tertile compared with the lowest tertile for Ca (all p ≤ 0.04). No significant differences were found in any of the main outcome variables between VD tertiles. Girls with CPP had significantly lower ISI (p = 0.04) and higher Ca levels (p = 0.005) than puberty-matched healthy girls.

Conclusion: Calcium levels may influence glucose metabolism as well as pubertal timing in healthy children and girls with CPP. The possible role of calcitropic hormones on activation of the pituitary-gonadal axis needs further elucidation.

Cryptic chromosomal deletions detected by array-CGH in four cases affected by central precocious puberty and neurodevelopmental disorders

Mariangela Cisterino; Alexandrə Made; Francesca Marabotto; Giulia Rossetti; Laura Losa; Arianna Zaroli; Chiara Visconti

Background: Central precocious puberty (CPP) may be associated with CNS abnormalities including neurodevelopmental disorders (ND), epilepsy (E), CNS structural malformation(s) and/or with dysmorphic features. In the literature, some chromosomal aberrations have been reported in patients with this association.

Objective and hypotheses: The aim of this study was to detect cryptic chromosomal anomalies in patients affected by CPP and CNS disorders using the array-CGH technique. Methods: We carried on the array-CGH analysis in 4 girls affected by CPP associated with one or more of following CNS anomalies: ND, E and structural abnormalities detected with MRI. The age at the onset of CPP ranged from 4 to 7.3 years.

Results: Case 1. A de novo distal deletion of the chromosome 9 short arm [del(9p)24.3-p23] was found, as in the cases affected by 9p- syndrome. To our knowledge, this is the second reported case of precocious puberty associated to 9p distal deletion. Cases 2 and 3. A deletion of the chromosome 8 short arm was found: in the first case the deletion was localized in the region 8p23.2 and in the latter in the region 8p23.3.1. In both these cases the deletion determines the lost of the gene CSMD1, which is known to be particularly expressed in the ovary during the embryogenesis. Moreover, a duplication of CSMD1 has been found in a girl affected by delayed puberty [1]. Case 4. A deletion of chromosome 8p22 was detected, which is cited in the Database of Genomic Variants as a normal variant. However, this deletion may be related with the age at menarche in normal population.

Conclusions: Our observations confirm the usefulness of the array-CGH analysis for detecting cryptic chromosomal aberrations in patients with CPP associated with CNS abnormalities and/or dysmorphic features. Further studies are needed to identify genes responsible for this association.

51st Annual Meeting of the ESPE

Horm Res 2012;78(suppl 1) 117
**P1-d3-374 Puberty and Neuroendocrinology 1**

**Longer androgen receptor CAG repeat alleles are associated with body fat and serum SHBG in boys**

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**Background:** Longer androgen receptor gene CAG trinucleotide repeat alleles, AR-(CAG)n, are associated with lower sensitivity of the androgen receptor (AR). Shorter AR-(CAG)n have been associated with premature adrenarche in children and with subfertility in adult men. However, it is not known if the length of CAG trinucleotide repeats AR-(CAG)n is associated with age at pubertal onset and body fat accumulation in puberty in normal children.

**Objective and hypotheses:** The objective was to evaluate associations of the AR-(CAG)n polymorphism with the development of pubic hair, circulating levels of androgens, and body fat in healthy boys.

**Methods:** A longitudinal study of 78 healthy boys from the COPENHAGEN Puberty study was conducted with clinical examinations and blood samples every half year from 2006 to 2011. We genotyped AR-(CAG)n and measured reproductive hormones. The boys were divided in quartiles (Q1-Q4) according to the number of CAG repeats; Q1: <20 CAG repeats; Q2-Q3: 21-23 CAG repeats and Q4: > 24 CAG repeats.

**Results:** Median AR-(CAG)n was 22 (range 17-30). The number of repeats was positively correlated with sum of four skin folds at a comparable age (10 years, p=0.026) and at a similar pubertal stage (3 months prior to pubic hair development, p=0.037). The number of CAG repeats was negatively correlated with age at pubarche (p=0.054). The mean age of pubarche was lower in boys with longer CAG repeats (Q4) compared to boys with short CAG repeats (Q1) (p=0.051). The number of CAG repeats was inversely correlated with serum SHBG levels at comparable age (p=0.015) but no significant correlations with circulating levels of androgens (DHEAS, Adione or Testosterone) or free androgen index (FAI) were observed.

**Conclusions:** The AR-(CAG)n polymorphism was positively correlated with body fat and negatively with SHBG levels and age at pubarche in healthy boys.

**P1-d3-375 Puberty and Neuroendocrinology 1**

**High urinary phthalate concentration associated with delayed pubarche in girls**

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1University hospital of Copenhagen, Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark; 2University Hospital of Copenhagen, Rigshospitalet, Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark

**Background:** Phthalates are a group of chemicals present in numerous consumer products. They have anti-androgenic properties in experimental studies and are suspected to be involved in human male reproductive health problems. A few studies have shown associations between phthalate exposure and changes in pubertal timing among girls, although controversies exist.

**Objective and hypotheses:** The objective was to investigate the association between phthalate concentrations and pubertal timing in girls.

**Methods:** We determined the concentration of 12 phthalate metabolites in first morning urine samples from 725 healthy Danish girls (aged 5.6-19.1 years) in relation to age, pubertal development (breast and pubic hair stage) and reproductive hormone levels (luteinizing hormone, estradiol and testosterone).

**Results:** Development, p=0.037). The number of CAG repeats was negatively correlated with age at pubarche (p=0.054). The mean age of pubarche was lower in boys with longer CAG repeats (Q4) compared to boys with short CAG repeats (Q1) (p=0.051). The number of CAG repeats was inversely correlated with serum SHBG levels at comparable age (p=0.015) but no significant correlations with circulating levels of androgens (DHEAS, Adione or Testosterone) or free androgen index (FAI) were observed.

**Conclusions:** The AR-(CAG)n polymorphism was positively correlated with body fat and negatively with SHBG levels and age at pubarche in healthy boys.

**P1-d3-377 Puberty and Neuroendocrinology 1**

**Kiss-1 and ERα mRNA expression and puberty onset in the female SD rats with disrupted positive feedback in HPG axis**

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**Background:** The causes of normal and precocious puberty are still unclear. We hypothesized that the positive feedback mediated by ERα-kiss-1 pathway in HPG axis is required for normal puberty and precocious puberty induced by estrogen agonists. To test this hypothesis, we determine 1. the effect of neonatal exposure to the ERα specific agonist PPT and the ERβ specific agonist DPN on puberty onset in female rats. 2. the effect of disrupted positive feedback in HPG axis on vaginal opening (OV) and mRNA expression of kiss-1 and ERα in hypothalamic in the female rats.

**Methods:** Group1 were randomly grouped into 3 subgroups and treated with PPT, DPN and placebo, respectively, between p.n. days 16 and 20. Group2, treated with testosterone enanthate sc injected on day 2 and then treated with methods same to group1. OV were observed from p.n. day 21 till the day of
In group2, in which the positive feedback in HPG axes were disrupted, no OV were observed till p.n. day 49, and neither PPT nor DPN could induce earlier OV in them. 2. mRNA expression of kiss-1 and ERα in hypothalamus of female rats with normal or disrupted positive feedback in HPG axes (Tab2). The mRNA expression of kiss-1 and ERα in hypothalamus of female SD rats with Normal or Disrupted positive feedback in HPG axes * p<0.01, vs control.

<table>
<thead>
<tr>
<th>Normal positive feedback</th>
<th>Disrupted positive feedback</th>
</tr>
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<tbody>
<tr>
<td>group</td>
<td>Kiss-1 mRNA</td>
</tr>
<tr>
<td>PPT</td>
<td>2.08 ± 0.37* (n=5)</td>
</tr>
<tr>
<td>DPN</td>
<td>1.43 ± 0.33* (n=5)</td>
</tr>
<tr>
<td>Control</td>
<td>0.60 ± 0.32 (n=5)</td>
</tr>
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Results: We hypothesized that constitutional delay of growth and puberty (CDGP) might be a mild phenotype of IHH and investigated the occurrence of defects in major causative IHH genes in patients with CDGP. Methods: Fifty six Brazilian patients (45 males) with CDGP were selected. Genomic DNA was extracted and the whole coding sequences of GnRHR, TACR3, TAC3, KiSS1R, FGFR1 and FGFR8 was analyzed. The control group consisted of 150 adults with normal pubertal development.

Results: We identified a novel variant c.1345G>T (p.A449S) in TACR3 in heterozygosis in a female patient with CDGP. Although the p.A449S variant was not observed in the control individuals, this amino-acid exchange involves a non-conserved residue and was predicted to be benign by in silico analysis using SIFT and PolyPhen. The recurrent c.137A>G (p.Q106R) in FGFR1 mutation was identified in heterozygosis in a male CDGP patient. This mutation has been previously associated with a partial GnRH receptor loss-of-function in patients with autosomal recessive IHH, but its role in CDGP is probably worthless. We also observed two new synonymous variations, one in FGFR1 c.1332A>G (p.P444P) and one in KiSS1R c.183G>A (p.S61S), both with no apparent effects on gene function. Known polymorphisms in TACR3 (rs2276973, rs17033889, rs5085919) and KiSS1R (rs73507527, rs350132, rs3746147 and rs10407968) occurred in a similar frequency in CDGP and control groups. No genetic abnormalities were found in TAC3 and FGFR8 in these CDGP patients.

Conclusion: Testosterone administration to female rats during the critical period may postpone sexual maturation as revealed by delayed OV. PPT, DPN may inhibit kiss-1 and ERα mRNA expression in hypothalamus in the female rats with disrupted positive feedback in HPG axes. PPT or DPN can induce earlier puberty onset in female rats through positive feedback mediated by ERα-kiss-1 pathway in HPG axes.

P1-d3-379 Puberty and Neuroendocrinology 1
Creating a European consortium to study GnRH deficiency (C.O.S.T. Action BM1105)
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Background: GnRH deficiency is characterized by absent puberty, infertility, and psychosocial morbidity. It is a treatable cause of infertility yet patients are often challenged to find appropriate medical expertise given the rarity of their condition (1/10’000). This disorder has a strong genetic component with 16 disease genes discovered via several approaches including cytogenetics (chromosomal aberrations), homoygosity mapping in consanguineous families, and candidate genes. To date, the pace of discovery has been piecemeal and the majority (2/3) of GnRH deficient patients have no known genetic cause.

Objective: To develop a European registry for patients with GnRH deficiency and create a network bringing together clinicians, translational investigators, basic scientists, bioinformaticians, geneticists, and genetic counselors to foster discovery in the field of human reproduction.

Methods: The Clinical Group will develop a web-based database for patient phenotypes and clinical guidelines; the Genetics & Bioinformatics Group will assist in using cutting edge genetic technologies and provide expertise in interpreting data; the Basic Research Group will help prioritize candidate genes identified via whole exome sequencing and explore the biology of novel genes using animal and cellular models; the Education and Training Group will coordinate training program for young investigators.

Results: Twenty European countries have joined the C.O.S.T. Action (funded 2012-2015) and it is still open for interested parties to join at http://www.cost-esf.org/domains_actions/bmbs/Actions/BM1105

Conclusions: We believe this European Consortium will help accelerate scientific discovery in the field of GnRH deficiency yielding important scientific insights including novel biomarkers and targeted therapies for infertility that will benefit patients and families.

P1-d3-380 Puberty and Neuroendocrinology 1
FGFR1 mutations in split hand/foot malformation with or without gonadotropin deficiency
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Background: The limb anomaly “split hand/foot malformation” (SHFM) is a malformation of the extremities that can be isolated or associated with other developmental abnormalities including cleft lip and palate, ectodermal dysplasia in EEC syndrome and in few cases with isolated gonadotropin deficiency with or without anosmia. Several loci are described in the isolated form of SHFM. Known causes of syndromic SHFM include TP63 mutations. SHFM associated with at least cleft palate, arhinencephaly or isolated go

Objective and hypotheses: During this work, we tested the hypothesis that SHFM associated with at least cleft palate, arhinencephaly or isolated gonadotropin deficiency may be due to FGFR1 mutations.

Methods: For this, we sequenced FGFR1 exons from DNA extracted from

blood lymphocytes of 13 patients having this phenotype without TP63 muta-
tion.

Results: 7 heterozygous missense mutations of FGFR1 were found in 7 pa-
tients. These mutations are located in the tyrosine kinase domain but also in the extracellular domain of FGFR1. One mutation occurred de novo. The family analysis for six other mutations showed a highly variable expressivity and incomplete penetrance of a phenotype ranging from normal to Kallmann syndrome with severe gonadotropin deficiency. MRI focused on the olfactory bulbs has not revealed agenesis of the olfactory bulbs in 3 cases.

Conclusions: FGFR1 must thus be tested in patients presenting at birth an
EEC syndrome or SHFM associated with a cleft palate and/or an arhinen-
cephaly and an isolated gonadotropin deficiency. This work shows the im-
portance of FGFR1 in the limbs development as it was already known for
FGFR2 and FGFR3.

P1-d3-381 Puberty and Neuroendocrinology 1
Response to GnRH agonist challenge is the
favorable tool to diagnose hypogonadotropic
hypogonadism in boys with delayed puberty
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Germany

Background: Diagnosis of idiopathic hypogonadotropic hypogonadism (IHH) in males with delayed puberty can be difficult. An exact distinction is important for counseling, treatment, and future fertility.

Objective and hypotheses: We prospectively assessed the accuracy of the GnRH agonist (triptorelin acetate) challenge in comparison to inhibin B (IHB) for diagnosing IHH. GnRH agonist challenge examines the integ-

rity of the pituitary gland and the testicles. INHB is a marker for Sertoli cell activity.

Methods: 37 prepubertal males, testicular volume ≤4 ml, age ranges between 13.6 and 16.5 years. 28 males spontaneously reached a testicular volume ≥8 ml (constitutional delayed puberty), 9 males did not during an 18 months follow-up (IHH). A GnRH agonist test was carried out in all patients at initial presentation. INHB was measured by ELISA (Beckman Coulter, Inc., USA). LH basal (LH1) and LH after 4 hours (LH2) were measured by CLIA (Sie-
mens Health Care Systems, Germany).

Results: ROC plot analysis of the area under the curve (AUC) for diagnosing
IHH was greater for LH2 (100%) followed by INHB (98%; 95%-CI:94%-100%) and LH1 (95%; 95%-CI:98%-100%). A LH2 concentration <6.3 IU/l had a sensitivity and specificity of 100%. For an INHB concentration <114.0 pg/ml the sensitivity was 100% and the specificity was 89.3%. For LH1 <9.2 IU/l sensitivity was 88.9%, specificity was 89.3%. The combination of an
INHB concentration <120 pg/ml and LH1 ≥9.2 IU/l resulted in a sensitivity of 88.9% and a specificity of 96.4%.

Conclusions: LH response 4 hours after triptorelin acetate (LH2) has an excel-
lent sensitivity and specificity to diagnose IHH. INHB has a strong sensitivity but limited specificity. We recommend the GnRH agonist challenge to dia-
gnose IHH in males with delayed puberty.

P1-d3-382 Puberty and Neuroendocrinology 1
Evaluation of gonadal function in 51
adolescents with history of orchiopexy
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Background: Despite timely and successful interventions, undescended tes-
ticle is still reported as a major cause of male infertility.

Objective and hypotheses: We evaluated the gonadial function of 51 adoles-
cents between December 2010 and July 2011, who had history of orchiopexy
due to undescended testes (35 unilateral and 16 bilateral).

Methods: The etiology of undescended testes, location of the testes prior to
orchiopexy, history of hCG use, age at orchiopexy, serum LH, FSH, total
testosterone (T), AMH, inhibin B levels and scrotal ultrasonography were
analyzed.

Results: The testes had an inguinal location in 49 (73%) of 67 undescended
testes. hCG treatment had been applied on 16 (%31) boys. Age at orchiopexy
was 4.3± 2.6 yr (range: 0.6-10 yr). Age at orchiopexy and at current evaluation
were similar between boys with unilateral and bilateral undescended testes. Serum LH, FSH, T, AMH and inhibin B levels were not significantly differ-
ent between boys with unilateral and bilateral undescended testes and they
were independent of age at orchiopexy . The testicile volumes had a positive correlation with serum LH and T levels, inverse correlation with serum AMH
levels. AMH levels positively correlated with inhibin B levels, negatively cor-
related with FSH levels. AMH levels did not correlate with T levels. Serum
inhibin B levels negatively correlated with FSH levels, and had no correlation
with any of the other parameters tested. When all the boys in the study were
evaluated, high FSH, reduced AMH and inhibin B were seen in 17.6%, 9.8 %
and 51% of the boys, respectively. Scrotal USG revealed 5 boys with testicu-
lar microtheliosis and 6 boys with varicocele.

Conclusions: Our study is in line with the previous reports that Sertoli cell
function is significantly affected in adolescent boys with undescended testes,
and serum inhibin B levels provide the best determinant of Sertoli cell func-
tion.

P1-d3-383 Puberty and Neuroendocrinology 1
Plasma kisspeptin levels in girls with
premature thelarche
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1Izmir University, Pediatric Endocrinology, Malatya, Turkey; 2Firat
University, Biochemistry Department, Elazığ, Turkey

Background: Premature thelarche (PT) is defined as isolated breast develop-
ment without secondary sex characteristics in girls below the age of eight. The mechanisms are not fully understood.

Objective and hypotheses: We aim to determine whether the level of kis-
speptin, which play a role in the release of gonadotropins, is associated with
PT.

Methods: The patient group included the children with PT aged 3-7 years
(n=20) and the control group included healthy children of the same age group
(n=20). In the patient and control groups, basal follicle-stimulating hormone
(FSH), luteinizing hormone (LH), estradiol (E2), prolactine (PRL), and sex
hormone binding globulin (SHBG) levels were measured using ICMA. Go-
adotropine-releasing hormone (GnRH) test was applied to the patient group,
and the peak levels of FSH and LH were determined. Kisspeptin levels were
measured using ELISA.

Results: While the plasma basal FSH, LH and E2 levels of the patient and
control groups were not significantly different, the PRL level was higher in
the patient group (p<0.05). In the patient group, kisspeptin levels were sig-
nificantly higher compared to the levels of the control group (2.96±1.21ng/dl vs.
1.19±0.41ng/dl; p<0.05), and kisspeptin levels showed a significant correla-
tion with PRL level (p<0.05).

Conclusions: These results suggest that PT may be related to a premature
increase of kisspeptin leading to premature activation of the HPG axis or via
kisspeptin-induced increase of PRL.

Poster Presentations
**P1-d3-384** Sex Differentiation 1

**Structural analysis of androgen receptor mutant proteins**

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1Universidade Estadual de Campinas - UNICAMP, Centro de Biologia Molecular e Engenharia Genética - CBMEG, Campinas, Brazil; 2Universidade Estadual de Campinas - UNICAMP, Departamento de Genética Médica, Faculdade de Ciências Médicas, Campinas, Brazil; 3Universidade Estadual de Campinas - UNICAMP, Departamento de Pediatría, Faculdade de Ciências Médicas, Campinas, Brazil; 4Universidade de São Paulo - USP, Unidade de Endocrinologia do Desenvolvimento, LIM42, Endocrinologia, Hospital das Clínicas, São Paulo, Brazil; 5Universidade Estadual de Campinas, Centro de Biologia Molecular e Engenharia Genética - CBMEG, Campinas, Brazil.

**Background:** The androgen insensitivity syndrome (AIS) is the most frequent cause of sex development disorders in 46,XY patients. Molecular analyses of androgen receptor (AR) gene including structural and functional studies of AR protein may confirm the clinical diagnosis.

**Objective and hypotheses:** The purpose of this research was to analyze structural modifications upon AR protein caused by four different mutations identified in Brazilian patients. All mutations were located in the AR ligand-binding domain. The mutations were: p.P694S, p.L768V, p.I898F and p.P904R.

**Methods:** A modeled protein for each mutant AR was built using the resolved 3D structure of human AR in complex with dihydrotestosterone (PDB-ID 2AM9) as template. Molecular modeling was performed using MODELLER webserver program. The modeled images were examined and edited by PyMOL®program and Millennium STING (CNPTIA Embrapa, Brazil).

**Results:** The mutation p.P694S was identified in a patient with mild AIS (MAIS). Structural analysis did not show any discrepancy in the internal contacts for wild type and mutant residues indicating no detectable structural changes. The mutation p.L768V was found in patients with partial AIS (PAIS). The structural analysis revealed that residue 898 has lost ten hydrophobic interactions and maintained three hydrogen bonds in the mutant protein. The mutations p.L768V and p.P904R were associated with complete AIS (CAIS). The number of internal contacts for mutant residue V768 increased from 9 to 15 and the number of hydrophobic interactions increased from 12 to 56. The number of internal contacts for wild type and mutant residues indicating no detectable structural modifications upon AR protein caused by four different mutations identified in Brazilian patients. All mutations were located in the AR ligand-binding domain. The mutations were: p.P694S, p.L768V, p.I898F and p.P904R.

**Conclusions:** This study demonstrated that the structural analysis of AR mutant proteins could be used to correlate genotypes to phenotypes associated with PAIS, CAIS or MAIS. However, each mutation described here will be further investigated for functional abnormalities.

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**P1-d3-385** Sex Differentiation 1

**Testis transcriptome analysis in cryptorchid boys: a new insight on the pathogenesis of azoospermia**

Faruk Hazdisselimovic; Nils Omar Hadzisselimovic; Philippe Demougin; Eduard Oakeley; 1Kindertagesklinik Liestal, Pediatrics, Liestal, Switzerland; 2Biocentrum Basel, Molecular Genetics, Basel, Switzerland; 3Biocentrum Basel, Molecular Biology, Basel, Switzerland; 4Novartis institutes for biomedical research, Molecular Biology, Basel, Switzerland.

**Background:** Despite timely and successful surgery, 32% of patients with bilateral and 10% with unilateral cryptorchidism will develop azoospermia. Cryptorchid boys at risk of azoospermia (HAZR) display a typical testicular histology; impaired transformation of gonocytes into Ad spermatogonia, reduced number of Sertoli cells and severe Leydig cell atrophy indicating a general testicular developmental delay. Aim: This study aimed to analyze data on whole genome expression signatures of undescended testes at risk of developing azoospermia.

**Patients and methods:** Whole genome expression analysis was performed using Affymetrix method at twenty-three testicular biopsies from 22 boys were analyzed (19 testes from 18 boys with cryptorchidism).

**Results:** Expression profiling identified 483 genes not or under-expressed in the azoospermia risk group compared with the low risk for azoospermia group. Annotated loci were associated with: spermatogenesis, EGR4, DDX4, Leydig cell; IGRF1 and Sertoli cell; RHOXF1, RHOX2, NRG1, ETV5 (Sertoli cell). EGR4, which is involved in regulating the secretion of luteinizing hormone, was virtually not expressed. IGRF1, RHOX1, RHOX2, DDX4 are LIH while NRG1 FSH dependent gene. Finally, decreases expression of developmental genes FGF9 and FGF22 was also observed in the HAZR group. Conclusion: In the HAZR group we observed impaired expression of gonadotropin dependent genes encoding transcription factors that have different roles in embryogenesis and development. Multiple differences in gene expression between the HAZR and LAZR groups, confirm the importance of an intact hypothalamic-pituitary testicular axis for fertility.
Mutations in the NR5A1 gene in patients with 46,XY disorders of sex development (DSD): high frequency of familial multi-generational occurrence

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Background: Nuclear receptor SF1/NR5A1 regulates the transcription of genes involved in reproduction, steroidogenesis and male sexual differentiation. Mutations in humans cause gonadal dysgenesis with/without adrenal failure in both 46,XY and 46,XX individuals.

Method: In a cohort of patients with familial 46,XY DSD, we identified 6 heterozygous NR5A1 mutations in 19 subjects from 5 unrelated families. We also identified a de novo heterozygous mutation in one patient with 46,XY DSD and no affected relatives.

Results: Low ovarian reserve with preserved fertility was detected in affected females. Extreme within-family variability was found in 46,XY affected patients, with phenotypes ranging from severe fetal undervirilization, prompting female sex of rearing to spontaneous pubertal development and even preserved fertility. Mutational analysis revealed in the first family a W279X heterozygous mutation and an intronic deletion (g3314-3317delCTTC) (IVS 4+8), and in the second a Y183X heterozygous mutation. These mutations had been previously reported. A novel R313H heterozygous variation was found in the third family, and a novel S303R was found in the fourth. A novel heterozygous R69H mutation was found in the only patient studied (46,XY DSD) from the last family, and a G77e de novo mutation in the sporadic case. All new mutations were predicted to affect protein function by prediction models (SIFT, Polyphen and MutationTaster).

Conclusions: We emphasize the extreme phenotypic variability that can be observed even in siblings with the same mutation. As previously reported, we found spontaneous puberty in 46,XY individuals raised as males, and for the first time we report preserved fertility in one of these affected individuals. Individuals with heterozygous NR5A1 mutations and mild phenotypes, such as isolated hypospadias in 46, XY patients, compensated ovarian dysfunction and early menopause in 46,XX subjects, might easily go undetected. A careful family screening of 46,XY as well as 46,XX individuals is recommended whenever an index case is detected.
quency of 7.5-15% in European patients (Köhler et al, 2009, Allali 2011). Thus, NR5A1 mutations are a frequent cause of XY DSD with hypospadias also in Egyptians. NR5A1 analysis should be considered in XY DSD with hypospadias. Early cryopreservation of sperms should be initiated as there is a risk of early gonadal failure.

P1-d3-390 Sex Differentiation 1
Gonadal dysgenesis and atypical expression of OCT-3/4 in testis of prepubertal patients with complete or partial androgen insensitivity syndrome
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Background: In the prepubertal (PP) human testis (TES), the androgen receptor (AR) is expressed in peritubular cells (PC) and interstitial cells (IC), including Leydig cells (LC) but not in Sertoli cells (SC). It has been reported that adult patients with complete androgen insensitivity (CAIS) have dysgenetic TES without differentiated germ cells (GC). The transcription factor OCT-3/4, specific of embryonic stem cells, is found in carcinoma in situ (CIS) and in gonadoblastoma cells.

Objective and hypotheses: To study OCT-3/4 expression in TES of PP patients with complete or partial (PAIS) androgen insensitivity as marker of malignancy risk.

Population and methods: We have studied 9 PP patients (CAIS: n=7; PAIS: n=2) gonadotropinized between 0.25 and 6.3 years old. In 5/9 subjects, diagnosis was supported by AR gene analysis (MT49V, R631X, E603X, L621P mutations and the del1550-1569ex1 deletion), while in 4 subjects by clinical hormonal data (including absence of SHBG response to testosterone stimulus, Belgorosy A JECM 1987). Histology, OCT-3/4, AR and ERu immunoexpression (n=9) and the testosteron secretion in primary cell culture (n=3) was studied. PP TES without endocrine pathology was used as control (C, n=10).

Results: Signs of testicular dysgenesis were found in 7/9 testes; CIS and/or OCT-3/4 positive expression in 5/9 samples; hyperplasia of LC in 4/9 testes. Positive AR LC immunoexpression was found in 3/9 samples but with atypical cytoplasm localization, different to C testis (nuclear). Expression of ERu in LC was positive, different from C (ERu negative). Testosterone secretion from cultured testes in vitro confirmed the presence of steroidogenic cells, although no response to hCG was found in patient cell cultures, different from C.

Conclusions: OCT-3/4, a marker of risk of gonadoblastoma, was frequently found in testis of PP patients with CAIS and PAIS. These results suggest that a testicular biopsy is recommended before postponing gonadectomy to adulthood.

P1-d3-391 Sex Differentiation 1
Gonadal histology in patients with complete androgen insensitivity syndrome (CAIS): influence of age, gonadal location and residual androgen receptor activity
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1Charles University, 2nd Faculty of Medicine, Department of Pediatrics, Prague, Czech Republic; 2Erasmus MC, Daniel den Hoed Cancer Center, Josephine Nefkens Institute, Department of Pathology, Rotterdam, Netherlands; 3Erasmus MC, Department of Clinical Genetics, Rotterdam, New Caledonia; 4University Hospital Ghen, Department of Pediatrics, Dino van Derkerk, Belgium; 5Erasmus MC – Sophia Children's Hospital, Department of Pediatric Urology, Rotterdam, Netherlands; 6Erasmus MC – Sophia Children's Hospital, Department of Pediatric Endocrinology, Rotterdam, Netherlands; 7Charles University, 2nd Faculty of Medicine, University Hospital Motol, Department of Pediatrics, Prague, Czech Republic

Background: Typical changes in gonadal histology of patients with CAIS have been described. These might develop primarily with age, and be influenced also by other factors such as gonadal location and residual androgen receptor (AR) activity.

Objective and hypotheses: To assess the influence of age, gonadal location, and residual AR activity on gonadal histology in patients with CAIS.

Methods: In total 39 gonads from 20 patients were available for assessment. Age at gonadectomy ranged from 3 months to 18.5 years. Twelve patients lacked any AR activity. Eight patients had some residual AR activity. Histological abnormalities were assessed on HE stained slides. Immunohistochemical detection of OCT3/4 and KITLG (malignant germ cell tumor markers) was done on 38 gonads.

Results: The total number of tubules containing germ cells declined with age. AR activity was an independent predictor for germ cell survival (p<0.001). OCT 3/4 positive germ cells were present in 13/38 (34.2%) gonads; KITLG was detected in matched areas in 6 of those (46.2%). However, neither OCT3/4 nor KITLG positivity was found to be dependent on age, residual AR activity or gonadal location. Tubular atrophy, Leydig cell hyperplasia, and Sertoli cell (SC) adenomas and interstitial fibrosis were dependent on age (p<0.001), i.e., predominantly found in pubertal or postpubertal gonads. Tubular atrophy and lymphatic enlargement were significantly more prevalent in patients without AR activity (p<0.01 and p<0.05, respectively). Hyalin deposits, SC nodules and eosinophilic changes of SC cytoplasm did not show any dependence on studied factors.

Conclusions: Survival of germ cells in general is dependent on age and AR activity. However, survival of pre-malignant germ cells is not. Most of the abnormal features develop with rising age. AR residual activity seems to have an impact only on some of these features. Gonadal location is probably not an independent predictor for observed changes.

P1-d3-392 Sex Differentiation 1
A CYBSA gene mutation explains the last unsolved case of our cohort of 30 patients with a diagnosis of 17α-hydroxylase/17,20-lyase deficiency
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Background: Combined 17α-hydroxylase/17,20-lyase deficiency is a rare, well defined disease, due to mutations of CYP17A1. Isolated 17,20-lyase deficiency is rarer, difficult to diagnose clinically and recent studies have shown that at least 3 genes in addition to CYP17A1 could be responsible for this disorder.

Objective and hypotheses: Using hormonal and sequencing data of our pa-
tients, we tried to precise the clinical, biological and genetic profiles associated to the different forms of this disorder.

Methods: We studied 30 patients from 26 families. The hormonal profile was first established in details, and then the candidate genes, CYP17A1, POR, AKR1C2 and CYB5A were sequenced in 5 patients. In the remaining patients, hormonal data were evocative of isolated 17,20-lyase deficiency. In 3 patients the p.R358Q mutation known to cause this isolated disorder was identified. One patient was homozgyous, the others being compound heterozygous, with the second mutation causing a complete combined deficiency. POR was then sequenced in the remaining patients, allowing us to identify mutations in 3 of them. We failed to found any mutation (or large deletion) in CYP17A1, POR and AKR1C2 in the last patient, but identified a homozgyous mutation, c.94delC, in CYB5A, finally solving this case.

Conclusions: Our study adds 7 cases to the few described cases of isolated 17,20-lyase deficiency and confirms that this disorder could be due to at least 3 different genes. Our detailed hormonal data show that ACTH test can distinguish POR deficiency from CYP17A1 or CYB5A deficiency: high progesterone (> 80 nm), pattern of non classical 21-OHD, and no stimulation of cortisol. At this time, with the exception of the dosage of methemoglobin, clear clinical or hormonal criteria differentiating cases due to CYP17A1 or CYB5A mutations remain to be defined.

P1-d3-394 Sex Differentiation 1
Variable phenotype of 46,XY DSD in three brothers with a novel mutation in NR5A1 gene
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Background: The NR5A1 gene (9q33.3) encodes a nuclear receptor that regulates many aspects of adrenal and reproductive development and function. Mutations in this gene can be associated with a wide range of reproductive phenotypes, including 46,XY Disorders of Sex Development (DSD), premature ovarian failure and spermatogenic failure. Heterozygous changes in NR5A1 can be inherited from a fertile mother in a sex-limited dominant fashion, thus mimicking an X-linked disorder, like partial androgen insensitivity.

Objective and hypotheses: To report three sibs with sex ambiguity due to a novel NR5A1 mutation.

Methods: The three 46,XY sibs, all raised as boys, were born to healthy non-consanguous parents and were first evaluated as newborns. The index case, now aged 12, had a single perineal opening and gonads were palpable in the labioscrotal folds. Spontaneous puberty began at the age of 11 years with high FSH and normal LH and testosterone levels. His 5-year-old brother had penoscrotal hypospadias and the gonads were palpable in the labioscrotal folds. In the first months of life he had high levels of LH and normal FSH and testosterone levels. An hCG stimulation test was negative. The younger brother, now 4 years old, had a microphallus and posterior labioscrotal fusion, and gonads were in the inguinal region. In the first months of life he had normal levels of FSH, LH and testosterone, and a positive response to hCG testing.

Results: No mutations were found in androgen receptor and SRD5A2 gene. Molecular analysis of NR5A1 in the three sibs revealed a p.C65Y mutation in exon 3, which was not previously described.

Conclusions: The recent focus on the molecular analysis of NR5A1 has solved many cases of 46,XY DSD labeled as idiopathic. This family adds evidence that mutations in this gene lead to variable genital and hormonal features.
Conclusions: Elevated TSH levels are associated with low birth-weight, both in infants with hyperthyrotropinemia and in neonates with normal thyroid function. A rapid recovery rate is expected in most cases. Hyperthyrotropinemia is apparently not stress related.

P1-d3-396 Thyroid 1
Detection of genetic abnormalities in children with congenital hypothyroidism using MLPA analysis
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Background: Thyroid dysgenesis (TD) is the most common cause of congenital hypothyroidism (CH). Among genetic factors that may contribute to TD etiopathogenesis, the highest importance is ascribed to mutations of transcription factors and TSHR-encoding genes.

Objective and hypotheses: Assessment of incidence of changes involving genes encoding PAX8, FOXE1, NKX2-1, TSHR and TPO detected by multiplex ligation-dependent probe amplification (MLPA) in CH and TD children.

Methods: Investigations included 46 children selected in mass screening tests between 1995 and 2009, in whom CH resulted from TD: ectopy (n=17), agenesis (n=22), hypoplasia (n=7). DNA was isolated from peripheral blood samples using Master Pure DNA Purification Kit (Epicentre Biotechnologies), 50-100 ng of genomic DNA was employed in MLPA analysis using a SALSA MLPA kit P319-A1 THYROID (MRC-Holland). Genetic changes were analyzed within selected fragments of the PAX8, FOXE1, NKX2-1, TSHR and TPO genes. Testing followed a standardized procedure provided by the manufacturer. MLPA analysis included: DNA denaturation and MLPA probe hybridization, ligation, PCR reaction, separation of reaction products by capillary electrophoresis and data analysis.

Results: Four types of heterozygous deletions in probe hybridization regions were identified for the following genes: PAX8, FOXE1, NKX2-1, TSHR and TPO in exon 2, FOXE1 in exon 1 and TPO in exon 16. Genetic abnormalities of selected gene fragments were identified in 6/46 subjects.

Conclusions: MLPA screening showed genetic abnormalities in 13% of CH and TD children, manifested as deletion at probe hybridization site. Precise determination of the character of such abnormalities and genotype-phenotype correlation requires extending the study of selected regions to include other molecular methods (Sanger sequencing). Table 1. Genetic abnormalities depending on TD type

P1-d3-397 Thyroid 1
Iodine deficiency in pregnant women living in the capital city of Turkey, who appear to be iodine-sufficient
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Background: Previous studies about current iodine status in Turkey have yielded contradictory results. Although urinary iodine concentration (UIC) in school-age children suggests sufficient iodine status, studies on neonatal TSH indicate that iodine deficiency (ID) is still a continuing problem.

Objective and hypotheses: We aimed to assess the iodine nutritional status of pregnant women living in the capital city of Turkey that appears to be sufficient in earlier studies. We hypothesized that if ID is an ongoing problem, pregnant women’s iodine intake is insufficient.

Methods: This was a hospital-based, non-interventional, prospective cross-sectional study. A total of 162 pregnant women at second trimester were examined regarding iodized salt use, UIC, goiter rate and thyroid functions. Goiter status was determined by palpation. UIC was measured using colorimetric method based on Sandell-Kolhoff reaction. Serum levels of thyroid hormones and TSH were measured by chemiluminescence immunoassays.

Results: While the proportion of iodized salt use was 80.2%, most women (72.8%) had an UIC below 150 μg/l. The median UIC was 80.5 (8.9–340.3) μg/l, indicating insufficient iodine intake. Total goiter rate was 15.4%. Preferential T3 secretion and relative hypothyroxinemia reflected by elevated molar ratio of FT3/FT4 were present in 89.5% of the women. About 12% had subclinical hypothyroidism or isolated hypothyroxinemia. These thyroid abnormalities supported that the pregnant women were greatly affected by ID.

Conclusions: Our study reveals that ID is a serious problem among pregnant women living in the capital city appearing iodine sufficient. These data confirm that iodine nutritional status in school-age children does not reflect the iodine supply of pregnant women. Nationwide surveillance studies should urgently performed for assessment and monitoring the iodine status of pregnant women directly. The Ministry of Health should provide iodine supplementation to pregnant women to protect them and their offspring against adverse consequences of ID.

P1-d3-398 Thyroid 1
Central regulation of TSH and mutations of the TSH receptor in children with subclinical hypothyroidism
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Background: The diagnosis of subclinical hypothyroidism (SH) during childhood is still controversial. Mutations in the TSH receptor (TSHR) gene (in exons 1,2,4,6 and 10) have been reported in children with SH. Loss-of-function mutations of the TSHR gene have been described in families with TSH resistance. Functional studies of the P68S mutation in exon 2 show loss of function of the TSHR gene.

Objective and hypotheses: To investigate the prevalence of exon 2 TSHR gene mutations in children with SH in relation to the central regulation of TSH secretion.

Methods: SH was diagnosed in 60 children with TSH > 5.0 mIU/ml (P) and low normal concentrations of T-4, FT-4 and T3. A 60 minute TRH test was performed on all patients using 7 mcg/kg TRH (max: 200 mcg) iv. Genomic DNA was isolated by standard methods from peripheral blood of the 60 P and 25 controls (C). Presence of the P68S mutation of the TSHR gene was confirmed by electrophoretic analysis of PCR products after restriction endonuclease digestion with XmnI using 2U of enzyme and 10 ll of PCR product.

Results: The P68S mutation of the TSHR gene was found in 10 P (16 % of the total P) with SH but not in any of the controls. Of the P with the P68S mutation, 5P had abnormal TRH-test results, 2 had normal TRH-test results, and 3 were relatives of patients with abnormal TRH tests without the P68S mutation. The range of pre-treatment TSH and FT-4 concentrations of the 10 P with the P68S mutation were: 3P with abnormal TRH tests: 3.9 – 5.0 mIU/ml and 0.9 – 1.48 ng/dl, respectively and in the 2P with normal TRH tests: 3.1 – 4.34 mIU/ml and 1.1 – 1.5 ng/dl, respectively. Of the 10P, 4P presented with goiter, 2P with premature adrenarche and 1P with polycystic ovarian disease which all resolved after thyroxine therapy.

Conclusions: The P68S mutation of the TSHR gene and TSH resistance is not rare in children with SH although other TSHR gene mutations may also be present. Thyroxine therapy in these children seems to be beneficial.
Obstetric outcome in CH adult females

Methods: Gynecologic history was evaluated in a semistructured questionnaire carried out by expert clinicians. The results were compared with data derived from national register of births. 155 girls with permanent CH between 20 and 30 years of age, born between 1978 and 1994, in 4 Italian regions, were recalled. 69 (44.6%) subjects (mean age 21.5 ± 4.4 yrs; 25 [36.6%] athyreosis, 32 ectopic gland [47%], 12 [17%] in situ gland) agreed to participate our study.

Results: 9 pts reported 10 pregnancies (15%), of which terminated by abortion (1 miscarriage: 6’th week of GA in 28.4 yrs pt with ectopic thyroid gland; 2 elective abortion: 3’rd week of GA in 28 years pt with athyreosis). The abortion rate was 1.45% for miscarriage (0.54% in control population), 2.9% for elective abortion (0.86% in control population). In the fullterm pregnancies the median of L-T4 dose needed in each trimester was 15% (10%-20%), 15% (10%-20%) and 20% (10%-30%) respectively. TSH values >2.5 mU/L were found in 7/7 cases in the first trimester, in 4/7 in the second, in 3/7 in the third. All newborn showed normal results at neonatal screening and at thyroid ultrasound. The table shows the data of live births subdivided according to maternal age.

<table>
<thead>
<tr>
<th>Subjects n,%</th>
<th>CH mothers’ gland morphology</th>
<th>Live births n,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20 yrs</td>
<td>27/89 (39%)</td>
<td>0/27 0.0%</td>
</tr>
<tr>
<td>20-25 yrs</td>
<td>26/93 (30%)</td>
<td>2/26 15.4% 8%</td>
</tr>
<tr>
<td>25-35 yrs</td>
<td>16/89 (23%)</td>
<td>2/16 12.5% 24%</td>
</tr>
</tbody>
</table>

Conclusions: These preliminary data seem to indicate a normal fertility in patients with CH. The abortion rate seems to be higher than in the control population. The monitoring of L-T4 dose adequacy in pregnancy is poor in our patients. These data warrant further investigation to evaluate the impact of genetic or therapeutic factors.

Short-term memory in children with congenital hypothyroidism

Objective and hypotheses: To evaluate intellectual outcome in patients with CH treated with an initial LT4 replacement dose between 10 and 15 μg/kg/die. Methods: One hundred ten patients (33 males) affected by CH, detected by neonatal screening program at an average age of 15.2±6.2 days, were longitudinally followed up to the age of 8 years. Thirty healthy children comparable for age, sex and socioeconomic status (SES) were enrolled in the study as controls. At 8 years of age, Intellectual Quotient (IQ) was assessed using the WISH-III. SES was evaluated by Grafar score revised with 1 reflecting the highest and 5 the lowest score.

Results: Mean verbal (VIQ 100.1±11), performance (PIQ 100.8±22) and full-scale (FSIQ 101.6±13.2) IQ scores in patients with CH were normal and comparable to the controls IQ scores (VIQ 101.9±12.1, PIQ 102.8±14.2, FSIQ 102.7±12.7 respectively). No relationship was observed between IQ scores and initial LT4 dose, whereas IQ scores were significantly related to SES (VIQ <0.01, PIQ<0.02, FSIQ<0.01). Additional variables influencing the IQ scores were the severity of CH at diagnosis (FT4 or T4 values) (VIQ p<0.01, PIQ p<0.01, FSIQ p<0.02) and the chronological age at diagnosis only for VIQ (p<0.04). However, when a General Linear Model was used only the SES resulted significantly related to IQ scores (VIQ p=0.01, PIQ p=0.02, FSIQ p=0.01).

Conclusions: Our results indicate that an initial LT4 dose between 10 and 15 μg/kg/day allows to achieve a normal IQ at the age of 8 years. The socioeconomic status seems to be the major factor affecting the intellectual outcome of CH patients.
**P1-d3-402 Thyroid 1**

**“Block-and-replace” method in pediatric Graves’ disease**

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**Background:** The “block-and-replace” method is still widely discussed in literature and comprises an association between high dose of antithyroid drugs (ATD) and L-thyroxine (L-T4) in order to block hormone synthesis and ensure a state of euthyroidism secondary to L-T4 replacement.

**Objective and hypotheses:** To compare efficacy of ATD+L-T4 therapy versus monotherapy in pediatric patients with unstable Graves’ Disease (GD).

**Methods:** 25 pediatric patients (23 female) diagnosed with GD at our center (average age at diagnosis: 8.6 years) with unstable response to ATD (elevated TSH and fT3, and low fT4 levels) were treated with ATD+L-T4 therapy. For each patient we evaluated: 1) percentage of state of hyperthyroidism, hypothyroidism and euthyroidism in both monotherapy with ATD and ATD+L-T4 therapy; 2) remission rate after combination therapy and efficacy of ATD+L-T4 therapy to postpone a definitive treatment; 3) onset of side effects during combination therapy.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism: major relapse</th>
<th>Hyperthyroidism: minor relapse</th>
<th>Hypothyroidism</th>
<th>Euthyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (IU/L)</td>
<td>ATD: 14.55 ± 12.74</td>
<td>ATD+L-T4: 1.84 ± 4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.08 ± 17</td>
<td>3.56% ± 10.77</td>
<td>6.7% ± 9.0</td>
<td>67.8% ± 18.59</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>n.s. (0.075)</td>
<td>&lt; 0.001</td>
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</tr>
</tbody>
</table>

1) 1 patient (4%) went into remission with the ATD+L-T4 therapy; 15 patients (60%) required a definitive therapy (4 radioiodine, 11 surgery). In the 4 patients undergoing radioiodine, the ATD+L-T4 therapy has delayed the administration of radioisotope for 4.9 years. In the 11 patients who underwent thyroidecomy, ATD+L-T4 therapy has delayed surgery for 2.9 years.

2) No serious side-effects during the ATD+L-T4 therapy were observed.

**Conclusions:** “Block-and-replace” therapy is indicated in those cases in which autoimmune hyperthyroidism is difficult to manage, when it is necessary to postpone both the use of radioactive iodine or surgery to a more appropriate age in order to contain the risks that these procedures entail.

**P1-d3-403 Thyroid 1**

**Subclinical hypothyroidism in obese children and metabolic implications**

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**Objective and hypotheses:** Aim of this study was to assess the prevalence of subclinical hypothyroidism in a paediatric population of overweight and obese children and its association with metabolic parameters and with metabolic syndrome (MS).

**Methods:** Clinical and metabolic evaluations in 600 overweight and obese children and adolescents (310 females, 290 males, 260 prepubertal, 240 pubertal, mean age: 10.7 ± 3.1 years) were performed. MS was defined according to paediatric NCEP-ATPIII criteria. TSH levels were evaluated both in quartiles and to a fixed cut-off level (pathological value > 3.500 µIU/ml). The presence of autoimmune thyroiditis was assessed in those subjects with abnormal TSH values.

**Results:** 75 subjects (12.5%) had abnormal TSH levels and 10 of them (13.3%) showed antithyroid antibodies. Age decreased (p<0.001) and the number of prepubertal children increased (p<0.006) with the rise of TSH levels. non-HDL cholesterol (p=0.03), triglycerides (p<0.0001), PNFI index (p=0.0001), fasting glucose (p=0.04), HOMA-IR (p=0.04), glucose peak (p=0.03) and glucose area (p=0.01) during OGTT increased with the rise of TSH levels. No differences in the prevalence of MS were found among the TSH quartiles and in subjects with altered TSH levels. A negative correlation between age and TSH levels was found (β= −0.122; p<0.003). Furthermore, positive correlation between TSH and non-HDL cholesterol (β=0.095; p=0.023), triglycerides (β=0.232; p<0.0001), PNFI (β=0.185; p<0.0001) and fasting glucose (β=0.107; p<0.01) was shown independently of confounding factors.

**Conclusions:** Subclinical hypothyroidism and autoimmune thyroiditis are frequent in overweight and obese children. The association between TSH levels and metabolic parameters suggests a role of the hypothalamus-pituitary-thyroid axis in the regulation of lipids and glucose metabolism. Conversely the absence of an association with the metabolic syndrome suggests that this axis may modulate specific metabolic alterations.

**P1-d3-404 Thyroid 1**

**Familial brain-lung-thyroid syndrome due to a new NKX2-1 mutation p.Q172L causing disabling benign hereditary chorea**

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**Background:** Brain-lung-thyroid syndrome is characterized by the triad of congenital hypothyroidism (CH), benign hereditary chorea (BHC) and surfac tant deficiency syndrome. It is caused by mutations in the homeobox containing transcription factor NK homeobox 2 / thyroid transcription factor 1 gene (NKX2-1/TTFF1).

**Objective and hypotheses:** Description of the variability of the neurologic phenotype.

**Methods:** Case report and direct sequencing of NKX2-1.

**Results:** A term newborn was detected by the neonatal screening with increased TSH (17 µIU/L). The confirmatory laboratory test revealed subclinical CH (TSH 25 µU/L, normal fT4) in the context of normal thyroid morphology. L-T4 was started immediately. Family history revealed that the patient’s father was suffering from CH due to athyreosis. L-T4 had been started at day 6 of life. Despite normal TSH and fT4 under substitution, he developed progressive hypothyronia from 10 month of life on, evolving to severe choreoathetotic cerebral palsy until the age of 5 years. The father is non-ambulatory and wheelchair chair dependent. The combination of BCH and CH suggested brain-lung-thyroid syndrome without pulmonary disease. Direct sequencing of NKX2-1 revealed a new heterozygous missense mutation (c.515A>T, p.Q172L) in father and daughter. The mutation lies within the homeodomain in exon 3. Pathogenicity of the mutation is further supported by in silico analysis. Genetic counselling of the family has taken place. Under close neurological follow-up, the daughter showed slight hypotonia only at 12 months. No pulmonary abnormalities occurred until now.

**Conclusions:** Our case report illustrates how haploinsufficiency of a new NKX2-1 mutation resulted in an unusually late onset of hypotonia evolving to particularly severe BCH. Thus, unexplained hypotonia in patients with initially isolated CH despite adequate substitutive therapy should evoke NKX2-1 mutations before onset of BCH.

**P1-d3-405 Thyroid 1**

**Transient neonatal hypothyroidism resulting from maternal ingestion of a traditional Korean seaweed soup**

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**Background:** Neonatal hypothyroidism secondary to neonatal exposure to iodine containing medication or skin preparation has been well described. Hypothyroidism resulting from maternal ingestion of high iodine containing food through breast milk is less well recognized.

**Objective and hypotheses:** A male infant presented at 21 days of age with unconjugated hyperbilirubinaemia starting at the third day of life. Both his parents originated from South Korea.

**Methods:** Investigation showed an unconjugated bilirubinaemia of 346 µmol/L with an unchanged TSH and free T4 levels. Bilirubin levels and TSH values were normal in the patient's father and maternal relatives and a representative of a local traditional Korean food. The patient's mother presented with mild hypothyroidism 2 months after delivery. The patient's father did not have any history of hypothyroidism.

**Results:** In silico transcription factor NK homeobox 2 / thyroid transcription factor 1 gene (NKX2-1/TTFF1) was analyzed in the affected family. No significant mutations in the coding region of NKX2-1 were observed. Further analysis of NKX2-1 expression by RT-PCR revealed a new isoform of NKX2-1 lacking an in-frame 18 bp deletion in the 3’UTR and a new heterozygous missense mutation (c.515A>T, p.Q172L) in father and mother.

**Conclusions:** Maternal ingestion of high iodine containing food through breast milk is less well recognized. Further studies are needed to evaluate the role of NKX2-1 in the pathogenesis of transient neonatal hypothyroidism.
### P1-d3-406 Turner Syndrome 1

#### Effects of karyotype on birth size, pretreatment growth, and response to GH treatment (GHTx) in Turner syndrome (TS): data from 1383 patients followed in a global observational study

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**Background:** TS phenotype varies greatly, at least in part due to karyotype, which ranges from complete loss of the 2nd sex chromosome (45X) to mosaic karyotypes (45X > 2nd cell line [46XX;46XY;47XXX];other), or structural rearrangements of 2nd X. While 45X is generally regarded as the most severe form of TS, data on karyotype-related differences in growth & response to GH are limited.

**Objective:** To evaluate karyotype effects on birth size, preTx height (H) & response to GH (1-styr & near-adult height [NAH]) in TS.

**Methods:** We assessed relationships between karyotype & growth in 1383 pts with TS. Differences among karyotype groups overall, & pairwise differences between 45X & each other group, were assessed by ANOVA. ANCOVA models were developed to assess factors (e.g. karyotype, baseline age, HSDS, target HSDS, age at GH start) significantly associated with 1-styr height gain & gain from baseline to NAH (HV~2cm/yr or bone age ≥14 yr).

**Results:** All karyotype groups were relatively small at birth, but 45X/46XX & other mosaic karyotypes were not as small as 45X. Isochromosome X was shortest at GH start. There were no significant differences among karyotypes for GH dose (0.31±0.08 mg/kg/w overall). 1yr GHTx data were available for 648 pts & NAH data for 388. Mean 1yr HSDS gain was greatest for pts with Y-containing karyotypes (0.9±0.4 SDS; N=9, not shown) but no other significant differences were seen. At NAH GHTx duration was shorter for 45X/46XX & 45X/47XXX so HSDS gain was lower, but NAHSDS not significantly different. By ANCOVA significant factors for NAH were shorter preTx HSDS, taller THSDS & longer GHTx. Karyotype was not significant.

**Conclusions:** Prenatal growth was less impaired with mosaic karyotypes vs 45X. However HSDS at GH start was similar across karyotypes, likely due to referral/selection bias for GH Tx. Karyotype did not appear to significantly affect GH response at 1 yr or NAH.

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### Poster Presentations

**P1-d3-407 Turner Syndrome 1

#### Patients with Turner syndrome and isolated SHOX deficiency have similar bone geometry at the radius

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**Background:** Girls with Turner syndrome (TS) have altered bone mineral density and geometry at the radius, which may pose an increased fracture risk in adulthood. The etiology of their low bone strength remains unknown. Since short stature homeobox (SHOX) gene plays a major role in long bone growth and patients with SHOX deficiency share some skeletal features with TS, SHOX gene has been suggested as a causal one.

**Objective and hypothesis:** We hypothesized that bone geometry and mineral density is similar between girls with TS and patients with isolated SHOX deficiency.

**Patients and methods:** Fourteen patients with SHOX deficiency (median age 11.8 yrs, range 6.0-36.8, 10 children and 4 adults) were examined by peripheral quantitative CT (pQCT) at the non-dominant forearm. Results were expressed as Z-scores using published reference data and then compared to the results of 67 girls with TS (median age 13.7 yrs, range 6.0-19.4). The differences from reference data were tested by one-sample T test and differences between TS and SHOX deficiency were tested by two-sample T test.

**Results:** Trabecular volumetric bone mineral density (vBMD) was decreased in TS (mean Z-score -0.7±1.3, p<0.001) but normal in isolated SHOX deficiency (Z-score 0.3±1.3, n.s., group difference p<0.01). Cortical vBMD was low in both groups (Z-score -1.6±1.5, p<0.001 and -1.9±2.3, p<0.01, respectively) without significant group difference. Both groups had increased total bone cross-sectional area (CSA) at the diaphysis (Z-score 0.7±1.7, p<0.01 and 2.3±1.6, p<0.001, respectively), but normal cortical bone CSA (0.0±1.0, n.s. and -0.7±2.3, n.s., respectively).

**Conclusions:** Patients with Turner syndrome and isolated SHOX deficiency have similar bone geometry at the radius. Our results support the hypothesis that skeletal abnormalities in patients with TS are caused by SHOX deficiency. Low trabecular vBMD in TS is probably a consequence of hypogonadism due to ovarian failure.
Background: Recent studies suggest that assessment with MRI is gold standard for evaluation of aortic dimensions and aortic valve leaflets.

Aims: To describe abnormalities detected on echocardiogram and cardiac MRI in Turner syndrome. MRI in adolescents and adults with Turner syndrome (TS) may have clinical implications.

Methods: We aimed to apply MLPA technique to detect X chromosome abnormalities in Turner syndrome (TS) and compare the results to conventional karyotyping.

Methods: We studied 33 females by MLPA and conventional cytogenetic. We studied 33 females by MLPA and conventional cytogenetic.

Results: MLPA. 23 patients had normal karyotype and only one had a doubtful MLPA result. Sensitivity of MLPA was 100% and specificity 95.6%.

Conclusions: We suggest that MLPA could represent a rapid, economic, automated, reliable and accurate method to diagnose Turner syndrome in girls with short stature, not requiring culturing cells. Of course, larger samples are needed.

Background: Turner syndrome is one of the most common genetic conditions affecting females, with an incidence of one in 2500-5000 live births. TS occurs when an entire X-chromosome is deleted, a portion of an X-chromosome is deleted, or the X-chromosome is deleted in a subset of cells (TS mosaicism). Clinical features include primary hypogonadism, renal abnormalities, structural cardiac problems, and short stature, but with early diagnosis and initiation of GH therapy, normal or near-normal adult stature can be achieved. It is estimated that one in 50-100 girls with short stature have TS. Experts in the field recommend that short girls be tested for this condition.

Methods: The MLPA kit (SALSA P095), used to detect the most frequent aneuploidies, includes 36 genomic targets, eight probes for chromosome 13, 18, 21 and X, and four probes for Y chromosome (MRC-Holland, Amsterdam, The Netherlands).

Results: 10 females had mosaic Turner syndrome and all were detected by MLPA. 23 patients had normal karyotype and only one had a doubtful MLPA result. Sensitivity of MLPA was 100% and specificity 95.6%.

Conclusions: We suggest that MLPA could represent a rapid, economic, automated, reliable and accurate method to diagnose Turner syndrome in girls with short stature, not requiring culturing cells. Of course, larger samples are needed.
Conclusions: Compared with 45X/46XX patients, GH treated 45X/46XY Turner girls had a greater height increase, especially after the start of puberty induction, and a smaller RHD, suggesting that the presence of the Y chromosome might improve the response to GH in Turner girls. Confirmation of these results in larger groups of patients is indicated.

P1-d3-412 Turner Syndrome 1
Ovarian tissue cryopreservation in girls with Turner syndrome: feasibility and acceptability
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Background: Ovaries of children and adolescents with Turner syndrome (TS) are considered by most pediatric endocrinologists to contain a minimal number of oocytes if any, precluding attempts to collect and store gonadal tissue for future procreation. There are clinical data in the literature suggesting that a yet undetermined proportion of Turnerian ovaries could harbor follicles. Our objective was to test if cryopreservation of ovarian tissue could allow fertility in girls with TS. The short term objective is to assess the feasibility of the procedure, the demand and the medical characteristics of TS patients volunteering for the protocol.

Methods: 18 girls with TS aged from 4 to 18 years referred in our center by pediatric endocrinologists for ovarian preservation were included. Clinical examination, blood samples for hormonal measurements and surgical procedure, were performed according the protocol; the tissue is shipped to an expert lab to be prepared in strips and frozen for cryopreservation; acceptability of the procedure was assessed through self-questionnaires.

Results: -14/18 of girls included are of puberty age with spontaneous puberty in 9/14 (64%); - karyotypes were: 45X; 3; 10 mosaicisms with absence or structural anomalies in one of the X chromosome; 5 mosaicisms with complete chromosomes; -serum FSH range from 4 to 109 U/L; AMH concentrations were measurable (>2 pmol/L) in 7/18 girls; - the number of strips made from the ovarian tissue varies from 3 to 23. None of the girls had any complications with surgery. In general, the procedure was considered as slightly aggressive.

Conclusions: Girls with TS volunteering for fertility preservation are mostly of post-pubertal age with spontaneous puberty. Surgery was not complicated and the overall procedure was considered as well accepted by the girls with TS.

P2-d2-413 Adrenals and HPA Axis 2
Early pubertal onset in congenital adrenal hyperplasia due to 21-hydroxylase deficiency
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Background: Diminished cortisol synthesis in patients with congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency results in increased ACTH secretion that enhances adrenal androgen production. Hyperandrogenism in childhood may cause premature pubarche, accelerated growth and consequently short final height. Whether patients with CAH exhibit early true puberty is a matter of debate.

Objective and hypotheses: The aim of the current study was to assess pubertal characteristics and growth patterns in children with CAH.

Methods: Fifty-five subjects (33F:22M) with CAH (22, salt wasting (SW); 15, simple virilizing (SV); 18, non classical (NC)) were enrolled. The design was a retrospective longitudinal study. All subjects underwent genetic analysis of the CYP21 gene.

Results: The most common mutation in the entire group was V281L (22%) followed by E2splice (21%), I172N (14%), 8del (11%) and others (32%). The mean age of onset of pubarche was earlier in females 5.6±2.3 years (1.9-10.0) than in males 7.3±2.4 years (1.8-11.3) in all forms of CAH. Onset of pubarche was earlier in SV (5.0±2.6 years) than in SW (6.9±1.6 years) and NC (7.1±2.4 years) forms. In females, despite early onset of telarche 8.9±2.1 years, the mean age of menarche was within the normal range 12.5±1.9 years, though highly variable 9.16.5 years. High variability in onset of gonadarche was shown in males as well 10.5±1.5 years. Seven patients were treated with LHRH-analogs due to early onset of puberty. Low final height was shown in both females 155.7±9.0 cm and males 164.4±8.0 cm.

Conclusions: Our results indicate that onset of puberty is earlier in children with CAH despite early initiation of therapy. Patients with SV form are at higher risk for earlier pubarche and advanced bone age (BA). The earlier timing of pubertal onset plays a critical role in final height attainment. Earlier diagnosis in children with SW form, stricter control and future medical therapy such as aromatase inhibitors may delay pubertal onset and improve the final height attainment in these patients.

P2-d2-414 Adrenals and HPA Axis 2
Prevalence and clinical presentation of adrenarche in healthy prepubertal children
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Background: Adrenarche refers to the onset of increased production of adrenal androgens, mainly DHEAS. Clinical signs of adrenarche include adult type body odor, oily hair, comedones, acne, and pubarche. Adrenarche is defined premature (PA) if clinical signs appear before the age of 8 years in girls and 9 years in boys and serum DHEAS concentration is ≥ 1 µmol/L. The prevalence and presentation of adrenarche in prepubertal children is not well known.

Objective and hypotheses: Our objective was to examine the prevalence and clinical presentation of adrenarche in a population based sample of prepubertal healthy Finnish children aged less than 9 years. We hypothesized that the prevalence of adrenarche is higher than generally thought in boys when studied in a population based setting.

Methods: Healthy prepubertal children (209 girls and 228 boys; age ≥ 7.6 ± 0.4 years and 7.6 ± 0.4 years, NS) taking part in the Physical Activity and Nutrition in Children (PANIC) Study were included in this study. Clinical signs of adrenarche were assessed by a physician. Serum DHEAS concentration is determined by enzyme immunoassay.

Results: In all studied children, girls had more often one or more signs of androgen action (24.5 vs. 9.6%; p<0.001) but biochemical adrenarche was less common in girls than in boys (8.1 % vs. 16.7%; p = 0.045). Only four girls (1.9%) and none of the boys had pubarche. Total prevalence of PA was 4.7 % (8.0% in girls and 1.8% in boys).

Conclusions: Though clinical signs of androgen action are more common in prepubertal girls than in boys, biochemical adrenarche is more common in boys. Thus, girls are more sensitive to adrenal androgens. This difference may be explained by their more active androgen receptor or by modulation of other hormones and factors, including gonadal steroids.
P2-d2-415 Adrenals and HPA Axis 2
The frequency of mutations in the CYP21A2 gene in the general population of Cyprus
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Background: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is the most common autosomal recessive disorder and is mostly attributable to mutations in the CYP21A2 gene. The carrier frequency of CYP21A2 mutations in the general population has been estimated to be 1:25 to 1:10.

Objective and hypotheses: So far the true carrier frequency for CAH due to 21-OHD has not been determined by comprehensive mutation analysis of the CYP21A2 in a specific European population and the present study aims in doing so.

Methods: The present study screened for mutations in the CYP21A2 gene a statistically valid number of 300 clinically asymptomatic subjects (150 males and 150 females) from the general population of Cyprus. The methodology used for the CYP21A2 genotyping involved multiplex ligation-dependent probe amplification (MLPA) and direct sequencing of PCR products of the CYP21A2 gene.

Results: Genotyping of the 600 unrelated alleles from the 300 Cypriot asymptomatic individuals revealed a carrier frequency of 9.83%. The most frequent mutations among the tested subjects of the present study were the mild p.V281L (4.3%), followed by p.Q318stop (2.5%), p.P453S (1.3%), p.V304M (0.8%), p.F482S (0.6%) and p.M283V (0.1%). In conclusion, the detected 9.83% CAH carrier frequency suggests one of the highest prevalence of CYP21A2 carriers reported by a genotyping analysis and the previously described major mutations are found to dominate the mutation spectrum of the Cypriot population. In addition, the rare V304M mutation which to our knowledge was reported only once before in a female patient of Asian origin seems to be quite frequent (0.83%) in the Cypriot asymptomatic population and imply a possible founder effect.

Conclusions: Knowing of the prevalence and the nature of the genetic defects in our population will be of immense help in our understanding and awareness of NC-CAH in females presenting with hyperandrogenemia.

P2-d2-416 Adrenals and HPA Axis 2
Idiopathic precocious pubarche and late-onset congenital adrenal hyperplasia: distinctive features
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Background: Precocious pubarche (PP) may reveal late-onset congenital adrenal hyperplasia (LO-CAH) in 5 to 20% of cases, and the ACTH stimulation test is usually recommended in children presenting with PP. This test is expensive and stressful and the results are normal in most cases.

Objective and hypotheses: Aim of our study was to determine clinical and plasma steroid precursors of LO-CAH among children presenting with PP. The methodology involved multiplex ligation-dependent probe amplification (MLPA) and direct sequencing of PCR products of the CYP21A2 in a specific European population and the present study aims in doing so.

Methods: The present study screened for mutations in the CYP21A2 gene a statistically valid number of 300 clinically asymptomatic subjects (150 males and 150 females) from the general population of Cyprus. The methodology used for the CYP21A2 genotyping involved multiplex ligation-dependent probe amplification (MLPA) and direct sequencing of PCR products of the CYP21A2 gene.

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Conclusions: Knowing of the prevalence and the nature of the genetic defects in our population will be of immense help in our understanding and awareness of NC-CAH in females presenting with hyperandrogenemia.
P2-d2-418 Adrenals and HPA Axis 2

Loss of length in patients with congenital adrenal hyperplasia is associated with elevated hydrocortisone dosage during the first year of life

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1Hadassah Hebrew University Medical Center, Division of Pediatric Endocrinology, Jerusalem, Israel; 2Ha’Emek Medical Center, Pediatric Endocrine Unit, Afula, Israel; 6Dana Children Hospital, Pediatric Endocrine Unit, Tel Aviv, Israel; 6Soroka University Medical Center, Pediatric Endocrine Unit, Beer-Sheva, Israel

Background: Decreased final height in congenital adrenal hyperplasia is mainly caused by advanced bone age and compromised pubertal growth. Elevated glucocorticoid levels are associated with decreased chondrocyte proliferation and linear growth.

Objective and hypotheses: We studied the correlation between hydrocortisone (HC) dosage and growth velocity during the fastest growth period the 1st year of life.

Methods: Ethnicity, mutation, clinical phenotype, HC dosage, and growth parameters at 1, 3, 6 and 12 months of age were assembled from 71 patients with salt-wasting CAH at 5 pediatric endocrine centers in Israel. Normal average 1st y growth from the Israeli Ministry of Health registry was used as the control data.

Results: Six-months-old CAH males and females were significantly shorter than controls (2.23 ± 0.01, 1.64 cm; p <0.05, respectively). This deficit increased further at 1y of age. A strong negative correlation was found in males between the HC dosage at 3 months and the length at 6 and 12 months and between the HC dosage at 6 months and the length at 12 months of age (r = -0.609, r = -0.517, respectively). A weaker yet statistically significant relation was found between HC dosages at 3-6 months, and length at 6-12 months for the entire group (r = -0.3, p <0.05).

Conclusion: Patients with CAH may lose significant height already at 6-12 months of age. Higher dosages of glucocorticoids are associated with a slower growth velocity during the 1sty of life mainly in boys. Intensive infantile optimization of the HC dosages may improve 1sty growth and final height in CAH patients.

P2-d2-419 Adrenals and HPA Axis 2

Assessment of adrenal function in female-to-male adolescents with gender identity disorder (GID)

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1University College London Hospital, Adolescent Endocrinology, London, United Kingdom; 2Barts and the London School of Medicine and Dentistry, Centre for Endocrinology, London, United Kingdom; 3St Bartholomew's Hospital, Department of Endocrinology, London, United Kingdom; 4St Bartholomew’s Hospital, Department of Otolaryngology, London, United Kingdom; 5St Bartholomew’s Hospital, Department of Neurosurgery, London, United Kingdom

Background: Most adolescents with GID have no overt functional or phenotypic abnormalities to explain their presentation. Currently all female to male (FtM) persons undergo detailed evaluation of adrenal function.

Objective: We aimed to determine whether subtle adrenal abnormalities were present in the above group and thereby develop a suitable investigation schedule.

Methods: Over the past 4 years, 56 biological females with mean age 16.57 years (13.5-18.4) were referred to the National GID service at the CHU Hédi CHAKER Sfax over a period of 22 years (1990 - 2011).

Results: During the period of study, we collected 10 cases of triple A syndrome with loss of length, small breasts, hypogonadism and delayed puberty. The serum 17-OHP was low in all cases. Synacthen test was conducted at 3 children, and it was negative in 2 cases. Plasma levels of ACTH was high in all cases. Mineralocorticoid deficiency was noted in 8 cases. The genetic study found the same mutation in AAAS gene (VS14+1G-->A) in 9 cases; it has not been performed in a patient. Our patients have been treated with hydrocortisone and Florinef, 5 patients had balloon dilatations and 1 patient had surgery with Heller’s myotomy. After an average decline of 5 years 7 months, growth retardation was identified in 5 cases, one patient was lost sight and a patient died of malnutrition.

Conclusions: The Allgrove syndrome is a serious disease, despite the surgical therapeutic, medical treatment of achalasia, treatment of adrenal insufficiency and the alacrimia seems to be the best alternative therapy in child.

P2-d2-420 Adrenals and HPA Axis 2

Allgrove syndrome: a study of 10 cases

Monica Hachicha1; Hajer Aloulou1; Safa Hadji Hmida1; Lamia Stahi2; Sana Kmha1; Imen Chachboub1; Hassen Kammoun1; Thounaya Kammoun1
1Hedi Chaker Hospital, Pediatric, Sfax, Tunisia; 2Hedi Chaker Hospital, laboratory of human medical genetics, Sfax, Tunisia

Background: Triple A or Allgrove syndrome is a rare autosomal recessive disease with alacrimia, achalasia cardia, and ACTH-resistant adrenal insufficiency.

Objective and hypotheses: Study the epidemiologic, clinical, biological, genetic, therapeutic and evolving aspect of Allgrove syndrome among our patients.

Methods: A retrospective study of cases of the Allgrove syndrome hospitalized in the pediatrics department at the CHU Hédi CHAKER Sfax over a period of 22 years (1990 - 2011).

Results: During the period of study, we collected 10 cases of triple A syndrome from 7 families. There were 3 girls and 5 boys. The average age was 3 years 5 months with extremes ranging from 13 months to 4 years 6 months. All our patients had a proven alacrimia. The adrenal insufficiency was inaugurated by a seizure related to hypoglycemia in 2 cases and a melanoderma in all patients. Achalasia was noted in 5 cases. The cortisol was low in all cases. Synacthen test was conducted at 3 children, it was negative in 2 cases. Plasma levels of ACTH was high in all cases. Mineralocorticoid deficiency was found in 8 cases. The genetic study found the same mutation in AAAS gene (VS14+1G-->A) in 9 cases; it has not been performed in a patient. Our patients have been treated with hydrocortisone and Florinef, 5 patients had balloon dilatations and 1 patient had surgery with Heller’s myotomy. After an average decline of 5 years 7 months, growth retardation was identified in 5 cases, one patient was lost sight and a patient died of malnutrition.

Conclusions: The Allgrove syndrome is a serious disease, despite the surgical therapeutic, medical treatment of achalasia, treatment of adrenal insufficiency and the alacrimia seems to be the best alternative therapy in child.

P2-d2-421 Adrenals and HPA Axis 2

Endonasal transphenoidal endoscopic pituitary surgery; Results in four paediatric patients with Cushing’s disease

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Background: Selective transphenoidal adenomectomy remains the accepted first line treatment for Cushing’s disease (CD), until recently by microscopic (sublabial) transphenoidal pituitary surgery. Endonasal transphenoidal endoscopic surgery is emerging as a less invasive treatment for pituitary adenomas with lower post-operative complications and morbidity.

Objective: There are no published series of the treatment of paediatric CD by endoscopic pituitary surgery and we report our centre’s preliminary results.

Methods: Four paediatric patients (median age 14.4yr; range 11.7-16.8yr) fulfilled standard diagnostic criteria for CD. Preoperatively no abnormality was identified on pituitary MR scanning in 3 (75%) patients. Bilateral parietal sinus sampling demonstrated central ACTH secretion (IPS/P ACTH ratio >2.0, pre or post-CRH) in 3 (75%) patients with laterisation of ACTH

(419-1166) and 840 (506-1426) nmol/l in the controls. The one GID adolescent with a baseline 17OHP of 8.4 nmol/l, greater than the normal laboratory range (<2) did not have an exaggerated rise on stimulation and other adrenal androgens were normal.

Conclusion: In our national cohort of FTM GID adolescents, we have not been able to demonstrate any variations, subtle or otherwise in adrenal steroid secretion to differentiate them from the control group. Baseline adrenal steroid profile may be evaluated but unless the androgens and precursor concentrations are elevated, Short Synacthen testing does not appear to be indicated.

Poster Presentations

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secretion (IPSG post CRH ≥1.4) in 2 patients. The same neurosurgeon and
dorsal nasal surgeon undertook all the operations. ‘Cure’ was defined as
a 0.90th cortisol level of <30nmol/L post-operatively on two separate oc-
casions associated with regression of the clinical features of CD. Results: Clinical recovery and biochemical ‘cure’ was achieved in 3 (75%) patients
and a corticotroph adenoma was confirmed histologically in all cured cases.
One case developed post-operative CSF leak requiring lumbar drain insertion
and patching. At a mean interval of 5.9 years (0.4-9.8) yr post-operatively,
cured patients have shown no recurrence. One patient, who had a large diffuse
adenoma requiring more extensive surgery has parhypopituitarism, another patient has GH and gonadotrophin deficiencies.

Conclusions: Our experience shows that endonasal transphenoidal endo-
coscopic surgery for removing corticotroph adenomas in children, in most cases
not visualized on MRI imaging, is minimally invasive and gave excellent post-operative recovery/results. In skilled hands this technique provides an
alternative to conventional transsphenoidal microscopic surgery in managing paediatric CD.

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### Table: Patients with Adrenocortical Adenomas

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical manifestation</th>
<th>Tumor size (mm)</th>
<th>Non-enhanced attenuation (HU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>14</td>
<td>female</td>
<td>hypercortisolism</td>
<td>23-25</td>
</tr>
<tr>
<td>Patient 2</td>
<td>2</td>
<td>female</td>
<td>virilization</td>
<td>36-37</td>
</tr>
<tr>
<td>Patient 3</td>
<td>4</td>
<td>male</td>
<td>hypercortisolism</td>
<td>38-64</td>
</tr>
<tr>
<td>Patient 4</td>
<td>5</td>
<td>male</td>
<td>hypercortisolism</td>
<td>4-5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>13</td>
<td>female</td>
<td>hypercortisolism</td>
<td>17-18</td>
</tr>
<tr>
<td>Patient 6</td>
<td>15</td>
<td>female</td>
<td>hypercortisolism</td>
<td>31-32</td>
</tr>
<tr>
<td>Patient 7</td>
<td>10</td>
<td>female</td>
<td>hypercortisolism</td>
<td>24-32</td>
</tr>
</tbody>
</table>

Conclusions: Pediatric adrenocortical adenomas have a higher non-enhanced density than adult. Only one patient with hyperaldosteronism has low non-enhanced attenuation (6 HU). We can suggest that the children with large adenomas and the clinical manifestation of virilization syndrome are at higher risk of developing adrenocortical carcinoma and should be watched more closely than other.

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### Table: Genotypes and Phenotypes

<table>
<thead>
<tr>
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<td>10</td>
<td>female</td>
<td>hypercortisolism</td>
<td>24-32</td>
</tr>
</tbody>
</table>

Conclusions: Our aim was to describe the mutational spectrum of CYP21A2 and evaluate genotype-phenotype correlations in a cohort of Portuguese patients with 21-OHD.

Methods: Molecular analysis of CYP21A2 was performed in 23 patients with clinical and laboratory 21-OHD. A variety of genotyping techniques were used.

Results: Genotyping was performed in 23 unrelated patients: 4 salt-wasters, 8 simple-virilizers, 11 non-classical patients. CYP21A2 mutations were detect-
ed in all patients. Homozygosity was found in 7 patients (30,4%), compound heterozygosity in 12 patients (52,2%) and heterozygosity for one mutation in 4 patients (17,4%). The most frequent mutations were V281L (37,8%), I172N (17,8%), I2 splicing (13,3%), Q318X (11,1%) and CYP21A2 deletions or gene conversions (6,7%). The overall concordance between genotype and phenotype was 73,9%, with complete concordance in the salt-waster pheno-
type.

Conclusions: The frequency of the underlying genetic defect in our patients has some differences compared with other Portuguese cohorts probably due to a smaller sample size and the use of different genotyping techniques. In most cases there was a good correlation between genotype and phenotype, which highlights the concept that the molecular analysis of CYP21A2 provides useful information in terms of prediction of disease severity, genetic and prenatal counseling. The discrepancies may be explained by novel mutations, incor-
correct genotyping, compound heterozygosity for two or more mutations and other genetic variations in androgen biosynthesis or sensitivity to androgens.
Objective: Five years ago, we have shown that 24-hour blood pressure profiles are altered in CAH patients with elevated systolic levels correlated with the degree of obesity, whereas normal-weight patients tended to diastolic hypotension (Völkl et al. 2006, JCEM).

Design: We included 39 children with CAH, aged between 6.9 and 17.1 years (median 13.5, n=21 females) into this single-centre, cross-sectional, prospective, observational study. All patients had proven CAH (genetic: 0; n=13; A, n=16; B, n=8; D, n=2), received steroid substitution therapy and underwent standardized 24-hour blood pressure (BP) monitoring (Mobil-O-Graph-TM, I.E.M., Solberg, Germany). N=26 of them had participated in the previous study. Eleven different variables of 24h-BP were analysed and compared with current German reference data.

Results: BMI SDS slightly improved over the five years, but are still significantly different from zero (from 1.09 SDS, 0.21;2.01, to now 0.61 SDS, -0.28;1.68). Daytime and night-time systolic BP did not change significantly and remained elevated (from 0.35 SDS, -0.10;0.85, to now 0.46 SDS, -0.47;1.2, and from 0.39 SDS, -0.03;0.68, to now 0.67 SDS, -0.13;1.1), whereas the lowered daytime diastolic BP improved (from -1.2 SDS, -1.5; 0.71, to now -0.62 SDS, -1.2;0.10, p=0.007) and stayed normal during the night (from -0.18 SDS, -0.86;0.11, to now 0.29 SDS, -0.40;0.83). Nocturnal dropping of systolic BP did not change (from 13.2%, 9.1;15, to now 12.6%, 6.1;17). The different parameters of systolic and diastolic BP were significantly correlated with BMI SDS and the skinfold thicknesses (r=0.323 to 0.660, p<0.05). There was no clear correlation with equivalent hypocortisone and fludrocortisone dosage, bone age, and various laboratory parameters (e.g. renin, 17OHP-progesterone).

Conclusions: Despite an improvement of BMI SDS among our CAH patients, these follow-up data confirm our previous findings of altered 24h-BP profiles with elevated systolic BP levels correlated with the parameters of overweight.

P2-d2-426 Adrenals and HPA Axis 2

Primary adrenal insufficiency caused by a new mutation on DAX1 gene

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Introduction: Primary adrenal insufficiency can be caused by a deficiency in steroid biosynthesis or abnormal adrenal gland development. One of the genes involved in adrenal development is DAX1 which also functions in gonadal and pituitary development. We aimed to present a new mutation on DAX1 gene in a patient with adrenal insufficiency and normal male sexual development.

Case: 33 days old male infant admitted with dehydration, hyponatraemia, hyperkalemia and prerenal kidney failure. His physical examination revealed severe dehydration with normal anthropometric measurements (length: 52cm (3-10%), weight: 3150gr(10%), head circumference:37 cm (25-50%)), normal male genital development and scrotal hyperpigmentation. Premnatal and natal histories were unremarkable. Parents were fourth degree relatives. Laboratory evaluation showed high ACTH level (>1250pg/ml). Baseline and corticotropin stimulated cortisol, 17-OH-progesterone, 1-4 androstenedione and DHEA-S levels were 1.3 μg/dl-6.6 μg/dl, 0.18 ng/ml-1.4 ng/ml, 0.06ng/ml-0.1ng/ml and 110 μg/dl-115.8 μg/dl. Plasma renin activity (32.32ng/ml/hr) was high whereas aldosterone level (7,1ng/dl) was relatively low. Karyotype of the patient was 46,XY. With the diagnosis of adrenal insufficiency hydrocortisone, fludrocortisone, and salt treatments were initiated, to which the patient responded well. Hormonal and clinical features of the patient suggested adrenal insufficiency was related to abnormal adrenal development rather than a steroid biosynthesis defect. Thus a new frame shift mutation on DAX1 gene (c.543delA hemizygous mutation) which can alter gene function and cause the disease has been identified.

Conclusion: In the patient presenting with adrenal insufficiency not related to steroid hormone biosynthesis deficiency suggested an abnormality in adrenal gland development and genetic evaluation revealed a new frame shift mutation on DAX1 gene. Although for the present time only adrenal gland seems to be affected, pituitary and gonadal functions will be followed in the patient.

P2-d2-427 Adrenals and HPA Axis 2

Congenital adrenal hyperplasia, CAH, a cohort of 606 patients, 1915-2011

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Background: Treatment of Congenital adrenal hyperplasia, CAH, with hydrocortisone was first performed in the 1950’s. The diagnostics for the disease have changed drastically with the development of molecular genetics and the introduction of screening. Neonatal screening for CAH, has been performed in Sweden since 1986, more than 2.5 million infants have been screened.

Objective and hypotheses: To investigate the effect of screening on a large clinical cohort of identified CAH patients and to analyze the change in clinical presentation observed over time in relation to disease severity and CYP21A2 genotype.

Methods: 609 patients known from the screening, laboratory follow-up, or CYP21A2 genotyping were included in the study. The sex of the patients, the CYP21A2 genotype or disease severity when known were recorded.

Results: Twenty-one patients born before 1950 were identified. The oldest patient was born 1915, with V281L genotype. In the 40’s 18% of the patients had the SW form of CAH. Four males born in the 20’s and 40’s had 46,XX caryotype. An increase in the number of identified patients was seen after 1970. The sex ratio was 1.6 in the 1970’s. Since the introduction of neonatal screening 312 patients were diagnosed. Among the patients detected by the screening the sex ratio was close to 1. Late clinical diagnosis of non classic (NC) CAH in females accounted for the female preponderance (ratio 1.35) observed also during the period 1980-1999. In 404 patients the CYP21A2 genotype was known. 86% of the patients diagnosed after the start of the screening and more than 70% of the patients had known CYP21A2 genotype. In 15% of the patients the disease severity was not known. 15% had the NC form, 20% SV, and 50% CAH.

Conclusions: 50% of the patients had SW CAH, increasing to 75% at the end of the study period. The majority of patients with the NC form were women with V281L genotype and late diagnosis, which accounted for the higher sex ratio seen over time.

P2-d2-428 Adrenals and HPA Axis 2

Novel mutation of SCNN1B gene in a patient with pseudohypoaldosteronism causing intermittent resolution of salt loss accompanied by life threatening fluctuations in electrolytes

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Background: Pseudohypoaldosteronism (PHA) is characterized by renal resistance to the action of aldosterone. Multiple target organ defects (MTOD) is the most severe form of PHA caused by loss-of-function mutation in the α, β or γ subunits of the epithelial sodium channel (ENaC), resulting in defective sodium transport in many organs containing the ENaC. Affected patients develop life-threatening salt loss, hyperkalaemia, and acidosis due to end-organ resistance to aldosterone.

Objective and hypotheses: We present a severe neonatal case of MTOD-PHA with a novel homozygous mutation in SCNN1B gene. We describe a novel finding of intermittent resolution of severe salt loss during episodes of sepsis with accompanying hypernatraemia.

Methods: A female neonate presented with hyponatraemia (117mmol/l), hyperkalaemia (8.5mmol/l) and high urinary sodium (180mmol/l). Renin and Aldosterone were elevated confirming the diagnosis of PHA. Proband’s electrolytes were normalised on sodium supplements (45mmol/kg/day) and sodi-
Surgical management of congenital adrenal hyperplasia in girls: a population-based study

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Background: Despite the long-term influence of feminizing surgery in girls with CAH and several consensus conferences, little is known of when and which type of surgery is performed at a population level.

Objective and hypotheses: Describe the surgical management of girls with classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Methods: We analyzed the first surgical procedure performed in a national population-based cohort of 166 girls born from 1996 to 2003.

Results: Genetic testing revealed pathogenic homozygous donor splice site mutation in intron 12 of SCN1B gene: c.1542+1G>A. Mutation is anticipated to cause skipping of exon 12 and defective subunit of ENaC. We describe a novel mutation in SCN1B gene of ENaC. The genotype of intermeddant resolution with sepsis could be secondary to the novel genotype or increased mineralocorticoid sensitivity. Other hormones controlling water balance may have a role. Functional studies of the genotype and further hormonal studies during episodes of resolution may help in determining the cause.

Conclusions: Our study demonstrates a wide heterogeneity of surgical techniques performed by a large number of institutions throughout the country despite the rarity of the condition. Similar studies should be undertaken in other countries to allow the establishment of evidence-based guidelines for the surgical management of CAH.

P2-d3-430 Adrenals and HPA Axis 3

The use of serum steroids in monitoring therapy of congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Background: The management of children with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) remains difficult, and assessing the adequacy of treatment has always been a matter of great concern to the clinician.

Objective: To assess the utility of serum steroids measurement in monitoring the treatment of patients with CAH 21OHD, and determine the valuable steroid parameters for optimal hydrocortisone replacement therapy.

Methods: Nineteen-one Patients with CAH 21OHD aged (3.67±1.54) yrs treated with hydrocortisone and fludrocortisone replacement were followed in an intervals of (0.55±0.23) yrs over a period of (1.47±0.7) yrs. At each visit, bone ages were estimated, peripheral blood were collected to test serum E1 (estrone), E2 (estradiol), T (testosterone), P (progesterone), DHEA-S (dehydroepiandrosterone sulfate), 17OHP (17-hydroxyprogesterone), Δ4-A (androstenedione) and FT (free testosterone) concentrations. The patients were classified as being in good control (GC) or in poor control (PC) based on biological criteria and bone age (G-P) advancement (ΔBA/ΔCA). Comparisons were carried out between the serum steroid concentrations of the two groups. The receiver operating characteristic curve (ROC) was used to determine the cut-off value for diagnosing ‘Poor Control’.

Results: Each of serum E1, E2, Δ4-A and 17OHP levels was higher in PC group than GC group (P<0.05). ROC analysis showed that any of serum 17OHP or and Δ4-A level was of significance in diagnosing ‘Poor Control’, with the diagnostic efficacy being serum Δ4-A, serum 17OHP and serum Δ4-A in combination with 17OHP in descending order. Serum Δ4-A of 3.9 nmol/L has 77.8% of sensitivity and 75% of specificity in diagnosing ‘Poor Control’. Serum 17OHP of 7.1 ng/ml has 66.7% of sensitivity and 77.8% of specificity in diagnosing ‘Poor Control’.

Conclusions: Serum Δ4-A/17OHP level can be used as the biochemical indicators to monitor the treatment of CAH 21-OHD.

P2-d3-431 Adrenals and HPA Axis 3

Screening for hypothalamic-pituitary-adrenal axis suppression in asthmatic children on corticosteroids is not possible when employing clinical and static biochemical parameters

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Background: It is impractical to test all asthmatic children for hypothalamic-pituitary-adrenal axis suppression (HPAS) with dynamic adrenal function tests.

Objective: To determine which parameter is the most useful screening test for HPAS in asthmatic children.

Methods: 143 asthmatic children, 5-18 years old, treated with corticosteroids were recruited. Height velocity (HV), weight velocity (WW), height standard deviation score (SDS), weight SDS, change in systolic blood pressure from...
Conclusions: revealed normal BP rhythm. According to the BP monitoring data the HCT dose was reduced/
cases the nocturnal dip was reduced and the dip episodes during the day were
between urinary cortisol level and 24-hour BP measurement parameters. In 6
and SBP load >95 centile, SBP load >90 centile, and SBP load >75 centile (SBP), day mean SBP, maximal SBP (r=0.73; 0.8; 0.68 respectively p<0.05); 
Results:

<table>
<thead>
<tr>
<th>Screening Variable</th>
<th>ACTH</th>
<th>11DOC</th>
<th>11DOC/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height SDS</td>
<td>0.12</td>
<td>0.186</td>
<td>-0.13</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.10</td>
<td>0.262</td>
<td>-0.01</td>
</tr>
<tr>
<td>HV SDS</td>
<td>0</td>
<td>0.999</td>
<td>0.07</td>
</tr>
<tr>
<td>WV SDS</td>
<td>-0.04</td>
<td>0.638</td>
<td>0.07</td>
</tr>
<tr>
<td>LSPB</td>
<td>0</td>
<td>0.992</td>
<td>0.05</td>
</tr>
<tr>
<td>cortisol</td>
<td>0.05</td>
<td>0.538</td>
<td>0.08</td>
</tr>
<tr>
<td>ACTH</td>
<td>0.10</td>
<td>0.248</td>
<td>0.04</td>
</tr>
<tr>
<td>DHEAS</td>
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<td>0.025</td>
<td>0.21</td>
</tr>
<tr>
<td>UFC (mmol/l)</td>
<td>0.08</td>
<td>0.373</td>
<td>0.19</td>
</tr>
<tr>
<td>UFC (mmol/lCr)</td>
<td>0.08</td>
<td>0.397</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The area under the receiver operating characteristics (ROC) curve for DHEAS in boys at 10-14 yrs (n=37) is the highest with 76 %.
The screening performance at 2.0 µmol/l: sensitivity 100 (95%CI 63.1-100.0) %, specificity 38 (95%CI 17.9-54.3) %, accuracy 51 (95%CI 31.9-65.6) %, positive likelihood ratio (LR) 1.6 (95%CI 1.4-2.4), negative LR 0.0 (95%CI 0.0-4.5).

Conclusions: No useful screening test for utilization at all ages could be identified.

P2-d3-433 Adrenals and HPA Axis 3
24-hour blood pressure monitoring: might be useful in the individualization of the hydrocortisone supplementation?

Background: Patients on lifelong HCT supplementation are at risk of long-term cardiovascular complications due to glucocorticoid exposure, while even slight disruptions in the cortisol diurnal rhythm may affect the blood pressure (BP) profile.

Objective and hypotheses: (1) to evaluate daily BP profile in AI patients on HCT supplementation, and its correlation with blood and urine cortisol level, (2) to attempt the use of 24-hours ambulatory BP monitoring for individualization the HCT supplementation.

Methods: The pilot study involved 15 (8 girls) AI patients (14 secondary AI due to combined pituitary hormone deficiency; 2 primary AI: congenital adrenal hyperplasia and after bilateral adrenalectomy due to primary pigmented nodular adrenal disease) at the mean age of 12.1 (4.96-17) years. 24-hour BP monitoring was performed using Ambulatory BP Monitor (Spacelabs90217,USA). It was set to take a reading every 15 minutes (day-6AM-10:59PM), and every 30 minutes (night). Cortisol level was assessed in blood samples (every 6 hours), and in 24-hour sample of urine.

Results: There were significant correlations between mean blood cortisol level and day mean arterial pressure (MAP) (r=0.76, p<0.05); mean systolic BP (SBP), day mean SBP, maximal SBP (r=0.73; 0.8; 0.68 respectively p<0.05); and SBP load >95 centile, SBP load >90 centile, and SBP load >75 centile for height (r=0.71; 0.7; 0.73 respectively p<0.05). There was no correlation between urinary cortisol level and 24-hour BP measurement parameters. In 6 cases the nocturnal dip was reduced and the dip episodes during the day were noticed. According to the BP monitoring data the HCT dose was reduced increased up to 20%, the hours of administration were changed. Re-evaluation revealed normal BP rhythm.

Conclusions: In patients on HCT supplementation MAP and SBP are correlated with blood cortisol level. The 24-hour BP monitoring may be used for the adjustment of the HCT supplementation in AI patients.

P2-d3-433 Adrenals and HPA Axis 3
A rare association: neonatal ovarian cyst and 21-hydroxylase deficiency

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Background: Neonatal ovarian cysts are rare in 21-hydroxylase deficiency (21OHD).

Objective and hypotheses: We describe a patient with classic form of 21OHD and endometrial bleeding at 2 months due to ovarian cyst.

Results: At 26 weeks of gestation, because of citoris enlargement in a 46 XX fetus, the diagnosis of classic form of 21OHD has been done and confirmed by molecular studies of CYP21A2 gene (homogygous for IVS2-13A>C-G). Because of late diagnosis, the mother was not treated with dexamethasone. At birth, the neonate had a male phenotype (Prader V), and high levels of testosterone (16 ng/ml), 17OH-progesterone (78 ng/ml) and renine (110 µl/l). Hydrocortisone and salt were given on day 2, then fludrocortisone on day 8. At 1 month, testosterone level was 0.5 ng/ml. At 2 months, because of vaginal bleeding and breast development, pelvic ultrasound was performed and showed enlarged uterus of 4 cm and a giant cyst of 6 cm diameter on left ovary. To prevent ovarian torsion, she underwent cystectomy. Estradiol level in cyst fluid was very high. Bloodling stopped 2 weeks following surgery. At 3 months, she still had breast buds; pelvic ultrasound showed a small cyst of 2 cm on right ovary, normal left ovary and enlarged uterus. Hormonal measurements showed normal levels of testosterone (0.1 ng/ml), LH (2.0 mIU/ml) and FSH (3.1 mIU/ml), and high estradiol level (365 pg/ml). At 4 months, we performed GnRH test: baseline LH 1.0 mIU/ml, peak LH 17.0 mIU/ml, and baseline FSH 10.0 mIU/ml, peak FSH 48.6 mIU/ml. Estradiol was normal (<30 pg/ml).

Conclusions: High steroid levels in neonatal period act as positive feedback on gonadotrop axis. Besides we speculate that glucocorticoid treatment mediated androgen suppression led to a marked rise in gonadotropin levels that favoured follicular growth and estrogen secretion. Interestingly, this severe virilised female neonate had higher FSH than LH levels when compared to usual higher LH levels in infant males, keeping the gender dichotomy in gonadotropin secretion.

P2-d3-434 Adrenals and HPA Axis 3
A review of patients with congenital adrenal hyperplasia

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Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder stemming from one of the enzymatic defects in cortisol biosynthesis from cholesterol. In the majority of instances the disorder comprises deficiency of 21-hydroxylase (21-OHD). This defect causes excessive androgen production from adrenal source, which leads to virilization of varying severity (Prader grade 1- 5) in female fetus.

Objective and hypotheses: To find out frequency of different types of congenital adrenal hyperplasia, rate of consanguinity, family occurrence, birth weight, final height and weight.

Methods: Medical records of patients with CAH between 1968 and 2011 in 3 endocrine centers were reviewed.

Results: Out of 617 patients, 39.6% had 21-hydroxylase deficiency (21-OHD). In 21-OHD group 73.7% had salt wasting, 20.8% simple virilizing (SV) and 5.5% non classic type. Frequency of other types were as follow:11-hydroxylase deficiency (11-OHD), 13.3%; 3—βhydroxysteroid dehydrogenase deficiency (3—βOHDD), 4%; lipoid adrenal hyperplasia, 1%; 17-hydroxylase deficiency, 0.6% and Antley-Bixler, 0.3%. Parental consanguinity was present in 62.6% and familial occurrence was reported in 42.6% of the patients. Sixteen girls had grade 5 virilization of Prader staging. The most prevalent Prader stage was 4 in 21-OHD-SW and 11-OHD. In
21-OHD-SV, 9 patients had Prader 4 & 5 virilization. The difference between midparental height and final height SDS was lowest in 21-OHD-SV (1.2 ± 1.1). Birth weight of all the patients was normal except 5 patients with 21-OHD-SW, 2 with 3—90HD and 2 with 21-OHD-NC that the mean ± SD was 1.2 — 2.25 kg. All the patients were assigned as their genetic gender except 5 patients due to delayed diagnosis or resentment of the parents. 

Conclusions: The prevalence of different types of CAH, grade of virilization, final weight and height and birth weight have detected among referral patients.

P2-d3-435 Adrenals and HPA Axis 3

DAX-1 (NROB1) mutations in four patients affected by adrenal hypoplasia congenita

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Background: X-linked Adrenal Hypoplasia Congenita (AHC) is an inherited disorder of adrenal gland development which is caused by mutations in DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) (NROB1) gene located on X-chromosome. DAX-1 (OMIM*300473) encodes for an orphan nuclear hormone receptor that functions as a transcription factor regulating expression of other genes.

Patients and methods: Four boys in a life-threatening state came to our attention and were suspected for AHC. Laboratory studies at diagnosis, in neonatal period, showed in all hypernatremia and hyperkalemia. Severely low serum cortisol levels and high plasma ACTH levels confirmed primary adrenal insufficiency. The hydrocortisone therapy with saline and glucose infusions were started immediately. In each of these patients, two exons of the DAX-1 gene were directly sequenced after polymerase chain reaction amplification of the entire coding region.

Results: Molecular analysis of the DAX-1 gene coding region and the adjacent splicing sites revealed one novel mutation (patient 1) and two already known mutations (patients 2, 3 and 4). In patient 1 we found a novel mutation c.T1118>G (p.Ile373Ser) in exon 1. This mutation was also found in the patient’s mother. In patient 2 and 3 a known mutation c.A1319>T (p.Asn440Ile) in exon 2 was identified. Both patients’ mothers were not available for the genetic testing. Both mutations are located in ligand-binding domain (LBD) that functions as a transcriptional repression domain. In patient 4 a known mutation c.C109>T (p.Glu37X) in exon 1 was found, that resulted in a premature stop codon generation destroying the N-terminal domain of DAX-1. This mutation was also found in the patient’s mother.

Conclusions: We show that molecular analysis of DAX-1 gene is very important for the confirmation of clinical diagnosis of adrenal insufficiency and highlights the role of further genetic counselling in families with AHC patients.

P2-d3-436 Adrenals and HPA Axis 3

Premature adrenarche: no difference between sexes with respect to metabolic complications and pubertal timing in children born appropriate for gestational age

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Background: Premature adrenarche (PA) refers to the isolated development of pubic and/or axillary hair under the age of 8 years in a girl and 9 years in a boy. In girls, PA has been associated with obesity, decreased insulin sensitivity, coronary arterial disease and polycystic ovary syndrome (PCOS) in adulthood. Data about PA in boys with respect to metabolic complications and pubertal timing are scarce and conflicting.

Objective and hypotheses: To determine whether premature adrenarche (PA) has a different impact on metabolic issues and pubertal timing in boys and girls born appropriate for gestational age (AGA).

Methods: Growth, puberty and metabolic work-up of 47 girls and 23 boys with PA born AGA followed-up from our outpatient endocrinology clinic between 2000-2009 were reviewed.

Results: Initial anthropometric measurements except for body mass index (BMI) standard deviation score (SDS) were similar in younger than in boys than girls (p = 0.01), bone age (BA) SDS, homeostasis model assessment-insulin resistance (HOMA-IR) and fasting plasma glucose / insulin ratio (FIRG) and plasma lipids were similar between sexes. Hormone levels except for significantly higher dehydroepiandrosterone sulfate (DHEA-S) levels in boys than girls (p<0.0006) were also similar between the sexes. BA SDS and BA / CA were significantly advanced (p<0.05) with respect to initial evaluation in 28 girls at onset of gonadarche unlike the case in 9 boys with PA (p>0.05).

Conclusions: PA in children born AGA does not herald any significant differences with respect to metabolic complications between sexes, and it appears to be a discrete process from onset of puberty in girls unlike boys, in whom it is likely a variant of normal puberty.

P2-d3-437 Adrenals and HPA Axis 3

Decline of cortisol levels over time before the first dose of intravenous immunoglobulin in patients with Kawasaki disease

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Objective: Cortisol is a stress hormone and is secreted in response to stress or inflammatory insults as a normal counter regulatory hormone. However, little is known about the changes in cortisol levels in Kawasaki disease (KD) before the first intravenous immunoglobulin (IVIG) treatment. The aim of this study was to investigate changes in cortisol levels before the first IVIG treatment, and to compare cortisol levels between responders and non-responders to initial IVIG.

Methods: Blood samples were obtained in 46 children (23 females) with KD before their first IVIG treatment. Serum cortisol levels, white blood cell (WBC) counts and C-reactive protein (CRP) levels were measured.

Results: Seventy seven blood samples were obtained from 46 patients before their first IVIG treatment. The day of illness was negatively correlated with the cortisol level (r=-0.47, P<0.001), but not with the WBC count or CRP level. Cortisol levels before IVIG treatment were higher in non-responders than in responders.

Conclusions: Cortisol levels in patients with KD decreased over time, despite the persistence of elevated WBC and CRP, before initial IVIG treatment. Cortisol levels were higher in non-responders than in responders to initial IVIG.

P2-d3-438 Adrenals and HPA Axis 3

Molecular characterization of 21-hydroxylase deficiency in patients with the congenital adrenal hyperplasia (CAH) in two Balkan countries

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Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused in 95% of cases by 21-hydroxylase deficiency. Molecular characterization of CYP21A2 gene mutations in many countries revealed specific mutations associated with different forms of the disease. No data are available for the Balkan region.

Objective and hypotheses: The aim of this study was molecular characterization of CAH in Macedonian and Serbian patients with all three forms of CAH and comparison with findings in other countries.

Methods: Methods used for the molecular characterization included: differential polymerase chain reaction (PCR), amplification created restriction site (ACRS), restriction endonuclease digestion, agarose and polyacrylamide gel electrophoresis.

Results: We studied 71 patients with 21-hydroxylase deficiency (53 Macedo-
Background: PCOS is a heterogeneous group of disorders. Recently some monogenic disorders were recognized to present as PCOS: NC-CAH forms, mutations in WNT 1 and lamin A/C gene, primary insulin receptors defects, lipodystrophies, as well as glucocorticoid(GC) resistance with mutations in the GC receptor. We hypothesized that using a candidate gene approach; new monogenic reasons for PCOS can be found in the GC sign pathway.

Objective: To study the role of GC signaling proteins in the etiology of PCOS. GC signaling was evaluated by GC sensitivity in vitro by F-Dex binding assays and by sequencing 3 genes from GC pathway: Glucocorticoid receptor gene (NR3C1) and 2 co-receptor protein genes FKBP4 and FKBP5.

Methods: Recruited 25 controls and 28 PCOS pts. Pts had ACTH stim test, 21-OH gene analysis, OGTT, lipid profile. GC sensitivity was evaluated by F-Dex binding assays. GC index was calculated as log of AUC from the difference in binding to the GC receptor between control and PCOS pts. NR3C1, FKBP4, FKBP5 genes were sequenced.

Results: Using the F-Dex binding assay, we found a cohort of PCOS patients that were GC resistant, with dec binding of F-Dex molecules to the GC receptor in comparison to controls( fig 1). GC receptor number was found to be no different from the control patients. From these results, we were able to calculate a glucocorticoid index(GI). We found that the degree of resistance, as calculated by this index, correlated with biochemical data such as degree of elevation of testosterone and other androgens, as well as pt phenotype.

Also, we found that this also correlated with the elevated stimulated ratios of delta 5/cortisol, DHEA/ delta 4, as well as DHEA/Testo. Mutational analysis is pending.

Conclusions: It was found that the degree of GC resistance, as calculated by the GI, correlates well with phenotype and biochemical data for these pts. This indicates that the cause of PCOS of this subgroup was due to a candidate gene in the GC receptor signaling pathway.

P2-d3-440 Adrenals and HPA Axis 3

Monogenic approach to PCOS

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Background: PCOS is a heterogeneous group of disorders. Recently some monogenic disorders were recognized to present as PCOS: NC-CAH forms, mutations in WNT 1 and lamin A/C gene, primary insulin receptors defects, lipodystrophies, as well as glucocorticoid(GC) resistance with mutations in the GC receptor. We hypothesized that using a candidate gene approach; new monogenic reasons for PCOS can be found in the GC sign pathway.

Objective: To study the role of GC signaling proteins in the etiology of PCOS. GC signaling was evaluated by GC sensitivity in vitro by F-Dex binding assays and by sequencing 3 genes from GC pathway: Glucocorticoid receptor gene (NR3C1) and 2 co-receptor protein genes FKBP4 and FKBP5.

Methods: Recruited 25 controls and 28 PCOS pts. Pts had ACTH stim test, 21-OH gene analysis, OGTT, lipid profile. GC sensitivity was evaluated by F-Dex binding assays. GC index was calculated as log of AUC from the difference in binding to the GC receptor between control and PCOS pts. NR3C1, FKBP4, FKBP5 genes were sequenced.

Results: Using the F-Dex binding assay, we found a cohort of PCOS patients that were GC resistant, with dec binding of F-Dex molecules to the GC receptor in comparison to controls( fig 1). GC receptor number was found to be no different from the control patients. From these results, we were able to calculate a glucocorticoid index(GI). We found that the degree of resistance, as calculated by this index, correlated with biochemical data such as degree of elevation of testosterone and other androgens, as well as pt phenotype.

Also, we found that this also correlated with the elevated stimulated ratios of delta 5/cortisol, DHEA/ delta 4, as well as DHEA/Testo. Mutational analysis is pending.

Conclusions: It was found that the degree of GC resistance, as calculated by the GI, correlates well with phenotype and biochemical data for these pts. This indicates that the cause of PCOS of this subgroup was due to a candidate gene in the GC receptor signaling pathway.

P2-d3-441 Adrenals and HPA Axis 3

Influence of ethnicity on the origin and clinical and hormonal characteristics of premature pubarche

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Background: Smallness for gestation, prematurity and obesity are known risk factors for premature pubarche (PP), whereas PP can be a forerunner of polycystic ovary syndrome in girls.
Objective: We wanted to analyze 1) whether Magreb girls with PP are at higher risk for exaggerated adrenarche and higher serum AMH concentrations, given their known risk for increased adiposity and insulin resistance, and 2) whether the presence of acne was associated with a more severe form of adrenarche.

Patients and methods: 27 (6 boys and 21 girls) children evaluated for PP in 2010 and 2011 at our hospital, in whom a non-classical form of congenital adrenal hyperplasia by ACTH testing and an adrenal tumor by ultrasound were excluded, were studied. Anthropometric data, basal and ACTH stimulated serum androgens, serum SHBG as marker of insulin resistance, serum AMH and bone age readings at the left hand and wrist were collected.

Results: Seven of the 11 Magreb children and 13 of the 16 Belgian girls who were investigated, had a premature adrenarche (PA), defined by a basal DHEAS > 6.0 mg/L. Mean gestational age, birth weight SDS, BMI SDS, serum AMH and SHBG concentrations were not different between the Magreb and Belgian girls with PA. Degree of pubic hair development, bone age advancement and the prevalence of acne were also similar. Children with associated acne (n=11) had similar basal serum DHEAS and bone age advancement.

Conclusion: Magreb girls with PP have a comparable frequency of premature adrenarche. No correlation between PA, insulin resistance and potential later risk for polycystic ovary syndrome was found. Serum AMH did not differentiate girls with PA from an idiopathic form of PP. Associated acne does not necessarily indicate an exaggerated form of PP.

P2-d3-442 Adrenals and HPA Axis 3
Phaeochromocytoma- clinical presentation of three cases
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Background: Classically phaeochromocytoma presents with headaches, palpitations, and diaphoresis in association with severe hypertension.

Objective and hypotheses: To present the heterogeneity of the clinical presentation of phaeochromocytoma in children.

Methods: We present 3 cases of phaeochromocytoma diagnosed in our department between 2007-2011.

Results: Case 1: a 5.8 years-old boy presented with headache and severe hypertension. High urinary levels of norepinephrine and normetanephrine were found. The abdominal magnetic resonance (MRI) showed bilateral adrenal masses. Right adrenalectomy and left sympathetic ganglionectomy were performed. An inactivating mutation in the exon 3 of VHL gene was found. Case 2: a 10.5 years-old girl with a retinal hemangioblastoma. The physical examination was normal. Von Hippel Lindau disease was suspected and an abdominal CT scan was performed as a screening and showed an adrenal mass. Urinary levels of norepinephrine and normetanephrine were elevated. MIBG scan showed a left adrenal mass while MRI showed bilateral adrenal masses. Right adrenalectomy and left tumorectomy were performed, then left adrenalectomy was performed. An inactivating mutation in the exon 2 of VHL gene was found in the index case, his mother and one of his brothers. Case 3: a 15-year-old patient presented with transient and acute left lumbar pain. His clinical examination including blood pressure was normal. An abdominal ultrasonography showed a left adrenal mass. The urinary norepinephrine and normetanephrine were high. The MIBG scintigraphy and the Ga 68 DOTATATE PET scan showed uptake in the left adrenal mass. Left adrenalectomy was performed.

Conclusions: Phaeochromocytoma can be nearly asymptomatic even in the presence of very high levels of catecholamine production.

P2-d3-444 Adrenals and HPA Axis 3
Molecular mechanisms of action of a novel point mutation in the human glucocorticoid receptor gene causing primary generalized glucocorticoid resistance
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Background: Primary Generalized Glucocorticoid Resistance (PGGR) is a rare genetic disorder characterized by partial, generalized target-tissue insensitivity to glucocorticoids. The molecular basis of the condition has been ascribed to mutations in the human glucocorticoid receptor (hGR) gene, which impair the molecular mechanisms of hGR action and decrease tissue sensitivity to glucocorticoids. We recently identified a novel case of PGGR caused by a novel mutation in the hGR gene.

Objective and hypotheses: To present the findings of the genetic testing of the above patient and the molecular mechanisms through which the natural novel hGR mutant impairs glucocorticoid signal transduction.

Methods and results: DNA was extracted from peripheral lymphocytes and the entire coding region of the hGR gene was amplified and sequenced. A single, heterozygous nucleotide (A to G) substitution was identified at nucleotide position 2177 (exon 8), resulting in histidine (H) to arginine (A) substitution at amino acid position 726 in the ligand-binding domain (LBD) of the receptor. In transient transfection assays, the mutant receptor hGRh726R demonstrated decreased ability to transactivate the MMTV promoter in response after stimulation (peak <500 nMol/L) in case n. and at the lower limit of the standard (555 nMol/L) in case 2 (table). These 2 pts started hydrocortisone replacement therapy (8 mg/m2/day) with prompt clinical improvement.

Conclusions: Our study shows that central AI could arise during follow-up of childhood ALL, even treated without cranial radio-prophylaxis. It is however not possible, given the small number of cases, to determine the risk factors and predict the time of onset of central AI in these pts. In our study 1 microgram ACTH test confirmed the suspicion of central AI in symptomatic cases.

P2-d3-443 Adrenals and HPA Axis 3
Central adrenal insufficiency (AI) after childhood acute lymphoblastic leukemia (ALL)
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Background: In patients with ALL adrenal insufficiency can arise in the induction phase, due to the high dose corticosteroid therapy, but rarely has been reported during post treatment follow-up.

Objective and hypotheses: We investigated the adrenal function of 7 patients (pts) with a suspected AI arisen during follow-up after treatment for childhood ALL.

Methods: We evaluated pituitary adrenal axis of a cohort of 56 consecutive pts in follow-up (3.6 ± 1.6 years) after childhood ALL (age at diagnosis 5.6 ± 3.4 years; protocol AIEOP ALL 2000, 10/56 treated with cranial radio-prophylaxis). 7.56 pts showed biochemical and clinical (2/7 pts) signs of suspected central AI (table) at median time of 2.4 years after treatment (range 1.2-5.5 years). All pts did not show signs of AI before and immediately after ALL treatment. In pts with suspected central AI, 1 microgram ACTH test was performed.

Results: In 5/7 cases the ACTH test ruled out AI. In the two symptomatic cases, reduced cortisol basal levels were confirmed with inadequate cortisol response after stimulation (peak <500 nMol/L in case n. 6 and at the lower limit of the standard (555 nMol/L) in case 2 (table). These 2 pts started hydrocortisone replacement therapy (8 mg/m2/day) with prompt clinical improvement.

Conclusions: In patients with ALL adrenal insufficiency can arise in the induction phase, due to the high dose corticosteroid therapy, but rarely has been reported during post treatment follow-up.
Conclusions: Mutations in the hGR gene impair glucocorticoid signal transduction and alter tissue sensitivity to glucocorticoids. The location of this mutation in the LBD of the receptor may predict impaired affinity for the ligand, while manifestation of the disease at the heterozygote state may indicate a dominant negative effect of the mutant GR upon the wild-type receptor.

Methods: Case records of all children diagnosed between January 1st 2000 and December 31st 2010 were reviewed. Diagnosis was confirmed by raised serum 17OHP progesterone level or characteristic urinary steroid profile. Clinical features, biochemical findings, treatment and outcome of all cases were reviewed.

Results: Forty two children (Male=21) were diagnosed over the decade studied [average 3.8 cases annually, range 1-6/year]. Fourteen of 42 [33%] were diagnosed in the first seven days of life which would precede a screening result [8 females with virilised genitaila, 4 males]. A further 15/42 children [14 male, 1 female] presented with salt losing crisis after the first week of life. The average incidence of CAH was calculated as 1 per 26,000 births which is an incidence of 0.44 per 100,000 population less than 14 years old.

Conclusions: Two thirds of children presenting with CAH in the Republic of Ireland between 2000 and 2010 would have been identified earlier through a newborn screening programme. The incidence of CAH is comparable to countries where newborn screening has been implemented successfully.

P2-d1-447 Autoimmune Endocrine Disease 2

Low-dose cyclosporine A in a patient with severe APS1-associated keratoconjunctivitis

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Background: Autoimmune Polyendocrine Syndrome type 1 (APS1, OMIM 240300) is a rare AR disorder caused by mutations in the AIRE gene, primarily involving the endocrine glands. The patients manifest also mucocutaneous candidiasis (CMC) and ectodermal dystrophies. Chronic keratoconjunctivitis is the most commonly observed ophthalmic finding in APS1 (25%-50%), its pathogenesis is unclear, appropriate intensive management is required. Corneal transplants and keratoplasty have little success, medical treatment often fails in severe keratoconjunctivitis/progressive forms.

Objective: To describe a case with APS1 with bilateral chronic keratoconjunctivitis, who developed severe photophobia and corneal ulceration, which was successfully treated with cyclosporine A orally.

Patient and methods: The patient, a 35-year-old male, was diagnosed in infancy with APS1 (CMC, Addison’s disease, hypoparathyroidism). Over time, he developed additional manifestations and severe phenotype (Table). Disabling chronic keratopathy appeared since 17 yr of age with dry eye, photophobia, decreased vision, corneal ulceration. Conservative management with lubricants and artificial tears, associated with topical corticosteroids, antibiotics and ophthalmic agents in the acute phase of keratitis did not prevent the progression of the ulcerative process. Addition of low-dose cyclosporine A orally (5 mg/kg/day), in a single dose for 4 months, led to arrest of the ulcerative process, reduce corneal edema and greatly improve photophobia. Minimum short-term side effects were noted (slight increase in creatinine, hypomagnesemia).

Results and conclusions: The benefit of systemic cyclosporine A in the short treatment of keratoconjunctivitis has been reported in isolated cases with APS1. Our experience confirms efficacy of immunosuppressive therapy and supports the use of oral cyclosporine A in selected patients with severe APS1-associated keratoconjunctivitis. Further studies are needed to confirm our preliminary findings.
P2-d1-448 Autoimmune Endocrine Disease 2

Clinical characteristics of patients with autoimmune polyglandular syndrome type 1 (APS 1)

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Background: Autoimmune Polyglandular Syndrome type 1 (APS 1) is a rare autoimmune-recessive disease caused by mutations in the autoimmune regulator (AIRE) gene. Although typical manifestations of APS 1 include candidiasis, hypoparathyroidism and Addison’s disease, other autoimmune conditions can be associated with the syndrome.

Objective: To present clinical course of APS 1 in patients from Caucasian population diagnosed at pediatric age. Patients: 8 patients (4 girls, 4 boys), currently aged 10-26 years, diagnosed in one department of endocrinology between year 2000 and 2010. Mean age of boys and girls is 21 and 15 years respectively. The diagnosis of APS 1 was made on the basis of presence of at least two cardinal features of the disease.

Results: In every patient mucocutaneous candidiasis and hypoparathyroidism were found. So far adenocortical failure was diagnosed in 4 cases. The disease manifested the earliest at the age of 15 months as the mucocutaneous candidiasis. The highest number of disturbances (6 in total) were diagnosed in 1 patient. In 2 patients 5 disturbances, in 2 patients 4 abnormalities, in 2 patients 3 components and in 1 patient 2 disturbances were diagnosed. Among rare abnormalities megaloblastic anemia, keratitis, asplenia, and central diabetes insipidus were found. In 1 boy profound short stature of undetermined etiology was noticed. In the studied patients the presence of the following autoantibodies: against 21-hydroxylase, against LKM (liver-kidney microsomes), against GAD65 and against IA2 were found. In every patient molecular analysis of AIRE gene was performed. The most common Finnish R257X mutation was found in 5 patients.

Conclusions: APS 1 is a rare syndrome in Caucasian pediatric population. In the studied patients many components of the syndrome manifested at early age. Patients with APS 1 require constant and regular specialist care with knowledge that new components of the APS 1 may appear over the years.

P2-d1-449 Autoimmune Endocrine Disease 2

Interruption of insulin treatment after anti-CD20 therapy in a boy with type 1 diabetes, thyroiditis and immune thrombocytopenia

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Background: Type 1 diabetes (T1D) is a component of autoimmune polyglandular syndrome (APS). No cure for T1D exists but immune modulatory approaches are under investigation. Specific antibody treatment targeting CD20 expressed at the surface of B-lymphocytes would increase C-peptide level and reduce daily insulin dose.

Objective and hypotheses: We report a case with APS type 3 associating thyroiditis, immune thrombocytopenia (ITP) and T1D where insulin therapy was stopped for 28 months after treatment with anti-CD20.

Results: ITP and thyroiditis were diagnosed in a 13-year-old Caucasian boy. L-T4 therapy was started. ITP was resistant to immunoglobulins and dependent on high dose steroids. Under such therapy, the patient developed T1D (Glycemia 22mmol/l, insulin 36.2U/l, HbA1c: 6.1 %, anti-GAD 3530E/l (>|9.5)) and insulin treatment was initiated. Because of persistent severe thrombocytopenia even under Cyclosporine treatment, anti-CD20 therapy was introduced for 2 months, allowing stabilization of thrombocytes >50G/l and stopping steroids 7 months later. Three and five months after anti-CD20 therapy, L-T4 and insulin could be stopped, respectively. Twelve months later normal C-peptide level, HbA1c (5.6%) and reduced anti-GAD (672E/l) were measured. A second anti-CD20 trial for relapse of ITP, thyroid-
Autoimmune Endocrine Disease 2

Multiorgan autoimmunity in a Turner syndrome patient with partial monosomy 2q and trisomy 10p
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Background: Turner syndrome (TS) is a condition caused by numerical and structural abnormalities of the X chromosome, characterized by a series of clinical features, the most common of which are short stature and gonadal dysgenesis. An increased frequency of autoimmune diseases as well as an elevated incidence of autoantibodies has been observed in TS.

Objective and hypothesis: What follows is a unique case of TS with chromosomal aneuploidy and rearrangements on chromosome 2 and chromosome 10 ([45, X]/46XX del2(q37.1), dup10 (p12.32)]. The patient presented partial empty sella and a coarctation of aorta with a cohort of multiorgan autoimmunity-related manifestations including Hashimoto’s thyroiditis, celiac disease, insulin dependent diabetes mellitus (Type 1 diabetes), alopecia, onychoodystrophy, possible autoimmune inner ear disease with sensorineural deficit, preclinical hyposurrealism. We aimed to investigate the genetic background that could be responsible for the complex association of multiorgan autoimmune manifestations in this patient.

Method: Screening of the autoimmune regulator gene (AIRE) and of protein tyrosine phosphatase non receptor type 22 (PTPN22) gene was performed on patient’s DNA. SNP-array analysis was conducted to verify in which extent autoimmunity-related genes could have been affected by the peculiar TS karyotype and identify genome-phenotype correlations.

Results: AIRE gene screening revealed heterozygous c.834 C>G polymorphism and IVS9+6 G>A variation, thus excluding autoimmune polyendocrine syndrome Type 1. Heterozygous R626W polymorphism of the PTPN22 gene was detected in patient’s DNA. SNP-array analysis revealed that autoimmunity-related genes could be affected by the partial monosomy 2q and trisomy 10p including IL2 receptor/CD25.

Conclusion: These data suggest that early genetic analysis is recommended in TS patients with complex associations of multiorgan autoimmune manifestations for their diagnostic classification as well as indicator of undiscovered pathogenetic mechanisms.

Autoimmune Endocrine Disease 2

Vitamin D status in children with Hashimoto’s thyroiditis
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Background: Vitamin D is involved in immune system and, in particular, on T cell-mediated immunity. Vitamin D receptor is profoundly present in the immature immune cells of thymus and the CD8. T cell-mediated immunity. Vitamin D receptor is profoundly present in the immature immune cells of thymus and the CD8.

Objective: To investigate vitamin D status in children with Hashimoto’s thyroiditis.

Subjects and methods: The study group consisted of 78 children with newly diagnosed Hashimoto’s thyroiditis and 74 subjects as the control group. Parameters of calcium metabolism, thyroid function tests and 25(OH) vitamin D levels were measured.

Results: Vitamin D deficiency rate was significantly higher in the Hashimoto group compared with the control subjects (73.1% vs 17.6%, p<0.0001). In the Hashimoto group, mean 25(OH) vitamin D levels were significantly lower compared with the control group (12.5±7.0 vs 22.3±7.9 ng/mL, p<0.001) and was inversely correlated with the AntiTPO levels (r = -0.30, p = 0.007).

Conclusion: The higher vitamin D deficiency rates besides lower vitamin D levels in the Hashimoto group together with the inverse correlation between vitamin D and AntiTPO suggest that vitamin D deficiency may have a role in the autoimmun process in Hashimoto’s thyroiditis in children.
ACTH 380 ng/l; aldosterone 10 pg/ml; antibodies against adrenal capsule positive; TSH 6.5; FT4 1.2; anti-thyroglobulin antibodies +; anti-GAD and anti-IgA + without clinical diabetes. Suspected type II polyglandular A-I syn-
drome. Conclusion: We remark on the vague symptomatology of the cases of-
ten leads to late and mistaken diagnostic approaches. Hyperpyrexia is a
common sign in these patients to suspect the disease. Addison’s disease
involves long-term monitoring given the association of other endocrine dis-

eases.

P2-d1-455 Autoimmune Endocrine Disease 2

Autoimmune polyendocrine syndrome type 1 due to homozygous missense mutation in autoimmune regulator gene AIRE in a consanguineous Saudi family

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Background: Autoimmune polyendocrine syndrome type 1 (APS-I) or auto-
imune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) is a rare autosomal recessive disease caused by mutations of the Autoimmune Regulator (AIRE) gene. Affected individuals typically have chronic mucocu-
taneous candidiasis, primary hypoparathyroidism, and primary adrenocortical insufficiency (Addison’s disease). Other autoimmune diseases such as auto-
immune hepatitis, diabetes mellitus, keratitis, and pernicious anaemia occur less frequently. The causative AIRE gene has important role in induction and
tolerance to self antigens

Objective and hypotheses: The objective of this study was to describe the
phenotypic features of APS-I in a consanguineous Saudi family and correlate it to their mutation in the AIRE gene

Methods: Clinical data were obtained from medical records of a Saudi fam-
ily with clinical and biochemical features of APS-I, DNA sequencing of the
AIRE gene was done for all family members

Results: Seven out of eight children fulfill the diagnostic criteria of APS-I, one sibling was disease free. The phenotypic presentation varies significantly
despite the fact that they all are pediatric and carries the same genetic muta-
tion. Both parents were clinically asymptomatic. Analysis of AIRE gene dem-
strated that the affected family member are homozygous for pathogenic
missense mutation (c.202A>G (p.T68A)). Subsequent molecular analysis of
the parents showed that they are heterozygous for (c.202A>G (p.T68A)).

Conclusions: Autoimmune polyendocrine syndrome type 1 (APS-I) is a rare
monogenic disease. Clinical presentation may vary among sibling with same
genetic mutation. Genetic counseling is highly recommended, since no spe-
cific therapy available

P2-d1-456 Autoimmune Endocrine Disease 2

A new mutation in autoimmune polyendocrine syndrome type 1

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Background: Autoimmune polyendocrine syndrome type 1 (APS-1) APECED is an autosomal recessive disorder that causes progressive endo-
crine tissue destruction, cell-mediated immune deficiency and ectodermal dystrophies. The disabling ocular manifestations include chronic persistent
keratoconjunctivitis, dry eye, tricycletis, retinal detachment , optic atrophy
and rarely cataract. We report a new AIRE gene mutation in a patient with
hypoparathyroidism, mucocutaneous candidiasis, ectodermal dysplasia, perni-
cious anaemia and cataract.

Case: A 9 years and 10 months of age female patient admitted because of alo-
pecia. In physical examination, weight 25.5kg(25–50 p), height 130.2cm(25–50
p). She had multiple local alopecia, bilateral loss of eyelashes and eyebrows,
oral candidiasis, ectodermal dysplasia of nails. The patient was first child of
firstdegree consanguineous Turkish parents. In history, it was learned that one
year ago, she admitted to an other hospital because of lethargy and convul-
sion. Laboratory results: Ca:3.5; P:9.3 mg/dl; PTH: 1.2(11-75)pg/ml. The diag-

nosis of hypoparathyroidism was made and racolat was given. The history of
unmetable diaper dermatitis and recurrent oral candidiasis during her infancy
were also taken. Laboratory Ca:9.1; P:9.3 mg/dl; ALP:345 IU/L; PTH:1pg/ml;
Hb:11.6 g/dl; MCV:102,3 fL. She was diagnosed as megaloblastic anemia.
Vit B12:34 pg/ml. She had normal cortisol (25 µg/dl) response to ACTH
stimulation test. Due to complaint of blurred vision ophthalmology consul-
tation was performed. Her best corrected visual acuity was 20/50 OD and
20/50 OS. Slit lamp examination showed a posterior polar cataract on left eye.
Genetic analysis: Sequencing of the entire coding region of the AIRE
gene led us to identify an homoyzgous mutation in exon 2:c.267_275del(p.
Tyr90_Arg92del).

Discussion: Although the association between APS 1 and keratoconjuctivitis has been frequently established our patient had unilateral posterior polar cata-
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ract which was very rare. The 9bp deletion c.267_275del(p.Tyr90_Arg92del)
has never been reported.

P2-d1-457 Autoimmune Endocrine Disease 2

HLA markers of autoimmune endocrinopathies

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Aim: to study associations of polymorphic alleles of HLA-DQB1, HLA-
DQA1 genes with diabetes mellitus 1 type (DM1), diabetes mellitus 1 type
in combination with autoimmune thyroiditis (AT), celiac disease (CD) in pro-
bands of Krasnodar region

Materials and methods: The alleles of HLA-DQB1, HLA-DQA1 genes in
110 children with DM1, 20 children with DM1 in combination with AT and
in 24 patients with celiac disease were determined. Genotyping of specificities
of HLA-system were conducted with use of commercial sets of «DNA-Tech-
ology» firm, Moscow.

Results: For patients with DM1 of Krasnodar Region following classic pre-
disposing alleles:DQA1*0301,DQB1*0201, DQB1*0302 and DQB1*0304
were typical. DQB1*0304 allele, revealed only in Russian populations and
was not meet in European populations, was selected in 4,5% patients with
DM1 of Krasnodar Region type, which is higher than in patients with DM1
in Moscow and Volgoda population. Combination of AT and DM1 was regis-
tered in 17%, CD in 0,3%. In Krasnodar population of patients with DM1
in combination with AT, DQB1*0303 allele was registered significantly more
frequent than in comparison with group of patients with diabetes without AT
(p<0,0206). For patients with celiac disease without diabetes it was typical:
DQA1*1301-38,2%, DQA1*0501-43,8% alleles and of DQB1*0201-41,8%,
DQB1*0302-10,4% genes. It was conducted a comparative analysis alleles
of DQA1 and DQB1 genes in groups of patients with CD and DM1. Protect-
ing DQA1*0103 allele for DM1 was registered more frequent at CD. Predis-
posing to DM1 DQB1*0302 allele was noted more frequent in patients with
DM1. DQB1 0601 allele also was revealed more frequent in patients with
DM1. OB1*0303 allele was registered 5 times more frequent at CD, protec-
tive for DM1 DQB1 0602/8 - 4 times more frequent was registered at CD.

Conclusions: Obtained results testify the necessity of further studying of
HLA-system genes at given diseases, in their combinations, including other
populations of Russian Federation.
Adult height prediction for Han children based on automated bone age determination

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Background: Adult height prediction (AHP) methods have been studied for more than 60 years, but until recently these have considered only Caucasian children.

Objective and hypotheses: To present an AHP method for Asian (Han) children based on automated bone age determination.

Methods: The Asian AHP model is constructed on the basis of an existing model for Caucasian children. This study determines the necessary adjustment to describe normal Chinese children. Bone age is determined by the model for Caucasian children. This study determines the necessary adjustment.

Results: The accuracy of the new AHP model for Asian children has been validated in untreated ISS children. The same accuracy is expected in clinical practice in this patient group because the method is based on automated bone age determination. However, the model has not yet been validated on an independent population of normal-stature Han children with known adult height, and the authors therefore solicit investigators in possession of such image data to join this research project for its completion and final publication.
A 23-day-old female neonate was admitted for respiratory distress and hypercalcaemia. Physical examination: hypotonia, coarse face, bell-shaped chest and respiratory distress requiring ventilatory support. The parents were third degree cousins. Laboratory evaluation: serum Ca: 19 mg/dl (N: 8.4-10.5), P: 2.6 mg/dl (N: 4.8-8.2), PTH: 1096 pg/m (N: 9-52) and urinary Ca/Cr: 0.5 mg/mg (N<0.86). At 25 days of age, the sc calcitonin (10 IU/kg-4 doses) and one day later, pamidronate (0.5 mg/kg) for 2 days were given, without immediate effect on serum Ca but increase in PTH to 1600 pg/ml. The po cinacalcet (30 mg/m2/day) had been started on 28th day of life. On the 4th day of cinacalcet treatment, which was 5 days after last pamidronate doses (4 x doses) and one day later, pamidronate (0.5 mg/kg) for 2 days were given, without immediate effect on serum Ca but increase in PTH to 1600 pg/ml. The po cinacalcet (30 mg/m2/day) had been started on 28th day of life. On the 4th day of cinacalcet treatment, which was 5 days after last pamidronate doses (4 x doses) and one day later, pamidronate (0.5 mg/kg) for 2 days were given, without immediate effect on serum Ca but increase in PTH to 1600 pg/ml. 

Conclusion: A completely inactive CaSR would be predicted consistent with a completely inactive CaSR in children born prematurely, appropriate and small for gestational age (SGA).

Methods: 63 patients (30m/33f), 43 born AGA and 20 born SGA, mean age 11.3 years, entered the study. Bone geometry was evaluated from digitalized X-rays taken at the level of the 2nd metacarpal bone. The following parameters were assessed: outer (D) and inner (d) diameter, cortical area (CA), medullary area (MA) and metacarpal index (MI). Bone quality was evaluated by ultrasound, measuring the amplitude dependent speed of sound (AdSoS), and bone transmission time (BTT). The results were evaluated according to bone age and expressed as SDS compared to a group of 325 control children born at term of normal weight and height, matched for age and sex.

Results: Children born AGA: D -1.19±1.10 (p<0.0005), d -0.72±0.97 (p<0.0005), MI 0.34±1.13 (p<0.05), MA -0.80±0.83 (p<0.0005), CA -0.90±0.81 (p<0.0005), AdSoS -0.09±1.09 (NS), BTT -0.62±0.99 (p<0.0005), Children born SGA: D -1.45±1.13 (p<0.0005), d -0.82±0.83 (p<0.0005), MI 0.23±0.83 (p<NS), MA -0.81±0.72 (p<0.0005), CA -1.19±0.71 (p<0.0005), AdSoS -0.09±1.03 (NS), BTT -0.93±0.96 (p<0.0005). No difference was seen between AGA and SGA, apart from the outer diameter (D) which was significantly smaller only in the group of males SGA.
Conclusion: Children born prematurely have smaller bones with normal thickness and low quality. Moreover, being born SGA seems to be a further negative factor in males only.

<table>
<thead>
<tr>
<th>N</th>
<th>D SDS</th>
<th>d SDS</th>
<th>MI SDS</th>
<th>MA SDS</th>
<th>CA SDS</th>
<th>AdSox SDS</th>
<th>BTT SDS</th>
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<tbody>
<tr>
<td>AGA m</td>
<td>20</td>
<td>-0.82</td>
<td>±0.91*</td>
<td>-0.82</td>
<td>±0.91*</td>
<td>-0.69</td>
<td>±0.91*</td>
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<tr>
<td>AGA f</td>
<td>23</td>
<td>-1.51</td>
<td>±1.34*</td>
<td>-1.01</td>
<td>±1.34*</td>
<td>-1.08</td>
<td>±1.34*</td>
</tr>
<tr>
<td>SGA m</td>
<td>10</td>
<td>-1.58</td>
<td>±0.85*</td>
<td>-1.19</td>
<td>±0.85*</td>
<td>-0.41</td>
<td>±0.85*</td>
</tr>
<tr>
<td>SGA f</td>
<td>10</td>
<td>-1.31</td>
<td>±0.76*</td>
<td>-1.20</td>
<td>±0.76*</td>
<td>0.22</td>
<td>±0.76*</td>
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<tr>
<td>AGA</td>
<td>43</td>
<td>-1.19</td>
<td>±0.97*</td>
<td>-0.98</td>
<td>±0.97*</td>
<td>0.62</td>
<td>±0.97*</td>
</tr>
<tr>
<td>SGA</td>
<td>20</td>
<td>-1.45</td>
<td>±1.13*</td>
<td>-1.19</td>
<td>±1.13*</td>
<td>-0.93</td>
<td>±1.13*</td>
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* p<0.05 against zero; ° p<0.05 for the difference between AGA and SGA

Objective and hypotheses: Activating mutations of CaSR gene have been shown to cause hypercalciuric hypocalcaemia. Such mutations of CaSR cause increased sensitivity to Ca++ and hence down regulation of PTH secretion.

Methods: This is a case series where we report a pedigree with clinical picture consistent with familial autosomal dominant hypoparathyroidism. The proband, an 18-month-old girl was found to have incidental hypocalcaemia. We identified 4 other affected individuals in the family, including the father of the proband and his twin brother. All affected individuals had mild hypocalcaemia, low normal serum parathyroid levels and borderline high phosphate levels. Treatment with vitamin D and oral calcium did not improve their serum calcium levels. The father of proband has shown evidence of hypercalciuria and has recently been treated for urolithiasis.

Results: Mutation analysis identified a novel activating mutation of the CaSR gene. The activating mutation of CaSR inhibits PTH secretion and renal calcium reabsorption despite hypocalcaemia. Treatment with vitamin D and calcium can increase hypercalciumia leading to nephrocalcinosis and renal impairment. Asymptomatic individuals should not be overtreated in an attempt to normalise serum calcium.

Background: Familial hypomagnesemia with secondary hypocalcaemia is a rare autosomal recessive disease that results in electrolyte abnormalities shortly after birth.

Objective and hypotheses: Characterization of 2 cases with familial hypomagnesemia.

Methods: 2 patients analysed by means of TRPM6 gene.

Results: Patient 1 was a girl born in 2006 after a normal pregnancy from two unrelated parents. At the age of two months she was admitted to another hospital because of generalized seizures. She presented hypomagnesemia and hypocalcaemia (0.68-9.28 mg/dl). Treatment started with anticonvulsive and magnesium supplementation. Patient 2 was the brother of patient 1. He was born in 2010 after an uneventful pregnancy. At two month’s age he had an episode of acute dyspnea. During hospital admission, hypomagnesemia with secondary hypocalcemia was determined at whole body, lumbar spine, femoral region and radius using DEXA, and bone remodeling markers were analyzed. All the investigations were performed at baseline and after one year. DEXA, and bone remodeling markers were analyzed. All the investigations were performed at baseline and after one year.

Conclusion: Children born prematurely have smaller bones with normal thickness and low quality. Moreover, being born SGA seems to be a further negative factor in males only.
the peripubertal period was modelled for each group from individual values, the aBMD at femoral region was significantly higher in RG compared to the other two groups from 12.5 to 14 yr and this difference lasted up to 18 yr. At radius and lumbar spine, no difference was demonstrated between groups. Moreover, the mean annual aBMD gain tended to be higher in RG compared to SW and CO only at femoral region and this gain lasted longer with time in RG.

**Conclusions:** High-impact activity had a clearly favorable effect on aBMD at mechanically loaded bones site throughout the peripubertal period.

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**P2-d2-467 Bone, Growth Plate and Mineral Metabolism 2**

**High prevalence of vitamin D deficiency in healthy school children aged 11-18 years**

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**Background:** Vitamin D is necessary for bone health, but little is known about the vitamin D status of school children in our country.

**Objective:** To determine seasonal levels of serum 25-hydroxyvitamin D [25(OH)D3] in healthy school children aged 11-16 years in our region (latitude: 40° N).

**Populations and methods:** The healthy school children aged 11-16 years, selected by stratified sampling, were visited during spring (n = 375) and autumn (n = 371) at schools. After physical examinations were done, blood samples were taken for 25(OH)D3, calcium, phosphorus, alkaline phosphatase (ALP) measurements. Serum 25(OH)D3 levels were categorized as <10 ng/ml (vitamin D deficiency) and 10-30 ng/ml (vitamin D insufficiency).

**Results:** The mean 25(OH)D3 levels were 10.1±4.2 ng/ml in girls and 11.4±6.8 ng/ml in boys during spring (p = 0.001) and 14.6±8.1 ng/ml in girls and 18.0±7.4 ng/ml in boys during autumn (p = 0.001). There was a negative correlation between 25(OH)D3 and PTH levels in both seasons. Threshold for vitamin D level which causes hyperparathyroidism was detected as 14.3 µg/l. The prevalences of vitamin D deficiency and insufficiency were 49% and 51% during spring and 20% and 74% during autumn, respectively. If the threshold is changed to <15 µg/l, 81% of the children is vitamin D deficient in spring and 49% in autumn. 25(OH)D3 level was significantly lower in girls than those boys, moreover, the girls who wear concealing clothing had significantly lower levels of 25(OH)D3. Hyperparathyroidism was detected 24% of the children. Hypocalcemia was not detected.

**Conclusions:** The prevalences of vitamin D deficiency and insufficiency among the healthy school children are high in our region. We recommend vitamin D supplementation to the school children in this age group. Gender and seasonal differences should be taken into account for the dose adjustments, since 25(OH)D3 levels were significantly lower both in girls and in autumn.

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**P2-d2-468 Bone, Growth Plate and Mineral Metabolism 2**

**Bone mass and quality in juvenile idiopathic arthritis: comparison of the role of bone mass determinants using DXA, PQCT and QUS**

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**Background:** There are few prospective data on bone mass and quality using dual energy X-ray absorptiometry (DXA), peripheral quantitative computerized tomography (pQCT), and quantitative ultrasound (QUS) in JIA patients.

**Objective and hypotheses:** To evaluate bone mass and quality in a large cohort of children and young JIA adults, and to identify the main predictors of reduced Bone Mineral Density (BMD) and bone quality using these techniques.

**Patients:** Two hundred and five patients (144 females, 61 males; median age at study entry 15.6 years, 144 oligoarticular, 46 polyarticular, 17 systemic, and 18 enthesitis-related-arthritis onset (ERA)), fulfilling the criteria for JIA were evaluated. Of these, 141 patients (112 females, 29 males; 83 oligoarticular, 27 polyarticular, 15 systemic, and 16 ERA) were followed longitudinally with a second scan. The data obtained were compared with 80 ages- and sex-matched healthy subjects.

**Results:** JIA patients showed a reduced spine BMD SDS value in comparison to controls (p < 0.005). JIA patients showed significantly lower levels of TrabBMD, muscle CSA, and SSIp, AD-SoS, and QUS z-score, but not CorBMD and CBA, and showed fat CSA significantly increased than controls. These data were confirmed also in longitudinal evaluation, with no differences in comparison to the first evaluation. JIA patients presented no more significant lower levels of SSIp than controls. Analyzing the therapies, a significant negative correlation among spine BMD values, TrabBMD, AD-SoS, and systemic corticosteroids exposure or number of intra-articular corticosteroids injections, and a positive correlation among TNF-alpha-blocking agents and spine BMD, TrabBMD, and AD-SoS were observed.

**Conclusions:** Patients with JIA have a low bone mass and, after a first increase due to the therapy, do not reach the normal condition over time despite the current more effective drugs. Our data show that the pronounced bone deficits in JIA are due to reduction in muscle cross-sectional area. Thus, bone alterations in JIA likely represent a mixed defect.

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**P2-d2-469 Bone, Growth Plate and Mineral Metabolism 2**

**Neonatal presentation of McCune Albright syndrome: therapeutic considerations for thyrotoxicosis and severe fibrous dysplasia**

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**Background and objective:** McCune Albright syndrome (MAS) in neonates is rarely reported, notably with hypercortisolism, hyperthyroidism, liver dysfunction, skin lesions, and fibrous dysplasia. We report a case with thyrotoxicosis and extensive, life-threatening fibrous dysplasia, and summarise clinical course and experience with Pamidronate.

**Case report:** A boy, born 2.3kg at 36wks, presented with high output cardiac failure on day 2. Ultrasound suggested Infantile Hepatic Haemangioendothelioma (IHE); heart failure was managed with diuretics, digoxin and ventilatory support pending definitive treatment of IHE. At transfer to our Paediatric Liver unit age 6wks he was emaciated, wt 1.8kg. Biochemistry showed high calcium 3.8mmol/l, high urea 8.5mmol/l and markedly raised liver enzymes. IHE may cause thyroid dysfunction, and tests revealed thyrotoxicosis (TSH <0.1mIU/L, T4 6.7pmol/l, T3 9pmol/l). Mother was euthyroid and TRAb negative. A large abdominal café au lait patch was noted. Treatment with prednisolone and thyroxine restored euthyroid status. Hypercalcaemia resolved with rehydration. Serum 25(OH)D3 was normal and PTH level appropriately suppressed until normocalcaemia restored. Skeletal survey showed osteopenia, multiple cortical erosions and fractures of ribs and long bones. Urinary phosphate fractional excretion was >90%. Cortisol levels were normal. Once euthyroid, cardiac failure resolved and liver function partially improved. Hepatic lesion was no longer thought significant. MAS seemed the underlying diagnosis; DNA from bone biopsy confirmed GNAS R201 mutation. Serial pamidronate doses were given over 4 months to improve bone disease, in particular the ribs. There was reduction in urinary NTX and phosphate, improved pain control, progressive weight gain and weaning off ventilatory support.

**Conclusions:** Reported neonatal MAS with severe fibrous dysplasia is rare. Treatment options are limited. Prognosis and value of bisphosphonate are uncertain.
were elevated (283 nmol/l, normal range: 75 - 250 nmol/l), but the infant had received usual rickets – prophylaxis, (800 IE Cholecalciferol) and parents denied any accidental Vitamin D- overdose. 1,25-(OH)2 Vit. D was in the upper normal range (61 pg/ml) and PTH was low (2,1 pg/ml, normal range: 16-65 pg/ml). Williams- Beuren syndrome and mutations in the calcium sensing receptor gene (CASR) were excluded. In order to further investigate 25-OH Vit. D metabolism, sequencing of CYP24A1 was performed and showed two mutations: c.443T>C and c.1186C>T. Only c.1186C>T has been described in the pathogenesis of infantile hypercalcemia so far, in both compound heterozygous and homozygous form. The c.443T>C mutation has been seen, so far, not described in relation to the disorder in question, however mutation analysis programs strongly suggest involvement in pathogenesis. A low-calcium diet was started. 25-OH-Vit. D normalized within weeks, while PTH remained low.

Discussion: Hypercalcemia is a rare symptom in infancy and childhood. Recently, Schlingmann et al. showed that mutations within CYP24A1 are found in individuals diagnosed with IH. Our patient was admitted under suspicion of Vitamin D intoxication, however proved to be a new case of compound heterozygosity.

Conclusion: CYP24A1 mutations should be considered in infants under rickets–prophylaxis that are suffering from hypercalcemia and regarded as a potential cause for increased 1,25- Vitamin D serum levels.

P2-d-471 Bone, Growth Plate and Mineral Metabolism 2

Serum vitamin D levels in children with recurrent otitis media

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Background: Recurrent otitis media is common in early childhood and basic preventive measures are undertaken with chemoprophylaxis, immune-prophylaxis, surgery and improvement of environmental risk factors for treatment without any sequels.

Objective and hypotheses: The objective of this trial is to evaluate serum vitamin D levels in cases with recurrent otitis media and to investigate the effect of vitamin D therapy on the risk of re-occurrence of the disease.

Methods: A total of 42 children between 17 years of age, diagnosed with recurrent otitis media were enrolled as the Study Group. A total of 54 children with similar demographic characteristics with an age range of 1-7 years and with no chronic systemic disease were enrolled as the Control Group (Group II). In patients with low initial serum vitamin D levels (vitamin D <15 ng/mL), vitamin D (5000 IU/day) was administered in addition to conventional treatment for otitis media while vitamin D treatment of 400 IU/day was administered in patients with initial serum 25OHD levels >15 ng/mL and all cases were followed-up in due course. The trial was approved by local ethics committee.

Results: Mean serum 25(OH) vitamin D in the study group was 11.2 ng/mL. In the control group, mean serum 25(OH) vitamin D level was 29.6 ng/mL. Serum 25(OH) vitamin D levels were under 20 ng/mL in 30% (n=16) of the control group. Comparison of serum 25(OH) vitamin D levels and PTH in Study and Control groups revealed a statistically significant difference (p<0.05). Treatment was initiated in cases diagnosed with vitamin D deficiency and patients were followed-up in due course. In 1-year follow-up of 21 patients with regular visits, no recurrence was observed during this period, apart from three cases where one attack was detected in two patients and 2 attacks were observed in one patient.

Conclusions: We believe that co-administration of supplementary vitamin D together with conventional treatments is appropriate in the management of upper respiratory infections like otitis media.

P2-d-472 Bone, Growth Plate and Mineral Metabolism 3

Vitamin deficiency in adolescents: 2 different patterns of radiological changes related to IGF-1 level, calcium intake and BMI

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Background: Vitamin D deficiency (VDD) is still a major public health problem specially during the winter. The symptoms and signs of VDD in adolescents are less specific and are easily missed.

Objective and hypotheses: Describe the clinical manifestation, radiological changes in relation to calcium intake and IGF-I level in adolescents with severe vitamin D deficiency (25OH Vit D < 10 ng/dL).

Methods: 40 adolescents with VDD were studied. Clinical exam included symptoms and signs related to VDD and dietary intake of calcium. Plain x rays of their knees, hips and wrists were studied. Endocrine tests included levels of Vitamin D, PTH and IGF-I.

Results: The manifestations of 40 adolescents with severe VDD included arthralgia in the knees hips and ankle joints and backache (32/40), difficulty in climbing stairs and/or running (9/40), muscle cramps (12/40) , facial twitches (4/40) and carpo-ural spasms (2/40). High Alkaline phosphatase (ALP)(40/40). Serum ALP and PTH were significantly high and serum phosphate significantly low in adolescents with VDD. Two patterns of radiological changes have been recorded in adolescents with VDD. Pattern I) (n = 8) appears as metaphyseal multi-locular cystic lesion with sclerotic margins, exoentric subcortical location without significant cortical erosions, periostal reaction, osteoporosis, or other metaphyseal manifestations. Pattern II) (n = 18) appears as generalized diminished bone density with prominent primary and 2nd bone trabeculations, widening of the metaphyseal zone with relatively more lucency (zone of poor ossification) with rather loss of all bone trabeculation. No cupping or fraying of the metaphyses was identified. Patients are treated with injecting a mega dose of cholecalciferol (10,000 IU/kg, max 600,000 IU) every 3-6 months according to their serum 25-OH-vitamin D measured every 3 months. Radiological changes improved after six months but complete cure took 2 years in patients with cystic lesions.

Conclusions: Repeated Mega doses of cholecalciferol are effective therapy for adolescents with VDD.

P2-d-473 Bone, Growth Plate and Mineral Metabolism 3

Body composition and bone mineral density in Egyptian children (Delta Region)

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Background: Weight gain is associated with changes in body composition during infancy. Clinical assessment of growth and nutrition status is enhanced by accurate measurement of body composition. Dual energy X-ray absorptiometry (DXA) provides an important means of quantifying total body regional fat mass, skeletal muscle mass and bone mineral mass and density.

Objective and hypotheses: This study aimed to assess body composition; total fat percent, arm fat percent, trunk/lower limb fat and trunk fat percent in different Egyptian pediatric age groups in Delta region.

Methods: A 310 healthy subjects (135 females and 175 males), ranging in age from one to 13 years included in this study. Subjects were categorized according to age into 4 groups. Total body composition and BMD of lumbar spine L2-L4 were measured by dual-energy x-ray absorptiometry (DXA) with a Lunar DEXA-3Q.

Results: The percentage of total body fat, percentage of arm fat and trunk fat / lower limb fat values in male groups tend to be high in infancy compared to early childhood; however they start to increase in late childhood and adolescence. Their is steady increase of total body BMD, arm and leg BMD and BMD of L2-L4 by advancement of age group in female groups. Total body BMD, arm and leg BMD and BMD of L2-L4 showed significant steady increase by age group advancement. Correlation studies show that there is significant positive correlation between age and BMD in both males and females and between age and percentage of total body fat in females. Moreover, percentage of total body fat and percentage of arm fat showed significant correlation with trunk fat / lower limb fat, L2-L4 and total BMD.
Conclusions: There is steady increase in fat mass in male by increasing age. While fat mass in female is high in infancy, decline in childhood, then steady increase in adolescence. There is significant difference between male and female infants and adolescence as regard fat mass and BMD.

P2-d3-474 Bone, Growth Plate and Mineral Metabolism 3

A rare and unexplainable case of hypocalcemic rickets with underlying double pathology; vitamin D deficiency and primary hypoparathyroidism

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Introduction: Hypocalcemic seizures in infants are most commonly secondary to Vitamin D deficiency, especially in families of Asian origin but can also result from primary hypoparathyroidism. The occurrence of both conditions simultaneously in a patient presenting with rickets is extremely rare.

Case report: We report a boy presenting to our hospital at the age of 5 months. He was born at term via C-section, birth weight 2.3kg. His parents were consanguineous with Asian backgrounds. He was exclusively breastfed until 4 months old. Development was normal. He presented with seizures secondary to severe hypocalcemia with corrected serum calcium 1.24mmol/l (normal range (NR) 2.12-2.62). His kidney function and magnesium was normal. PTH levels were undetectable on 2 occasions (<0.3 pmol/L, NR 1.3-7.6). His total 25OH Vit D <10nmol/L (NR > 80 nmol/L). Alkaline phosphatase was 2553 IU/L (NR 200-1100). X-rays showed severe osteopenia and rickety changes. There was no calcium loss in his urine. Initial treatment with ergocalcferol was ineffective in raising calcium levels but once alphacalcidol was added, marked improvement occurred. After 3 months, there was radiological healing of his rickets.

Discussion: In vitamin D deficiency, serum calcium falls, stimulating PTH release. PTH is required to release calcium from the bone, retain calcium in the distal tubules and activate conversion of 25OH Vit D to 1-25 OH Vit D. The action of PTH causes the rickety changes seen on Xray. In our patient, PTH remains undetected (even once Vit D replete) and therefore we cannot explain the phenomenon of rickets changes.

Conclusions: In hypocalcemic seizures, Alphacalcidol should be prescribed until vitamin D and PTH levels are available. Further molecular study may be warranted to explore the possibility of bone resorption and deposition without PTH in our patient.

P2-d3-475 Bone, Growth Plate and Mineral Metabolism 3

Increasing prevalence of vitamin D deficiency in Korean children and adolescents: results from the Korea national health and nutrition examination surveys (KHANES) 2008 - 2010

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Background: Vitamin D plays an important role in bone metabolism and its deficiency causes rickets in children. Recently, medical researchers became interested in nonskeletal effects of vitamin D deficiency such as increase in the risk of cardiovascular diseases, diabetes mellitus, infection, and autoimmune diseases.

Objective and hypotheses: This study was performed to investigate the prevalence of vitamin D deficiency and yearly change in its prevalence among Korean children and adolescents, and to identify association of vitamin D deficiency with metabolic risk factors.

Methods: We assessed the data of 2,934 Korean children and adolescents aged 10 to 18 years obtained from the Korea National Health and Nutrition Examination Surveys 2008-2010. We analyzed the measured serum 25-hydroxyvitamin D levels and defined vitamin D deficiency as 25-hydroxyvitamin D <20 ng/mL.

Results: Overall, vitamin D deficiency was found in 70.1% of children and adolescents in the years 2008-2010. The prevalence of vitamin D deficiency was higher in 2010 than in 2008 (74.77% vs. 61.26%, respectively). Vitamin D deficiency was more prevalent among girls (75.1%) than boys (65.8%), and in urban (37.2%) than rural areas (31.1%). We divided the children into three groups by age (group I 10 to 12-years old, group II 13-15, and group III 16-18). The mean 25-hydroxyvitamin D level was lowest in group III. 25-hydroxyvitamin D level was negatively correlated with diastolic blood pressure.

Conclusions: Vitamin D deficiency is very common among Korean children and adolescents, especially among late adolescents and its prevalence increased between 2008 and 2010. Serum 25-hydroxyvitamin D level was inversely correlated with diastolic blood pressure. The nonskeletal consequences of vitamin D deficiency in children and adolescents should be further investigated.

P2-d3-476 Bone, Growth Plate and Mineral Metabolism 3

Kenny Caffey syndrome

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Background: Kenny-Caffey Syndrome is an extremely rare hereditary skeletal disorder, in most cases it is autosomal dominant trait,others are inherited as autosomal recessive trait.

Objective: Is to report an Egyptian patient with Kenny -Caffey Syndrome.

Methods: An 8.5 year old Egyptian patient who had convulsions at the age of 5 days. At the age of 4 years, the parents noticed that the child was markedly stunted.

On examination, the child was short (height=106.5 cm, SDS = -4.0), weight=20 kg with a SDS of weight for height of +2.1, head circumference = 47.5cm with a SDS of skull circumference for height of -3.1 and his sitting height was 60 cm (sitting height SDS = -3.96). He had a widely open anterior fontanel and metopic suture. He had some dysmorphic features (hypertelorism, long nose and high arched palate), with brachydactyly and short 4th and 5th metacarpals and slight bowing of both tibiae.

Investigations showed low serum calcium, high serum phosphorous together with low serum parathyroid hormone. His peak growth hormone was 1.2 ng/ml and 4.5 ng/ml after clonidine and insulin provocation tests respectively. Plain-x-rays of extremities showed slender long bones together with cortical thickening and short metacarpals. CT brain revealed bilateral basal ganglia calcification. Hypoparathyroidism with the typical picture of growth retardation, cortical thickening of the long bones, and delayed closure of the anterior fontanel suggested the diagnosis of Kenny Caffey syndrome.

Results: Mutation in the TBCE gene is the tubulin specific chaperone E, Mutation analysis done and all exons were sequenced,excited to identify a homozygous change that is not known single nucleotide polymorphism(SNP), causing replacement of a conserved amino acid,this fits with our expectation that mutation should nevertheless preserve some activity of the protein. The patient is currently on growth hormone therapy along with calcium and vitamin D lsupplementation.

Conclusions: Early detection & diagnosis improves quality of life and prevents comorbidities.

P2-d3-477 Bone, Growth Plate and Mineral Metabolism 3

PRKAR1A mutation in a Japanese patient with pseudohypoparathyroidism type Ia-like phenotype

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Background: Pseudohypoparathyroidism type Ia (PHP-Ia) is an autosomal dominant disorder characterized by unresponsiveness to several hormones and unique body phenotype referred to as Albright’s hereditary osteodystrophy (AHO). Although PHP-Ia is usually caused by abnormalities of GNAS mediating the G-protein-coupled receptor (GPRC) signaling, molecular bases remain to be clarified in a small fraction of patients. In this regard, PRKAR1A has been identified as a causative gene for resistance to multiple hormones and acrodysostosis reminiscent of endocrine and skeletal abnormalities in PHP-Ia.

Objective and hypotheses: To report a Japanese female patient with PHP-Ia-like phenotype and PRKAR1A mutation.

Methods: This patient had PHP-Ia-like phenotype including mildly elevated
serum PTH and TSH values and AHO-like clinical features such as short stature, round face, brachydactyly, and mental retardation. Results: While neither mutation nor imprinting defect was identified in GNAS, a novel de novo heterozygous missense mutation (p.T239A) was identified at the cAMP-binding domain A of PRKAR1A. Western blot analysis using primary antibodies for the phosphorylated cAMP responsive element (CRE)-binding (CREB) protein showed markedly reduced CREB phosphorylation in the forskolin-stimulated lymphoblastoid cell lines of this patient. CRE-luciferase reporter assays indicated significantly impaired response of protein kinase A to cAMP in the HEK293 cells expressing the mutant p.T239A protein. Conclusions: The results indicate that PHP-ia-like phenotype is caused by a heterozygous PRKAR1A mutation impairing cAMP-mediated GPCR signaling. Since GNAS and PRKAR1A are both involved in the GPCR signal transduction cascade and have some different characters, this would explain the phenotypic similarity and difference between GNAS abnormalities and PRKAR1A mutations.

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**Sexual dimorphism in bone geometry of adult patients with classical congenital adrenal hyperplasia: data using peripheral quantitative computed tomography**


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**Background:** Long-term glucocorticoid treatment may influence bone and muscle development in patients with congenital adrenal hyperplasia (CAH). Objective: The aim of this study was to evaluate bone mineral density (BMD), bone geometry and muscle mass. **Methods:** Seventy-three adult patients with classical CAH due to 21-hydroxylase deficiency were included. BMD, bone geometry and muscle mass were measured at the non-dominant forearm using peripheral quantitative computed tomography (pQCT). Glucocorticoid equivalent doses throughout childhood were calculated and at time of pQCT androgen levels were measured. Anthropometric data of patients were compared with a reference population by standard deviation (SD) scores (mean±SD). **Results:** In males, mean SD score for trabecular BMD (-0.33±0.71) was reduced, whereas mean cortical BMD (1.05±1.11) was elevated. In females beside normal values of BMD SD scores (trabecular, cortical, mean total) (0.86±1.12) and medullary cross-sectional area (CSA) (1.12±1.17) were significantly increased (p<0.001). In all patients SD scores for cortical thickness (-0.65±0.91) and muscle CSA (-0.83±0.91) were reduced. Treatment duration was associated with lower trabecular BMD (r=-0.56, p<0.001). As a consequence of suppressed androgenization and simple virilising CAH a lower muscle CSA SD score was observed (OR: 0.58 and 0.46, respectively, p<0.05). **Conclusion:** There was a sexual dimorphism with enlarged total and medullary CSA in females, whereas in males trabecular BMD was reduced and cortical BMD elevated. Cortical thickness and muscle CSA were reduced in CAH patients with possible long-term impact on bone development. Monitoring of bone and muscle development might be warranted.

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**Biochemical markers of bone turnover in Turkish children and adolescents**


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**Background:** The measurements of biochemical markers of bone turnover is important in the evaluation of bone health and diseases. Since rate of bone growth shows variations in different age groups, genders, and ethnicities, age-specific reference ranges for biochemical markers should be established. **Objectives:** To determine biochemical markers of bone turnover in healthy Turkish children and adolescents aged 11 to 18 years and evaluate in relation to their ages and pubertal development. **Population and methods:** Serum osteocalcin and alkaline phosphatase (AP) as bone formation markers and C-terminal telopeptide of type I collagen crosslinks (CTX) as a bone resorption marker were measured in 746 healthy children and adolescents (349 girls and 397 boys). **Results:** In girls, all the markers of bone turnover changed significantly with pubertal stage, were maximal at midpuberty, and decreased to the lowest level by Tanner stage 5. We observed similar changes in boys, but the levels of all these markers were significantly higher in boys than those girls, especially between 12 and 17 years old. Serum osteocalcin, AP, and CTX reached a peak at ages 11 and 12 years in girls, however they reached a peak at ages 13 and 14 years in boys. There was a negative correlation among the age and all of these markers. **Conclusions:** We established age- and gender-specific reference ranges of these bone turnover markers in Turkish children and adolescents first time. We observed a marked effects of ages and pubertal development on bone turnover markers in both girls and boys. Our data will be useful in the clinical investigation on bone turnover in bone health and diseases.

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**A novel mutation in CYP24A1 in familial “idiopathic” infantile hypercalcemia**


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**Background:** Idiopathic Infantile Hypercalcemia (IIH), Lightwood like but without facial dysmorphism or heart murmur is a disorder so far not well understood. High level of 1,25-OH-D2-D was previously frequently observed and recently, mutations in CYP24A1, which encodes 25-hydroxyvitamin D24-hydroxylase, the enzyme of 1,25-dihydroxyvitamin D3 degradation have been identified in several IIH cases. **Objective:** The objective of our study was thus to investigate CYP24A1 in a cohort of children affected by IIH with high level of 1,25-OH2-D2-D. **Result:** Direct sequencing of CYP24A1 revealed a new homozygous mutation in two siblings, inherited from both heterozygous parents. **Clinical and biological analysis showed that the older brother which received a milk enriched with 1500 IU /d of vitamin D showed at 6 months of age a decrease appetite, weight stagnation, and intermittent vomiting revealing severe hypercalcemia (3.68 mmol /l), with very low PTH (1 ng/l), mild elevated 25-OH-vitD (140 nmol /l), and normal 1,25-OH2-vit D levels and hypercalcuria (Ca/creat at 4.3) explaining the nephrocalcinosis. Stop of vitamin D supplementation, hyperhydration and furosemide and locasol therapy allowed a normalization of the serum calcium level within 3 weeks. Subsequently, the child received milk enriched with 6000 IU/d and vitamin D levels were adequately, except after the summer when 1,25-OH-vitD levels raised up to 360 nmol /l in spite of sun protection. Based on these observations, the younger brother did not received vitamin D but only enriched milk (600 IU/d) and moderate elevated 1,25-OH-vitD levels (maximum 140 nmol /l). In addition, renal ultrasound revealed no nephrocalcinosis. **Conclusion:** Our study confirms that CYP24A1 plays a causal role in some IIH cases and that preventive measures including vitamin D suspension could protect affected relatives particularly in the infant period.