Poster Presentations

P1-d1-153 Adrenal 1
Glucocorticoid receptor gene variants are not associated with variation of 17-hydroxyprogesterone levels in newborn screening cards

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17-hydroxyprogesterone (17-OHP) screening for classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is part of many newborn screening programs worldwide. However, sensitivity does not reach 100%, mainly due to a high variability of 17-OHP levels in healthy newborns and thus high cut-off values. Recently, the glucocorticoid receptor (GR) N363S variant has been linked to relatively low degree of virilization and serum 17-OHP in untreated female CAH patients at the time CAH was diagnosed clinically. To test whether GR variants underlie variation of 17-OHP screening values, we compared genotypes for three functional GR variants (N363S, ER22/23EK, BclI) with 17-OHP in 1000 screening cards randomly selected from routine screening program. 17-OHP was measured by conventional immunnoassay (TRFIA) and tandem mass spectrometry (LC-MS/MS) method, which has been shown to increase screening specificity by avoiding cross-reactions of the 17-OHP antibody and additionally quantifying cortisol and 21-deoxycortisol. There was no significant association of GR variants with 17-OHP values obtained from TRFIA. We also did not detect differences regarding GR genotypes and steroid profile in LC-MS/MS. Lack of significant genotype effects remained stable even after subgrouping samples for gestational and postnatal age. However, retrospective genotyping of five CAH children reported previously with screening failure revealed N363S heterozygosity in two of them, remarkably, the only two babies reported so far suffering from severe salt-wasting CAH that was not detected by newborn screening. To conclude, functional GR variants do not influence neonatal steroid hormone concentrations including 17-OHP in healthy individuals. Nonetheless, a possible impact on screening sensitivity in subjects affected by CAH remains to be investigated.

P1-d1-154 Adrenal 1
Pitfalls in the molecular diagnosis of 21OH deficiency (21OHD) due to point mutations identification without further characterization of gene deletions/duplications/conversions

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Aim: Study of the impact of a partial molecular diagnosis of CAH-21OHD based on point mutations detection without complementary analysis of gene deletions/duplications/conversions.

Subjects/Methods: 534 Spanish patients (67 classic (CL), 360 paediatic and 107 adolescent/adult nonclassical (NC) forms) carrying V281L, P30L or Q318X mutations analysed by Southern (BglII and TaqI CYP21A2 probe) and semiquantitative primer extension (Exzietu et al 1995, 2002, 2006).

Results: Twenty percent (69/351) of the NC patients with apparent homozygosity for V281L (V281L/0, absent signal of normal allele), showed a pattern suggestive of hemizygosity due to hybrid gene or large conversion. Considering those patients whose alleles were segregated by parental DNA analyses (n=179), 27 gene deletions and 16 large conversions were detected (24%). With respect to the P30L-carrying alleles (n=63), a pattern of conversion at the 5' promoter was detected in 21 (33%). According to the severity of the allele, CL forms were detected in 13 (compound heterozygosity with severe alleles); the remaining, with V281L, showed NC disease. The severe mutation Q318X was detected in non-deficient alleles carrying gene duplications (Q318Xdup). This allele was infrequent in 21OHD patients, only one NC patient was carrying Q318Xdup-V281L. Most patients (89%) were carrying the Q318X in single genes, the double mutant alleles 655G+Q318X, Q318X+R356W and 1172N+Q318X were also detected. Q318Xdup, however, was detected at a frequency of 1.4% in general population (2:143 partners of 21OHD patients), not different than that of the Q318X severe allele (1:143).

Conclusions: 1) V281L hemizygosity due to gene deletions or conversions should be distinguished from homozygosity (two mild alleles). 2) One third P30L alleles are carrying a 5' conversion involving the promoter region, a severe allele associated to virilizing forms. 3) Carrier detection in general population requires the detection of gene duplications, specially if Q318X is detected.

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at age 19 (>300 fold higher than normal). Urinary Na/K ratios normalized gradually with age in all patients. Growth curves of the patients were reflective of the severity of PHA and compliance with salt therapy. Functional expression studies in oocytes showed that ENaC with gGl327Cys mutation, as observed in patient A, showed nearly 40% activity of the wild type ENaC. In contrast, stop mutation as in patient B reduces ENaC activity to less than 5% of the normal. Our results demonstrate distinct genotype-phenotype relationships in PHA patients. The degree of ENaC function impairment affects differently the renin-aldosterone system and urinary Na/K ratios. The differences observed are age dependent and PHA form specific.

**P1-d1-156 Adrenal 1**

**Compound heterozygosity (Phex911le and Arg448His) in the CYP11B1 gene resulting in late-onset Congenital Adrenal Hyperplasia in Danish trizygotic triplets**

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Congenital Adrenal Hyperplasia (CAH) is in 90% of cases caused by mutations in the 21-hydroxylase encoding gene (CYP21). In 5% CAH is caused by mutations in the 11beta-hydroxylase encoding gene (CYP11B1). 11beta-hydroxylase deficiency is associated with cortisol insufficiency, symptoms of androgen excess and, in two thirds of the patients, hypertension. We hereby present compound heterozygosity in the CYP11B1 gene in three 8.3 years old Danish children who were trizygotic triplets (two boys and one girl) who presented with pubic hair (stage PH2-3) and penile growth (stage G2) in the boys, and with breast development (stage B3) in the girl. They all showed accelerated growth, markedly advanced bone age, and were normotensive. The 24-hour urinary steroid profile revealed elevated tetrahydro-11-deoxycortisol (THS) excretion, highly indicative of 11beta-hydroxylase deficiency. Blood samples showed slightly elevated basal ACTH, highly elevated 11-deoxycortisol and androstendione levels. ACTH-infusion test revealed insufficient cortisols response, and elevated deoxycortisol responses. Molecular analysis demonstrated compound heterozygosity in all three triplets based on the presence of two different mutations in the CYP11B1-gene (Phexle and Arg448His) inherited from unrelated mother and father respectively, of which the Phex911le mutation has not previously been described. Intriguingly, the girl presented with breast development and a pubertal response to LHRH stimulation (mx LH 13.4 U/l) at first presentation, suggesting idiopathic central precocious puberty with the potential risk of overlooking the underlying diagnosis, whereas the two boys had prepubertal LH responses in accordance with peripheral precocious puberty. In conclusion, late onset CAH in children with central precocious puberty should be considered, if signs of androgen excess are pronounced, even in the absence of hypertension. Furthermore, we describe a new mutation in the CYP11B1 gene as a source for 11beta-hydroxylase deficiency.

**P1-d1-157 Adrenal 1**

**Atypical clinical presentation in a girl with type 1 autoimmune polyglandular syndrome**

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Autoimmune Polyglandular Syndrome type 1 (APS1) is a monogenic autoimmune disorder associated with organ and non-organ-specific autoimmune manifestations. Diagnosis (at least two of the following disorders): chronic mucocutaneous candidiasis, hypoparathyroidism and/or primary adrenal insufficiency. The AIRE gene has been mapped on chromosome 21q22.3. We describe a 14.1yrs old girl from Northern Italy who developed subsequent y,mucocutaneous candidiasis(1yr), T1DM(3.6yrs), intestinal dysfunction (3.9 yrs), epilepsy(4.7yrs), hypoparathyroidism(3.5yrs), ocular myasthenia(5.6yrs) and precocious puberty (6.6yrs). Autoimmune pattern showed the presence of ICA, GADA, IAA, thyroid peroxidase(TPO), L-aminooacid deboxarlsyase(ADDC), thyrotropin and thyroxine(T4), androgen excess and, in two thirds of the patients, hypertension. We hereby present compound heterozygosity in the CYP11B1 gene in three 8.3 years old Danish children who were trizygotic triplets (two boys and one girl) who presented with pubic hair (stage PH2-3) and penile growth (stage G2) in the boys, and with breast development (stage B3) in the girl. They all showed accelerated growth, markedly advanced bone age, and were normotensive. The 24-hour urinary steroid profile revealed elevated tetrahydro-11-deoxycortisol and androstendione levels. ACTH-infusion test revealed insufficient cortisols response, and elevated deoxycortisol responses. Molecular analysis demonstrated compound heterozygosity in all three triplets based on the presence of two different mutations in the CYP11B1-gene (Phexle and Arg448His) inherited from unrelated mother and father respectively, of which the Phex911le mutation has not previously been described. Intriguingly, the girl presented with breast development and a pubertal response to LHRH stimulation (mx LH 13.4 U/l) at first presentation, suggesting idiopathic central precocious puberty with the potential risk of overlooking the underlying diagnosis, whereas the two boys had prepubertal LH responses in accordance with peripheral precocious puberty. In conclusion, late onset CAH in children with central precocious puberty should be considered, if signs of androgen excess are pronounced, even in the absence of hypertension. Furthermore, we describe a new mutation in the CYP11B1 gene as a source for 11beta-hydroxylase deficiency.

**P1-d1-158 Adrenal 1**

**Is fasting early morning salivary cortisol a reliable method with which to investigate children treated with inhaled steroids for potential adrenal suppression?**

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Introduction: Recently there has been concern about adrenal suppression in children treated with inhaled steroids for asthma. However it remains unclear at what dose of inhaled steroids this may occur and how best to screen or investigate when there is clinical concern. Measuring salivary cortisol is an attractive potential method as it is cheap & non-invasive.

Aims: To establish whether measurement of fasting early-morning salivary cortisol is useful clinical tool for assessing possible adrenal suppression in asthmatic children treated with inhaled steroids.

Methods: Basic laboratory work to validate a salivary cortisol assay, establish its relationship to plasma cortisol, establish the stability of samples in the post and to investigate potential interference with the assay by inhaled steroids was undertaken. Age-specific reference ranges for fasting early morning cortisol were then established in 155 healthy children. These were compared with samples obtained from 55 children treated with varying doses of inhaled steroids.

Results: Fasting early morning cortisol levels were inversely related to the dose of inhaled steroid ( r = 0.36, p = 0.007). The higher the dose of inhaled steroid, the lower the cortisol. This was apparent at total daily doses of 800mcg budesonide/beclometasone & 400mcg fluticasone. In children on inhaled steroids 18.5% had salivary cortisol levels < 2.0 nmol/l (approximates to plasma cortisol <150 nmol/l) compared to 7.8% of controls (p<0.05). And 7.8% of asthmatics had a cortisol <1.5nmol/l (approximates to plasma cortisol <100 nmol/l) compared to 3.2% of controls.

Conclusions: A dose related reduction in early morning salivary cortisol was found in children on inhaled steroids (at relatively modest doses). Significantly higher proportions of children on inhaled steroids had cortisol levels below that which may trigger clinical concern but there was considerable overlap with controls. Whilst potentially useful in identifying individuals who may need further investigation salivary cortisol alone may not reliably predict adrenal suppression.
Enhanced cortisol catabolism due to induction of cytochrome P450 3A4 in epileptic children taking oxcarbazepine (OXC)

Wolfgang Hägler1; Stefan Wudy2; Michaela Hartmann1; Gerhard Luef; Markus Rauchenauer2
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In contrast to other antiepileptic drugs, OXC so far has not been known to induce hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who was also treated with OXC for temporal lobe epilepsy. He required high supplementation doses of hydrocortisone (>20mg/m²/day). Nevertheless, he had grossly elevated ACTH levels (>300pg/mL) and hyperpigmentation. An i.v. hydrocortisone-test confirmed enhanced cortisol (F) elimination (90min post-injection concentration of 40% on OXC vs. 59% off OXC) and excessive amounts of urinary 24h 6β-OH-cortisol/F. After switching from OXC to levetiracetam, all symptoms, weight, biochemical features and hydrocortisone dose (0mg/m² vetiracetam, all symptoms, weight, biochemical features and hydrocortisone injection concentration of 40% on OXC vs. 59% off OXC) and excessive metabolism. Our index-patient was a 16y-old boy with Addison’s disease, who induce hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-induced hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 16y-old boy with Addison’s disease, who-

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<table>
<thead>
<tr>
<th>All units in ug/day</th>
<th>Controls (20.27 [4.21] y)</th>
<th>OXC (15.13 [0.44] y)</th>
<th>% Diff. in OXC</th>
<th>Index-Patient on OXC (16.4y)</th>
<th>Index-Patient off OXC (16.9y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum C21 (log)</td>
<td>10177.0 [3349.9]</td>
<td>7876.6 [4402.0]</td>
<td>-22.5</td>
<td>17276.5 [10936.5]</td>
<td>13250.5</td>
</tr>
<tr>
<td>Fcomb/BSA</td>
<td>61.27 [15.97]</td>
<td>41.21 [15.97]</td>
<td>-32.8</td>
<td>77.14 [83.77]</td>
<td></td>
</tr>
<tr>
<td>Sum C19 (log)</td>
<td>3.74 [0.36]</td>
<td>3.47 [0.17]</td>
<td>-7.2</td>
<td>4.06 [3.85]</td>
<td></td>
</tr>
<tr>
<td>6-OH-F</td>
<td>249.35 [130.21]</td>
<td>320.38 [142.05]</td>
<td>+28.5</td>
<td>2192.31 [294.77]</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>6β-OH-F / F</td>
<td>4.67 [0.17]</td>
<td>+101.3</td>
<td>13.37 [1.80]</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>6β-OH-F/ (THF+aTHF)</td>
<td>0.07 [0.02]</td>
<td>+114.2</td>
<td>0.36 [0.04]</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>6β-OH-F/ (THF+aTHF+ TME)</td>
<td>0.03 [0.01]</td>
<td>+133.3</td>
<td>0.18 [0.02]</td>
<td></td>
</tr>
</tbody>
</table>

***p<0.002, ** p=0.015, *p=0.02

Although the sum of urinary C21 steroids, Fcomb/BSA and the sum of C19 steroids were not significantly reduced in patients on OXC, the cytochrome P450 3A4 elimination pathway towards 6β-OH-cortisol was significantly increased. These results confirm that OXC increases elimination of glucocorticoids via induction of cytochrome P450 3A4. Neurologists should be aware that epileptic patients on OXC who also take glucocorticoids may require greater glucocorticoid doses to reach the desired treatment effect.

P1-d1-160 Adrenal 1
Pitfalls in the diagnosis and treatment of Familial glucocorticoid deficiency

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Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterised by ACTH resistance with severe glucocorticoid deficiency but no intrinsic mineralocorticoid defect. An ACTH receptor (MC2R) mutation is found in 25 % of cases (FGD 1), while some cases of FGD 2 show a mutation in the MRAP gene, which encodes a protein involved in the trafficking of MC2R. Between 1974-94 eight patients (5 M: 3 F) from 4 families in the West of Scotland were diagnosed with FGD. The diagnosis was confirmed by molecular genetic analysis in 5, of whom 3 are the survivors of affected kindreds. None of the families were consanguineous. Key features are highlighted in the table.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Presentation</th>
<th>Gene/Mutation</th>
<th>Initial Plasma Nat</th>
<th>Follow up Plasma Nat</th>
<th>Initial presenting features</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>M</td>
<td>5.6 y</td>
<td>MC2R/S741</td>
<td>125</td>
<td>119</td>
<td>Hypoglycaemia, convulsions, hyppertension</td>
<td>Epilepsy; cognitive impairment</td>
</tr>
<tr>
<td>1.2</td>
<td>F</td>
<td>3.5 y</td>
<td>NK</td>
<td>128</td>
<td>NA</td>
<td>Hypoglycaemia, fever, acidosis, vomiting, diahrhoea, convulsions, coma</td>
<td>Died 3.5 y; adrenal cortex hypoplastic</td>
</tr>
<tr>
<td>1.3</td>
<td>M</td>
<td>7 m</td>
<td>MC2R/S741</td>
<td>134</td>
<td>133</td>
<td>Hypoglycaemia, vomiting, diarrhoea, convulsions, coma</td>
<td>Well</td>
</tr>
<tr>
<td>2.1</td>
<td>M</td>
<td>5w 3d</td>
<td>NK</td>
<td>133</td>
<td>122</td>
<td>Jaundice, diarrhea, dehydration, hypoglycaemia</td>
<td>Died 10 w; adrenals considered normal size at post mortem</td>
</tr>
<tr>
<td>2.2</td>
<td>M</td>
<td>10 d</td>
<td>NK</td>
<td>129</td>
<td>136</td>
<td>Jaundice, sudden collapse, hypoglycaemia</td>
<td>Died 3.2 y; very small adrenal glands at post mortem</td>
</tr>
<tr>
<td>2.3</td>
<td>F</td>
<td>2 w</td>
<td>MC2R/S741</td>
<td>138</td>
<td>133</td>
<td>Hypoglycaemia, prolonged jaundice, poor feeding, heart murmur</td>
<td>Well</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>3 m</td>
<td>MC2R/S741</td>
<td>129</td>
<td>133</td>
<td>Hypoglycaemia, convulsions, hyppertened nephries, collapse</td>
<td>Well</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>3 w</td>
<td>MRAP/M11</td>
<td>134</td>
<td>132</td>
<td>Hypoglycaemia, jaundice, lethargy, shock, neonatal hepatitis, hypoglycaemia</td>
<td>Well</td>
</tr>
</tbody>
</table>

† NR 135-145 mmol/L.

Age at diagnosis during life ranged from 11 days to 6.6 years (median 80 days). Three patients have died; two were diagnosed posthumously (1.2, 2.1) and one (2.2) died despite being on glucocorticoid therapy. Of the remaining five patients, 4 are alive and well, but one (1.1) has cerebral damage related to severe hypoglycaemia and prolonged seizures during childhood. Hypopituitarism without associated hyperkalaemia was noted at presentation in 7 of 8 patients (renin normal in 3, elevated in 2, not measured in 3). All surviving patients required at least one hospital admission with adrenal decompensation, with hyponatraemia. Additional disorders included severe eczema (2), asthma (3), congenital heart lesions (3), and renal anomalies (2). FGD is a potentially lethal disease which can present at post mortem (when the diagnosis may be missed) and is associated with morbidity and death despite treatment. FGD should be considered in children presenting with unexplained hypoglycaemia, low sodium levels but normal potassium. We stress the importance of teaching families “sick day” rules for increasing hydrocortisone therapy during illness and providing them with an Adrenal Insufficiency card detailing the emergency treatment required.
An association between exaggerated adrenarche (EA), low birth weight (BW) and later development of hyperinsulinaemia and ovarian hyperandrogenism has been widely reported. In order to identify possible risk factors for future morbidity, the Scottish Paediatric Endocrine Group conducted a 3-year prospective study (2004-07) investigating the insulin status, steroid biochemistry and pelvic ultrasound appearance of patients presenting with a clinical diagnosis of EA. Fifty patients [42F:8M] were recruited. A 10 ml blood sample (fasted) was taken for biochemical analysis. Height, weight, bone age, blood pressure (BP) and pubertal status were assessed. Girls underwent pelvic ultrasound examination. Mean (SD) age at presentation was 7.7 (0.99) yr for girls vs 8.8 (0.67) yr for boys. BW was similar to the population mean (girls -0.05 SD; boys -0.53 SD). Both sexes were above average in height (Ht SDS girls vs 8.8 (0.67) yr for boys. BWt was similar to the population mean (girls 13.0 -- N Reference 38 40 -- 5.0 40-100 follicular development. hyperandrogenism on imaging, the raised AMH suggests advancement of the overweight status of the subjects. Despite no evidence of ovarian low BWt in our cohort. While the biochemistry was largely unremarkable, markedly elevated. In contrast to previous studies, we found no evidence of ovarian was in the upper range of normal in girls and slightly raised in boys, with DHAS and androstenedione were only modestly elevated. Of note, insulin †ur - unrecordable.

X-linked adrenal hypoplasia congenita (AHC) is a rare hereditary disorder affecting the hypothalamic-pituitary-adrenal and the hypothalamic-pituitary-gonadal axis and is caused by mutation or deletion of the NROB1 gene. This gene encodes the DAX-1 protein that has been classified as an orphan member of the nuclear receptor superfamily. AHC is characterized by adrenal insufficiency in infancy and early childhood followed by premature menarche, pubertas aegrotans and later development of hyperinsulinaemia and ovarian hyperandrogenism. The aim of this study was to evaluate the clinical, endocrine and molecular characteristics of X-linked AHC patients, diagnosed between 1984 and 2007 in Israel. Twelve patients from 5 families who were followed up for up to 23 years were studied. The diagnosis of AHC was confirmed by hormonal and molecular studies in all patients. Most of the boys presented with signs of adrenal insufficiency such as salt-wasting and failure to thrive during the neonatal period. Aldosterone deficiency usually preceded cortisol deficiency requiring early mineralocorticoid therapy. Serum cortisol levels performed in the first weeks of life ranged from very low to high levels (≤ 0.1 to < 64.4 μg/dL). Five boys showed signs of precocious sexual development during infancy and childhood (e.g. enlargement of penis and testes). In 4 patients the initial diagnoses were erroneous. Molecular analysis of the NROB1 gene identified point mutations in 6 patients including a novel splice site mutation in one family (V511-G to C). In six patients from two families who manifested impaired mental development, contiguous gene deletion was found. This study highlights the protein manifestations of X-linked AHC due to different molecular defects and emphasizes the value of genetic testing in boys presenting with salt-wasting with or without cortisol deficiency. A high index of suspicion is required in order to avoid misdiagnosis and to facilitate appropriate management.

The main objective of this study was to determine the efficacy and safety of pamidronate in improving bone mineralization and reducing fracture incidence in osteogenesis imperfecta (OI).

Material and Methods: Intravenous pamidronate was administered to 64 children (from 21 months to 10 years old) with severe OI, in a 1 mg / kg single daily dose for 3 sequential days in 4 months intervals, over a 24-48 months duration. Clinical status, biochemical characteristics including bone turnover markers, the bone mineral density of the lumbar spine and femoral neck, and radiologic changes were assessed regularly during treatment.

Results: The number of fractures decreased from median of 8 (range 4-11) to 0 fractures/year (range 0-4) (P<0.05). After 16 months of treatment, there was significant improvement in bone mineral density (BMD-DXA) z-scores of the lumbar spine from median of -5.90 (range -7.01 to -4.76) to -2.70 (range -4.46 to -1.98) (P<0.001). Serum alkaline phosphatase (ALP) (bone formation marker) decreased from a median of 731.0 U/L (range 95-286 U/L) (P<0.001), implying a significant reduction in bone turnover and its resorption and increase in bone mineralization. There was no improvement either in their height growth velocities or in their standard deviation scores. Mobility and ambulation improved in all but 5 children, (Poster Presentations)
P1-d1-164 Bone 1

A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene: Unusual heterogeneity of phenotypes

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Loss-of-function mutation in the calcium-sensing receptor gene (CASR) decreases the sensitivity of the G protein-coupled parathyroid CaSR and leads to PTH-induced hypercalcemia and hypocalciuria. Heterozygous mutations cause familial benign hypocalciuric hypercalcemia (FHH), which in most patients is characterized by moderate, asymptomatic hypercalcemia. In contrast, homozygous mutations lead to severe primary hyperparathyroidism in neonates with severe hypercalcemia and failure to thrive. Here we report on two related consanguineous families in which a novel CaSR mutation led to both autosomal recessive and dominant FHH. Fourteen subjects of two related families of Arab-Bedouin descent were analyzed. The proband (case 1) was a 5-yr-old male with a history of recurrent hospital admissions due to abdominal pain and hypercalcemia (13 mg/dl), which was first discovered at the age of 2 yrs. Due to increased levels of intact PTH (100 pg/ml, <65 pg/ml), parathyroidectomy was recommended. Further analysis revealed low urinary excretion of calcium (FeCa < 0.001), which suggested a diagnosis of FHH. Sequencing of CaSR revealed a novel missense mutation in exon 4 of both alleles that predicts a Gln459Arg replacement in the fifth extracellular loop of the protein. The mutant protein was normally expressed in HEK293 cells but retained only 30 to 50% of the calcium-dependent activity of wild-type CaSR. Both parents and two sibs were heterozygous for this mutation and were normocalcemic. In family 2, the proband (case 2, first cousin of case 1), as well as the father and two sibs, were heterozygous for the Gln459Arg mutation. Serum 25(OH)D was normal in tested subjects. We hypothesize that Gln459Arg causes only a very modest reduction in CaSR activity, which may explain the mild biochemical findings in the heterozygous family members and the moderate phenotype of the homozygous proband. Our study demonstrates the heterogeneity of the biochemical phenotypes of CaSR mutations, even within a family, and provides evidence for FHH inheritance as an autosomal recessive disorder.

P1-d1-166 Bone 1

The effect of vitamin D therapy on serum adiponectin levels in children with vitamin D deficiency rickets

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Objective: Adiponectin and its receptors are known to be expressed in osteoblasts. Vitamin D deficiency rickets (VDDR) is a bone disease related to increase in osteoblastic activity. The aim of this study is to investigate the effect of vitamin D therapy on serum adiponectin levels in children with VDDR.

Methods: VDDR was diagnosed with clinical, biochemical, and radiological findings in children who were otherwise healthy. Patients were treated with 300,000 U D3 (IM.) and calcium lactate. The success of therapy was measured by increase in osteoblastic activity. The aim of this study is to investigate the effect of vitamin D therapy on serum adiponectin levels in children with VDDR.

Results: The study was completed with twenty-one patients. The median (range) chronological at referral was 7.3 (2-16) months. Weight and height but no BMI were significantly increased after four weeks of therapy (66±0.53 vs 68±0.56 cm, p<0.001; 7.65±1.66 vs 8.2±1.51 kg, p<0.001; 17.3±1.45 kg/m2, p<0.05, respectively). Biochemical markers measured before and after therapy are shown in the Table. The adiponectin levels decreased significantly after vitamin D therapy (p<0.001).

Conclusion: The findings of this study indicate that serum adiponectin level increases in children with VDDR. We concluded that the increase in adiponectin levels might results from increased osteoblastic activity in VDDR.
Vitamin D (Vit D) is one of the important elements for bone metabolism. The need for Vit D is increased during periods of rapid growth. In order to determine the frequency of Vit D deficiency and its correlation with different factors, 313 healthy children and adolescents (192 females and 121 males aged 8 — 18 years) with no Vit D supplement after infancy, were enrolled into the study. The serum levels of 25-hydroxyvitamin D3 [25(OH)D3] (RIA), iPTH (IRMA), calcium, phosphorus, phosphate alkaline, creatinine, sodium, potassium, fasting blood sugar, T4, T3uptake and TSH were measured. Vit D status was designated according to the serum level of 25(OH)D as: severe deficiency < 12.5 nmol/L; deficiency, ≥12.5 and <25; insufficient, ≥25 and <50; normal ≥50 and <250. Frequency of different states of Vit D is illustrated in the table. The concentration of 25(OH)D in males (mean±SD, nmol/L) (65.8±68.1) was significantly more than females (31.1±44.3) (P<0.0001), and it was significantly higher in prepubertal than pubertal subjects 77.2±77.9 and 37.6±49.4 respectively (P<0.0001). Vit D had significant negative correlation with BMI and height SDS (P=0.002 and P=0.006 respectively), but did not have significant seasonal variation. The subjects with low 25(OH)D did not have any sign of rickets in wrist and hand radiograph. Conclusion: Subclinical Vit D deficiency was more prevalent in females and during puberty. More obese and taller children need more Vit D. Normal values of Vit D should be reevaluated according to the level that causing PTH rising.

### Table 1: Vit D state

<table>
<thead>
<tr>
<th>Vit D state</th>
<th>Female %</th>
<th>Male %</th>
<th>total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe deficient</td>
<td>37.5</td>
<td>5.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Deficient</td>
<td>29.2</td>
<td>24.0</td>
<td>27.2</td>
</tr>
<tr>
<td>Insufficient</td>
<td>17.7</td>
<td>38.8</td>
<td>25.9</td>
</tr>
<tr>
<td>Normal</td>
<td>15.6</td>
<td>32.2</td>
<td>22.0</td>
</tr>
</tbody>
</table>

Pseudohypoparathyroidism (PHP) is characterized by hypocalcemia, hyperphosphatemia, and elevated PTH levels due to end-organ resistance to this hormone. Patients with PHP type Ib (PHP-Ib) typically exhibit isolated renal resistance to PTH and lack features of Albright’s osteodystrophy (AHO) and show epigenetic abnormalities of GNAs. Microdeletions at the NESP55 differentially methylated region (DMR) of this gene and the closely linked STX16 (encoding syntaxin-16) are detected in patients with autosomal dominant PHP-Ib (AD-PHP-Ib). We now investigated Turkish PHP-Ib patients with respect to GNAS imprinting and searched for previously described microdeletions. Seven patients (4 F, 3 M) with PHP-Ib from six families were included in the study (Table). Clinical findings at presentation included symtoms related to hypocalcemia, i.e. seizures, tetany or spasms in five patients and routine laboratory examination without any hypocalcemic symptoms was observed in two patients. With the exception of two affected siblings from a consanguineous kindred, all patients were apparently sporadic cases. Differential methylation status at each of the four GNAS DMRs through nucleotide sequence analysis of bisulfit-modified genomic DNA revealed that two of the patients had broad methylation defects, which is consistent with observations in sporadic PHP-Ib. The two patients from the consanguineous family showed an isolated A/B methylation defect and both turned out to be carriers of the 3 kb STX16 microdeletion, which they had inherited from their healthy mother, who showed a normal GNAS methylation pattern. We did not detect any methylation defect at this locus in the remaining three patients. About half of the investigated patients thus showed no epigenetic GNAS abnormalities indicating other genetic or epigenetic defects are the cause of PTH-resistance in these patients.

### Table 2: Clinical Findings

<table>
<thead>
<tr>
<th>Patients</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
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<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presentaion</th>
<th>Seizure</th>
<th>Seizure</th>
<th>Basal ganglia Calcification</th>
<th>Tetany</th>
<th>Tetany</th>
<th>Spasm</th>
<th>Routine lab</th>
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</thead>
<tbody>
<tr>
<td>Ca (8.4-10.5 mg/dl)</td>
<td>4.6</td>
<td>5.5</td>
<td>8.6</td>
<td>yes</td>
<td>yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PO4 (2.5-4.9 mg/dl)</td>
<td>9.4</td>
<td>7.1</td>
<td>5.3</td>
<td>9.1</td>
<td>7.6</td>
<td>6.8</td>
<td>7.8</td>
</tr>
<tr>
<td>ALP (250-600 U/L)</td>
<td>253</td>
<td>1043</td>
<td>928</td>
<td>639</td>
<td>499</td>
<td>468</td>
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<tr>
<td>PTH (9-52 pg/ml)</td>
<td>1153</td>
<td>174</td>
<td>137</td>
<td>86.9</td>
<td>84.2</td>
<td>381</td>
<td>465</td>
</tr>
<tr>
<td>U Ca/Cr</td>
<td>0.006</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-OH D (10.50 nmol/l)</td>
<td>52.89</td>
<td>50</td>
<td>5.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylation Pattern</td>
<td>Broad defect</td>
<td>Normal</td>
<td>Normal</td>
<td>Isolated A/B defect</td>
<td>Isolated A/B defect</td>
<td>Normal</td>
<td>Broad defect</td>
</tr>
<tr>
<td>3 kb deletion in STX16</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Incidence of vitamin D deficiency rickets in eastern part of Turkey

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Turkey, especially its eastern part, has been accepted as endemic for vitamin D deficiency (VDR). In a study performed by our team in the region at the beginning 2000's, the incidence of VDR was 6.09% in children aged between 0-3 years. In 2005, Ministry of Health initiated free vitamin D supplementation campaign in nationwide for every infant to eradicate the VDR.

In this study, we aimed to investigate the incidence of VDR in children aged between 0-3 years. Between March 2007 and February 2008, 49133 children aged 0-3 years who were brought to different pediatric outpatient clinics in Erzurum were evaluated for VDR. The diagnosis of VDR was made by radiological and biochemical findings in the cases who were initially suspected of having clinically VDR. During one year period, 39(0.079%) of the 49133 were diagnosed as having VDR. Of these, 29 (74.4 %) were boys and 10 (25.6 %) were girls. The majority (69%) of the cases with VDR was diagnosed in spring, 25% of cases (10/39) was under 6 months of age. While 23 (59%) of the cases were breastfed, the others were fed with cow’s milk and/or additional food. None of the cases with rickets was taking vitamin D supplementation. 53% of the mothers were illiterate. 89% of families of the cases had lower income than minimum rate. 89% of cases had veiled mothers. The most frequent physical findings were rachitic rosary, enlargement of the wrists and craniotabes. Laboratory findings of the cases were compatible with VDR; serum Ca 7.5±1.9 mg/dL, PO4 4.4±1.3 mg/dL, ALP 134±823, 25OHD3 5.8±2.9 ng/mL, iPTH 240±106 pg/mL. We concluded that although VDR has been a continuing childhood health problem, nationwide free vitamin D supplementation campaign initiated by the Government appear to be effective on the way of completely eliminating of VDR.

Contribution of vitamin D supplementation to the vitamin D status of infants in the age of fortified milk

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In line with European guidelines, infant formulas in France have been vitamin D fortified with a vitamin D3 content of approximately 400 IU/L. New guidelines for the prevention of vitamin D deficiency and rickets have subsequently been published and the vitamin D supplement for formula-fed infants was reduced to 800 to 400 IU per day. To contribute to the evaluation of the need for vitamin D supplementation, a study was conducted to assess vitamin D3 intake (cholecalciferol) provided by sunlight exposure and fortified milk, and intake provided by supplementation prescribed in form D2 ergocalciferol.

This study was conducted in a population of Caucasian infants. The vitamin D2 intake, the type and quantity of milk received, time spent outdoors and clothing modalities were recorded. Vitamin D status was assessed on serum 25(OH)D2 and 25(OH)D3 levels were evaluated by HPLC followed by radioimmunoassay. Forty-five term infants between the ages of 2 and 14 months (26 boys and 19 girls) were included, during winter for 19 cases and during summer for 26 cases. The contribution of 25(OH)D2 vitamin D supplementation (mean: 18 ng/mL; range: 2-47) was approximately equal to vitamin D intake from sun exposure and 25(OH)D3 fortified milk (mean:21 ng/mL; range: 5.5-32). Three infants had a low 25(OH)D2 level and one had a level of 2 ng/mL. The 25(OH)D3 level was low (< 10 ng/mL) for 5/36 infants, all included during summer. The most original result of this study is the equivalent contribution to the infant’s vitamin D status of D3-intake by fortified milk and sun exposure and D2-intake by the prescribed vitamin D supplement. The vitamin D status appeared to be satisfactory under these conditions, with extreme values within the locally defined normal range.

Study of osteogenesis in achondroplasia:
From laboratory to clinical evidence

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¹G. Gaslini Institute, Pediatrics University of Genoa, Genoa, Italy; ²G. Gaslini Institute, Orthopedic Surgery, Genoa, Italy; ³San Martino Hospital, Endocrinology, Genoa, Italy; ⁴IRCCS G. Gaslini Institute, Epidemiology and Biostatistics Section, Genoa, Italy; ⁵IRCCS G. Gaslini Institute, Pediatrics, University of Genova, Genoa, Italy

Achondroplasia is the most frequent osteodysplasia, characterized by a disorder of chondroblastic proliferation and maturation in cartilaginous growth plate of long bones. The achondroplastic osteoblasts also presented an impaired osteogenesis. They incorporate a large amounts of calcium already in the early stages of the process and they express a low level of alkaline phosphatase with a consequent inadequate mineralization. We have clinically verified if achondroplastic patients submitted to lengthening of the lower limbs (ACH) present some ossification difference in respect of a control group of patients submitted to lengthening for congenital limb-length discrepancy (CG). We analyzed an index of bone consolidation, internationally used, expressed by the ratio between the number of days between the application and removal of fixators and centimeters obtained (healing index, HI). We tested and compared the ossification times in achondroplastic patients (ACH) and in (CG). We considered 26 ACH (11 males and 15 females) and 25 CG (12 males and 13 females) undergo at surgery between 1994 and 2007. The lengthening skeletal segments were respectively 26 femurs and 10 tibiae in ACH and 18 femurs and 16 tibiae in CG. To assess the increase in length, measurements were performed on X-rays of the lower limbs of individual patients. The mean HI, estimated for the 26 femurs of ACH, is 37.3 ± 7.9 days for cm (range 27.1-65.5), while for 14 femurs of CG is 56.9 ± 17.7 days for cm (range 36.5-91.6). The mean HI, estimated for the 10 tibiae of ACH is 40.4 ± 7.8 days for cm (range 30.1-52.3), while in 13 tibiae of CG patients is 69.7 ± 14.6 days for cm (range 46.4-93.7). The lowest HI found in ACH compared to CG is essentially a result of altered osteogenesis as demonstrated in cell cultur.
obese children. The lack of physical activity, which plays an important role in the pathogenesis of obesity, has, however, no influence on bone strength in this group.

**P1-d1-174 Bone 1**

**Spontaneous corticosteroid-induced osteoclastogenesis in children with 21-hydroxilase deficiency**

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1University of Bari, Department of Biomedicine of Development Age, Bari, Italy; 2University of Bari, Department of Human Anatomy and Histology, Bari, Italy

21-OHD children need chronic glucocorticoid (cGC) therapy since diagnosis (at birth or later) to replace congenital deficit of cortisol synthesis. cGC therapy is the most frequent and severe form of drug-induced osteoporosis, but its pathogenesis is not completely understood. GCs cause a rapid decline in bone mass due to a striking remodelling imbalance of osteoclasts (OCs) and osteoblasts (OBs) number, by exerting an anti-apoptotic effect on OCs in vivo. Furthermore, GCs enhance expression of RANKL, a cytokine involved in OCs formation and activation, and inhibit in vitro the production of osteoprotegerin (OPG), the decoy receptor for RANKL, by stromal cells and OBs. We investigated the osteoclastogenetic potential of peripheral blood mononuclear cells (PBMCs) obtained from 18 children (9 F), aged 3-16 years, affected by 21-OHD (molecular diagnosis) on long-term GC therapy and from 25 children (C) who never received GCs and without risk factors for osteoporosis. PBMCs from pts (9 with classical forms) and C were cultured for 15-17 days in presence/absence of recombinant human M-CSF (25 ng/ml) and RANKL (30 ng/ml). At the end of the culture period, mature OCs were identified as tartrate-resistant acid phosphatases positive (TRAP+) multinucleated cells, containing three or more nuclei. Freshly isolated PBMCs from pts and C were stained for CD11b, CD14, and CD51/CD61 and analyzed by flow cytometry. Spontaneous osteoclastogenesis, without adding M-CSF and RANKL, and significantly higher OCs reabsorbing activity occurred in 21-OHD pts. Conversely, MCSF and RANKL were essential to trigger and sustain osteoclastogenesis of PBMCs from C. Furthermore, only in 21-OHD pts we identified a percentage of CD51/CD61 positive cells, which are OCs precursors (OCPs) strongly committed. In conclusion, our data showed that cGC therapy increases the number of circulating OCPs, enhancing spontaneous OCs formation in vitro and therefore suggests a new mechanism of cGC-induced osteoporosis.

**P1-d1-175 Pancreas 1**

**MODY type 2 in Greig Cephalopolysyndactyly syndrome (GCPS) as part of a contiguous gene deletion syndrome**

Amnon Zung; Sagi Josefisberg Ben-Yehoshua

1Kaplan Medical Center, Pediatric Endocrinology Unit, Rehovot, Israel; 2Kaplan Medical Center, The Institute of Clinical Genetics, Rehovot, Israel

GCPS (OMIM 175000) is a rare syndrome affecting limb formation and craniofacial development, with an autosomal mode of inheritance. The syndrome originates from a deletion in the 7p13 region, including the GLI3 gene. Atypical cases with additional symptoms including mental retardation (MR) are related to the loss of genes closely linked to the GLI3 locus. We describe a 7-year old girl with GCPS based on typical signs of macrocephaly, a broad nasal bridge, hypertelorism, syndactyly of hands and preaxial polysyndactyly of the feet, with MR. Following febrile convulsion-associated hyperglycemia, several fasting blood glucose measurements were in the range of 126-136 mg/dL. OGTT showed impaired glucose tolerance, and first-phase insulin response (1-3 min in IV-GTT) was 23 mU/L, compatible with impaired secretory defect of insulin. Her HgA1C was 6.9 (normal range 4.5-5.7), and Islet-cell, insulin and GAD autoantibodies were negative. At the time of the workup, the patient reported polyuria, polydipsia and a new onset of nocturnal enuresis. Glybenclamide was initiated, and over a period of 16 months of treatment her HgA1C was normalized but her fasting glucose levels are still elevated. Cytogenetic study has shown a deletion in 7p13-15 area that has been reported to include both GLI3 gene and the closely located glucokinase gene (GCK), which accounts for the presentation of MODY type 2. Although GCK gene was found to be deleted in five patients with atypical GCPS in a previous study, only one of them presented with borderline high blood glucose levels. We describe the first case of MODY type 2 in a patient with GCPS due to contiguous gene deletion syndrome.

**P1-d1-176 Pancreas 1**

**Using an insulin secretagogue to target the insulin secretory defect in Turner’s syndrome**

La Rosa Clementina; Gerard Conway

University College Hospital, Endocrinology, London, United Kingdom

Women with Turner syndrome (TS) have been shown to have an 11.5% relative risk of type 1 diabetes mellitus (DM) and 4.3% relative risk of type 2 DM. However it has been recently highlighted that the defect of glucose homeostasis has been observed even in younger, non-obese women with TS and that the pattern of insulin secretion seems more likely to be due to beta-cell dysfunction or insufficiency, reminiscent of maturity onset diabetes of the young. Consequently traditional categorisation of DM may not be completely appropriate in TS. We hypothesised that Repaglinide, an insulin secretagogue targeting the first phase of insulin response (FPIR), should be a treatment suitable for diabetic TS patients and therefore tested its effect. We measured FPIR to intravenous glucose overload (IVGTT) in eight diabetic TS patients. All subjects exhibited reduced FPIR compared to non-diabetic Turners women in a reference population. Repaglinide was administrated for 11-14 weeks in association with other anti-diabetic drugs and an IVGTT was repeated after optimising drug dosage. As showed in Table 1, a significant improvement in glucose homeostasis was observed with a rise in FPIR by over 50% after treatment (P<0.028) and significant reduction in Glucose AUC (AUCG P< 0.046).

<table>
<thead>
<tr>
<th>Date shown as Mean (SD)</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>33.1 (8.4)</td>
<td>29.5 (8.1)</td>
</tr>
<tr>
<td>Fasting BM (mmol/L)</td>
<td>10.6 (5.2)</td>
<td>7.3 (2.5)</td>
</tr>
<tr>
<td>Fasting Insulin (mU/L)</td>
<td>11 (6.3)</td>
<td>14.6 (7.9)</td>
</tr>
<tr>
<td>AUC glucose</td>
<td>156.8 (42)*</td>
<td>119.8 (35)*</td>
</tr>
<tr>
<td>FPIR</td>
<td>22.3 (12.3)*</td>
<td>57.4 (31.8)*</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.3 (1.9)</td>
<td>7.5 (1.3)</td>
</tr>
</tbody>
</table>

* P < 0.05

The drug was generally well tolerated, diarrhoea was observed as side effect, only in two cases. We have shown that the early beta cell insufficiency in TS is responsive to medical therapy. Our experience has emphasized the theory that TS patients exhibit a particular form of diabetes which requires specific treatments of which Repaglinide has been shown to be effective.

**P1-d1-177 Pancreas 1**

**Resistin, adiponectin, leptin levels in adolescents with cystic fibrosis**

Maria Cristina Maggio; Daniela Gucchiardino; Mirella Collura; Francesca Pardo; Andrea Liotta; Silvano Bertelloni; Eleonora Gucchiardino; Saviero Tesa; Giovanni Consello

1University of Palermo, DUM, Palermo, Italy; 2ARNAS Palermo, Centro Regionale per la Fibrosi Cistica, Palermo, Italy; 3University of Pisa, Department of Reproductive Medicine and Pediatrics, Pisa, Italy; 4ARNAS Palermo, Analysis Laboratory, Palermo, Italy

Patients with Cystic Fibrosis (CF) have significant improved survival and quality of life, due to progress in diagnosis and follow-up. However, especially in adolescents, several complications related to endocrine and nutritional assess are described, since CF is characterized by weight loss and chronic low-grade inflammation.

Leptin plays an important role in energy balance and may be affected by hormonal and metabolic derangement associated with chronic disease. Serum concentrations of leptin are decreased in children with CF and associated with
clinical conditions and body composition. Adiponectin is higher in adult CF patients but no data are reported in CF children. Furthermore no data are available in literature about Resistin levels in CF. 24 patients (14 M; 10 F, age 13.3±2.6 years) affected by mild CF disease were selected between the patients in peripuberal age following these criteria: no CFRD, no pathological OGTt; no acute disease; no lung and/or bowel transplantation; good nutritional status. These patients were compared with health controls matched for sex and age. BMI: CF 17.92±3.29 (M/F: 17.26±2.97; 18.84±3.65), Controls 22.42±1.15. HOMA IR: CF 1.2±0.76, Controls 1.84±0.78. HOMA B%: CF 1.49±1.14, Controls 2.38±1.25. Leptin (ng/ml): CF 8.71±7.97 (M/F: 3.67±5.49; 10.44±11.88), Controls: 3.97±4.52 (M/F: 3.97±4.52; 6.16±6.73). Adiponectin (µg/ml): CF 11.3±8.44 (M/F: 11.81±3.99; 8.56±4.17), Controls 8.63±1.67 (M/F: 7.8±1.52; 9.88±1.04). Resistin (ng/ml): CF 2.05±0.87 (M/F: 2.07±0.89; 2.11±1), Controls: 0.93±0.42 (M/F: 0.92±0.29; 0.92±0.62). HOMA IR and HOMA B% are in the normal range but lower than in controls, expression of a lower insulin-resistance with a lower insulin secretion. Leptin levels and BMI had a statistically significant correlation (r 0.72, p 0.000069). Adiponectin levels were mildly lower in CF than in controls, possible expression of low malnutrition typical of CF. Furthermore these are the first data in pediatric CF. Resistin levels more elevated in CF than in controls may be explained by chronic inflammatory pattern typical of these patients.

**P1-d1-178** Pancreas 1

**Molecular characterisation of patients with hyperinsulinism and deafness**

Klaus Brusgaard\(^d\); Muhammed Alawi\(^d\); Lone Svargo\(^d\); Henrik Christensen\(^e\)

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Congenital hypoglycemic hyperinsulinism (CHI) is a clinical and genetic heterogeneous entity. Clinical manifestations can vary from serious life threatening to milder difficultly identifiable cases. Children who don’t react adequate to medical treatment are subject to pancreatic recession. The molecular ethiology are from recessive mutations of the ABCB8 (SUR1) and KCNJ11 (Kir6.2) to dominant mutations of the GCK or GDH genes. Focal dysplasia characterised by loss of maternal Chromosome 11 and hereby ABCB8 and KCNJ11 is a common cause of CHI. ABCB8 and KCNJ11 is localised to chromosome 11p15. Interestingly the USH1C gene is localised upstream of ABCB8. Usher syndrome type I caused by mutations in USH1C is an autosomal recessive sensory defect involving congenital profound sensorineural deafness, vestibular dysfunction, and blindness due to progressive retinitis pigmentosa. Three Saudi Arabian patients were submitted to be analysed for Congenit Hyperinsulism associated with deafness. The adjacent genes USH1C and ABCB8 both were analysed using quantitative realtime PCR and microsatellite markers to establish if any Loss of heterozygosity (LOH) was present. Finally, all individual exons of the ABCB8, KCNJ11 and USH1C genes were amplified by PCR. The microsatellite D11S902 was absent. By PCR of ABCB8 it was shown that only exon 23 to 39 is present. In the same way only exon 1 and 2 of the USH1C gene was shown to be present. All of KCNJ11 is present. By sequencing, a homoygous contiguous partial gene deletion was identified, starting in USH1C intron 2, c.90+592, and ending in ABCB8 intron 21. We here report the analysis of patients with a complex phenotype that can be explained by a large deletion involving two genes.

**P1-d1-179** Pancreas 1

**Protein sensitive hyperinsulinaeum hypoglycaemia due to a novel mutation in the short-chain L-3-hydroxyacyl-CoA dehydrogenase (HADH) gene with normal acylcarnitines and urine organic acids**

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Short-chain L-3-Hydroxyacyl-CoA dehydrogenase (SCHAD), encoded by HADH (formally known as HADHSC) catalyses the penultimate reaction in the β-oxidation of fatty acids. So far, three patients with mutations in HADH and hyperinsulinaemic hypoglycaemia (HH) have been reported. The acylcarnitine profile in these reported patients showed increased hydroxybutyryl-carnitine and raised urinary 3-hydroxyglutarate. We report a novel mutation in HADH associated with protein sensitive HH. The index case was born at term with a birth weight of 3500 grams to consanguineous parents. There was no neonatal hypoglycaemia. At 4 months of age she had a tonic-clonic convulsion due to hypoglycaemia. Her hypoglycaemia responded to diazoxide but episodes of hypoglycaemia recurred even on diazoxide but consuming high protein feeds. Investigations confirmed HH (blood glucose level of 1.8mmol/l with simultaneous serum insulin level of 58mU/l) associated with undetectable serum ketone bodies. The acylcarnitines and urine organic acids were normal on each occasion tested. This patient was severely protein sensitive (accounting for the unexplained episodes of hypoglycaemia) but not leucine sensitive with a normal serum ammonia level. As the parents were consanguineous and the proband was negative for mutations in the ABCB8, KCNJ11 and GLUD 1 genes the HADH gene was sequenced. A novel variant M188V (562A>G) in exon 5 of the gene was identified. The parents and two unaffected siblings were all heterozygous for the variant. The variant is novel and the residue is conserved across species. We conclude that patients with HH due to mutations in the HADH gene may have normal acylcarnitines and urine organic acids. Mutations in the HADH gene are associated with increased protein sensitivity. Understanding the molecular mechanisms of how mutations in the HADH gene cause HH and protein sensitivity will provide new insights into pancreatic beta-cell physiology.
Parents shoulder much of the burden of care in childhood diabetes. The aim of the study was to assess parent well-being of adolescents with Type 1 Diabetes and determine the relationship with metabolic control and adolescent quality of life (QOL). Clinical data and centrally analysed HbA1c were collected on 2,062 adolescents, aged 11-18 years and from 1,863 parents from 21 centers in 19 countries in Europe, Japan, North America and Australia. Adolescents completed QOL, well-being and Life Ladder questionnaires. Parents completed the WHO5 well-being and Family Burden questionnaires. Mean HbA1c was 8.2% (±1.4%), higher in girls 8.3 (±1.5%) than in boys 8.1 (±1.3%), p<0.001. Cronbach’s alpha coefficient value of WHO-5 scale was 0.872. Mean parent well-being score was 64.±1.2 (range 0-100). Good well-being (>50) was reported by 1,497 parents (80%), poor well-being (<50) by 366 parents (20%) and likely depression (mean score <70) by 128 parents (8%). Paternal well-being scores were significantly greater than maternal scores (68±16.8 vs 63.6 ±19.2 p=0.001). Adolescents of parents with poor well-being (<50) had significantly lower HbA1c values (8.1% ±1.4 vs 8.4% ±1.4 p<0.005), greater well being (p=0.001), less impact of diabetes (p=0.001), less worries (p=0.001), less self-reported problems with parent over involvement (p=0.01), greater health perception (p=0.001) and global quality of life (p=0.001), and less physical and psychological symptoms (p=0.001) than adolescents of parents with poor well-being (<50). There were significant centre differences with greater parent well-being in centres with lower HbA1c values (p=0.001).

Family burden was lower in parents with higher well-being scores (p<0.001). The WHO-5 well-being questionnaire provides a short, easy to score, valid tool and enables ongoing assessment of parent well-being which is an important part of diabetes care.
Type 1 diabetes (T1D) is a multifactorial autoimmune disease with both environmental and genetic component contributing to the disease outcome. Several studies have suggested that reactive oxygen species (ROS) are involved not only in diabetes development but also in severe microangiopathic complications like diabetic retinopathy (DR) and diabetic nephropathy (DN). The aim of our study was to investigate well described polymorphic markers in antioxidant enzymes superoxide dismutase (MnSOD) and glutathione-S-transferases M1 and T1 (GSTT1 and GSTM1) in association with microangiopathic complications. One hundred and twenty five patients with T1D were included in the study. They were divided in two groups: patients with chronic complications of diabetes (case subjects) and without complications (control subjects), matched by sex, age and duration of diabetes. Genotyping was conducted using Real-time PCR for Ala(-9)Val polymorphism in MnSOD and multiplex PCR for determination of GSTM1 and GSTT1 null genotypes. Pearson Chi- Square test revealed significant differences in allele and genotype frequencies for the two groups. We found a positive association of MnSOD genotype Val/Val (OR = 2.52, 95% CI = 1.02-6.24, p = 0.042) and GSTM1 null genotype (OR = 2.5, 95% CI = 1.051-6.33, p = 0.035) with diabetic retinopathy but not with diabetic nephropathy. Polymorphisms in antioxidant enzymes could be useful genetic markers for identification of patients with T1D at an increased risk for developing diabetic retinopathy.

**P1-d1-187 Pancreas 1**

**Insulin sensitivity, adiponectin and resistin are related with the inflammatory status and pulmonary function in cystic fibrosis (CF)**

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Chronic inflammation, as in CF, may be associated with insulin-resistance. We evaluated insulin-resistance and a possible role of adiponectin, and resistin in the regulation of glucose metabolism in CF. Fifty-seven patients, subdivided in 3 groups: prepubertal (aged 6-11,7yr, n = 6), pubertal (aged 12.1-17.4 yr, n = 19) and adult (aged 19-39.3 yr n=32), and healthy subjects as adult controls (aged: 21-31,1 yr, N = 28) were enrolled. C-peptide, glycated Hb, C reactive protein (CRP), resistin, adiponectin were assayed in serum. OGTT was done in prepubertal patients and both OGTT and IVGTT in the elder patients.

OGTT was classified according to the CF International criteria, the First Phase Insulin Response (FPIR) and HOMA index according to ISPED criteria. Acute insulin response (AIR) was assessed also as well as FEV1, Schwachman score and genotype. Data are mean±SEM. HOMA was altered in one patient, glucose/insulin ratio in 13 patients. Based on OGTT, 26 patients were normal, 21 had impaired glucose tolerance (IGT), 8 had CFRD without fasting hyperglycaemia and one had CFRD with fasting hyperglycaemia. Based on FPIR, 15 patients were normal, and 42 had insulin deficiency. Crosstabulation analysis according to age showed the highest prevalence of normal OGTTs in puberty and IGT increased with age. FPIR decreased from puberty to adulthood. Considering genotype, heterozygosis for the ΔF 508 mutation was protective towards developing insulin deficiency with respect to homozygosis, confirmed by a significant difference in FPIR and AIR. Adiponectin was lower in the homozygotes. In adult patients adiponectin was lower compared with controls (10757,8±807 vs 15445,7±1691mg/ml, p<0,05), whereas resistin was increased (4,39±0,4 vs 3,04±0,3ng/ml). In CF, resistin correlated with CRP (R=-0,5; p = 0,00) and FEV1 (R = -0,47; p = 0,00).

In conclusion, CF patients develop insulin deficiency, can present mild insulin
resistance and show changes in adiponectin and resistin concentrations related to the inflammatory status and pulmonary function. This work was supported by the Italian Cystic Fibrosis Research Foundation (grant FFC182006) with the contribution of “Intreo S.p.A.”

P1-d1-188 Pancreas 1
Overweight at disease onset and during therapy in pediatric patients with type 1 diabetes
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Increased weight gain has been reported in type 1 diabetes prior to disease onset (accelerator hypothesis) and as a side-effect of intensified insulin therapy. Pediatric studies are complicated by the age- and gender-dependence of the BMI and by the time-trend of increasing obesity prevalence in recent years in many countries. By March 2008, the DPV database includes 11604 pediatric patients with type 1 diabetes, age <20 years, on stable insulin regimen, accumulated between 1995 and 2007 in 201 diabetes centers in Germany and Austria. Standardized patient records are documented locally at each participating center. Data are pseudonymized and transferred for central analysis twice yearly. Potentially inconsistent data are reported back to the centers for verification. BMI reference values on more than 34000 German children and adolescents are used to calculate BMI-SDS-LMS (Cox transformation according to Cole et al.). At diabetes onset, BMI-SDS was increased to +0.43±0.70 in patients <5 years of age, compared to +0.36±0.89 (5-10 years), +0.24±1.0 (10-15 years) and +0.18±0.98 in patients 15-20 years (p=0.0001). BMI-SDS at onset was higher in boys compared to girls (p=0.0001) and higher in patients with migration background (p=0.02). During the subsequent course of diabetes female gender, duration of disease and migration background were independently related to increasing BMI-SDS (all p<0.0001) in a multiple regression model. BMI-SDS was higher in children on insulin pumps (+0.54) compared to intensified (+0.49; p<0.05) or conventional insulin therapy (+0.41; p=0.0001). In conclusion, at disease onset, children and adolescents with type 1 diabetes are more overweight compared to healthy controls and gain additional weight during the following years. The insulin regimen is likely one factor involved, in addition to demographic variables. Optimization of diabetes management should limit weight gain during therapy.

P1-d1-189 Pancreas 1
The correlation between measures of insulin sensitivity in youth: Comparing the hyperinsulinemic-euglycemic clamp, the FSIVGTT and the OGTT
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Given the rise in childhood obesity and its association with insulin resistance, valid methods to measure insulin sensitivity (IS) in youth are needed. Surrogate estimates from the oral glucose tolerance test (OGTT) have not been well studied in children and adolescents. The objective of this study was to examine the correlation between four different methods of measuring IS in a group of children: the hyperinsulinemic-euglycemic clamp, the frequently sampled intravenous glucose tolerance test (FSIVGTT), various indices derived from the OGTT, as well as fasting indices (HOMA-IR, QUICKI, and fasting insulin [INSO]). Twenty healthy children (mean SD age: 9.02 years) were studied: 9 boys and 11 girls. Their mean (SD) BMI z-score was 1.5 (0.8). No participant had impaired fasting glucose/glucose tolerance or diabetes. Each child underwent a 3-hour hyperinsulinemic-euglycemic (40 mU/m2/min) clamp, an insulin modified minimal model FSIVGTT, and a 3-hour OGTT. Correlations were established using Spearman’s rank correlations. Two clamp derived formulas were considered, that were highly correlated (r=0.85). The index derived from the FSIVGTT was highly correlated with both clamp measures (r=0.69, 0.74). We considered 9 different indices derived from the OGTT. Of these, 4 indices showed high correlation with the clamp results: ISI Matsuda (r=0.63, 0.69), ISI Belfiore (r=0.62, 0.64), SI_OGTT (r=0.62, 0.62) and Log sum insulin, the most closely correlated index (r=0.67,0.80). Fasting indices of IS had slightly lower correlations with clamp results: HOMA-IR (r=0.55, 0.56), QUICKI (r=0.55, 0.57), and INSO (r=0.59, 0.63). Measurement of IS using the clamp, the FSIVGTT, and OGTT derived indices were highly correlated in this group of children. In particular, the Log sum insulin index was the most strongly correlated with clamp results, and appears to provide more valid information than either HOMA-IR or QUICKI. This suggests that OGTT derived indices provide valid, clinically feasible methods of estimating IS in youth.

P1-d1-190 Pancreas 1
Autoantibody-associated severe hypoglycemia mimicking hyperinsulinemia in neonatal lupus
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Neonatal lupus is an autoimmune disease associated with intrauterine transferred maternal antibodies. The clinical picture is characterized by cutaneous lesions, cardiomyopathy, heart block, hepatic disease, and hematologic cytopenias due to pre- and postnatal immune reactions to the maternal antibodies. We report a child with neonatal lupus presenting congenital heart block, cholestatic hepatitis, hematologic cytopenias, and severe hypoglycemia. Pacemaker therapy was required 24 hrs after birth, due to aspasia several transfusions were necessary. Apart from the known symptoms of neonatal lupus our child presented persisting severe hypoglycemia. Investigations were performed several times including blood glucose, insulin, 3-beta-OH-butyrate, lactate, free fatty acids, glucagon, IGF-1 and other parameters being involved in glucose homeostasis. Glucagon tests showed normal results. All findings were consistent with hyperinsulinemia but the blood levels of insulin during hypoglycemia were low or not detectable. Therapy was carried out with intravenous glucose administration for the first weeks of life followed by oral feeding highly supplemented with simple and complex carbohydrates. This supplementation was necessary until the blood levels of maternal autoantibodies decreased. From the age of 6 month on no hypoglycemic crises occurred under formula feeding. According to the laboratory findings and the clinical course the child with neonatal lupus suffered from autoantibody triggered hypoglycemia. This resulted in an activated insulin signaling pathway with no findings suggesting any other metabolic or endocrine disorder. Insulin receptor activating antibodies are described in patients with systemic lupus erythematosus and could be the reason for the child’s symptoms. With ongoing molecular biological investigations we try to elucidate the underlying mechanism.

P1-d1-191 Pancreas 1
Multiple autoimmune events after allogeneic bone marrow transplantation (BMT) in a girl with atypical cystic fibrosis (CF)
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Several cases of autoimmune activation after BMT are reported. Different pathogenetic mechanisms have been suggested to explain this phenomenon as the transfer from donor to recipient or the result of an immunologic dysregulation. In our patient, a 15 year-old girl with atypical CF, multiple autoimmune events occurred after allogeneic BMT. These involved glomerulonephritis, pseudogout, pericarditis, pleuritis, pancreatitis, autoimmune diabetes and hypothyroidism. This girl, with a genetically confirmed atypical CF, had been an alloimmunized child. The occurrence of multiple autoimmune events after allogeneic hematopoietic stem cell transplantation in this patient is consistent with a target organ-specific autoimmune response.
A novel mutation of the thyroid transcription factor-1 gene causing congenital hypothyroidism with resistance to TSH

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Resistance to TSH (RTSH [MIM 275200]) is a heterogeneous condition defined by variable degree of insensitivity to biologically active TSH molecule. The etiology of RTSH has been poorly clarified, although some cases were reported to be associated with gene mutations in TSHR, PAX8, or GNAS1. In this report, we describe a patient with RTSH who had a novel mutation of TITF1. The patient was Japanese boy with congenital hypothyroidism detected by newborn screening. He suffered from recurrent lower respiratory infection in his infancy and subsequently developed choreoathetosis. Computed tomography of the brain showed normal brain structure. Molecular analysis for TITF1 revealed a novel heterozygous deletion/insertion mutation (c.470_479delinsGCG, p.P157RfsX38) that was predicted by in silico analysis to disrupt the coding sequence of TITF1 gene.

In conclusion, changes of the expression of costimulatory molecules on the thyroid follicular cells suggest the different degree of the activation and stimulation of the cells during the development of the pathologic process within thyroid gland.

Alopecia areata: A novel association with resistance to thyroid hormones in a family with novel TRβ mutation

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Resistance to thyroid hormone (RTH) syndrome is a rare genetic disorder caused by thyroid hormone b receptor mutations. Up to date 124 mutations in the TRb gene identified in more than 500 different families. Goiter, learning disabilities, psychological abnormalities, sinus tachycardia, hearing deficits, short stature, growth delay are among the most common symptoms in patients with RTH. Alopecia areata (AA) is an autoimmune disease of the hair follicle, frequently associated with other autoimmune disorders, one of the most common of which are thyroid diseases like Hashimoto’s thyroiditis and Graves’ disease. To our knowledge AA associated with RTH has not been reported previously. Here we describe a family having RTH syndrome, caused by a novel TRβ mutation, coexisted with alopecia areata in all affected members of the family. Index case is a 4/12-years-old boy with RTH due to a novel heterozygous missense mutation of the TR β gene, (I353V), and diffuse, patchy alopecia without autoimmune thyroid disease. This mutation was also detected in his father, elder brother and an aunt who were affected by RTH (carrying the same mutation) and also have alopecia areata. We speculate that alopecia areata might be a new phenotypic feature of RTH.


**P1-d1-195 Thyroid 1**

**Levothyroxine administration through nasogastric feeding tubes**

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In neonates, levothyroxine may be administered through a nasogastric feeding tube. Although in several EU countries registered L-thyroxine solutions are commercially available, this is not the case in the Netherlands. Current practice in the Netherlands is therefore to make a suspension of ground tablets in water and to administer it on a metal spoon, because there are anecdotal reports that levothyroxine may adsorb to plastics. This study evaluates the adsorption of levothyroxine to nasogastric feeding tubes. Two 15 mcg/mL suspensions were prepared in the pharmacy laboratory (levothyroxine tablets suspended in water and in 4.2% bicarbonate, respectively). These were compared to a 15 mcg/mL reference solution of pharmaceutical grade levothyroxine dissolved in water pH>10. Levothyroxine concentrations of the samples were quantified using HPLC both directly after preparation, and after one hour instillation in a gastric feeding tube (Vygon Corp. Nutrisafe 2, PUR, 8 Ch.). All samples were homogenized and diluted with method 1 (1:1) before HPLC analysis. The experiment was repeated with a levothyroxine solution commercially available in Germany (L-thyroxin Henning 100 mcg/mL, Sano-Aventis). The solubility of levothyroxine tablets suspended in water was very poor, with a levothyroxine recovery of 1,8 mcg/mL (12%) compared to the reference solution. In alkaline (pH8) solubility was increased. Instillation of the two tablet suspensions in nasogastric feeding tubes led to a further loss of the drug to 1,4 mcg/mL (9%). This phenomenon was not observed in the commercial solution, with 98% drug recovery after instillation in a gastric feeding tube. The current practice of levothyroxine administration to young children in the Netherlands leads to a large variability in the amount of drug actually delivered to the patient. This is most likely caused by poor solubility of the drug in water or by negative influences of the tablet matrix, and not by adsorption to plastics. Access to the commercial preparation is desirable.

**P1-d1-196 Thyroid 1**

**Polymorphism A/G at position 49 of exon 1 of the CTLA-4 gene and antithyroid antibodies in children with Hashimoto’s thyroiditis**

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The genetic predisposition to antithyroid antibodies production is well known. The CTLA-4 gene is considered as one of the strongest genetic factors determining this predisposition. The aim of the study was to evaluate the association between the polymorphism A/G at position 49 of exon 1 of the CTLA-4 gene and antithyroid antibodies level in young patients with Hashimoto’s thyroiditis.

**Material and methods:** One hundred caucasian children were examined: 45 with Hashimoto’s thyroiditis (average age 14,9 years, range: 8,1-17,9), and 55 healthy controls matched for age, sex and ethnic origin. In children in the control group the thyroid dysfunction and antithyroid antibodies presence were excluded, as well as other autoimmune diseases. TSH value, and antibodies (anti-TPO ab and anti-Tg ab) were measured. These were classified according to the following criteria: less than 1 mcg/L was considered as normal, 1-2 mcg/L as increased, and more than 2 mcg/L as high.

**Results:** The prevalence of high TSH value and high anti-TPO ab and anti-Tg ab in the study group was: TSH: 24% (11 patients), anti-TPO ab: 33% (15 patients), anti-Tg ab: 38% (17 patients).

**Conclusion:** Our results confirm the high prevalence of high anti-TPO ab and anti-Tg ab in children with Hashimoto’s thyroiditis. They also suggest a genetic predisposition to this antibody production, which cannot be confirmed by the CTLA-4 gene polymorphism A/G at position 49 of exon 1 of the CTLA-4 gene.

**P1-d1-197 Thyroid 1**

**Multiple endocrine neoplasia 2A: Prophylactic intervention**

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A good genotypic-phenotypic correlation is observed in type 2 multiple endocrine neoplasm (MEN). Specific mutations of the RET proto-oncogene give rise to syndromatic variants of MEN [MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTMC)], and are in turn correlated to the prognosis, patient age at onset of the tumor, and aggressivity of the lesion. The current recommendations comprise prophylactic thyroidectomy in carriers, at an early age: grade II risk (mutations in codons 618, 620, 630, 634, 891) between 2-5 years of age; and grade III risk (mutation in codon 918) between 1-6 months of age. We present two families with MEN 2A (the index case in both instances being the mother with medullary thyroid carcinoma and mutation in C634R in exon 11 of the RET proto-oncogene), in which carrier studies were made among the relatives (father and offspring). In the first family prophylactic radical thyroidectomy was performed in the first daughter at 28 months of age, revealing a 3-mm microcarcinoma and C cell hyperplasia in part of the thyroid parenchyma. In a second daughter thyroidectomy was performed at 23 months of age, with the identification of hyperplasia. In the second family prophylactic radical thyroidectomy at 18 months of age revealed a 2-mm medullary microcarcinoma in the right thyroid lobe, and C cell hyperplasia in the rest of the parenchyma. Despite the very young age of the patients in our series, malignancy was already seen to be present. The discovery of new mutations and the study of new affected families will improve genotypic-phenotypic correlation, and will allow us to establish more precise interventional protocols Cooperation among different specialists is needed (pediatric endocrinologists, endocrinologists, pediatric surgeons and geneticists) to ensure very close follow-up of the MEN 2A index cases, and early intervention.

**P1-d1-198 Thyroid 1**

**Relationship between sodium iodide symporter and thyroid peroxidase and proapoptotic (TIAR and TIA-1) markers detection in thyrocytes from adolescents with thyroid diseases**

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The course of Graves’ disease (GD) is associated with the inflow of lymphocytes to the thyroid gland and dysregulation of the immune system characterized by reaction to thyroid antigens (peroxidase, thyroglobulin, TSH receptors and Na⁺/I⁻-symporter). After activation they shift to the inflamed thyroid gland, thus leading to the production of cytokines which can stimulate activity of thyrocytes and increase expression on intracellular proapoptotic markers such as TIAR and TIA-1. The aim of this study was to estimate sodium iodide symporter (NIS) and thyroid peroxidase (TPO) expression in thyrocytes from patients with GD and no-toxic multinodular goitre (NTMNG). The investigation was performed on thyroid cells isolated from postoperation thyroids of GD patients. In the first family prophylactic radical thyroidectomy was performed in the first daughter at 28 months of age, revealing a 3-mm microcarcinoma and C cell hyperplasia in part of the thyroid parenchyma. In a second daughter thyroidectomy was performed at 23 months of age, with the identification of hyperplasia. In the second family prophylactic radical thyroidectomy at 18 months of age revealed a 2-mm medullary microcarcinoma in the right thyroid lobe, and C cell hyperplasia in the rest of the parenchyma. Despite the very young age of the patients in our series, malignancy was already seen to be present. The discovery of new mutations and the study of new affected families will improve genotypic-phenotypic correlation, and will allow us to establish more precise interventional protocols Cooperation among different specialists is needed (pediatric endocrinologists, endocrinologists, pediatric surgeons and geneticists) to ensure very close follow-up of the MEN 2A index cases, and early intervention.
Congenital central hypothyroidism (CCH) is a rare disease occurring in 1:20,000 to 1:50,000 newborns. Congenital central hypothyroidism is usually missed by newborn screening because of low TSH levels. However, delayed substitution of L-thyroxine may lead to psychomotor delay of the affected newborns. We report on the first child of nonconsanguinous German parents, who developed a newborn infection within the first week of life and was treated with intravenous antibiotics for 5 days. After initial improvement, feeding problems occurred and the child became progressively sleepy. This was first thought to be due to the infection. On day 19 thyroid function tests revealed isolated central hypothyroidism with elevated basal and TRH stimulated prolactin levels excluding an inactivating TRH receptor gene mutation. All other hormone axes were normally functioning. Further mutational analysis revealed a frame-shifting 1bp deletion (C105V) in exon 3 of the TSHβ subunit gene. The child was immediately started on a replacement therapy. Several studies concerning overt as well as subclinical hypo and hyperthyroidism are associated with impaired diastolic function and exercise testing. Hypothyroidism was diagnosed by neonatal screening and LT4 treatment was initiated within the first month of life and carefully adjusted to maintain TSH levels in the normal range and free thyroxine in the high-normal range. The purpose of this study was to clarify the phenotype of patients with a R450H mutation of the TSH receptor (TSHR) gene; the mutant receptor has previously demonstrated moderately impaired function in vitro and the mutation has been frequently identified in Japanese patients with resistance to TSH. We performed a clinical investigation of five homozygous and three heterozygous Japanese patients. Six of eight patients were referred because of hyperthyrotoxpinemia on neonatal screening. In the two remaining patients with heterozygous mutation, hyperthyrotoxpinemia was diagnosed after screening. At the first examination, serum TSH/FT4 levels of the homozygous and heterozygous patients were 12.6-38.5 µU/mL (elevated)/1.1-1.3 ng/dl (normal) and 4.7-7.4 µU/mL (slightly elevated)/1.1-1.7 ng/dl (normal), respectively. Treatment with L-T4 was started in all homozygous patients, and then an intravenous dose of L-T4 was necessary to maintain a normal TSH. During adolescence, one homozygous patient had high level of TSH (54.8 µU/mL) with low level of FT4 (0.6 ng/dl; normal range, 0.7-1.5) in the absence of L-T4 for one month, suggesting that patients with partially compensated TSH resistance might develop uncompensated resistance to TSH if untreated. Only one heterozygous patient was treated with L-T4 from the age of 25 months because of TSH levels higher than 10 µU/ml (11.9 µU/ml). In the two remaining heterozygous patients, the TSH levels were 3.1-7.5 µU/ml after the age of 6 months. For the eight patients, ultrasonography and 123I scintigraphy showed thyroid glands of normal size and location. Clinical assessments including intelligence tests of all affected patients revealed normal growth and development. Early treatment should be recommended for patients with hyperthyrotoxpinemia and a homozygous R450H mutation, even when thyroid hormone levels are normal. However, further research is needed to decide whether replacement therapy is necessary for heterozygous patients.

**Long-term levothyroxine therapy in young adults with congenital hypothyroidism: Effects on cardiovascular system**

**Background:** Congenital hypothyroidism is a common thyroid disorder in children, and is routinely treated with life-long levothyroxine (LT4) replacement therapy. Several studies concerning overt as well as subclinical hypothyroidism and hyperthyroidism have demonstrated that thyroid hormones may impact on the cardiovascular system, however, there are no data concerning the effects of long-term LT4 administration in patients with congenital hypothyroidism. **Objective:** The aim of the study has been to evaluate whether long-term LT4 replacement therapy in young adults with CH is associated with cardiovascular abnormalities. **Patients and Methods:** Thirty young adults with congenital hypothyroidism aged 18±0.2 years and 30 age and sex-matched controls underwent cardiac and carotid Doppler ultrasound and symptom-limited cardiopulmonary exercise testing. Hypothyroidism was diagnosed by neonatal screening and LT4 treatment was initiated within the first month of life and carefully adjusted to maintain TSH levels in the normal range and free thyroxine in the high-normal range. **Results:** Compared with controls, hypothyroid patients exhibited left ventricular (LV) diastolic dysfunction, impaired exercise capacity, and increased intima-media thickness. At multiple regression analysis, the number of episodes of plasma TSH levels >0.5 mU/ml and >4.0 mU/ml from the age of 1 year onward and mean TSH plasma levels during puberty were the independent predictors of diastolic filling and cardiopulmonary performance indexes. **Conclusions:** Long-term LT4 treatment in young adults with congenital hypothyroidism is associated with impaired diastolic function and exercise capacity, and increased intima-media thickness. Such abnormalities occur despite careful replacement therapy and appear related to unphysiological fluctuations of TSH levels, with attendant episodes of subclinical hyperthyroidism and, more frequently, subclinical hypothyroidism.
symptoms of hyperthyroidism. After 2 years, D-T4 treatment was switched to TRIAC. During the long course of both treatments, thyroid hormones, TSH, heart rate, thyroid size, and markers of peripheral thyroid status (SHBG and Alk Phos) were monitored. It was concluded that compared to D-T4, TRIAC treatment is more effective in suppressing TSH and lowering thyroid hormone levels in PRTH. However, both treatments were unable to completely normalize thyroid hormones and reduce thyroid size. The effects of treatment on symptomatology was also modest.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>D-T4 treatment</th>
<th>TRIAC</th>
<th>Off treatment</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (µg/dl)</td>
<td>19.7</td>
<td>22.9±1.4</td>
<td>13.1±2.1</td>
<td>19.8</td>
<td>(5.1-13.5)</td>
</tr>
<tr>
<td>s-T4 (ng/dl)</td>
<td>2.6</td>
<td>3.96±1.5</td>
<td>2.28±0.4</td>
<td>4.2</td>
<td>(0.9-2.0)</td>
</tr>
<tr>
<td>T3 (ng/ml)</td>
<td>3.61</td>
<td>3.11±0.5</td>
<td>2.04±0.5</td>
<td>2.21</td>
<td>(0.8-2.0)</td>
</tr>
<tr>
<td>s-T3 (µg/ml)</td>
<td>12.7</td>
<td>14.7±3.4</td>
<td>5.98±0.8</td>
<td>6.98</td>
<td>(2.6-5.1)</td>
</tr>
<tr>
<td>TSH (U/L)</td>
<td>3.09</td>
<td>2.47±0.8</td>
<td>1.41±0.4</td>
<td>1.42</td>
<td>(0.27-6.3)</td>
</tr>
<tr>
<td>Thyroid volume (ml)</td>
<td>9.0</td>
<td>17.0±7.1</td>
<td>14.2±2.7</td>
<td>12.5</td>
<td>(5-10)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>116</td>
<td>111±10</td>
<td>102±5</td>
<td>96</td>
<td>(85-91)</td>
</tr>
</tbody>
</table>

* Mean of multiple measurements during treatment periods has been given for all parameters.

**P1-d1-204 Thyroid 1**

### Raised TSH serum levels in children born prematurely: A follow-up study

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Non autoimmune hyperthyrotropinemia has been previously reported with a certain prevalence among children born prematurely. The natural history of the raised TSH in these children is however not known. We evaluated therefore 26 children born at a gestational age of 32.1 ± 1.6 weeks, appropriate for weight and length, for the first time at the age of 8.1 ± 2.1 years (phase 1) and subsequently at the age of 10.5 ± 1.9 years (phase 2). At each visit T3, T4, TSH, TPO-Ab, TG-Ab were evaluated and a thyroid ultrasound was performed. Iodine deficiency was excluded. Mean TSH serum level was similar in both phases (6.27 ± 1.0 vs 3.0 ± 0.9 mU/l; NS; normal range: 0.4-4.3 mU/l), however it was above the upper normal limit in 4 (15.4%) subjects at phase 1 (range 3.9-5.2 mU/l) and in 6 (23.7%) subjects at phase 2 (range 3.7-5.2 mU/l). There was no statistical difference between the two frequencies. During the follow-up 17 patients remained euthyroid, 2 showed a persistently raised TSH, 3 normalized and in 4 TSH increased above the upper normal limit. Free T4 and T3 were always in the normal range (phase 1 vs phase 2: T4 12.6±1.9 vs 12.2±1.6 µg/dl; T3 4.2±0.4 vs 3.9±0.4 pg/ml) and TPO- and TG-Abs were absent. Ultrasound showed a normal thyroid structure in all children. Mean thyroid volume increased during the follow-up study (p=0.025), but remained significantly lower than that of matched controls (phase 1: 1.07 ± 0.49 SDS; p=0.000; phase 2: -0.25 ± 0.54 SDS; p=0.005). We confirm that hyperthyrotropinemia is a transient condition in low birth weight children born prematurely, with a thyroid volume similar to its normal structure would support the concept of a partial refractoriness of the gland to TSH action or secretion of TSH isofoms with reduced bioactivity.

**P1-d1-205 Thyroid 1**

### Presentation and course of differentiated thyroid carcinoma in pediatric patients: Comparison between pre-pubertal children and adolescents

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Background: Differentiated thyroid carcinoma (DTC) is uncommon in children and adolescents. Since the prevalence is low in prepuberty, clinical studies tend to include prepubertal children within the larger group of adolescents. Objective: To analyze separately the clinical characteristics of prepubertal patients diagnosed with DTC, and to determine whether differences exist in their presentation, course, and outcome.

Methods: The records of 27 patients (10 prepubertal;17 pubertal) diagnosed and followed in our tertiary pediatric endocrine clinic from 1986 to 2007 were reviewed. Age at diagnosis, extension of tumor, treatment modality, course, and outcome were analyzed.

Results: At diagnosis the prepubertal group was distinct with regard to the prevalence of positive family history (p=0.037) and degree of tumor invasi; we have excluded the future hormone extension (p=0.012), lymph nodes involvement (p=0.09) and lung metastases (p=0.09). The extent of surgery and permanent post-operative complications (vocal cord paralysis, 2 patients; hypoparathyroidism, 2 pati-
Growth hormone (GH) is known about plasma ghrelin in these children during the first years of life and in controls from 2 months to 17 years. Subjects and methods: Forty children with PWS (24 boys 16 girls, median age 3.6 years [0.2 - 17.2 years], median BMI 0.3 Z-score [-4.0 - 4.4]) were compared to 84 controls (57 boys 27 girls median age 4.2 years [0.3 - 17.1] median BMI 0.1 Z-score [-1.5 - 1.9]). Children with PWS were then divided into 2 groups according to age and GH treatment.

Results: Median plasma ghrelin levels were significantly higher in children with PWS compared to controls at any age (568 vs. 173, p<0.0001) and decreased with age in both groups (p<0.0001). In the whole group of PWS, we found an inverse relationship between ghrelin and BMI Z-score (p=0.0032), insulin (p<0.0001), HOMA-IR (p=0.0027), leptin (p=0.0027) and lean mass (p=0.04). Plasma ghrelin levels were significantly higher in children with PWS than in controls, both in the youngest children below 3 years who were not receiving GH (771 vs. 233 p<0.0001) and in the children older than 3 years all of whom were treated with GH (428 vs. 159 p<0.0001). In young children with PWS, we did not find any relationship between ghrelin and BMI Z-score, insulin, HOMA-IR.

Conclusion: Plasma ghrelin levels in children with PWS are elevated at any age, particularly during the first years of life, thus preceding the development of obesity.

Placental Thyroid Hormones and Autoimmune Thyroid Disease

Both proapoptotic cytokines, IL-6 and IL-1beta are considered to be involved in the pathogenesis of autoimmune thyroid disease as well as in nonendocrine autoimmune diseases such as juvenile chronic arthritis (JCA). The cytotoxic mechanisms lead to thyroid damage and hypothyroidism (hypoT) in the course of chronic autoimmune thyroiditis (AIT) or thyroid stimulation in Graves disease (GD), leading to hyperthyroidism (hyperT). Serum concentrations of IL-6 and IL-1beta in patients with hypo- and hyperthyroidism (vs control) were evaluated at the onset of disease (before treatment introduction). We studied 26 children: 9 children with hyperT (7 girls and 2 boys), age 11.3+/-3.2 years, mean hormone values: TSH 44.9 uIU/mL, fT4 0.61 ng/dL, T3 3.23 pg/mL; ATP0 2691.7 IU/mL, 12 children with hypoT (9 girls and 3 boys, age 12.7+/-5.1 years; mean hormone values: TSH 0.0 uIU/mL, fT4 4.06 ng/dL, T3 18.95 pg/mL; TRAb 29.7 U/L). A group of 5 healthy subjects (2 girls and 3 boys, age 13.1+/-6.4 years) were also studied as a control group. Serum concentrations of IL-6 were low in all groups (below 1.6 pg/mL) compared with high values in sera and synovial fluids of patients with JCA (in many of them more than 100 pg/mL). IL-1beta levels were significantly higher in hypoT (2.23 pg/mL) compared to control (1.43 pg/mL) (p=0.037) and were not significant to hyperT (1.59 pg/mL). There was no difference between hypoT and hyperT. These results support the significant role of IL-1beta in the late hypothyroid phase of AIT rather than in late phase of extremely severe GD thus supporting the involvement of different proapoptotic factors in these autoimmune thyroid disorders of young patients.

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visceral fat (VFat) by magnetic resonance, and obtained birthweight (BW) and gestational age (GA) from hospital records. As expected, mean IMT was increased in the patients, as compared to IMT values in 16 asymptomatic age-matched young women (0.47 ± 0.01 mm vs. 0.38 ± 0.02 mm, p<0.001). BW and VFat were significantly correlated with IMT in young women with androgen excess, but the former association was no longer present after adjusting for GA. In multiple regression analyses, both GA (β=−0.39 to−0.48, p<0.01 to p<0.001) and VFat (β=0.29 to 0.38, p<0.05 to p<0.01), but not BW or serum androgens were independent predictors of IMT, explaining together 27% and 30% of left and mean IMT variance, respectively. GA (β=0.60, p<0.0001), but not BW, visceral fat or serum androgens, was also an independent predictor of the improvement in IMT over 18 mo, following specific therapy for the hyperinsulinemic hyperandrogenism in these subjects. Our results suggest that visceral adiposity is associated with increased cardiovascular risk in non-obese young women with androgen excess and point to a hitherto unknown association between early development, independent of size at birth, and vascular risk in these subjects.

**P1-d2-210 Obesity and Fat 2**

Adiponectin as an early indicator of insulin resistance in non-obese prepubertal children born large for gestational age

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Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrinology Unit, Istanbul, Turkey; Istanbul University, Istanbul Faculty of Medicine, Social Pediatrics Unit, Istanbul, Turkey; Istanbul University, Istanbul Faculty of Medicine, Physical Therapy and Rehabilitation Department, Istanbul, Turkey

Being born large for gestational age (LGA) has an increased risk of developing insulin resistance. Hyperadiponectinemia is associated with insulin resistance. The aim of this study was to evaluate insulin resistance, body composition and adipose tissue levels in LGA born non-obese children at prepubertal ages. Twenty-one (8F,13M) LGA born non-obese children (mean age 5.5 ± 2.3 years) were evaluated with regard to fasting insulin, insulin, IGFBP-1, leptin, adiponectin levels and body composition by DEXA. Their data were compared to that of non-obese 58 (26 F, 32 M) appropriate for gestational age (AGA) children (mean age 5.9 ± 0.9 years). Insulin resistance was evaluated as HOMA-IR. LGA children were taller than AGA children (p<0.005) but had similar weight SDS (0.31 ± 0.7 vs -0.02 ± 0.7) and BMI SDS (-0.01 ± 0.8 vs -0.25 ± 0.7). There were no significant differences in leptin, IGFBP-1, glucose and insulin levels, HOMA-IR and body composition between LGA and AGA born children. However, adiponectin level was significantly lower in LGA born (8.6 ± 4.3 µg/mL) than AGA born (21.9 ± 3.3 µg/mL) children after controlling for age, sex and BMI SD (p=0.008). In conclusion, LGA children have lower adiponectin levels than AGA children in spite of similar BMI and insulin levels. Adiponectin is a better indicator of insulin resistance in LGA born children at prepubertal ages. Being born LGA is per se a risk factor for hyperadiponectinemia even in the absence of obesity.

**P1-d2-211 Obesity and Fat 2**

Familiar history of premature cardiovascular events as a sole and independent risk factor for increased carotid intima-media thickness

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University of Chieti, Department of Pediatrics, Chieti, Italy

Atherosclerosis represents a progressive and slow process that seems to start in early childhood. Several studies have shown significantly increased carotid intima media thickness (cIMT) in children with type 1 diabetes, hypertension, familiar hypercholesterolemia and obesity, but no data are available in pre-pubertal children with a sole familiar history of premature cardiovascular events (FHPCE). The aim of this study was to evaluate cIMT in children with a parental history of premature cardiovascular risk and the relationship between cIMT and other known risk factors (insulin resistance (IR), oxidant status and lipid profile) involved in structural vascular changes. In 16 pre-pubertal children (5M/11F, mean age 7.9 ± 2.4 years) with FHPCE, anthropometric measurements and inflammatory markers (high-sensitivity C-Reactive Protein, hs-CRP) were evaluated and compared with 17 healthy pre-pubertal subjects (4M/13F, mean age 6.6 ± 2.2 years). Basal insulin (I) and glycaemia (G) were evaluated and IR indices (HOMA-IR and G/I ratio) were calculated in all children. High resolution ultrasound techniques was used to evaluate cIMT. Children with FHPCE had an increased cIMT (0.39 ± 0.02 vs 0.32 ± 0.06 mm; p=0.001) compared to healthy subjects. hs-CRP was not different between the two groups (0.65 ± 0.62 vs 0.47 ± 0.38 mg/L; p=0.7). Furthermore, no significant differences were found in term of fasting insulin levels (6.96 ± 3.62 vs 8.66 ± 5.22 µU/mL; p=0.5). G/I (16.28 ± 10.63 vs 13.38 ± 9.55; p=0.4) and HOMA-IR (1.29 ± 0.82 vs 0.57 ± 0.38; p=0.055) between children with FHPCE and controls. In addition, in children with FHPCE, cIMT was not correlated to indices of IR, oxidant status and lipid profile. In conclusion, pre-pubertal children with precocious history of cardiovascular risk presented an increased cIMT that is not influenced by alteration in glucose metabolism and/or oxidant-antioxidant status and/or lipid profile; in these children probably here- ditary and genetic predisposition play a pivotal role in the pathogenesis of increased cIMT.

**P1-d2-212 Obesity and Fat 2**

The relationship between growth of infants and ghrelin and adiponectin levels in breast milk: A longitudinal investigation

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To investigate the relationship between ghrelin and adiponectin levels and growth of infants, total and free ghrelin and adiponectin levels were studied in human milk and fasting serum samples of 25 healthy lactating women aged 20-31 years who had vaginal delivery and their infants at the 1st and 4th months of delivery. All ghrelin and adiponectin levels were analyzed by Linco’s ghrelin radioimmunoassay (RIA) and adiponectin ELISA kit. Body weight and body mass index (BMI) of infants and their mothers at birth and during study period were also recorded. While there were no significantly changes in 1st and 4th months total ghrelin levels (TGHRL) of breast milk and the serum of infant (p>0.05), maternal TGHRL was found significantly decreased at 4th month (p<0.05). Free ghrelin levels (FGHRL) in breast milk, and both infant and maternal serum FGHRL, were found significantly increased at 4th month (p<0.05). The mean adiponectin level in the serum of infant was decreased at the 4th month (p<0.05). The other adiponectin levels were found unchanged during the study period. While breast milk TGHRL were similar with maternal serum levels at the 1st and 4th months, both breast milk FGHRL were found significantly higher than infant and maternal serum FGHRL (p<0.05). BMI at birth was negatively correlated with breast milk TGHRL of 1st month (r=−0.41, p<0.05). There was a positive correlation between breast milk FGHRL of 1st month and delta body weight, BMI at 4th month and maternal delta FGHRL (r=0.44, p=0.05; 0.47, p=0.05; 0.44, p<0.05 r respectively). Breast milk FGHRL of 4th month was positively correlated with delta body weight of infant (r=0.43, p<0.05). No relationship could be determined between breast milk ghrelin and adiponectin levels and infant or maternal serum levels at 1st and 4th months (p>0.05). These findings suggest that the ghrelin in breast milk might have a significant role in growth of infants without any effect on serum ghrelin levels of infants. However the adiponectin levels in breast milk seems to be unrelated to growth of infant in early life.
**P1-d2-213 Obesity and Fat 2**

B-type natriuretic peptide (BNP) in asymptomatically obese children: Is there a relationship with cardiac parameters?

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1Gulhane Military Medical Academy, Pediatric Endocrinology, Ankara, Turkey; 2Gulhane Military Medical Academy, Pediatric Cardiology, Ankara, Turkey; 3Gulhane Military Medical Academy, Pediatrics, Ankara, Turkey; 4Gulhane Military Medical Academy, Medical Clinical Biochemistry, Ankara, Turkey

Objective: Obesity in childhood and, in particular, adolescence is associated with increased risk for cardiovascular disease in adulthood. Here, it was aimed to assess (1) the changes in Nt-pro-BNP, epicardial fat tissue, and intima-media thickness of carotid artery in childhood obesity, all of which can be used for early diagnosis of cardiovascular complications in adults, (2) the correlations of these markers within each other and with other parameters in the follow-up of childhood obesity.

**Patients and Methods:** Twenty cases (10 male, mean age: 10.25±0.53) with the diagnosis of obesity (patients group) and 20 cases (10 male, mean age: 10.47±0.78) with innocent murmurs (control group) were recruited for the study. In all of the patients, the serum Nt-pro-BNP were measured and, by using transthoracic echocardiography, the thicknesses of epicardial fat tissue and intima-media of carotid artery were evaluated.

**Results:** The mean left ventricular mass index was detected 39.87±8.5 gr/m2 in the obesity group and 36.7±6.28 gr/m2 in the control group (p=0.05). The mean Nt-pro-BNP was measured 109.25±48.53 pg/mL in the obesity group and 51.96±22.36 pg/mL in the control group (p=0.001), while the mean thickness of epicardial fat tissue was detected 5.66±0.32 mm in the obesity group and 2.9±0.09 mm in the control group (p=0.001) and the mean carotid artery intima-media thickness was found 0.07±0.01 mm and 0.05±0.01 mm in the control group (p=0.001). No statistically significant correlation was found between NT-pro-BNP levels and left ventricle mass index, left and right ventricle diastolic functions, epicardial fat and intima thickness in obese patients (p>0.05; r: -0.29, -0.24, -0.18, -0.32, 0.19, respectively).

**Conclusion:** In childhood and adolescent obese patients, there is not a correlation between NT-pro-BNP and cardiac parameters. Because of this reason, there is no need to routinely monitor NT-pro-BNP levels during clinical follow-up of asymptomatic obese patients.

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**P1-d2-215 Obesity and Fat 2**

Increased RBP-4 and lipocalin in children born after IVF: An early marker of the insulin resistance phenotype?

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Assisted reproduction techniques (IVF) have been widely used during the last few decades. However, few long-term prospective studies of children conceived after IVF have been performed. Our earlier findings demonstrated elevated blood pressure and triglyceride levels in IVF children compared to controls, while no differences in the occurrence of the metabolic syndrome were observed. However, nontraditional metabolic risk factors, such as leptin, adiponectin, high sensitivity CRP (hsCRP), interlinkulin-6 (IL-6), and retinol binding protein 4 (RBP-4) and neutrophil gelatins-associated lipocalin (NGAL) have not been studied in IVF children as yet. The aim of the study was to study the non-traditional metabolic risk factors in children born after IVF. Patients and methods: 100 children born after IVF (47 boys) and 59 spontaneously conceived controls (30 boys), aged 4-14 y, were studied prospectively. All children underwent physical examination and had fasting glucose, insulin, lipid profile, IL-6, hsCRP, adiponectin, leptin, NGAL, and RBP4 determined. Results: As a group, IVF children had significantly higher RBP4 (p=0.009) and NGAL (p=0.028) levels than controls. Studied in subgroups, when SGA children and twins were excluded, IVF girls had higher RBP4 (p=0.019) than controls. Furthermore, IVF singletons at puberal age had significantly higher hsCRP (p=0.048) and IL-6 (p=0.032) levels than controls. There were no statistically significant differences in leptin and adiponectin levels between the two groups. In our study, significantly higher RBP4 and NGAL levels in IVF children than controls were found, suggesting an early insulin resistance phenotype in these children. These results are in accordance with our previous findings of higher triglycerides and blood pressure in IVF children than controls. Further prospective studies should be performed in IVF children to determine the natural course of their metabolic risk factors profile.

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**P1-d2-216 Obesity and Fat 2**

Cannabinoid receptor, CB1, expression in primary adipocyte cultures in lean and obese pre-pubertal children, in relation to High Molecular Weight (HMW) adiponectin, HOMA Insulin Resistance (IR) and waist circumference

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Childhood obesity is associated with insulin resistance and the metabolic syndrome. CB1 is an endocannabinoid receptor associated with increased food intake and lipogenesis. HMW adiponectin is an adipokine biomarker for insulin sensitivity. We aimed to study the expression levels of CB1 in pre- (p) and mature (m) adipocytes from obese and lean children in association with HMW adiponectin, HOMA IR and waist circumference. Primary cultures of pre- and mature adipocytes were developed from routine surgical biopsies of subcutaneous abdominal adipose tissue from 36 healthy lean (BMI <85%) and 17 healthy obese (BMI ≥95%) pre-pubertal children in 2 groups (group A: 2 months-7 yrs and group B: 8-12 yrs). CB1 expression was studied at the mRNA (mR) level with RT-PCR and at the protein level (P) with western immunoblotting. Serum HMW adiponectin and insulin were measured by ELISA and waist circumference (WC) was measured on the day of surgery. CB1 was significantly (S) increased at the P in the mature adipocytes of the
older lean while it was 5 decreased in the mature adipocytes of the obese in both age groups. Serum HMW adiponectin was 3 decreased and HOMA IR was increased in the lean and obese of the older age group. WC was at a high risk percentile in the obese boys. In the obese subjects the decreased HMW adiponectin levels in association with the increased HOMA IR and high risk WC might play a role in the development of metabolic syndrome. The increased CBI P in the older lean pre-pubertal children may reflect a physiologic mechanism which enhances fat deposition in preparation for the increased energy expenditure of puberty. The reduced mR and P of CBI in the older obese pre-pubertal children though may be the body’s attempt to reduce lipogenesis in the abdominal region to limit the development of excessive insulin resistance during puberty.

**P1-d2-217 Obesity and Fat 2**

**Echocardiographic epicardial adipose tissue in obese children: A new indicator of insulin resistance**

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**Aim:** Until now, the association between subepicardial fat (SAT), insulin resistance, and intima-media thickness has not been evaluated in obese children. In this study, we evaluated whether echocardiographic SAT is related to insulin resistance, and intima-media thickness in obese children.

**Patients - methods:** A total of 46 obese patients (10.2 ± 2.46 years of age, 25 male patients) and 30 age- and gender-matched control subjects (10.8 ± 3.11 years of age, 13 male patients) were included in this study. The criterion for diagnosing obesity was defined as the body mass index (BMI) being over 97% percentile of the same gender and age. Serum triglyceride, low- and high-density lipoprotein, cholesterol, and glucose and insulin levels were measured in the fasting state. The estimation of insulin resistance was made using a homeostasis model assessment index. Each subject underwent a transthoracic echocardiogram and the SAT thickness was measured during end-diastole from the parasternal long-axis views.

**Results:** The obese patients had significantly higher SAT thickness and intima-media thickness values compared to the subjects in the control group (5.7 ± 1.4 vs. 3.02 ± 0.66 mm, 0.78±0.015 vs. 0.05±0.01, p=0.001, respectively). Simple linear regression analysis showed no significant correlation between epicardial adipose tissue and BMI SDS (r=0.08, p=0.585), insulin resistance(r=0.170, p=0.264) whereas there was significant correlation between epicardial adipose tissue and intima-media thickness (r=0.379, p=0.02) with an optimal cut off point, 4.1 mm SAT thickness determined insulin resistance with 90% sensitivity and 61% specificity.

**Conclusions:** Our study showed that the epicardial fat was significantly related to intima-media thickness. Assessment of SAT(>4.1 mm) and intima-media thickness during routine echocardiographic examination, a non-invasive method, might be used in predicting insulin resistance in obese children easily and reliably.

**P1-d2-218 Obesity and Fat 2**

**BMI changes during childhood and adolescence as predictors of amount adult subcutaneous and visceral adipose tissue in men - The good study**

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It is unclear how body mass index (BMI) changes during childhood and adolescence predict adult body composition. We have investigated the impact of BMI changes during different developmental periods for adult body composition and fat distribution. Detailed growth charts were retrieved for the men participating in the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study (n=612). Body composition was analysed using Dual X-Ray Absorptiometry and adipose tissue areas using abdominal computer tomography at 18-20 years of age. The main finding in the present study was that the amount of adult subcutaneous adipose tissue of the trunk was predicted both by BMI changes during late childhood (4-10 years of age) and adolescence (10-19 years of age) while the amount of adult visceral adipose tissue was predicted by BMI changes specifically during adolescence. Subjects with increases in BMI Z-score of >1 SD during late childhood had larger amount adult subcutaneous adipose tissue (+83%; p< 0.001) than subjects with unchanged BMI Z-score, but unaffected amount of visceral adipose tissue. In contrast, during adolescence, subjects with increases in BMI Z-score of >1 SD had both larger subcutaneous (+138%; p<0.001) and visceral adipose tissue areas (+91%; p< 0.001) than subjects with unchanged BMI Z-score. BMI increases during adolescence predicted the amount of adult visceral adipose tissue independent of prepubertal BMI. Early childhood (1-4 years of age) BMI changes were positively associated with adult lean mass but not with adult fat mass. The amount of visceral adipose tissue in men was associated with BMI changes specifically during adolescence, while the amount of subcutaneous adipose tissue was associated with BMI changes during both late childhood and adolescence.

**P1-d2-219 Obesity and Fat 2**

**LDL receptor mutation in a Druze kindred - Clinical, biochemical and genetic characteristics**

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Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutation in the LDL receptor gene. The heterozygous frequency is about 1/500 and the homozygous frequency is about 1/1,000,000. Through siblings aged 6, 7 and 9 years from a Druze kindred presented with cutaneous and tuberous xanthomas, and with failure to thrive. LDL-cholesterol levels ranged between 800-900 mg/dl. Analysis of mutation in the LDL receptor gene was done for 48 members of the extended family. Genomic DNA was extracted from the family member’s peripheral blood, and from cord blood of a newborn sibling. LDLR exon 4 was sequenced directly. The Y188X mutation was detected in three index patients. Restriction enzyme analysis confirmed the DNA sequence in these patients. We identified other heterozygous family members as Y188X carriers. Using the cord blood of the new-born sibling we done for 48 members of the extended family. Genomic DNA was extracted from the family member’s peripheral blood, and from cord blood of a newborn sibling. LDLR exon 4 was sequenced directly. The Y188X mutation was detected in three index patients. Restriction enzyme analysis confirmed the DNA sequence in these patients. We identified other heterozygous family members as Y188X carriers. Using the cord blood of the new-born sibling we diagnosed him as heterozygous for the LDL receptor mutation. Interestingly 3 infants with normal LDL-cholesterol levels were diagnosed as heterozygous carriers based on DNA analysis. Identifying the mutation in this large Druze family enabled us to diagnose carrier children who would otherwise be...
It has been reported that soy protein maintains a cholesterol-lowering effect, which was attributed to its isoflavone content. We evaluated the effects of soy-derived isoflavones in children with FH. 12 FH children (8 F/4M; aged 5.3-11.2 y) have completed a randomized placebo-control study. Inclusion criteria were cholesterol above 200 mg/dl and LDL above 130 mg/dl on two measurements. Children with endocrine diseases and those with BMI-SDS > 95th percentile were excluded. Isoflavones’ containing candies were used to improve compliance. Following an AHA step-1 diet over 12 weeks, participants with persistent hypercholesterolemia were randomly assigned to three 8-weeks long interventions: placebo, 16mg/d and 48mg/d of isoflavones, with a washout time of two weeks between the intervention periods. A monthly report of intake was obtained throughout the study. By candies counting we ascertained a compliance of at least 80%. Isoflavones had no effect on thyroid hormones, sex-hormones and SHBG. Mean TSH level was significantly decreased during 16mg-isoflavones intervention. Neither low nor high doses of isoflavones had any effect on lipid profile in the children (Table). Isoflavones have no effect on lipid profile in children with FH. Although considered the preferred approach in children, dietary intervention yielded no benefit in our study. Table: The difference (%) in lipid profile was significant during 16mg-isoflavones intervention. 

<table>
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<th>Dietary Intervention</th>
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<th>16mg isoflavones</th>
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<td>5 ± 10</td>
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<td>5 ± 9</td>
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</table>

O.Si.M.E. study Group: Domenico Viggiano, Antonio Fasolino, Norma D’Alessio, Natalia Avellino, Maria Carmela Vorga, Antonio Giosef Prisco, Felice Sorrentino. All the members are Primary Care Pediatricians of the National Health Service Unit “Salerno 1”, Italy.

The presence of metabolic syndrome (MS) in children and its close relationship to obesity are well demonstrated. The majority of studies have been hospital-based or have dealt with racially heterogeneous cohorts, and thus are not exempted from selection bias or confounding factors. Furthermore, the prevalence in free-living individuals is not correctly estimated. Other studies aimed to identify one or some clinical or biochemical predictive factors for MS, such as Body Mass Index (BMI), Waist Circumference (WC), Waist-to-Height Ratio (WHR) or Insulin-Resistance (IR), the latter showing probably the best correlation. We conducted a primary care-based study in order to determine the prevalence of MS and to identify its predictive factors a cohort of free-living obese children and adolescents. 415 subjects were enrolled with obesity as unique selection criterion. The entire cohort was screened for MS (at least two findings other than obesity: fasting hyperglycaemia (FH), low levels of HDL cholesterol (LHC), hypertriglyceridaemia (HTg) and hypertension (HT)). The overall prevalence of MS was 30.8%, with a significant difference between prepubertal and pubertal subjects. The major findings (other than obesity) were LHC (46.2%), HT (23.6%), HTg (22.2%) and FH (16.6%). The influence of puberty was evident only concerning the prevalence of LHC (42.4% vs 55.1%; p = < 0.01). The only significant clinical parameter related to MS was WHR, directly related with IR (assessed by HOMA) and with the same predictive power for MS, as indicated by ROC curve (figure). Our data clearly indicate that MS can be present in a significant percentage, even in a cohort of “healthy” obese children and that a simple, no-cost parameter easily assessed at the time of clinical evaluation could identify at risk subjects.

The association between depressive symptoms in childhood and overweight in adolescence. The TRAILS study

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In view of the increasing prevalence, important predictors of overweight in childhood and adolescence need to be determined. Depressive symptoms have been described both as a predictor and as a complication of overweight. Therefore, the aim of this study is to assess the association between depressive symptoms in childhood and overweight in adolescence. The TRAcking Adolescents Individual Lives Survey (TRAILS) is a population-based cohort study among 2000 adolescents, presently aged 14–16 years. These adolescents have been assessed biennially from the age of 11 years. At ages 11 and 15, we have assessed weight, height, skinfold thicknesses, and depressive symptoms through the Youth Self Report and Child Behavior Checklist questionnaires. Body fat percentage and waist circumference have been obtained at age 15. Results regarding the first 1037 participants showed that at age 11, clinical depressive symptoms were present in 10.9% of girls and 9.9% of boys. Mean BMI at age 15 was 21.28 kg/m2 in girls and 20.18 kg/m2 in boys. Results from linear regression analyses showed no significant results in boys. The
Obesity and Fat 2

Genetic studies in severe childhood obesity
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The prevalence of obesity is increasing in children worldwide. Although environmental factors have had a large impact, there is much evidence to support the fact that 40-70% of body fat is inherited. To identify the genetic basis of severe childhood obesity, we have recruited over 3000 severely obese children (BMI sds>3) to the Genetics of Obesity Study (GOOS). 50% of the GOOS cohort have a positive family history of obesity, consistent with the major genetic component to the obesity in these children. Taking a candidate gene approach, we have identified seven monogenic causes of obesity. Pathogenic mutations in the melanocortin 4 receptor gene (MC4R) represent the commonest monogenic cause of obesity (103/2609 unrelated probands) and are present in 4-5% of children with severe obesity. Mutations in the leptin and leptin receptor gene are found in up to 3% of children. Most of these mutations are found in homozygous form and are associated with hypergonadotropic hypogonadism and impaired linear growth. Heterozygous mutations in pro-opiomelanocortin (POMC)(Y221C, H143Q, R236C) are found in 1% of patients and result in hyperphagia and an increase in lean mass as well as fat mass. The detailed clinical phenotype of these disorders is reported. Only 16% of patients in the GOOS cohort have any form of developmental delay or syndromic features. Rare mutations in TrkB are associated with developmental delay, hyperactivity and impaired short-term memory. In summary, we report the genetic findings in the GOOS study which account for 7% of the patients recruited. There is considerable heterogeneity in the metabolic and endocrine phenotypes associated with severe childhood obesity, suggesting that other genes and mechanisms remain to be found.

Obesity and Fat 2

Increased ADMA levels in obese children: A foretelling marker for atherosclerosis?
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ADMA (asymmetrical dimethylarginine), the metabolite of methylated L-arginine, is a competitive nitric oxide (NO) synthase antagonist. It decreases the production and availability of endothelium-derived NO. Increased ADMA concentrations have been described in adults with obesity, hypertension and arteriole occlusive diseases. This study was planned to search whether increased ADMA levels are present in obese children, related with insulin resistance and high atherosclerosis risk. In this prospective study, 65 obese and 45 non-obese children were evaluated. Fasting glucose, insulin, lipid profile, lipoprotein a, apolipoprotein A and B levels were measured. Height and body weight were taken, physical examination and pubertal staging were performed. Mean age of patients was 11.13±2.86 and 10.41±2.66 years in obese and controls respectively (p<0.05). The mean BMI of obese patients was 28.01±4.19, and 18.18±2.99 kg/m² in controls (p<0.05). The mean ADMA level was 0.95±0.40 in obeses, which was significantly higher than 0.68±0.21 micromol/L in controls (p=0.001). The mean HDL was lower, LDL was significantly higher in obeses than controls (HDL: 51.1±12.09 and 59.5±13.62 mg/dL (p<0.001), and LDL: 91.38±26.58 and 80.88±21.17 mg/dL (p=0.025) in obese and control groups respectively). HOMA-IR was also significantly higher in obeses than controls; 3.42±1.80 and 2.61±1.31 respectively (p=0.03). The mean FGR was significantly lower in obeses as 7.36±5.24 vs 9.97±7.67 in controls (p=0.044). ADMA levels were correlated with presence of acantosis nigricans (Pearson correlation=0.207, p=0.032) and lipoprotein a (Pearson correlation=0.207, p=0.032) levels. ADMA levels were not correlated with BMI, FGIR and pubertal stage. There was also no correlation between ADMA and HOMA-IR levels. In conclusion, ADMA levels in obese children are higher than non-obese controls. It is positively correlated with presence of AN, which is an indicator of insulin resistance at tissue level and lipoprotein a, of which high level is a risk factor for cardiovascular diseases.

Obesity and Fat 2

Multiplex analysis of serum cytokines in obese children before and after weight loss
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The prevalence of the metabolic syndrome in childhood obesity has been related to subclinical inflammation. The objective was to examine inflammatory markers, adipokines, and their potential relationships with leptin and insulin resistance in a pediatric sample with varying levels of obesity. Furthermore we tested if successful weight reduction leads to reduction of inflammatory markers. High-throughput xMAP multiplex immunobead assay technology (Luminex Corp., Austin, TX) was used to simultaneously test 9 cytokines and chemokines such as interleukin (IL)1beta, IL6, IL8, IL10, monocyte chemoattractant protein 1 (MCP1), interferon IFNgamma, and tumor necrosis factor (TNF)-alpha, adiponectin and resistin. Furthermore serum insulin, glucose and leptin levels were measured. Parameters were quantified in the sera of 52 children (56% female), age 10.9±2.4, participating in a 1-year obesity lifestyle intervention including nutrition training, behaviour- and exercise therapy. At baseline there were significant correlations between resistin and levels of several cytokines (IFNg, IL1b, IL6, IL8, and TNFa, p<0.05), as well as between insulin resistance (HOMA) and IL1b or IL10. After 1 year of intervention, changes of BMI (delta BMI-SDS) correlated positively with changes in IL1b, IL8, and IL10 (p<0.05). Successful BMI reduction (delta BMI-SDS>0.4) was accompanied by reduction of IL1b, IL8, IL10, TNFa and HOMA and an increase of adiponectin levels. These data show that multiplexed analysis of serum biomarkers is useful for the evaluation of prognostic markers of childhood obesity and the metabolic syndrome. Inflammatory parameters were related to weight status, weight change and insulin resistance. Successful weight reduction improves chronic inflammation and thereby reduces the risk for the metabolic syndrome and cardiovascular disease in obese children.

Obesity and Fat 2

Oral glucose ingestion increases acylated ghrelin levels in prepubertal obese children
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Maria Teresa Muñoz; Jesús Argente
Hospital Niño Jesús, CIBER 08/03, UAM, Endocrinology, Madrid, Spain

Ghrelin is involved in carbohydrate metabolism and its levels are influenced by glucose ingestion. Although its different fractions, acylated (AG) and non-acylated (NAG), appear to have different effects in this phenomenon, this process is not clearly defined. Our aims were to determine the effect of an oral glucose tolerance test (OGTT) on the dynamics of total and acylated circulating ghrelin levels in prepubertal obese children and to analyze its relationship with changes in glucose and insulin concentrations. Seventy Caucasian prepubertal obese children (Tanner 1, 48 males and 22 females, mean BMI

Poster Presentations
4.00±1.42) were included in the study. An OGTT was performed (glucose 1.75 g/kg, maximum 75 g), obtaining venous blood samples at 0, 30, 60 and 120 minutes for glucose, insulin, total ghrelin (TG) and AG quantification. Circulating levels of TG and AG were determined by RIA (Linco, USA). Analysis was performed by using ANOVA test for repeated measurements. TG levels decreased whereas AG levels and the AG to TG ratio increased throughout the OGTT, as did glucose and insulin (Table: mean±SD). A positive correlation between the AG to TG ratio and insulin levels was found exclusively at 0′ (r=0.38; p<0.001). A negative correlation between insulin and total ghrelin level at 0, 30 and 120 minutes was found (r=-0.26, r=-0.26 and r=-0.27 respectively, p<0.05). No correlations between glucose and TG, AG or the AG/TG ratio were seen at any timepoint.

Changes in blood glucose and insulin levels are associated with modificati-
on in serum ghrelin levels with the fractions AG and NAG being oppositely affected. This opposite effect could suggest that these fractions have distinct effects in glucose metabolism.

### P1-d2-227 Obesity and Fat 2
**Bioenergetics intragastric balloon for treatment of morbid obesity in Prader-Willi syndrome: A long term study**
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¹Bambino Gesù Hospital, Research Institute, Paediatric and Autonomic Endocrine Diseases Unit, Rome, Italy; ²Bambino Gesù Hospital, Research Institute, Paediatric Surgery Unit, Rome, Italy; ³Bambino Gesù Hospital, Research Institute, Anaesthesiology Unit, Rome, Italy; ⁴Bambino Gesù Hospital, Research Institute, Endocrinology Unit, Rome, Italy; ⁵Bambino Gesù Hospital, Research Institute, Epidemiology Unit, Rome, Italy

Obesity in Prader Willi Syndrome (PWS) is progressive and severe. A dra-
stic body weight reduction is mandatory to reduce the risk of cardio-respira-
tory and metabolic complications. The insertion of a Bioenergetics Intragastric Balloon (BIB) in the gastric cavity represents an effective alternative to the more complex and invasive bariatric surgery. Recently, we reported the risks and benefits of BIB for treatment of morbid obesity in 12 PWS patients during 6 months. The aim of this study was to assess long-term effects of BIB treatment. Five patients out of them (3M, 2F, aged 16±1.8 yrs (8.1÷30.1yrs), underwent multiple treatment with BIB. Two patients repeated the treatment twice, other two three times and one four times. We obtained excellent results in the two youngest patients. One was treated for the first time at 8 yrs (BMI: 44.6 ±36.7 kg/m²); 6 years later he stopped his fourth treatment with a BMI of 33.8 ±26.8 kg/m². The second patient (9.4 yrs) had a BMI of 39.1±26.7 kg/m²; at the end of his second treatment BMI was 23.6 ±18.2 kg/m². At 14.6 yrs, 2 yrs after last BIB, BMI was 29 ±18.7 kg/m². The third patient (12.4 yrs) inserted BIB three times in 3 years: starting BMI was 39.3 ±24.6 kg/m²; and at the end of his third treatment, slightly increased to 40.2 ±28.5 kg/m². At 17.5 yrs, his BMI was 49.1 ±33.1 kg/m². He underwent a bilio-pancreatic diversion. In the two oldest patients (20.6 ±18.1 yrs and 30.1 yrs), only a slight BMI reduction was obtained with their first balloon and a BMI stabilization was observed during the subsequent treatments. During the free intervals or after treatment interruption BMI tended to increase in every patient. However, some complications occurred: acute gaseous gastric distension due to ingestion of a fizzy drink; balloon rupture; recurrent diarrhoea and aerophagia. This study shows that, when non-invasive pharmacological therapies fail, BIB may be effective to control body weight in PWS patients with morbid obesity, particularly if started in early childhood. Careful clinical follow-up and close collaboration with parents are crucial to avoid severe complications caused by unrestrained food intake despite BIB.

### P1-d2-228 Obesity and Fat 2
**Is Leptin level associated with vascular changes related to early atherosclerosis in obese adolescents?**
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¹Ministry of Health, Department of Pediatrics, Manisa, Turkey; ²Celal Bayar University, Faculty of Medicine, Pediatric Endocrinology, Manisa, Turkey; ³Celal Bayar University, Faculty of Medicine, Department of Radiology, Manisa, Turkey; ⁴Celal Bayar University, Faculty of Medicine, Department of Biochemistry, Manisa, Turkey

**Objective:** Atherosclerotic Cardiovascular Diseases is the most common cause of death in the world. A high leptin concentration, in particular, is found in obese individuals and is strongly associated with vascular changes related to early atherosclerosis. We investigated relationship between leptin levels and atherosclerotic markers, homocysteine levels and artery carotid intima media thickness. We aimed to determine whether high leptin levels is associated with vascular changes related to atherosclerosis in obese adolescents children.

**Subjects and Methods:** Forty-eight obese children were included in this study. Thirty healthy children defined as a control group. Lipid profile, atherosclerotic markers, leptin and homocysteine levels and common carotid artery intima media thickness determined in both groups. Adolescents who were above the 95 percentile for Body Mass Index (BMI) were diagnosed as being obese.

**Results:** Leptin levels were significantly higher in obese adolescents than control group (p<0.001). Lipid profiles, atherosclerotic markers, and homocystein levels did not differ significantly between obese and control groups. Artery carotids intima media thickness in obese children significantly higher than controls (p=0.01). There were positively moderate correlations between leptin levels and Apo A in obese children (r=0.39, p=0.006). There were no similar correlation in healthy adolescents. We found positively moderate correlations between leptin levels and carotid artery intima media thickness in obese children (r=0.26, p=0.02).

**Conclusion:** Common carotid artery intima-media thickness (IMT) is considered a factor of cardiovascular risk and an early marker of coronary artery disease. Thicker IMT and positively relationship between leptin level and IMT in obese children suggest that high leptin level is associated with vascular changes related to early atherosclerosis. However, positively relationship between leptin level and Apo A level in obese children could be preventive vascular changes related to early atherosclerosis.
The increase in fat mass in obesity results not only from hypertrophy but also from hyperplasia from differentiation of preadipocytes into mature adipocytes. Current information on transcriptional control of adipocyte differentiation is mainly derived from mouse model systems. We differentiated human preadipocytes of the SGBS cell line into mature adipocytes and characterized the alterations in gene expression patterns using whole genome microarrays. 248 genes were significantly (fdr 0.05) regulated more than 5-fold during the differentiation process. Amongst these genes we recovered well known markers of adipogenesis such as adiponectin, C/EBPβ or PPARγ. Altogether 31% of the upregulated genes were associated with lipid metabolism. Principal component analysis and cluster analysis correctly grouped early differentiation stages vs. late differentiation stages. SGBS data were then compared to microarray data from differentiating mouse 3T3-L1 cells and human primary adipocytes. SGBS expression patterns showed highest similarity with primary subcutaneous adipocytes, as expected. These data indicate that SGBS cells are a valid model of human adipogenesis. We applied microarray data from differentiating mouse 3T3-L1 cells and human primary adipocytes (ACACB, ME1, PPP2R5A, DBI, AKR1C2, CDO1, ACY1, EPHX1) vs. mature adipocytes (ACACB, ME1, PPP2R5A, DBI, AKR1C2, CDO1, ACY1, EPHX1) during adipogenesis. There was, however, a lower but existent expression of most of these genes in primary human fibroblasts, mononuclear cells and/or HCAECs as an endothelial cell model. Prospectively, the SGBS model is a valid model of human adipocyte differentiation and can be applied to identify new regulators of adipogenesis as well as adipocyte biology.

**P1-d2-230 Obesity and Fat 2**

**Expression profiles of human adipocyte differentiation using the SGBS cell model**

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The rise in fat mass in obesity results not only from hypertrophy but also from hyperplasia from differentiation of pre adipocytes into mature adipocytes. Current information on transcriptional control of adipocyte differentiation is mainly derived from mouse model systems. We differentiated human pre adipocytes of the SGBS cell line into mature adipocytes and characterized the alterations in gene expression patterns using whole genome microarrays. 248 genes were significantly (fdr 0.05) regulated more than 5-fold during the differentiation process. Amongst these genes we recovered well known markers of adipogenesis such as adiponectin, C/EBPβ or PPARγ. Altogether 31% of the upregulated genes were associated with lipid metabolism. Principal component analysis and cluster analysis correctly grouped early differentiation stages vs. late differentiation stages. SGBS data were then compared to microarray data from differentiating mouse 3T3-L1 cells and human primary adipocytes of different fat depots. SGBS expression patterns showed highest similarity with primary subcutaneous adipocytes, as expected. These data indicate that SGBS cells are a valid model of human adipogenesis. We applied this microarray model to select 12 genes whose expression in vitro may be applicable to classify pre adipocytes (CXCL6, GATA6, LNX) vs. differentiating (GPX3) vs. mature adipocytes (ACACB, ME1, PPP2R5A, DBI, AKR1C2, CDO1, ACY1, EPHX1). Evaluation of mRNA expression by real-time PCR confirmed highest expression of CXCL6 and GATA6 in pre adipocytes, an early induction of expression for PP2R5, CDO1 and ACACB and an increase in expression of AKR1C2 and EPHX1 during adipogenesis. There was, however, a lower but existent expression of most of these genes in primary human fibroblasts, mononuclear cells and/or HCAECs as an endothelial cell model. Prospectively, the SGBS model is a valid model of human adipocyte differentiation and can be applied to identify new regulators of adipogenesis as well as adipocyte biology.

**P1-d2-231 Obesity and Fat 2**

**Estrogens influence endothelial function as measured by Peripheral Arterial Tonometry (PAT) index**

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**Background:** Factors like obesity, insulin resistance and estrogens are known to influence arterial endothelial function.

**Objective:** To study the effect of estrogens on the endothelial function in 7th grade school children (12.2, 0.57 yrs).

**Methods:** Endothelial function in 102 healthy subjects was evaluated by using Endo-PAT device; then divided into 4 quartiles (Q) on the basis of their PAT index. Q1 PAT < -1SDS (n=16,boys 10), Q2 PAT Mean to -1SDS (n=39,boys 16), Q3 PAT Mean to +1SDS (n=33,boys 13) and Q4 PAT > +1SDS (n=14,boys 5). Height, weight, waist circumference (WC), body fat, BMI, BP, lipids, glucose, insulin, adiponectin, estrone (E1), estradiol (E2), DHEAS & estrone sulfate (ES) were measured.

**Results:** Fasting glucose, insulin, QUICKI, lipids were normal in all 4 quartiles. BMI, WC, body fat increased with quartiles. Q2 levels in Q3 & Q4 were higher & lowest in Q1 (<0.01 respectively). The E1 levels were lower in Q1 highest in Q3 (<0.01). ES levels were the highest in the Q3 & were different from Q1 (p<0.001) (Table). DHEAS levels were not statistically different in any of these groups. The adiponectin levels were increased and highest in the Q4 quartile (p <0.01).

**Conclusion:** Better endothelial function was associated with higher BMI, Estrone, Estradiol, Estrone Sulfate. These findings underline the role of estrogens in maturation of endothelial function during childhood.

**P1-d2-232 Obesity and Fat 2**

**Effect of growth and body mass on intestinal absorption vs. endogenous synthesis of cholesterol in children and adolescents**

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Serum concentrations of plant derived sterols (phytosterols) are closely related to intestinal cholesterol absorption whereas cholesterol precursors such as lanosterol are markers for endogenous cholesterol synthesis. Studies from adults indicate that cholesterol absorption and synthesis are associated with body weight. The aim of our study was to investigate the relation between growth and BMI with sterol metabolism in children. We determined free and esterified phytosterol concentrations of brassicasterol, stigmasterol, campesterol and beta-sitosterol as well as lanosterol (marker of endogenous cholesterol synthesis) and total cholesterol in 521 serum samples (285 girls, 256 boys, aged of 7 to 17 years) with BMI-SDS range from -3.2 to 3.1 applying liquid chromatography-tandem mass spectrometry. Samples were categorized in two age groups (<12 and ≥12 years). Ratios of phytosterols and lanosterol to total cholesterol were calculated. Serum concentrations of phytosterols and campesterol did not differ between boys and girls. Phytosterol and total cholesterol levels were significantly lower, whereas the lanosterol concentrations were significantly higher in older boys≥12 years (P<0.001). For girls we did not identify age-dependent changes in phytosterol concentrations. In both sexes, phytosterol levels significantly decreased from pubertal stage 1 to 5 (P<0.001). BMI showed the strongest influence on serum sterol concentrations in girls and boys. Lean children (BMI-SDS ≤ -1) had significant higher phytosterol concentrations and lower lanosterol concentrations compared to children wit BMI-SDS > 1. In contrast the total cholesterol concentration was not associated by BMI in this normal population childhood cohort. In summary, endogenous cholesterol synthesis appears to increase during pubertal development. The BMI showed the strongest modulating effect on sterol homeostasis with higher phytosterol and lower lanosterol concentrations in lean children representing markers of cholesterol absorption and endogenous cholesterol synthesis, respectively.
**P1-d2-233** Obesity and Fat 2

**Metabolic syndrome and impaired glucose tolerance in obese children and adolescents are modulated by the rs997509 ENPP1 gene variant**

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The nucleotide pyrophosphatase/phosphodiesterase-1 (ENPP-1) gene encodes a membrane-bound glycoprotein that inhibits the insulin-receptor tyrosine kinase activity, resulting in reduced insulin sensitivity. Variants on the ENPP-1 have been associated with insulin resistance. Since insulin resistance is a pivotal factor in the development of metabolic syndrome, we aimed to test the association between the rs997509 ENPP-1 gene variant (C/T), previously associated with type 2 diabetes in adults. We screened 409 obese children (213 girls). All of them underwent a standard oral glucose tolerance test (OGTT); baseline measurements included blood pressure, plasma lipids and fasting insulin levels. The homeostasis model assessment of insulin resistance (HOMA-IR) and whole body insulin sensitivity index (WBISI) were calculated. A general linear model (GLM) was generated to assess differences between groups. Non-normally distributed values were log transformed before the analysis, but geometrical means are shown. A logistic regression equation was generated to predict the natural log of the odds for a subject to show IGT or metabolic syndrome according to genotype. Thirty seven percent of obese subjects showed the metabolic syndrome and 5.3% the IGT. Obese children carrying the rare allele (T) showed higher insulin (p=0.001), HOMA-IR (p<0.001) and lower WBISI values (0.04) (table 1). Moreover, subjects carrying the rare allele showed a higher prevalence of metabolic syndrome (p=0.005; OR: 2.4, 95% CI: 1.3-4.3) and of impaired glucose tolerance (p<0.001; OR 4.7, 95% CI: 1.9-11.4) than subjects homozygotes for the common allele. We conclude that the rare allele of ENPP1 rs997509 variant can predispose obese children to develop the metabolic syndrome and IGT.

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<th>CC (357)</th>
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<td>BMI (Kg/m2)</td>
<td>32.5 ± 5.4</td>
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<td>z-score BMI</td>
<td>5.4 ± 2.1</td>
<td>5.3 ± 2.5</td>
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<td>Gliceaemia (mg/dl)</td>
<td>79.8 ± 8.9</td>
<td>81.5 ± 9.1</td>
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<tr>
<td>Insulin (IU/l)</td>
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<td>41.6 ± 30.0</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA</td>
<td>6.2 ± 4.1</td>
<td>8.3 ± 5.2</td>
<td>&lt;.001</td>
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<tr>
<td>WBISI</td>
<td>2.5 ± 2.0</td>
<td>2.0 ± 1.5</td>
<td>0.04</td>
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</table>

**P1-d2-234** Obesity and Fat 2

**Genetic variation in LRP5 associates with metabolic characteristics in prepubertal children**

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Premature adrenarche (PA) associates with unfavourable metabolic characteristics. Wnt signaling has an effect on adrenocortical function. We hypothesized that low-density lipoprotein receptor-related protein 5 (LRP5), involved in Wnt signaling and cholesterol metabolism, has a role in the polygenic pathogenesis of PA and associates with metabolic characteristics in PA and healthy children. We performed a cross-sectional association study in 73 children with PA and 96 healthy age- and gender-matched controls. Three single nucleotide polymorphisms (SNPs) in LRP5 were analyzed by direct sequencing: c.1647C>T (p.F549F), c.3357A>G (p.V1119V) and c.3989C>T (p.A1330V). Baseline hormonal and lipid measurements and an oral glucose tolerance test were performed. There were no significant differences in the LRP5 SNP distributions between the control and PA groups. However, SNP A1330V associated with higher dehydroepiandrosterone sulfate (DHEAS) levels in the control subjects (A/A vs. A/a; mean, 0.8 vs. 1.4, p = 0.012). It associated also with higher levels of total cholesterol (TC, 4.2 vs. 4.7, p = 0.023) and low-density lipoprotein (LDL, 2.4 vs. 2.9, p = 0.018) in the control group, as did SNP V1119V (TC, p = 0.029; LDL, p = 0.022). SNP F549F associated with higher systolic blood pressure in the control group (100 vs. 108, p = 0.041) and SNP V1119V with higher systolic blood pressure in the PA group (103 vs. 108, p = 0.018). There were no differences in the parameters of glucose metabolism, serum HDL or triglyceride levels between the genotype groups. In conclusion, genetic variation in LRP5 did not predispose to PA. However it associates with serum DHEAS level, lipid pattern and systolic blood pressure in healthy prepubertal children indicating its possible role in the development of metabolic syndrome.

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**P1-d2-235 Reproductive Endocrinology 1**

**A complex submicroscopic chromosomal imbalance in Xp 21.2 with microduplication and one microtriplication containing the DAX1 gene in a patient with 46,XY/47,XY,+mar karyotype with partial gonadal dysgenesis and gonadoblastoma**

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Introduction: Models with transgenic animals demonstrated that DAX1 gene located at Xp21.2 chromosome (DDS locus) acts as an anti-testicular factor when extra copies were expressed.

Objective: To analyze the dosage effect of DAX1 gene in a patient with partial 46,XY gonadal dysgenesis and a 46,XY[11]/47,XY,+mar karyotype.

Patient: A non-syndromic 14 yr old patient was referred with primary amenorrhea and absence of breast development. External genitalia showed clitoromegaly (4.6X1.0 cm), a single perineal opening and non palpable gonads. LH and FSH levels (33 and 72 UI/L, respectively) and testosterone levels (479 ng/dL) were elevated. Pelvic ultrasound showed a 2 mL uterus and no gonads. She underwent laparoscopy and a gonadoblastoma in the right dysgenetic testis and ipsilateral epididymes and deferent duct and a left dysgenetic testis with ipsilateral Fallopian tube were identified.

Methods: FISH was performed using the clone RP11-89L23 which binds to the Xp21.2-2region containing DAX1 and contiguous MAGEB genes. MLPA (Kit P185 Intersex) was performed to screen DAX1, SOX9, WNT4 and SRY genes dosage. Array-CGH was carried out using BAC/PAC clones that provide tiling-path coverage of X chromosome with a 100-200 kb resolution.

Results: FISH identified duplication in Xp21 region in the marker chromosome. MLPA revealed the presence of extra copies of DAX1 gene. CGH-array revealed a duplication of 1.6 Mb (16 clones) in Xp21.2 and a triplication of 1.8 Mb (20 clones) in Xp21.2. This last clone contains DAX1 and MAGEB genes but the absence of triplication of RP11297K22 clone which contains the MAGEB genes indicates that only DAX1 is triplicated.

Conclusion: We described a non-syndromic girl with a 46,XY/47,XY,+mar karyotype containing a duplication in Xp21.2 region causing partial gonadal dysgenesis. Our findings support that DAX1 gene overexpression and not MAGEB genes cause gonadal dysgenesis.

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**P1-d2-236 Reproductive Endocrinology 1**

**Voice changes during testosterone treatment in female-to-male transsexual adolescents**

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At the end of male puberty the pitch of the voice drops as a result of androgens. The maximum voice change occurs between Tanner stages G3 and G4.
Female-to-male transsexual adolescents of the VUmc receive testosterone esters i.m. every 2 weeks in an increasing dose from the age of 16. The goal of the study was to investigate the influence of exogenous testosterone on the fundamental voice frequency (f0). 25 Female-to-male transsexual adolescents were included in the study. F0 was measured every 3 months from start of the androgen treatment by means of electroglottography during speech and reading. In addition serum testosterone concentrations were gathered at these time points. Fundamental frequencies were analyzed longitudinally and were related to testosterone levels and the testosterone dose given. During the first 6 months of the androgen treatment a maximal decrease in f0 was observed. A logarithmic relation was found between serum testosterone concentrations and the f0. The voice break occurs at the beginning of the induction of the male puberty, not at the end as is observed in biological males. This early change might be caused by the high serum testosterone levels within the first 72 hours after injection. There might be a certain threshold level at which the enlargement of the vocal tract and resulting lowering of the voice frequency takes place. Increasing doses of testosterone do not result in an additional effect on the f0. Another explanation might be the continuous presence of androgens in the blood during exogenous testosterone administration. In the onset phase of the biological male puberty testosterone levels fluctuate over the day, in this way the vocal tract is not exposed to continuous levels of testosterone and the subsequent change of the voice frequency will take longer than in biological male puberty.

P1-d2-237 Reproductive Endocrinology 1

46, XY partial gonadal dysgenesis: A cohort of 29 patients with clinical, biological, histological and genetic studies

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46, XY gonadal dysgenesis (GD) is an anomaly of testicular development which leads to a defect in the masculinisation of the foetus. The aims of this study were to describe patients with partial GD (circumstances of diagnosis, anatomy of external and internal genitals, biology, histology), their management (sex of rearing, attitude toward gonads, genitoplasty), their puberty and to evaluate mutation frequency of SF1, DAX1 and SOX9. Data from 29 patients born between 1966 and 2006 were analysed. Inclusion criteria were ambiguous genitals, karyotype 46, XY without mosaicism, strong arguments for GD (persistent Müllerian structures, gonadal histology and/or low serum testosterone or anti-mullerian hormone (AMH) level) and no evidence of renal or adrenal disease. Diagnosis was suspected at birth for 24 patients. External and internal genitals varied from very masculinised to very feminine with only clitoral enlargement. Sexual hormones (testosterone, AMH) could be normal in the first year of life but AMH was the more often disturbed. Testosterone response after hCG stimulation was variable (35% < 1 ng/ml but normal > 3 ng/ml in 39%). 11 patients were raised as males and 18 as females. The number of genitoplastic procedures was higher for males (2,8) than for females (2,2). Gonads were dysgenetic testes with peripheral tubules like embryonic cords and a thin albuginea, but few had only a reduced tubule density. 3 patients had a gonadoblastoma (aged 4 months, 1,5 and 7 years). Mutation screening is in progress but at least 4 patients without adrenal deficiency had a SF1 mutation and another a deletion of 9p. Phenotype of partial 46, XY GD is variable and diagnosis can be difficult when biochemistry is normal with no histological analysis (patients raised as males). SF1 mutations in isolated GD seem to be frequent.

P1-d2-238 Reproductive Endocrinology 1

A novel heterozygous GATA4 mutation in a patient with 46,XY DSD

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GATA4 is a transcription factor of the zinc-finger family, which plays a role in murine gonad and heart development. GATA4 is expressed in the urogenital ridge and the heart during embryonic development. The GATA4 knockout mouse presents with a severe heart defect and absence of the gonads. Recently, heterozygous GATA4 mutations were described in few patients with androgen insensitivity syndrome and testicular feminization. We report a novel heterozygous GATA4 mutation in a patient with 46,XY DSD. The patient was a young man with worldwide hypoplasia, bifid scrotum, palpable gonads, pulmonary stenosis and patent ductus arteriosus at birth. No Muellerian structures were visible by ultrasound. The patient was born small for gestational age (1600g) at 34 weeks of gestation. Testosterone increased normally from 2.0 to 7.1 ng/ml after HCG stimulation at 3 months. The mutation p.A200P/WT was absent in 100 healthy controls. Functional characterization of the mutation is in progress. Mutations of the androgen receptor and 5alpha-reductase gene were excluded in the patient. Mutations in GATA4 seem to be rather rare in patients with 46,XY DSD, but should be considered in patients with associated heart defects. However, the minor heart defect in the patient might also be an independent association.

P1-d2-239 Reproductive Endocrinology 1

A 46 XY Girl with fragile X syndrome and a novel nonsense mutation (Q116X) in the AR gene

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Background: Premature ovarian failure is well known to be associated with Fragile X Syndrome, but not sex reversal. Androgen insensitivity syndrome is the most common cause of sex reversal in a male and is caused by androgen receptor (AR) gene mutations. The AR gene is located on the X chromosome at Xq11-12 consisting of eight exons. There can be a wide variety of phenotypic abnormalities ranging from partial androgen insensitivity syndrome to complete androgen insensitivity syndrome based on the functional abnormality caused by the AR gene mutation.

Objective: To describe a novel mutation in AR gene that resulted in complete sex reversal in a 46XY girl with Fragile X Syndrome.

Methods: We describe a 15 month girl with Fragile X Syndrome (>200 CGG Repeats), of Bangladeshi origin who came to medical attention for development delay and macrocephaly at which time the karyotype was noted to be 46 XY. The child has normal female genitalia with no clitoromegaly, no palpable testes in the labia or inguinal canal. Neither uterus nor ovaries were seen on MRI of the pelvis. HCG stimulation test revealed a good testosterone response from 45 ng/dl to 699ng/dl. The AR gene was subsequently analyzed.

Results: Sequencing revealed the presence of a novel CAG to TAG nonsense mutation (Q116X) in exon 1 of the AR gene. This mutation results in a glutamine substitution which induces a stop codon.

Conclusions: To our knowledge this is the first report of this Q166X mutation in the AR gene. This is the first account of androgen insensitivity syndrome and Fragile X Syndrome to be reported in the same patient. The mutation in the AR gene causing the complete androgen insensitivity in our patient is thought to be unrelated to the Fragile X Syndrome however the FMR1 gene is located on Xq27.3.
The first and the rate-limiting step in the biosynthesis of hor- mones in all ste- roidogenic tissues, conversion of cholesterol to pregnenolone, is catalyzed by the cholesterol side-change cleavage cytochrome P450 (P450scce) encoded by a single gene CYP11A1. Until now mutations in CYP11A1 gene were reported in 6 patients, all of whom presented with adrenal insufficiency within the first 4 years of life, gonadal failure in both sexes and severely under-androgenized external genitalia (Prader stages 1-2) in 46,XY individuals. Here we identi- fied and functionally characterized a novel homozygous mutation in P450scce, which was associated with late-onset adrenal insufficiency and sexual ambi- guity in a male. A 46, XY patient was born at term to consanguineous parents. At birth he presented with hypospadias and bilateral cryptorchidism and was assigned as male. At 5 yrs the hypospadias and cryptorchidism were surgically corrected without any complications. At 9.5 yr the patient presented with le- thargy, hyperpigmentation, salt loss, undetectable adrenal steroids and eleva- ted ACTH. Hormonal replacement with hydrocortisone and 9α-fluorocortisone was started. A defect at the early step of the biosynthesis of adrenoc and nadal steroids was suspected. Results of STAR gene sequencing were normal. Direct sequencing of CYP11A1 gene revealed a novel homozygous L222P mutation. To clarify effect of this mutation on P450scce function, HEK293 cells were transfected with human adrenodoxin, adrenodoxin reductase and STAR expression plasmids together with either wild-type (p450scc-WT), mutant P450scce (P450scc-L222P) or empty expression plasmids. P450scce activity was determined by measuring concentration of pregnenolone syn- thesized from cholesterol in the medium. P450scce activity of P450scc-L222P mutant was ~7% compared to P450scc-WT. The results confirm functional significance of L222P mutation and illustrate existence of a milder phenotype associated with P450scce deficiency.

### Table 1

<table>
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<td>Wnt4 gene</td>
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A new mutation within the Wnt4 gene was identified in 1 out of 4 patients. Our data confirm that Wnt4 is involved in the regulation of Müllerian duct development and ovarian androgen biosynthesis. The absence of mutation in 3 out 4 adolescents with MRKH and hyperandro- genism does not exclude a potential abnormality within the Wnt4 transduc- tion signal. We suggest that in adolescent girls with primary amenorrhea, Müllerian duct abnormality and hyperandrogenism, a Wnt4 mutation should be sought.

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**P1-d2-242 Reproductive Endocrinology 1**

**Testicular function in sons of women with polycystic ovary syndrome (PCOS) from infancy to adulthood**

*Sergio Recabarren 1*, *Teresa Sir-Petermann 2*, *Rafael Ríos 3*, *Manuel Maliqueo 4*, *Pedro Rojas-Garcia 5*, *Monica Recabarren 2*, *Roy Rodolfo A. 5*

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**Context:** PCOS is a common endocrine-metabolic disorder with strong familial aggregation. It has been demonstrated that an important proportion of male members of PCOS families exhibit insulin resistance and related meta- bolic defects. However, the reproductive phenotypes in firstdegree male rela- tives of PCOS women have been less described.

**Objective:** To evaluate the pituitary-testicular function and Sertoli cell func- tion in sons of women with PCOS during different stages of life: early infan- cy, childhood and adulthood.

**Design:** Eighty four sons of women with PCOS (PCOSS) and 60 sons of control women without hyperandrogenism (CS), matched for age, were studied. In infants, children and adults of both groups, the pituitarygonadal axis was evaluated by a GnRH agonist test (leuprolide acetate, 10 μg/kg s.c.). Serum anti-Müllerian hormone (AMH) and inhibin B were used as Sertoli cell markers. Serum concentrations of gonadotropins, steroid hormones, sex hormone binding globulin (SHBG), inhibin B and AMH were determined by specific assays.

**Results:** Basal concentrations of gonadotropins, sex steroids and inhibin B were similar between PCOSS and CS during early infancy, childhood and adulthood. Stimulated gonadotropin and sex steroid concentrations were also similar in both groups in the three study periods. However, AMH serum concentrations were significantly higher in PCOSS compared to CS during early infancy [925.0 (457.3 - 1401.7) pmol/l vs 685.6 (417.9 - 1313.2) pmol/l, p = 0.039] and during childhood [616.3 (304.6 - 1136.9) pmol/l vs 416.5 (206.7 - 801.2) pmol/l, p = 0.007].

**Conclusions:** We conclude that AMH concentrations are increased in pre- pubertal sons of women with PCOS, suggesting that these boys may show an increased Sertoli cell number or function during infancy and childhood. Supported by FONDECYT Grants 1050915 and 1030487

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**P1-d2-243 Reproductive Endocrinology 1**

**Induction of fertility in hypogonadotropic hypogonadism - Is age at treatment important?**

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Past management strategies for induction of puberty for boys with hypogo- nadotropic hypogonadism have utilised oral, intramuscular or subcutaneous androgens, with gonadotrophin therapy at time of desired fertility, usually around 25-30 years Standard treatment utilises HCG 1500IU x2/week twice weekly with addition of rFSH 150 IUx3/week Mean sperm density at concep- tion approximates 5x106/ml 1.2. Further cycles of gonadotropins result in more rapid induction of spermatogenesis via an imprinting effect. Long term suppression of the hypothalamic pituitary testicular axis ensures persistence of pre-pubertal gonadotropins and lack of spermatogenesis. We hypothesised that pubertal induction with HCG and rFSH for boys with hypothalamic hypogonadism would result in effective testicular growth and normal sperma-
Congenital cryptorchidism and dioxin levels in breast milk and placenta

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Dioxins are persistent fat-soluble environmental toxicants. They accumulate into human body especially via fatty foods. In animal studies, fetal and lactational exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has been shown to affect the male reproductive system, including testicular descent. The aim of this study was to evaluate whether congenital cryptorchidism is associated with dioxin levels in placenta and breast milk samples reflecting fetal exposure. The study included breast milk and placenta samples representing cryptorchid or healthy boys attending the Danish-Finnish cohort study on the incidence and risk factors of cryptorchidism. The boys were born 1997-2001. They were examined for cryptorchidism at birth and at 3 months. Breast milk samples were collected as additive aliquots between the ages of 1 and 3 months. Altogether 280 placenta samples [112 Finnish (56 cases and 56 controls), 168 Danish (39 cases and 129 controls)] and 130 breast milk samples [65 Finnish (33 cases and 32 controls), 65 Danish (29 cases and 36 controls)] were analysed for the 17 toxic polychlorinated dibenzop-dioxins and dibenzofurans (PCDD/Fs).

Placenta Samples: In Danish boys, median (range) PCDD/F WHO-TEq* (pg/g fat) was 11.8 (7.4 - 27.4) in cryptorchid boys and 10.9 (4.2 - 31.3) in healthy boys, p=0.25. In Finnish boys, the level was 9.8 (4.0 - 22.4) in cases and 8.5 (3.0 - 30.6) in controls, p=0.39.

Breast Milk Samples: The median (range) PCDD/F WHO-TEq (pg/g fat) in Danish cryptorchid boys was 15.5 (9.0 - 28.3) and 11.4 (4.7 - 26.0) in controls, p=0.02. In Finnish boys, there was no significant difference between cases and controls 11.0 (4.0 - 25.2) vs. 10.5 (3.9 - 31.9), p=0.75. In conclusion, in the Danish group, but not among Finnish boys, congenital cryptorchidism was associated with increased maternal breast milk levels of PCDD/Fs. No association was found between placental dioxins and congenital cryptorchidism.

*WHO-recommended 2,3,7,8-TCDD equivalent quantity for PCDD/Fs.

Methods: Eighty-nine boys who had an orchidopexy were subjected to bilateral testicular biopsy. Histological analysis of 78 biopsies indicated three types of spermatogonia. After puberty, sperm concentrations were analysed and correlated to bilateral testicular histology. The aim was to define the risk of future infertility via a testis biopsy program for boys with cryptorchidism.

Findings: In patients with unilateral cryptorchidism 70% of scrotal testes had spermatogonia. In this study, sperm concentrations and postpubertal hormone levels were correlated to bilateral testicular histology. The aim was to define the risk of future infertility via a testis biopsy program for boys with cryptorchidism.

Background: Early surgical correction of an undescended testis is performed to prevent the development of male infertility. However, in boys with cryptorchidism early successful surgery cannot prevent infertility if they lack Ad spermatogonia. In this study, sperm concentrations and postpubertal hormone levels were correlated to bilateral testicular histology. The aim was to define the risk of future infertility via a testis biopsy program for boys with cryptorchidism.

Methods: Eighty-nine boys who had an orchidopexy were subjected to bilateral testicular biopsy. Histological analysis of 178 biopsies indicated three groups of high, intermediate, and low risk of infertility according to the presence of Ad spermatogonia. After puberty, sperm concentrations were analyzed and correlated to plasma gonadotropin and testosterone levels.

Findings: In patients with unilateral cryptorchidism 70% of scrotal testes had an impaired transformation of Ad spermatogonia, indicating that cryptorchidism is a bilateral disease. Sperm concentrations correlated to the number of Ad spermatogonia found at the time of orchidopexy (p=0.0005). All males in the high risk of infertility group were oligospermic (mean: 8.9 x 106 sperm/ ejaculate) and 20% were azoospermic. These patients had 25 times less sperm...
compared to the group with presence of Ad spermatogonia in both testes. (p=0.00000002) Correlations between testicular histology and postpubertal hormone levels confirmed a relative gonadotropin deficiency in the majority of males with cryptorchidism.

Interpretations: Ad spermatogonia proved to be a discriminating factor for the fertility outcome in cryptorchidism. Gonadotropin treatment following orchiopexy should be considered in cryptorchidism when no Ad spermatogonia are found in undescended gonads and scrotal testis have Ad germ cell counts <0.005 per tubule.

Results: IS was strongly correlated to breast stage (p = 0.01) in girls, and with genital stage (p = 0.001) as well as testicular volume in boys (p = 0.001). IS showed a curvilinear pattern with high pre-pubertal levels followed by a decline towards mid-puberty, after which an increase was seen in late puberty. In gender and puberty adjusted models IS was significantly correlated with age (p = 0.007), TBF% (p = 0.002) and IGF-I levels (p = 0.004), respectively. No correlation was found to VO2max (p = 0.597). In a multivariate analysis (GLM) including all factors IS remained significantly associated with puberty, age, TBF% and IGF-I, but not with VO2max or gender (adjusted R2 = 0.435). No correlations was found between GH-R genotypes and IS.

Conclusion: Adolescent insulin sensitivity was strongly dependent on pubertal development, age, TBF% and IGF-I, but not on VO2max or GH-R genotypes.

**P1-d2-247 Reproductive Endocrinology 1**

**Regulation of spermatogenesis in McCune-Albright syndrome: Lessons from fifteen year follow-up**

Filippo De Luca; Valérie Mitchell; Małgorzata Wasniewska; Teresa Arrigo; Maria Francesca Messina; Mariella Valenzise; Luisa De Sanctis; Najiba Lahou

1University of Messina, Department of Pediatrics, Messina, Italy; 2CHRU-Faculty of Medicine, Laboratory of Spermiology and Histology, Lille, France; 3University of Turin, Department of Pediatrics, Turin, Italy; 4CHU Cochin-saint Vincent de Paul, Laboratory for Hormone Biology, Paris, France

Sexual precocity in McCune-Albright syndrome (MAS) has been reported in only 15 % of boys, and little is known on the long-term evolution of MAS in males. In a boy with MAS we studied spermatogenesis, testis histology and immunohistochemistry with the aim to shed light on seminiferous tubule activity. A boy who presented at the age of 2.9 years with sexual precocity, monolateral macroorchidism, increased testosterone levels and suppressed gonadotropins, was followed up until the age of 18. Throughout follow-up testicular asymmetry persisted and gonadotropin and testosterone pattern did not change. At the age of 18, inhibin B was undetectable while alpha-inhibin nonreactive inhibin was within normal range. Anti-Mullerian hormone level was slightly subnormal. Sperm cells were 3 900 000 per ejaculate. Histology of both testes showed spermatogonia, spermatocytes, and, in some tubes, matured spermatooza. Sertoli cells were markedly stained with anti-inhibin alpha-subunit antibody in both testes. There was no immunostaining of Sertoli, Leydig or germ cells with anti-betaA or anti-betaB antibody. MAS R201H mutation was identified in both testes. Conclusion: The 15-year follow-up in this boy with MAS demonstrated that autonomous testicular activation and gonadotropin suppression persisted over time. This provides an interesting model of active spermatogenesis despite long-term FSH suppression. It also suggests that FSH is needed for full expression of the inhibin betaB-subunit gene, expression previously reported in normal adult subjects in germ and Leydig cells.

**P1-d2-248 Reproductive Endocrinology 1**

**Changes in insulin sensitivity during puberty: Relation to body composition, physical fitness and the GH-IGF-I axis**

Kasper Sorensen; Lise Akselgaard; Henrik Laerfers; Anders Juul

Copenhagen University Hospital, Growth and Reproduction, Copenhagen, Denmark

Background: In adults, insulin sensitivity (IS) is dependent on body composition, physical fitness and the GH-IGF-I axis. Marked changes in IS occur during puberty, but the primary determinants remain unclear.

Objective: The objective of the present study was to evaluate IS during puberty in relation to physical fitness (VO2max), total body fat percentage (TBF%), and IGF-I levels in healthy children and adolescents, and to evaluate the potential influence of a common polymorphism in growth hormone receptor (GH-R), the exon 3 deletion.

Population and Methods: Hundred and thirty-two healthy Caucasian subjects (70 girls) aged 8.5 – 16.1 years were recruited as a part of the COPENHAGEN Puberty study. TBF% was evaluated with a whole body DEXA scan. Physical fitness was determined by cycle ergometry with direct measurement of maximal oxygen uptake. IS was calculated from a standard 2-hour oral glucose tolerance test. The GH-R genotypes were determined by multiplex PCR.

**P1-d2-249 Reproductive Endocrinology 1**

**Premature thelarche — Characteristics at presentation and clinical follow-up**

Liat de Vries; Anat Gusz-Mark; Liora Lazar; Adi Reches; Moshe Phillip

Schneider’s Children Medical Center in Israel, Institute of Diabetes and Endocrinology, Petah-Tikva, Israel

Premature thelarche (PT) is defined as isolated breast development without any other signs of sexual maturation. The possible progression of PT towards true precocious puberty (PP) is not well established. The study aimed at investigating the clinical course and growth pattern of girls with PT. The charts of 140 girls with PT followed in our clinic between 1995 and 2005 were reviewed. Data on general and endocrine evaluation, course of growth and puberty, and the outcome of PT were collected. Analysis was conducted by age at appearance of PT: birth (n=58), 1-24 months (n=62), or 2-8 years (n=20). PT was diagnosed at mean age of 2.1±1.8 years. It was bilateral in 108 girls (93 Tanner stage 2; 15 -Tanner stage 3), and unilateral in 32 girls (all -Tanner stage 2), (p=0.02). The prevalence of unilateral PT was similar in all age groups. Anthropometric, bone age and laboratory parameters on admission were comparable in the 3 age groups, except for a lower weight SDS (p=0.05) on admission, significant increase in height-SDS during follow-up, (0.06±0.75 to 0.36±0.87 p<0.04), and higher FSH (basal and GnRH stimulated) (p<0.05), in girls with PT who presented before the age of 2 years. Mean duration of follow-up was 3±2.5 years (0.2-9.3). PT regressed in 51 girls, persisted in 46, progressed in 6 and had a cyclic pattern in 13. A progressive or cyclic course was significantly more prevalent among girls with PT presented after the age of 2 year (55%) compared to girls who presented at birth (13.3%) or at age 1-24 months (5.9%), (p<0.001). Only 7 girls (5.9%) progressed to PP: three with PP appearing at birth, one - at 6 months, and three between 4.4-6.08 years. Our data confirm the benign nature of PT. Late presentation (after age 2 years) is associated with a higher rate of either progressive or cyclic course. Neither age at presentation nor course of thelarche appears to be associated with different anthropometric, biochemical or bone age characteristics.
These were re-rated by the authors not knowing the chronological age, man- 
BA or BoneXpert’s rating. All the new ratings (large circles in Figure) were 
within 1.5 years of the automatic BA values except 5. In 2 of these, man- 
BA placed large emphasis on the accelerated carpal bones, which BoneXpert 
ignores.

The accuracy (standard deviation) between manual and automatic rating is 
0.69 years [0.65; 0.72] 95% CI; mean difference 0.08 years (n.s.), bias 0.08 
y/y (n.s.).

Conclusion: BoneXpert’s ability to process virtually all image automatically, 
to avoid errors, and to obtain good agreement with an operator for BA range 
2 - 16 years suggests that the method is efficient and reliable in children with 
precocious puberty.

P1-d2-251 Reproductive Endocrinology 1
Extractive testosterone: Contribution to the 
diagnostic evaluation of female precarious 
pubertal development
Ana Maria Sequera; Gabriela Ruibal; Maria Jose Iparaguirre; 
Liliana Munoz; Martha Suarez; Silvia Martin; Hugo Boquete; 
Mirta Mirasi; Hugo Fideleff

Hospital Dr. T. Alvarez, Medicine - Endocrinology Unit, Buenos Aires, Argentina; Hospital de Niños, Endocrinology Unit, Cordoba, Argentina

Early modifications of testosterone (T) of ovarian origin occur during female 
pubertal development as a result of LH pulsatility onset (Mitamura R et al, 
JCEM 2000). To analyze the usefulness of T measurement, with extractive 
methodology (Text) in female pubertal pathology and to compare its diagno-

P1-d2-252 Reproductive Endocrinology 1
Ovarian volume correlates with inhibin B levels 
in girls with central precocious puberty but not 
in girls with premature telarche
Gianpaolo De Filippo; Alfredo Nazzaro; Domenico Randina; 
Fortunato Lzonardo; Gioacchino Scarano; Maria Rita Melone; 
Enrico Spinosa

1Gaetano Rummo Hospital, Pediatric Endocrinology Unit, Benevento, 
Italy; 2Gaetano Rummo Hospital, Reproductive Medicine Unit, 
Benevento, Italy; 3Federico II University, Department of Clinical and 
Experimental Medicine, Naples, Italy; 4Gaetano Rummo Hospital, 
Medical Genetics Unit, Benevento, Italy

Precocious puberty (PP) in girls is defined as the appearance of secondary sex 
characteristics before the age of 8 years, mostly caused by premature idi-

P1-d2-253 Reproductive Endocrinology 1
Usefulness of GnRH infusion test in the 
diagnosis of boys with delayed puberty
Romina Ginsparg; Rodolfo Rey; Silvia Gottlieb; Ana Keselman; 
Alicia Martinez; Maria Gabriela Balleleri; Horacio Domene; 
Maria Gabriela Ropelato

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Endocrinológicas, Buenos Aires, Argentina; 2Dept Histología, Biología 
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Aires, Argentina; 3Hospital de Niños Ricardo Gutierrez, División 
Endocrinología, Buenos Aires, Argentina

In boys who present with delayed puberty, the differential diagnosis between 
hyponadotropic hypogonadism (HH) and constitutional delay of puberty 
is difficult. Although different tests have been proposed to distinguish these 
two conditions, no consensus exists. The aim of this study was to evaluate 
the usefulness of the GnRH infusion test to predict complete or partial HH in 
boys presenting with delayed or arrested puberty. Thirty-five boys (16.4 ± 3.0 
yr; 30 with no delay and 5 with arrested puberty) were submitted to IV GnRH 
infusion (0.83 µg/min for 120 min). LH and FSH were determined by 
IFMA at 0, 15, 30, 45, 60 and 120 min. Final diagnosis of complete HH (n=17) 
was done when tests were < 4 ml at 18 yr. Partial HH (n=11) was diagnosed when 
tests enlargement started but remained arrested for a period of at least 1 yr 
and did not reach 15 ml. ROC curves were used to determine the optimal cut-

Poster Presentations
LR+ was 8. The ratio between peak LH and peak FSH did not add any further useful information. In conclusion, in a boy with delayed or arrested puberty, a low basal FSH value confirms a diagnosis of HH with high accuracy, and no further test is necessary. A subsequent GnRH infusion test is useful to confirm HH when peak LH or peak FSH are below selected cut-off values. When both peak values are above cut-off values in the same patient, a constitutional delay of puberty can be diagnosed with high accuracy.

P1-d2-254 Reproductive Endocrinology 1
Prevalence of polycystic ovary syndrome in young women who had idiopathic central precocious puberty, according to Rotterdam criteria
Roberto Franceschi1; Rosella Gaudino1; Alma Marcolongo2; Maria Chiara Galli1; Franco Antoniazzi1; Franco Borotto1; Luciano Tato1
1Paediatric Unit, Policlinico G.B. Rossi, Department of Mother and Child, Biology-Genetics, Verona, Italy; 2Obstetrics and Gynaecology Unit, Department of Mother and Child, Biology-Genetics, Verona, Italy

Many studies report the occurrence of polycystic ovary syndrome (PCOS) following idiopathic central precocious puberty (ICPP) and it is unclear whether the association of PCOS with ICPP is any more frequent than PCOS would be expected by chance. The aims of this study were to find the prevalence of PCOS in a cohort of women who had ICPP as defined by the Rotterdam 2003 criteria, and to detect any predictive factors of PCOS at the time ICPP was diagnosed. Forty-six young women who had been treated with GnRH analogues during infancy, were observed at gynaecological age of 6.23 ± 3.3 yrs post-menarche. We found that 15% of the women had oligomenorrhea, 28% had clinical hyperandrogenism, 48% biochemical hyperandrogenism and 50% polycystic ovary morphology (PCOM). 41% had PCOS according to the Rotterdam 2003 criteria. The prevalent phenotype of PCOS was characterized by clinical and/or biochemical hyperandrogenism and PCOM. We did not find any predictive factors for PCOS at the time ICPP was diagnosed. In conclusion, patients with ICPP are prone to developing PCOS. The prominent phenotype in our cohort was due to PCOM associated with clinical and/or biochemical hyperandrogenism. Further follow-ups of these young adult patients will clarify whether this phenotype persisted and if it will have important long-term implications in terms of increased risk of infertility or metabolic complications.

P1-d2-255 Reproductive Endocrinology 1
Functional magnetic resonance imaging (fMRI) in children, promising preliminary data
Henrica de Bie1; Kim Oostrom1; Dick Veltman1; Henriette Delenarre - van de Waal1
1VU Medical Centre, Pediatric Endocrinology, Amsterdam, Netherlands; 2VU Medical Centre, Pediatric Psychology, Amsterdam, Netherlands; 3VU Medical Centre, Psychiatry, Amsterdam, Netherlands

Paediatric brain imaging studies have shown an increase of cortical grey and white matter volume across the age range of 4 to 20 years. In children with endocrine abnormalities, aberrant hormone activities may interfere with brain development. Especially thyroid hormone, corticosteroids and growth factors, receptors of which are found throughout the brain are suggested to be important in this respect. Functional MRI (fMRI) allows non-invasive assessment of brain functioning and is an exciting tool in neuroscience: It allows us to “watch the brain thinking”. Especially in longitudinal designs, it may also contribute to better understanding of functional brain maturation. At present, one of the critical limitations is that fMRI is extremely sensitive to motion and therefore difficult to perform in young children. To address this issue, we developed an extensive subject preparation protocol including a mock MRI session in a dummy scanner. Using this protocol, we were able to collect fMRI data also from very young children (range, 4.5 - 7.2 years). Preliminary analyses revealed robust activity of the parahippocampal and hippocampal areas during encoding versus baseline (picture).

P1-d2-256 Reproductive Endocrinology 1
Effect of prenatal and early postnatal androgens on adult hippocampal pyramidal neuron count and morphology of preoptic area in rats
Masalić Candemir1, Serap Semiz2, Esat Adigüzel2, Gülçin Abbar1, Nilüfer Gökcin Yonguç Damiro1
1Pamukkale University, School of Medicine, Pediatric, Denizli, Turkey; 2Pamukkale University, School of Medicine, Pediatric Endocrinology, Denizli, Turkey; 3Pamukkale University, School of Medicine, Anatomy, Denizli, Turkey; 4Pamukkale University, School of Medicine, Histology, Denizli, Turkey

Hippocampus is implicated on functions related to learning, and memory and hippocampal morphology exhibits sexual differences. Mechanisms were not clearly exhibited. Hippocampus is developmentally sensitive to gonadal steroids especially in prenatal period. Preoptic Area (POA) is the critical neural substrate underlying gender specific behaviors and morphologically different in both sexes. It is known that androgens assessed in prenatal period enlarge.
POA volume and contribute to male specific behaviors. Although previous studies demonstrated that administration of testosterone to newborn rats changes astrocyte morphology in POA, there is no information whether these differences continue in adulthood. In our study we investigated the effects of testosterone propionate (TP) administered in prenatal and postnatal periods on pyramidal cell count of hippocampus and morphology of astrocyte in rats. Wistar Albino rats were used in the study. Rats were assigned to one of 3 treatment regimes, including prenatal and postnatal group (maternal TP assessed in pregnancy), prenatal and postnatal androgen group (maternal TP assessed in pregnancy and TP assessed in postnatal period), and postnatal androgen group (TP assessed in postnatal period). Sesame oil was injected to male and female control group. When the rats were adult, they were sacrificed under general anesthesia after intracardiac perfusion. Acquired sections were exposed to cell count in hippocampal pyramidal layer with cresyl violet and astrocyte morphology in POA visualized by glial fibrillary acidic protein (GFAP) immunocytochemistry. Hippocampal cell count was higher in the male controls than in the female controls (p=0.01). The cell count in both prenatal and postnatal androgen groups was higher than that in female controls (p<0.03). In prenatal and postnatal androgen group, primary and secondary astrocyte branching count was higher than that in both postnatal and male control groups. No statistical study was done because most sections couldn’t be visualized by GFAP immunocytochemistry.

**P1-d3-257** GH Treatment 2

**Effect of growth hormone treatment on insulin secretion and sensitivity in relation to growth of children born small for gestational age**

Burak Salgin
University of Cambridge, Paediatrics, Cambridge, United Kingdom

NESGAS is a randomised multi-centre trial to evaluate the safety and efficacy of GH treatment at varying doses in short SGA children without catch-up growth. The aim of this analysis was to explore the relationship between changes in insulin secretion and sensitivity, IGF-I levels and growth over one year of treatment.

We studied 32 (22 males, 10 females) pre-pubertal children born SGA (aged 3.9-9.9 years) who had failed to show catch-up growth and were naive to GH treatment. Fasting blood samples were taken to assess IGF-I, glucose and insulin levels. Subjects underwent a short intravenous glucose tolerance test to measure acute insulin secretion, and HOME was used to calculate insulin sensitivity.

The disposition index gave an estimate of insulin secretion for the degree of insulin sensitivity. These measurements were repeated after treatment with GH (67µg/kg/day) for 12 months. GH treatment resulted in increases in SDSs for height (-3.1±0.6 to -2.2±0.7, p<0.001), height velocity (+1.2±1.2 to 3.6±1.9, p<0.001) and IGF-I levels and growth over one year of treatment.

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P1-d3-259 GH Treatment 2

**Three years safety and efficacy of a novel once-a-week sustained release rhGH (LB03002) in treatment-naive prepubertal children with GHD**

Ferenc Peter; Conrad Savoy; Hyi-Jeong Ji; Paul Saenger

Buda Children’s Hospital, for Biopartners-LG Life Sciences GH Study Group, Budapest, Hungary; Biopartners GmbH, Baar, Switzerland; LG Life Sciences Ltd., Seoul, Republic of Korea; Accelis Ltd., Budapest, Hungary; Albert Einstein College of Medicine, New York, United States

LB03002 is a novel once-a-week subcutaneous sustained release rhGH. Less frequent administration could provide a considerable improvement on patients’ compliance and convenience.

Previously untreated children with GHD (N=51; boys/girls: 30/21; median age at baseline: 7.3 ± 2.19 years) were randomized into 4 groups in a parallel, assessor-blinded, phase II/III study. They were treated for the 1st year with either daily rhGH 0.03mg/kg/day or with LB03002 at any of the 3 different
doses: 0.2mg/kg/week, 0.5mg/kg/week or 0.7mg/kg/week. Patients treated in the 1st year with LB03002 at 0.2mg/kg/week or at 0.7mg/kg/week were switched for the 2nd and 3rd year to 0.5mg/kg/week. Patients on LB03002 at 0.5mg/kg/week were treated with unchanged dose for the entire 3 years, whilst patients treated with daily rhGH for 2 years, were switched for the 3rd year to LB03002 at 0.5mg/kg/week. LB03002 in all dose groups was safe and well tolerated over the 3 years treatment period. All laboratory parameters for safety assessment, including fasting glucose and hemoglobin A1c, did not show any clinically significant changes from baseline. There were no obvious safety concerns, such as puberal advancement or acceleration of bone age. Occasional injection site re- actions were mostly mild and transient and resolved within 2 to 3 days post-dose without intervention. No patient discontinued the study over the 3 year period.

In children with GHD, prolonged administration of once-a-week LB03002 over 3 years was shown to be safe and well tolerated. Sustained growth over the entire 3 year period was observed, similar to the growth pattern known for daily rhGH. A pivotal phase III study in children with GHD (n=169) or TS (n=149).

**Background** The PREDICT study investigates the relationships between genomics and biomarkers before and during GH therapy in children with GHD or TS.

**Objective:** Analysis of genetic polymorphisms in relation to changes in IGF-I SDS after one month of GH treatment in previously untreated children with GHD (n=169) or TS (n=149).

**Methods:** Blood was taken for measurement of serum IGF-I and for DNA extraction at baseline, and after one month of GH treatment (Saizen®, Merck Serono) for a repeat IGF-I level. DNA was analysed for 1536 single nucleotide polymorphisms (SNPs; Illumina® platform) in 98 candidate genes. Baseline and one month IGF-I levels were analysed centrally and corrected to age and gender-related standard deviation scores (SDS). Genotypes were compared with changes in IGF-I SDS by ANOVA, corrected for multiple testing based on the number of linkage disequilibrium blocks within each gene, with significance defined as p<0.05.

**Results:** IGF-I genotypes were correlated with change in IGF-I SDS for six of the 98 selected genes, including SH2B2 (involved in JAK2 activation) and two isoforms of PI3 kinase (A and G). In TS, genotypes were correlated with change in IGF-I SDS for 12 genes that were distinct from those identified in GHD, including PI3 kinase B, receptors for oestrogen, insulin and retinoic acid, the signalling molecules AKT2 and GRB10, and two genes related to the GH axis: GHRH and POU1F1.

**Conclusions:** Multiple genetic polymorphisms (different in GHD and TS) in pathways related to the control of growth are associated with an early response to GH, as defined by change in IGF-I. These genotypes should be tested for their effect on growth and metabolic responses to GH to enhance our ability to predict the diverse effects of GH.

### Table 1: Height SDS (mean ± SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>End of 1st year</th>
<th>End of 2nd year</th>
<th>End of 3rd year</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB03002: 0.2 mg/kg/week (N=13)</td>
<td>5.00 ± 1.61</td>
<td>-3.97 ± 1.28</td>
<td>-3.10 ± 1.24</td>
<td>-2.56 ± 1.26</td>
</tr>
<tr>
<td>LB03002: 0.5 mg/kg/week (N=13)</td>
<td>3.94 ± 0.81</td>
<td>-2.55 ± 0.61</td>
<td>-1.87 ± 0.56</td>
<td>-1.49 ± 0.75</td>
</tr>
<tr>
<td>LB03002: 0.7 mg/kg/week (N=13)</td>
<td>4.64 ± 1.32</td>
<td>3.03 ± 1.11</td>
<td>2.22 ± 1.13</td>
<td>1.66 ± 1.26</td>
</tr>
<tr>
<td>Daily rhGH: 0.03 mg/kg/day (N=12)</td>
<td>4.52 ± 1.39</td>
<td>3.06 ± 1.27</td>
<td>2.17 ± 1.08</td>
<td>1.89 ± 1.05</td>
</tr>
</tbody>
</table>

*In cooperation with Biopartners* and LG Life Sciences’ GH Study Group

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**P1-d3-261 GH Treatment 2**

**Genetic polymorphisms associated with changes in serum insulin-like growth factor-I (IGF-I) in prepubertal children with growth hormone deficiency (GHD) or Turner syndrome (TS) after one month of GH therapy:**

*The PREDICT Study*

P1-d3-261

**Background**

The metabolic effects of GH vary, and GH sensitivity is unique among different populations of children. GH axis: GHRH and POU1F1.

**Objective:** Analysis of genetic polymorphisms in relation to changes in IGF-I SDS after one month of GH treatment in previously untreated children with GHD (n=169) or TS (n=149).

**Methods:** Blood was taken for measurement of serum IGF-I and for DNA extraction at baseline, and after one month of GH treatment (Saizen®, Merck Serono) for a repeat IGF-I level. DNA was analysed for 1536 single nucleotide polymorphisms (SNPs; Illumina® platform) in 98 candidate genes. Baseline and one month IGF-I levels were analysed centrally and corrected to age and gender-related standard deviation scores (SDS). Genotypes were compared with changes in IGF-I SDS by ANOVA, corrected for multiple testing based on the number of linkage disequilibrium blocks within each gene, with significance defined as p<0.05.

**Results:** IGF-I genotypes were correlated with change in IGF-I SDS for six of the 98 selected genes, including SH2B2 (involved in JAK2 activation) and two isoforms of PI3 kinase (A and G). In TS, genotypes were correlated with change in IGF-I SDS for 12 genes that were distinct from those identified in GHD, including PI3 kinase B, receptors for oestrogen, insulin and retinoic acid, the signalling molecules AKT2 and GRB10, and two genes related to the GH axis: GHRH and POU1F1.

**Conclusions:** Multiple genetic polymorphisms (different in GHD and TS) in pathways related to the control of growth are associated with an early response to GH, as defined by change in IGF-I. These genotypes should be tested for their effect on growth and metabolic responses to GH to enhance our ability to predict the diverse effects of GH.

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**P1-d3-262 GH Treatment 2**

**Lipolytic and anabolic effects of growth hormone are dissociated**

*Ralph Decker; Kerstin Albertsson-Wikland; Andreas EM. Nierop; Berit Kristrom; Zeen Hochberg; Johanna Dahlgren*

1International Pediatric Growth Research Centre, Department of Pediatrics, Gothenburg, Sweden; 2Muvara bv, Multivariate Analysis of Research Data, Data Theory, Leiderdorp, Netherlands; 3Pediatric Endocrinology University of Umea, Department of Pediatrics, Umea, Sweden; 4Division of Pediatric Endocrinology, Rambam Medical Centre, Haifa, Israel

**Context:** There is a broad variation in longitudinal growth response during GH treatment among prepubertal short children. Yet, growth is but one of the several effects of GH.

**Hypothesis:** The metabolic effects of GH vary, and GH sensitivity is unique to each child.

**Objectives:** To investigate whether diverse metabolic functions respond accordingly to growth.

**Design:** A randomized, prospective, multicentre trial was performed for a 2 years period, with two treatment regimens a) individualized GH dose, six different dose groups ranging 17-100 µg/kg/d (n=87) and b) standard GH dose
of 43 μg/kg/d (n=41). Patients: 128 prepubertal short children, 75% of them diagnosed as GH deficient and 25% as idiopathic short stature.

**Results:** The anabolic and carbohydrate variables IGF-1, IGFBP-3, FFM, and insulin target in the same direction as height gain. All variables in this vector bundle show high mutual correlations. IGF-1 increment correlated with height gain (r=0.54, p<0.0001). Stepwise regression showed that 71% of the variation in IGF-1 was explained by height gain (49%), changes in insulin (9%), free fat mass (FFM) (5%), and biceps skinfold (4%), with only a small impact of the GH dose (4%). Changes in IGF-1 solely accounted for all explainable changes in FFM (31%). FFM, but not total fat mass, increased after 2 years of GH treatment in doses >40 μg/kg/d (p<0.001). The anabolic component is dose-dependent. Independent samples t-test shows dose-dependency of ΔIGF-1 (p<0.0001), ΔIGFBP-3 (p<0.001), AFFM (p<0.001), and Δinsulin (p<0.05) comparing low (17, 33, 40 μg/kg/d) to high GH dose groups (50, 66, 100 μg/kg/d). Variables derived from adipose tissue (total fat mass, leptin, skinfold measurements, triglycerides) and lipid metabolism (cholesterol, HDL, LDL, triglycerides, lipoprotein(a), apolipoprotein A-II) form up a lipolytic bundle. This lipolytic effect is unrelated to the anabolic component and dose-independent.

**Conclusions:** The lipolytic and anabolic effects of GH are dissociated. The threshold of GH’s lipolytic effects is much lower than its anabolic and growth effects.

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**P1-d3-263 GH Treatment 2**

**Regional variation in growth hormone (GH) dose and response in GH-deficient (GHD) children: Does higher dose equal greater response or more adverse events?**

Christopher J. Child; Alan G. Zimmerman; Elena F Shavrikova; Jan Lebl; Charmian A. Quigley; Werner F Blum

1Eli Lilly and Company, Lilly Research Laboratories, Windlesham, United Kingdom; 2Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, United States; 3Pharma Support Inc, Statistics, St Petersburg, Russian Federation; 4Charles University, 2nd Faculty of Medicine, Prague, Czech Republic; 5Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany

The GH dose used in the USA for the treatment of GHD children is typically greater than in other countries. To evaluate if a higher dose leads to better growth response and/or more adverse events (AEs), prospective observational study data were compared for 5 countries (Czechia (CZ, N=30), France (FR, 286), Germany (GE, 764), Spain (SP, 169), USA (744)). Patients (pts) included were GH naïve at baseline (BL) and had a 1st-yr height (HT) velocity (HV) value available.

Table: Demographics, GH dose and height response (mean±SD, unless otherwise stated).

<table>
<thead>
<tr>
<th>Country</th>
<th>BL age (yr)</th>
<th>Median Max GH peak (μg/L) [Q1, Q3]</th>
<th>BL HT SDS</th>
<th>BL GH dose (mg/kg/week)</th>
<th>1st-Yr Δ HT SDS, Lower—Upper (95%) CI</th>
<th>1st-Yr HT HVL (cm/yr), Lower—Upper (95%) CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CZ</td>
<td>8.2±1.1</td>
<td>3.6 [2.0, 6.2]</td>
<td>-2.3±1.1</td>
<td>0.17±0.04</td>
<td>0.60±0.38, 0.46±0.74</td>
<td>9.0±1.8, 8.3±7.9</td>
</tr>
<tr>
<td>FR</td>
<td>10.6±3.5</td>
<td>6.6 [4.2, 9.0]</td>
<td>-2.3±0.7</td>
<td>0.24±0.05</td>
<td>0.60±0.41, 0.55±0.64</td>
<td>9.3±2.6, 9.0±9.6</td>
</tr>
<tr>
<td>GE</td>
<td>9.4±3.7</td>
<td>6.7 [4.9, 9.4]</td>
<td>-2.5±1.0</td>
<td>0.19±0.05</td>
<td>0.59±0.48, 0.56±0.63</td>
<td>8.8±2.5, 8.7±9.0</td>
</tr>
<tr>
<td>SP</td>
<td>10.7±3.4</td>
<td>5.9 [3.9, 8.2]</td>
<td>-2.6±0.9</td>
<td>0.21±0.05</td>
<td>0.62±0.50, 0.55±0.70</td>
<td>9.0±2.7, 8.6±9.4</td>
</tr>
<tr>
<td>USA</td>
<td>10.6±3.9</td>
<td>7.0 [4.0, 9.7]</td>
<td>-2.2±1.1</td>
<td>0.31±0.08</td>
<td>0.60±0.48, 0.56±0.63</td>
<td>9.4±2.9, 9.1±9.6</td>
</tr>
</tbody>
</table>

The BL age and maximum stimulated GH values were lower in CZ than in the other 4 countries, while mean GH dose at BL was 29% greater in USA than in FR and up to 82% greater than the other countries, but growth response (1st-Yr Δ HT SDS and HVL) was similar in all countries.

Rates of the following potentially GH-related AEs were assessed in patients with ≥ 1 follow-up visit: arthralgia, diabetes mellitus, edema, gynecomastia, hypothyroidism, increase in nevi, pseudotumor cerebri, recurrent otitis media, scoliosis and slipped capital femoral epiphysis. The rates of patients with ≥ 1 AE varied only slightly (CZ 3.3%, FR 3.4%, GE 9.8%, SP 4.7%, US 6.9%) and that of the USA was lower than GE.

Although BL age and severity of GH deficiency are among the important factors in growth response, the higher dose administered in the USA appears to elicit no better growth response, nor does it appear to cause additional AEs.

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**P1-d3-264 GH Treatment 2**

**The GH/IGF-I axis in children with Noonan syndrome compared to girls with Turner syndrome**

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Noonan syndrome (NS) shares several features similar to Turner syndrome (TS), the latter being an entity with decreased growth hormone (GH) sensitivity. To investigate the GH/IGF-I axis in children with NS and compare this to girls with TS. To correlate to patients’ genotype and response to GH treatment. Data of GH/IGF-I axis in 25 NS children (10 mutation-positive for PTPN11 gene), with data in 40 TS girls and 45 healthy volunteers, all prepubertal. The 24-hour GH profiles were evaluated at baseline. IGF-1 and IGFBP-3 were measured and compared in NS or TS before and during the first year of GH treatment. Abnormal GH secretion patterns were found in both NS and TS patients. GH maximum was increased in both groups (44±23 μUL, p<0.01 in NS; 51±47; p<0.001 in TS, compared to 30±23 μUL in the healthy children). The baseline GH secretion was increased in NS (1.4±0.6μUL/UL) and TS (2.4±3.1μUL/UL) compared to healthy children (1.1±1.2 μUL, p<0.01 for both). Pre-treatment IGF-1 levels were lower in NS (+1.7±1.3 SDS) compared to TS (0.6±1.8 SDS, p<0.0001). Baseline GH levels, IGF-1/IGFBP3 ratio, and chronological age at start accounted for 59% of the variance in first year growth response in NS. For the TS group, dose (p = 0.0091) and mean 24 hour GH (p = 0.0225) explained 28% of the variance in growth response. Changes in IGF-1 at 10 days and after the first year of treatment correlated positively in NS with changes in height SDS after the first year (r=0.70 and r=0.60 respectively, p<0.01 for both). In the TS group, changes in IGF-1 and IGF-1/IGFBP3 ratio did only correlate with changes in height SDS after one year (r=0.50 and p=0.46, respectively, p<0.05 for both). No correlation was found between mutation in the PTPN11 gene and GH/IGF-I status in NS but correlated to growth response, with a better response in the group without the mutation (0.6±0.3 SDS versus 0.9±0.4 SDS, respectively p<0.05). Both NS and TS children showed a disturbed GH secretion pattern, but growth and short term GH response on GH treatment correlated differently in NS than in TS.

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**P1-d3-265 GH Treatment 2**

**Serum proteomic profiles of response to growth hormone (GH) treatment in children with idiopathic short stature (ISS)**

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The etiology of idiopathic short stature (ISS) is heterogeneous and affected children have a broad spectrum of responsiveness to growth hormone (GH) therapy. At present no markers of GH responsiveness are available to facilitate treatment decisions for this patient population. Therefore, this pilot study sought to identify and characterize as yet unknown serum proteins that might be predictive of, or associated with, response to GH in such children. Ten children with ISS ages 7.0-14.8 yr at baseline were studied in a sub-study of the GenESIS observational research program. Serum samples were collected prospectively at baseline and following 6-14 months of GH treatment. Patients were classified on the basis of their annualized change in height SDS (ΔHSIDS) during GH treatment as good responders (ΔHSIDS ≥ 1.0) or poor responders (ΔHSIDS <0.5). Sera were immuno-depleted for high abundance proteins and analyzed using fluorescence 2-dimensional differential in-gel electrophoresis (2D-DIGE) and Matrix Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectroscopy (MALDI-TOF-MS) peptide profiling. Ge-
In clinic-based populations of children with short stature it is anticipated that social competence and cognitive development may be compromised.

We hypothesis that GH treatment will have a differential cognitive and QoL effect on GHD and ISS children. Ninety-nine children between the ages of 3 and 11 years at start of treatment were followed for two years in a randomised control study. The population was grouped into ISS (n=67) and GHD (n=33). Child and parent completed measurements included IQ (WISC) and Child Behaviour Checklist (CBCL); "I think I am"; the Silhouette Apperception Test (SAT) and the Birslen depression scale. Time points were at baseline 12 and 24 months. At baseline, the GHD group had significantly lower Performance IQ (p=0.02), lower Perceptual Organisation (p=0.02), and lower Performance Speed (p=0.004) than the ISS group. Following 24 months of GH treatment, improvements in full IQ for the GHD children (p=0.002) and Performance IQ (p=0.001) led to no significant difference between ISS and GHD. Verbal IQ showed no difference between the groups at baseline and increased significantly in both groups (p=0.015 & p=0.041). Those children who increased Full IQ the most were those whose baseline FIQ was below 90 IQ points. Perceptual organization remained significantly lower in the GHD group. There was a significant reduction in childhood depression at 12 and 24 months (P<.01; P<.004). Improvement were found only in the ISS group (P<.20; P<.016). Behavioural problems (CBCL) reduced more in the ISS group (p > .001) in several areas. ISS children become less withdrawn, and less aggressive at 12 and 24 months (P=.002; P=.015 and P=.000; P=.001), and their tendency to externalise their problems reduced (P=.006 and P=.004). Similar findings were not found in the GHD group. We conclude that cognitive improvements could be found in the GHD group whereas QoL improvements were made in the ISS group.

**P1-d3-268 GH Treatment 2**

Differential effects of GH in children with Growth Hormone Deficiency (GHD) or Idiopathic Short Stature (ISS): Evidence from an insulin-like growth factor-based GH-dosing study

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We recently demonstrated that in IGF-based GH dosing in short children with low IGF-1 levels, the IGF-1 target chosen determines GH dose and growth response. To assess the response of children with ISS versus GHD to IGF-1-based GH dosing, we studied prepubertal short children with low IGF-1 (<1 SDS) in a 2-yr open-label, randomized, IGF-1 concentration-controlled clinical trial. The patient population (n=165) was subdivided, based on peak GH values in an arginine-L-dopa stimulation test, into GHD (n=63; peak GH=7 ng/mL or less; n=102; GH>7 ng/mL) groups. In both groups the GH dose was targeted to either 0 SDS (G0T) or ~2 SDS (G2T) and the targeted mean IGF-1 levels were achieved within 6-9 months and did not differ between GHD and ISS. In both groups, G2T patients required significantly higher GH doses (median 119 and 65 mcg/kg/d for ISS and GHD) than G0T patients (median 26 and 33 mcg/kg/d for ISS and GHD). At 2 yrs, significantly greater changes in height SDS (ASHDS) were observed in G2T patients and ΔHSDS values were significantly greater for GHD than ISS in both IGF-1 target groups: ΔHSDS of 2.04 for GH and 1.33 for ISS groups in G2T, and 1.41 for GH and 0.84 for GHD in G0T. Patients with the lowest baseline GH and IGF-1 levels had the greatest ΔHSDS during treatment, whereas patients with the highest baseline GH and IGF-1 levels had the least ΔHSDS. Bone age changes during the 2-yr trial did not differ between groups. IGF-based GH dosing is clinically feasible in both GHD and ISS patients, although GH dose requirements and auxological outcomes are distinct among these groups. When targeted to an IGF-1 SDS of 0, patients with ISS require slightly lower GH doses, but exhibit lower growth rates. However, when targeted to an IGF-1 SDS of ~2, patients with ISS require dramatically higher GH doses, and still display reduced growth rates compared to GHD. Our findings suggest a certain degree of both GH and IGF-1 insensitivity in patients with ISS that requires specific management strategies to optimize growth during GH therapy.