Poster Presentations

P1-d1-163 Adrenals and HPA Axis 1

The role of S-palmitoylation of human glucocorticoid receptor in mediating the non-genomic actions of glucocorticoids

Nicolas C. Nicolaides[1]; Michael M. Roberts[1]; Tomoshige Kino[2]; Eleni Katsantoni[1]; Amalia Sertedaki[3]; George P. Chrousos[3]; Evangelia Charmandari[3]

[1]Biomedical Research Foundation of the Academy of Athens, Division of Endocrinology and Metabolism, Athens, Greece; [2]National Institutes of Health, Unit on Molecular Hormone Action, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland, United States; [3]University of Athens Medical School, Division of Endocrinology and Metabolism, First Department of Pediatrics, Athens, Greece

Background: In humans, glucocorticoids (GCs) regulate a broad spectrum of physiologic functions and exert both genomic and non-genomic actions through their ubiquitously expressed glucocorticoid receptor (hGR). The rapid non-genomic actions of GCs are likely to be mediated by membrane hGRs that transduce the glucocorticoid signal via activation of kinases. S-palmitoylation plays an important role in plasma membrane (PM) localization and occurs through a highly conserved 9 amino acid motif in the ligand-binding domain (LBD) of steroid receptors. A highly homologous sequence is present in the LBD of the hGRα protein, suggesting that the hGR might also undergo S-palmitoylation.
Objective and hypotheses: To determine the role of S-palmitoylation of hGR in mediating rapid glucocorticoid signaling following translocation and binding to the PM.
Methods: In vitro studies were performed to determine the specific residues within the 9 amino acid motif of the LBD of hGRα that are crucial for rapid glucocorticoid signaling. Specifically, we determined whether mutation of the amino acids at position -2, 0 and +5/6, relative to cysteine in the 9 amino acid motif, significantly reduces localization of the receptor to the PM, S-palmitoylation, association with caveolin-1, and MAPK and PI3K activation.
Results: Both the wild-type and the mutant receptors hGRαY663A, hGRαC665A and hGRáLL670/671AA showed similar distribution to the PM. Addition of 2-bromopalmitate, an S-palmitoylation inhibitor, did not prevent PM localization of the receptor or colocalization with caveolin-1. Compared with the wild-type hGR, all hGR mutant receptors resulted in decreased activation of MAPK signaling from 60 min onwards. A similar reduction in wild-type GR-induced MAPK signaling at 60 min was observed after treatment with 2-bromopalmitate.
Conclusions: S-palmitoylation facilitates sustained activation of the MAPK pathway. Further studies are required to confirm that the hGR protein mediates this effect through the 9 amino acid motif in the LBD.

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Arterial hypertension in children: alterations in mineralocorticoid and glucocorticoid axis and their impact on pro-inflammatory, endothelial damage, and oxidative stress parameters

Carmen Campino[1]; Rodrigo Bancalari[2]; Alejandro Martinez-Aguayo[2]; Marlene Aglony[2]; Hernan Garcia[2]; Carolina Avalos[2]; Lilian Bolte[2]; Carolina Loureiro[2]; Cristian Carvajal[1]; Lorena Garcia[3]; Sergio Lavanderos[3]; Carlos Fardella[1]

[1]Pontificia Universidad Catolica, Endocrinology, and Millennium Institute of Immunology and Immunotherapy, Santiago, Chile; [2]Pontificia Universidad Catolica, pediatrics, Santiago, Chile; [3]Universidad de Chile, School of Chemical Sciences, Santiago, Chile

Background and aims: The pathogenesis of arterial hypertension and its impact and determining factors with respect to cardiovascular damage in children is poorly understood. We evaluated the prevalence of alterations in the mineralocorticoid and glucocorticoid axes and their impact on pro-inflammatory, endothelial damage and oxidative stress parameters in hypertensive children.
Methods: 306 children (5-16 years old);
Group 1: Hypertensives (n=111);
Group 2: normotensives with hypertensive parents (n=101);
Group 3: normotensives with normotensives parents (n= 95).
Fasting blood samples were drawn for hormone measurements (aldosterone, plasma renin activity (PRA), cortisol (F), cortisone (E)); inflammation variables (hsRCP, adiponectin, IL-6, IL-8, TNF-α); endothelial damage (PAI-I, MMP9 and MMP2 activities) and oxidative stress (malondialdehyde). Familial hyperaldosteronism type 1 (FH-1) was diagnosed when aldosterone/PRA ratio >10 was associated with the chimeric CYP11B1/CYP11B2 gene. The 11β-HSD2 activity was considered altered when the F/E ratio exceeded the mean + 2 SD with respect to group 3.
Comparison between the groups was done by Kruskal-Wallis Test.
Results: HF-1 was only detected in group 1; 4/115 children (3.4%). The F/E ratio was elevated (>4.3) in Group 1= 18/115 (15.6%); Group 2= 5/101 (4.9%) and Group 3= 5/95 (5.3%).
The comparison between groups 1, 2 and 3 showed differences in levels of F (ug/dl): 9.9[6.7-14.4]*, 8.5[6.2-11.1], 8.4[6.3-10.4]; F/E ratio: 2.9[2.2-3.9]*, 2.8[2.2-3.3], 2.6[2.0-3.2]; hsCRP (mg/L): 1.2[0.4-2.3]*,**, 0.5[0.2-1.6], 0.5[0.2-1.3]; PAI-I (ng/ml): 22.2[13.4-31.7]*, 18.8[9.8-27.3], 14.9[8.9-23.3] and MMP-9: 2.2[1.3-3.0]*, 1.8[1.2-2.5], 1.6[1.2-2.3].*p<0.05 group 1 vs group 3, **p<0.05 group 1 vs group 2.
Conclusions: In hypertensive children, HF-1 and deficient 11β-HSD2 activity were detected in addition to increases in inflammation, subclinical and endothelial damage. These results highlight the importance of blood pressure measurement in the child population.

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The effect of anastrozole on the predicted height of obese boys with premature adrenarche, delayed gonadarche and advanced bone age

Nordie Anne Bilbao; Mariano Castro-Magana

Winthrop University Hospital, Pediatric Endocrinology, Mineola, United States

Background: Premature adrenarche is the early onset of secretion of adrenal androgens. The adrenal androgens dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione are converted to estrogens by the enzyme aromatase. There is a significant association of premature adrenarche with obesity. The increased aromatase activity in obesity enhances estrogen production. Since estrogens are responsible for skeletal maturation, its increase can result in an advanced bone age and a decreased height prediction. Estrogens exert a negative feedback to the pituitary LH and FSH secretion, thus causing delayed gonadarche. Aromatase inhibitors, which block the aromatase enzyme, decrease estrogens and increase testosterone, resulting in slower progression of skeletal maturation, and possibly increased predicted height.
Objective and hypotheses: We hypothesize that treatment with anastrozole increases the predicted height of obese boys with premature adrenarche, who have delayed gonadarche and advanced bone age. We aim to compare the

predicted height at baseline and after two years of treatment with anastrozole in these boys.
Population and methods: The study is conducted on twenty boys 8-12 years old diagnosed with prior premature adrenarche from years 2006 through 2011, who received anastrozole, 1 mg/day, for two years. These boys also have advanced bone age, delayed gonadarche in relation to the bone age and obesity (BMI +2 SDS). Predicted heights (determined from the Bayley-Pinneau tables) at baseline and two years after treatment were obtained and compared using the paired t- test.
Results (Preliminary): There is a significant increase in the mean predicted height of 6.8 cm two years after treatment with anastrozole compared to the predicted height at baseline in obese boys with premature adrenarche, delayed gonadarche and advanced bone age (p=0.02).
Conclusions: Anastrozole increases the predicted height in obese boys with premature adrenarche, delayed gonadarche and advanced bone age.

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

Gerard Noppe[1]; E.F.C. van Rossum[2]; F.J.W. Koper[2]; E.L.T. van den Akker[3]
[1]Erasmus MC, Sophie children's hospital, Pediatrics and internal medicine, Rotterdam, Netherlands; [2]Erasmus MC, Internal Medicine, Rotterdam, Netherlands; [3]Sophia children's hospital, Erasmus MC, Pediatrics, endocrinology, Rotterdam, Netherlands

Background: Congenital Adrenal Hyperplasia (CAH) is characterized by cortisol deficiency and androgen excess. Treatment titration is aimed at preventing androgen excess on the one hand, and iatrogenic Cushing's syndrome on the other. CAH is associated with poor health at adult age, partly due to overtreatment. Measuring cortisol in scalp hair is a new technique providing the clinician with a value representing long-term systemic cortisol exposure.
Objective: We studied whether hair cortisol is a valuable monitoring-tool in the follow-up of children with CAH.
Methods: We collected hair samples from 23 CAH patients, 5-17yr/o, and 23 age and gender matched healthy controls. Cortisol was extracted from hair using methanol and cortisol levels were measured using an ELISA kit. Anthropometric characteristics were measured, hydrocortisone (HC) dose documented and androgen levels obtained from routine laboratory measurements.
Results: Hair cortisol levels were significantly higher in patients compared to controls (31.12 pg/mg vs. 14.85 pg/mg, p<0.001). Body mass index (BMI) standard deviation score (SD), weight-for-height and waist circumference were significantly higher in patients. In controls, hair cortisol was correlated with BMI SD (r=0.51, p=0.021), weight for height (r=0.57 p=0.009) and inversely with height SD (r=-0.45, p=0.045). No correlations were found between hair cortisol and anthropometric measurements, HC dose or androgen levels in patients. Cumulative HC dose was found to be positively correlated with weight for height and BMI SD when adjusted for salivary 17α-hydroxyprogesterone levels.
Conclusions: Hair cortisol measurement, provides us for the first time with the possibility of measuring long-term hydrocortisone exposure in CAH patients. Hair cortisol measurement could be a useful novel monitoring tool in addition to current CAH monitoring tools.

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

Hedi Claahsen - van der Grinten[1]; Dehzad Farhang[1]; Karin Kamphuis - van Ulzen[2]; Chris de Korte[3]
[1]Radboud University Nijmegen Medical Centre, Department of Pediatric Endocrinology, Nijmegen, Netherlands; [2]Radboud University Nijmegen Medical Centre, Department of Radiology, Nijmegen, Netherlands; [3]Radboud University Nijmegen Medical Centre, Department of Clinical Physics Laboratory, Nijmegen, Netherlands

Background: TART are one of the most important and frequently detected complications in adult male CAH patients. Because of their central localization of these benign tumours near the mediastinum testis, compression of the seminiferous tubules may lead to obstructive azoospermia and irreversible damage of the surrounding testicular tissue.Therefore, it may be important to detect and treat the tumours in an early stage. Ultrasound is a good method for detection and follow-up of TART, especially when they are not palpable by physical examination. TART is already detected in children, however the age of first detection is not known and screening of TART is not routinely performed.
Objective: Aim of our cross sectional study was to define the prevalence and age of first detection of TART in a group of 42 paediatric male CAH patients age 0 – 19 years old.
Methods: All male classic CAH children who are regularly followed at our outpatient pediatric endocrine clinic were included. Ultrasonographic evaluation was performed by an experienced radiologist (KK) with a high-frequency linear array transducer L 17 / 5 (17 MHz) in two directions (transversal and sagital).
Results: Below the age of 10 years no TART were detected (16 patients). Above the age of 10 there was a clear increase in prevalence of TART: 10 – 12 years 28% (2 of 7 patients), 13 – 14 years 50% (4/8), 15 – 16 years 75% (3/4). Above the age of 16 TART were detected in 100% of the patients (7/7). The tumors were usually small (< 1 cm) mostly bilateral and generally not detectable by palpation.
Conclusion: In patients with classic CAH TART is already present in childhood with increasing prevalence in puberty. Based on our results we recommend regularly ultrasound from the age of 10 years in all boys with classic CAH.

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

Marta Ciaccio; Maria Sonia Baquedano; Roxana Marino; Natalia Perez Garrido; Pablo Ramirez; Juan Cáceres; Euardo Chaler; Mercedes Maceiras; Juan Manuel Lazzati; Marco Aurelio Rivarola; Alicia Belgorosky
Hospital de Pediatria "Prof Dr Juan P Garrahan", Endocrine Service, Buenos Aires, Argentina

Background: 3βHSD2 deficiency is characterized by salt loss, incomplete masculinization in males and mild or absent virilization in females.
Objective: To characterize a novel misssense mutation (G250V) in 3β-HSD2 gene.
Methods: We report a 7-month-old 46,XX girl referred because of precocious pubarche and postnatal clitoromegaly. Consanguinity was reported. She showed low serum cortisol, 4.8ug/dl, high ACTH, 2888 pg/ml, DHEAS, 53000 ng/ml and 17OHP 141 ng/ml, and plasma renin, 424 ng/ml. These data suggested 3βHSD2 deficiency. Enzymatic activity was analyzed by in vitro analysis of a mutant recombinant enzyme generated by site-directed mutagenesis after its transient expression in COS cells.
Results: A novel homozygous c.749G→T mutation in 3β-HSD2 gene resulting in a G250V change was found. This G is highly conserved in vertebrate 3β-HSD2 and is located in the substrate-binding domain of the enzyme. In silico PolyPhen and SIFT analysis predicted G250V to be a damaging substitution. Enzyme activity using 0.5 μM pregnenolone (P5) in the medium during 6h revealed relative conversion rates of P5 to P4 of 78±4% and 21±1% in WT and G250V-3β-HSD2 enzymes respectively. Using 0.5 μM DHEA, the relative conversion rate of DHEA to Δ4 during 6 h was 87±8% and 23±7% in WT and G250V-3β-HSD2 enzymes, respectively. Immunofluorescence studies showed that both WT and mutant G250V-3β-HSD2 protein colocalized with endoplasmic reticulum.
Conclusions: We identified a novel G250V 3βHSD2 gene mutation which causes an incomplete loss of enzymatic activity. Flux via the adrenal "backdoor" pathway, which converts 17OHP to DHT has been recently implicated in disorders of androgen excess. We hypothesized that this alternate pathway could not be activated in fetal 3β-HSD2 deficiency due to very low intraadrenal 17OHP substrate level explaining mild virilization or even normal sexual differentiation in females. Postnatal 3β-HSD1 activity might explain high serum 17OHP and clitoris stimulation.

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Is the ACTH test useful in the diagnosis of late onset congenital adrenal hyperplasia?

Diana Bareño; Diego Yeste; Laura Losada; Maria Clemente; Mariam Albisu; Antonio Carrascosa
Hospital Vall D´Hebron, Endocrinology Paediatric, Barcelona, Spain

Background: Premature pubarche is the most common manifestation of non- classical congenital adrenal hyperplasia (NCAH); however, accelerated growth rate or bone age, hirsutism and clitoral hypertrophy in prepubertal children can be further forms of clinical presentation. The adrenal stimulation test with ACTH is the recommended method for diagnosing NCAH.
Objective and hypotheses: To define the specificity and sensitivity of basal plasma concentrations of 17-OH-progesterone (17OHPG), androstendione, DHEA-S and testosterone as predictors of NCAH.
Patients and methods: Retrospective cohort study of patients undergoing ACTH for suspected NCAH (n = 280). NCAH was defined by a post-ACTH 17OHPG plasma level "d10 ng/ml and confirmed by molecular genetic analysis. Univariate descriptive analysis and ROC curves were applied to determine the sensitivity and specificity of each of the parameters evaluated.
Results: Indications for ACTH test and their results are shown in the table I and Table II, respectively.

Clinical finding	male	female	AGE	17-OHPG response to 60 min> 10 ng / ml	
	n	n	(Mean-Range)	n	%
Pubarche	43	210	72 ± 1.9 (0.5-11)	27	10.7
Hirsutism	0	11	6.0 ± 2.9 (0.9-2.9)	1	9.1
Accelerated bone age	0	1	8.8	0	-
Accelerated growth	2	2	6.5 ± 2.2 (3.4-8.8)	1	2.5
Clitoral hypertrophy	0	11	2.3 ±2.8 (0.1-9)	0	-

Diagnosis	17OHPG BASAL	17OHPG 60 MIN	Genetic Analysis
17OHP < 10 ng/ml (n=251)	0.54 ± 0.37 (0.01- 1.98)	32.4±17.22 (0.10-14.6)	Not performed
NCAH (n=29)	8.73 ± 6.15 (2.0-25.2)	32.4±17.22 (13.8-100)	Confirmed in all patients

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

P1-d1-170 Adrenals and HPA Axis 1

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

Dulanjalee Kariyawazam[1]; Albane Simon[2]; Kathleen Laborde[3]; Jérome Le Chenadec[4]; Sophie Parat[5]; Paul Czernichow[6]; Stéphane Blanche[7]; Michel Polak[1]
[1]Necker Enfants-Malades Hospital, Paediatric Endocrinology Gynaecology and Diabetology Unit, Paris, France; [2]Versailles Hospital, Department of Paediatrics, Le Chesnay, France; [3]Necker Enfants-Malades Hospital, Department of Physiology, Paris, France; [4]INSERM, CESP U1018, Equipe VIH et IST, Paris, France; [5]Necker Enfants-Malades Hospital, Neonatalogy Unit, Paris, France; [6]Necker Enfants-Malades Hospital, Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant, Paris, France; [7]Necker Enfants-Malades Hospital, Immunology and Hematology Unit, Paris, France

Background: Ritonavir-Lopinavir (r-LPV) is a human immunodeficiency virus 1 (HIV-1) protease inhibitor boosted by ritonavir, a cytochrome P450 inhibitor. It is prescribed in newborns as a post exposure prophylaxis in prevention of materno-foetal transmission, and in infected children as an antiretroviral treatment.
We showed that r-LPV treatment in newborns, compared to Zidovudine, is associated with increased 17 OH Progesterone and DHEA-S levels (JAMA. 2011 Jul 6;306(1):70-8). Premature newborns treated with r-LPV experienced life threatening insufficiency. R-LPV interaction with P450 cytochrome and specific adrenal susceptibility during neonatal period were suggested.
Objective and hypotheses: To better describe the adrenal function impairment due to r-LPV and to obtain data in short term exposed newborns and long term exposed adolescents.
Methods: 2 newborns treated with r-LPV for 4 weeks and 3 HIV infected adolescents long term treated with r-LPV were analysed. Basal and ACTH stimulated plasmatic hormonal concentrations (cortisol, 17-hydroxyprogesterone, 17-hydroxypregnenolone, DHEA, DHEA-S, androstenedione, testosterone) and ionogram were assayed.
Results: Adrenal function tests were abnormal in all patients. In newborns, high levels of 17 hydroxypregnenolone (> 30 nmol/L at basal level and >100 nmol/L after ACTH stimulation) and of DHEA-S (> 6 000 ng/mL) were found. 17OHProgesterone was normal. DHEA-S levels normalized after treatment completion. In adolescents, all had elevated androstenedione levels (> 6,3 nmol/L), 2 had elevated 17 hydroxypregnenolone and 1 had elevated DHEA-S (7176 ng/mL). All patients had normal basal and stimulated cortisol and mineralo-corticoid secretion.
Conclusions: The impact of r-LPV on adrenal function is confirmed. Short exposure on immature adrenal of newborns and long exposure on adolescents provoke adrenal anomalies with a preservation of cortisol secretion. These results suggest a 3βHSD2 block profile which needs to be further defined.
*D.K and A.S contributed equally and should be considered as joint first author.

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Oxidative stress and the regulation of ALADIN, the triple A syndrome gene product, and its protein partner ferritin heavy chain

Rathi Prasad; Adrian J Clark; Helen L Storr
William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Centre for Endocrinology, London, United Kingdom

Background: Triple A syndrome is a rare cause of adrenal failure accompanied in some by a progressive neurodegenerative process. The *AAAS* gene product is the nuclear pore complex protein ALADIN, of unknown function, which interacts with Ferritin Heavy Chain (FTH1). ALADIN is implicated in the nuclear localisation of FTH1 and apoptosis of neuronal cells induced by hydrogen peroxide is significantly reduced by transfection of *AAAS* or *FTH1* and maximally by both genes together, implicating oxidative stress in the pathogenesis of the disease.
Objective and hypotheses: To investigate the expression of ALADIN and FTH1 in response to oxidative stress in H295R adrenocortical tumour cells and SH-SY5Y neuroblastoma cells (chosen as representative of the cell type affected by AAAS).
Methods: Both cell lines were treated with H2O2 with subsequent quantification of mRNA and protein expression of ALADIN and FTH1.
Results: *FTH1* mRNA expression was significantly increased following treatment with a peak response at 6 hours in H295R cells (increased by 135%, n=3, $p<0.05$) and SH-SY5Y cells (increased by 96%, n=3, $p<0.001$). However in the SH-SY5Y cells, *AAAS* mRNA expression was significantly reduced in response to oxidative stress (reduced by 56%, n=3, $p<0.05$). Indeed, after treatment protein expression of ALADIN in SH-SY5Y cells is significantly reduced (reduced by 46%, n=3, $p<0.005$). In comparison *AAAS* mRNA expression and ALADIN protein expression are maintained in H295R cells with treatment.
Conclusions: FTH1 has potent ferroxidase activity and its mRNA expression is upregulated in both cell lines following application of oxidative stress. Interestingly, ALADIN appears to be downregulated both at transcriptional and translational levels in SH-SY5Y cells in response to oxidative stress whereas levels are maintained in H295R cells. Further exploration of the mechanisms of this differential regulation may have implications for susceptibility of these tissues to oxidative stress.

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Nicotinamide nucleotide transhydrogenase gene knockdown impairs redox homeostasis in H295R cells

Eirini Meimaridou; Julia Kowalczyk; Claire Hughes; Paul Chapple; Adrian Clark; Lou Metherell
WHRI, Endocrinology, London, United Kingdom

Background: Mutations in nicotinamide nucleotide transhydrogenase (NNT) and glutathione peroxidase 1 cause familial glucocorticoid deficiency in humans, characterized by a deficiency of glucocorticoids alone. Patients often present with hypoglycaemia which, if unrecognized and untreated, can lead to neurological impairments and may be life-threatening. NNT, a highly conserved gene, encodes an integral protein of the inner mitochondrial membrane. Under most physiological conditions, this enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of NADPH. Detoxification in mitochondria of reactive oxygen species (ROS) by glutathione peroxidases depends on this NADPH for regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) to maintain a high GSH/GSSG ratio.
Objective: To study the effects of NNT ablation in an adrenocortical cell line.
Methods: Generation of NNT knockdown (NNT-KD) and control (SCR) H295R cell lines by lentiviral delivery of shRNAs. Detection of superoxide production by Mitosox red staining. Western blotting of the apoptosis marker cleaved PARP. GSH/GSSG ratio measured by the luminescent GSH/GSSG-Glo Assay.
Results: Using shRNAs targeting NNT we achieved a 70% knockdown of NNT and >80% reduction in protein levels in H295R cells. NNT-KD cells showed not only increased levels of ROS and apoptosis but also a lower GSH/GSSG ratio when compared to SCR cells (18.82±8.75 vs 29.95±4.77; $p<0.001$) implying these cells also have an impaired redox potential.
Conclusion: Taken together with the fact that NNT mutations give rise to FGD, these results suggest that, in humans, NNT is of primary importance for ROS detoxification in adrenocortical cells, highlighting the susceptibility of the adrenal cortex to this type of pathological damage. Over time patients may develop other organ pathologies related to impaired anti-oxidant defence and will therefore need careful monitoring.

P1-d1-173 Adrenals and HPA Axis 1

In vitro expression of rare CYP21A2 mutations associated with non-classic CAH

Svetlana Lajic[1]; Michela Barbaro[1]; Fernanda Soardi[2]; Maricilda Palandi de Mello[2]; Anna Wedell[1]
[1]Karolinska Institutet, Molecular medicine and surgery, Stockholm, Sweden; [2]Universidade Estadual de Campinas, Laboratorio de Genetica Molecular Humana, Campinas Sao Paolo, Brazil

Background: Mutations in CYP21A2 are the most common cause of congenital adrenal hyperplasia (CAH). Rare, family or population specific mutations, account for around 5% of all CYP21A2 mutations. In order to enable genetic counseling in these families the disease phenotype associated with the rare mutations can be verified by in vitro functional analyses of the mutated P450c21 protein.
Objective: To verify the disease phenotype associated with five rare missense mutations identified in the CYP21A2 gene in patients investigated for non-classic CAH.
Methods: We have performed functional studies of five rare missense mutations (R233G, A265S, R341W, R366C, M473I) identified in the CYP21A2 gene in patients investigated for non-classic CAH. The mutant proteins were expressed in vitro in eukaryotic COS-1 cells and the enzyme activities toward the two natural substrates 17OHP and progesterone were determined in order to verify the disease-causing state of the mutations. Four mutations known to cause non-classic CAH (P30L, V281L, P453S, P482S) were also expressed in the same system.
Results: All mutations had in vitro enzyme activities above 5% for 17OHP classifying them as non-classic mutations (relative activity for conversion of 17OHP/progesterone: R233G, 8%/2%; A265S, 90%/104%; R341W, 5%/4%; R366C, 38%/28%; M473I, 85%/65%). By comparing the in vitro function of the rare mutations with the activity for the known non-classic mutations (relative activity for conversion of 17OHP/progesterone: P30L, 2%/1%; V281L, 18%/18%; P453S, 38%/24%; P482S, 61%/54%) we could establish a gradient for the classification of non-classic mutations. Data from the functional studies correlated with the patient phenotypes. The A265S mutation should most probably be considered as a polymorphism, while the M473I could represent a very mild mutation.
Conclusion: Genotype-phenotype relationships in CAH due to 21-hydroxylase deficiency can be improved by combining information gained from clinical, functional and structural studies.

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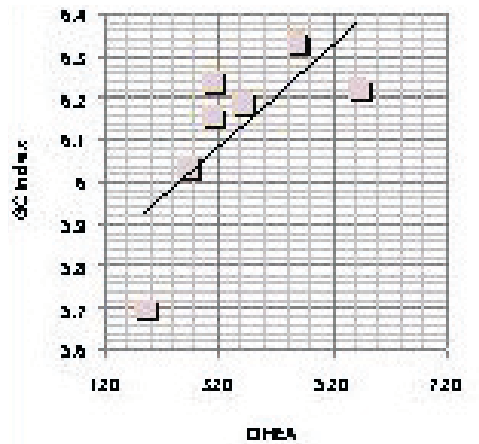
New monogenic cause of adrenarche

Aristotle Panayiotopoulos[1]; Felicitas Lacbawan[2]; Josef Michl[3]; Amrit Bhangoo[1]; Svetlana Ten[1]; Steven Ghanny[1]
[1]SUNY Downstate Medical Center, Pediatric Endocrinology, Brooklyn, NY, United States; [2]SUNY Downstate Medical Center, Molecular Pathology, Brooklyn, NY, United States; [3]SUNY Downstate Medical Center, Pathology, Brooklyn, NY, United States

Background: The cause of adrenarche (Ad) is unknown. New evidence points out Ad can be precursor of metabolic syndrome, adrenal or pituitary tumors, Cushing disease, PCOS. Presence of monogenic disorders in etiology of Ad such as Non Classical-CAH, glucocorticoid resistance (GCR), cortisone reductase deficiency, DHEA sulphotransferase deficiency, polymorphism of ACTH receptor, Androgen receptor, can indicate that there are other yet not identified monogenic disorders can present as Ad.
Objective and hypotheses: To study the role of Glucocorticoid (GC) signaling proteins in the etiology of Ad. GC signaling was evaluated in vitro by F-Dex binding assays and by sequencing genes from GC pathway.
Methods: We recruited 25 controls and 10 children (boys n=4, age 7 yrs) with Ad. Ad was defined as elevation of DHEA and/or DHEAS in children before 8 years with or without body odor, pubic, axillary hairs. ACTH test, 21 hydroxylase gene analysis, bone age evaluation. GC sensitivity was evaluated by F-Dex binding assays. GC index (GCI) was calculated as log of AUC from the difference in binding to the GC receptor between control and Ad patients.
Results: NC CAH was excluded by analysis of 21 hydroxylase gene. 7 pts were GCR compared to controls when evaluating GCI. GCI was reproducible in the same patients on a different days, total number of GC receptors were not different from controls. Severity of phenotype and elevation of DHEA, ACTH, Cortisol correlated with GCI. Ad group had significantly higher DHEA, 17-OH Pregnenalone, DHEAS, 17-OH Pregnenalone /F and others, comparing to the control without Ad and normal GCI.
Conclusions: There is subgroup of Ad patients who are characterized by GCR with elevated GCI. GCI correlated with the severity of phenotype. Correlation of GCI with Biochemical Steroids level and ratio indicate that the candidate gene is in GC signaling pathway. Mutation analysis of GCR, FKBP5 and FKBP4 are pending.



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Genetic analysis of 16 patients with familial glucocorticoid deficiency from 1 centre

Leyla Akin[1]; Louise A Metherell[2]; Selim Kurtoglu[1]; Claire R Hughes[2]; Mustafa Kendirci[1]; Nihal Hatipoglu[1]; Adrian J.L Clark[2]
[1]Erciyes University Faculty of Medicine, Pediatric Endocrinology, Kayseri, Turkey; [2]Queen Mary University of London, Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, United Kingdom

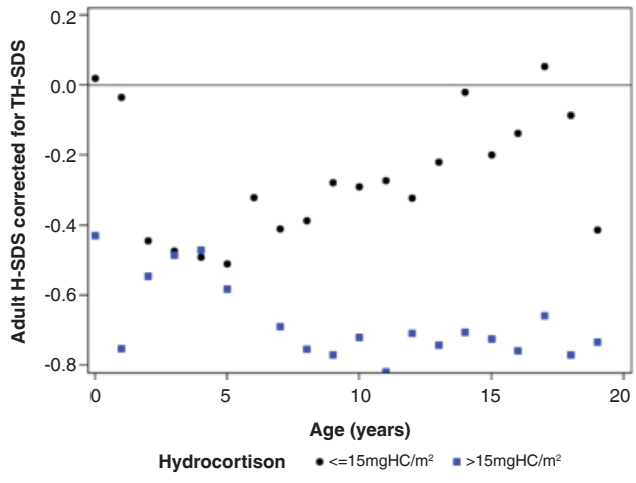
Background: Familial glucocorticoid deficiency (FGD) is a rare inherited disorder characterized by isolated glucocorticoid deficiency and ACTH resistance. Approximately 50% of cases result from mutations in the ACTH receptor (melanocortin 2 receptor, MC2R), MC2R accessory protein (MRAP) and the steroidogenic acute regulatory protein (StAR). Recently mutations in three further genes have been reported; one encodes a DNA replication protein, mini-chromosome maintenance 4, and the other two, nicotinamide nucleotide transhydrogenase and glutathione peroxidase 1, are antioxidant genes.
Objective and hypotheses: We searched for underlying mutations in 16 patients with clinically diagnosed FGD from 11 families.
Methods: The clinical diagnosis of FGD was made in patients who presented with symptoms of glucocorticoid deficiency associated with low serum cortisol and high ACTH levels in the absence of mineralocorticoid deficiency. MC2R, MRAP and STAR genes were sequenced in all patients.
Results: We found 9 mutations in 16 patients. In MC2R we found a novel homozygous mutation, p.L225R, in one patient and the known mutation, p.D103N, was homozygous in three siblings. In MRAP we found a novel homozygous p.L53P mutation in two siblings, the previously seen IVS3ds+1 del G mutation was homozygous in two cousins, and a homozygous V26A mutation was found in one patient whose father has FGD due to the same mutation. STAR gene sequencing was normal in all patients. Seven of 16 patients had no mutation in MC2R, MRAP or STAR.
Conclusions: Mutations in MRAP or MC2R were detected in 9 of 16 patients with clinically diagnosed FGD. This is in accordance with the literature reporting that about half of all cases result from mutations in MC2R, MRAP or STAR. The remaining patients may harbour mutations in one of the newly described FGD-causing genes.

P1-d1-176 Adrenals and HPA Axis 1

Longitudinal growth, adult height and hydrocortisone dose in 549 adult patients with congenital adrenal hyperplasia due to 21-Hydroxylase deficiency

Klaus Mohnike[1]; Helmuth G. Doerr[2]; F.-Wilhelm Roehl[3]; Katja Lieber[1]; Susann Empting[1]; Berthold Hauffa[4]; Wolfgang Hoepffner[5]; Michael Ranke[6]; Felix Riepe[7]; Stefan Riedl[8]; Juergen Braemswig[9]; AQUAPE-Study group[10]
[1]OvG University Magdeburg, Department of Pediatrics, Magdeburg, Germany; [2]University Erlangen, Department of Pediatrics, Erlangen, Germany; [3]OvG University Magdeburg, Institute of Biometry and Med. Informatics, Magdeburg, Germany; [4]University Essen, Department of Pediatrics, Essen, Germany; [5]University Leipzig, Department of Pediatrics, Leipzig, Germany; [6]University Tuebingen, Department of Pediatrics, Tuebingen, Germany; [7]University Kiel, Department of Pediatrics, Kiel, Germany; [8]University Vienna, Department of Pediatrics, Vienna, Austria; [9]University Muenster, Department of Pediatrics, Muenster, Germany; [10]Germany

Background: CAH due to 21-hydroxylase deficiency results in a mean reduction of adult height-SDS of -1.38 (systematic review by Muthusamy K, 2010).
Objective and hypotheses: To achieve normal growth, prevent overweight, infertility and testicular tumours it is recom-mended to treat with physiologic hydrocortisone doses and to avoid under- or overtreatment.
Methods: In this observational study, we correlated hydrocortisone doses (<15 mg/m2/d versus ≥ 15 mg/m2/d) with height-SDS at yearly intervals up to adulthood. 549 adult individuals (344 females) from 28 centres (79.8 % born after 1980) with 10.865 examinations were analysed. 52% of patients were treated with daily hydrocortisone doses >15 mg/m2. ANOVA test was performed to check the influence of target height (TH), clinical form of CAH, onset of puberty.
Results: Corrected for TH, 31 out of 325 (9.54 %) had a reduced adult height < -2 SD. Length at birth was normal (+0.21, range -2.31 to 2.46 SDS), and slightly reduced (-0.34; -2.9 to +2.9) during infancy. From 3 - 4 years, mean H-SDS increased to a peak at 8 years (+0.72 SDS, range -2.7 to +6.3). In salt wasters, mean age at onset of puberty was not different from reference group. However, early puberty (m < 9y.,f < 8y.) has been documented in 38 out of 208 children. H-SDS corrected for TH-SDS was significantly different with regard to the two HC dose groups has been found for different age groups: 1 year and 6 to 18 years (fig.).
Conclusions: Our data show that HC doses of <15 mg/m2/d during infancy and childhood result in normal height in adulthood. In salt wasters, mean age at onset of puberty was not different from reference group.



P1-d1-177 Adrenals and HPA Axis 1

Psychological characteristics of adolescents with Cushing's disease

Natalya Udalova[1]; Valentina Peterkova[1]; Ekaterina Svistunova[2]
[1]Endocrinology Research Centre, Pediatric Endocrinology, Moscow, Russian Federation; [2]The Moscow City Teachers' Training University, Psychology, Moscow, Russian Federation

Objective and hypotheses: The aim of our study was to identify psychological characteristics of adolescents with Cushing's disease comparing to healthy population. Additionally the study is aimed to draw a comparison of psychological state of the patients after different ways of treatment: proton therapy and transsphenoidal adenomectomy (TA).
Methods: 34 patients with Cushing's disease (18 female and 16 male), mean age 17,6 ± 2.8 yrs (ranging from 13,2 to 22,1) were examined. 20 patients underwent proton beam irradiation of the pituitary gland and 14 patients underwent TA. Patient's average age when the disease had been manifested was 10 years old (ranging from 7 to 15 years old). The control group comprised 34 healthy subjects comparable to the study group. The following techniques were used: PedsQL™4.0 Generic Core Scales; The Quality of Life Index (generic version); questionnaire for gender role and body identity; Bekhterev Institute Personality Inventory (BIPI); pro-active techniques "Double human image"; "Color relation test" and the Luscher color test.
Results: Patients with Cushing's disease show higher level of psychoemotional disorders comparing to healthy counterparts (68 vs. 18%).In most cases we dealt with passive protest reactions (p=0,042). 72% of female patients (9% in Control Group) exhibited masculine traits of sexual self-identification while 54% male patients (32% in Control Group) exhibited mostly androgynous traits. Female patients exhibited negative aspects of body identification through face and body type emotional denial. The psychological status of the patients after TA was significantly better in comparison with the patients after proton therapy therapy (p=0,039). They were highly motivated to continue the treatment and were satisfied with quality of life.
Conclusions: Our study revealed significant psychological differences in patients with Cushing's disease disease in comparison to healthy population. Psychological status in patients after TA was remarkably more favorable than after proton therapy.

P1-d1-178 Adrenals and HPA Axis 1

Premature adrenarche may be an independent negative risk factor for atherogenesis in girls

Ahmet Uçar; Nurçin Saka; Firdevs Baþ; Rüveyde Bundak; Feyza Darendeliler
Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrine Unit, Istanbul, Turkey

Background: In girls, premature adrenarche (PA) refers to isolated pubic or axillary hair growth under 8 years. PA has been associated with metabolic complications in adulthood.
Objective and hypotheses: We aimed to evaluate whether the metabolic risk factors for girls with PA is increased when compared to body mass index (BMI) matched peers.
Methods: Forty-seven girls with PA and 45 BMI- matched prepubertal peers were compared with respect to anthropometric, hormonal measurements and insulin sensitivity (IS) screened by fasting glucose insulin ratio (FGIR) and homeostasis model assessment (HOMA), fasting lipids and atherogenic index of plasma (AIP). Correlations of these parameters within both groups were assessed.
Results: AIP was significantly lower in girls with PA than controls ($p<0.001$). In girls with PA, AIP significantly correlated with BMI standard deviation score (SDS) ($r= 0.40$, $p=0.006$), weight SDS ($r=0.4$, $p=0.005$), FGIR($r= -0.39$, $p=0.022$) and HOMA-IR ($r= 0.37$, $p= 0.029$).
Conclusions: PA may be an independent negative risk factor for atherogenesis. The risk of atherogenesis in girls with PA is increased in relation to decreasing IS and increasing BMI. The reduced risk of atherogenesis cannot be directly attributed to premature onset of DHEA-S secretion from the adrenals.

P1-d1-179 Adrenals and HPA Axis 1

Cyclical cushing syndrome in a 5-year-old girl with primary non-pigmented nodular adrenal hyperplasia in absence of PKA1R1A mutations

Elizaveta Orlova[1]; Maria Kareva[1]; Galina Polyakova[2]; Maria Melikyan[1]; Polina Bogdanova[1]; Valentina Peterkova[1]
[1]Endocrinological Research Centre, Institute of Pediatric Endocrinology, Moscow, Russian Federation; [2]M. F. Vladimirsky Regional Clinical Institute, Hystopathology, Mocow, Russian Federation

Background: Cyclical Cushing Syndrome (Cyc CS) is an unusual form of hypercotrisolism associated with ACTH-producing adenomas either with primary pigmented nodular adrenal disease (PPNAD) or with adrenal hyperplasia. PPNAD is a part of Carney Complex (CNC) or McCune-Albright Syndrome in most cases. Only few cases of CycCS due to micronodular adrenal hyperplasia in children have been reported to date.
Case report: A 5-year old girl born to non-consanguineous parents was admitted to our clinic with clear «cushing» phenotype. She manifested at the age of two with the episode of bulimia, rapid weight gain and "moon" face. The symptoms regressed spontaneously within 1-2 months and recurred again in the next 2-3 months. More than five cycles appeared during three year period. Munchausen syndrome was excluded. Laboratory data confirmed ACTH-independent intermittent hypercortisolism (Table 1).

	Relapse period	Remission period	Normal range
F 0800h (nmol/l)	519	175	123 - 626
F 2400 h (nmol/l)	583	188	46 - 389
ACTH 0800 h (pg/ml)	2.4	7.0	8 -66
ACTH 2400 h (pg/ml)	4.1	9.0	
24-h-UFC (nmol/l)	599	52	46 - 413
24-UFC (Liddle's test) (nmol/l)	1236		
DHEA-S (ng/ml)	173	15	1000 - 5000
Ts (ng/ml)	0.1	0.06	0.1 - 22

CT-scan revealed mild bilateral adrenal hyperplasia without adrenal mass. Liddle's test was suggestive for the diagnosis of PPNAD. We did not find other components of CNC or McCune-Albright syndrome. Genetic analysis did not detect PKAR1A mutations. The girl underwent laparoscopic bilateral adrenalectomy. Histological examination revealed non-pigmented micronodular cortex dysplasia. She has been doing well on hydrocortisone replacement during two year follow up, new manifestations of CNC were not seen.
Conclusions: We described a case of CycCS due to non-pigmented nodular adrenal hyperplasia in a child without addition signs of CNC. PKAR1A mutations also were not found. Interestingly, the patient always had very low androgen levels. We suppose that this case possibly belongs to different group of adrenal hyperplasias in childhood distinct from PPNAD, and other genes than PKAR1A probably underlies this adrenal abnormality.

P1-d1-180 Adrenals and HPA Axis 1

Comprehensive characterisation of two 17α-hydroxylase isozymes reveals differential enzymatic properties

Silvia Parajes[1]; Angela Taylor[1]; Aliesha Griffin[1]; Ian Rose[1]; Irene Miguel Escalada[2]; Naeem Shafqat[1]; Lawerence Sacco[1]; Joachim Grötzinger[3]; Wiebke Arlt[1]; Ferenc Müller[2]; Nils Krone[1]
[1]University of Birmingham, Centre for Endocrinology, Diabetes and Metabolism, Birmingham, United Kingdom; [2]University of Birmingham, Developmental genetics, Birmingham, United Kingdom; [3]Christian-Albrechts Universität, Institute of Biochemistry, Kiel, Germany

Background: Zebrafish is emerging as an effective system in endocrinology. Zebrafish synthesise steroids in the interrenal (counterpart of the mammalian adrenal), gonad and brain. Human 17α-hydroxylase (hCYP17A1) facilitates the 17α-hydroxylase and 17,20-lyase reactions in the adrenal and gonad. Two zCyp17a (zCyp17a1;zCyp17a2) enzymes exist in zebrafish.
Objective: To characterise the expression pattern and function of the two zCyp17a enzymes.
Methods: zCyp17a expression was determined by RT-PCR in embryos and adult tissues. Functional assays were performed in COS7 cells overexpressing zCyp17a or hCYP17A1. 17α-hydroxylase activity was assessed by the conversion of pregnenolone into 17α-hydroxypregenenolone and progesterone into 17α-hydroxyprogesterone. The 17,20-lyase activity was measured by the conversion of 17α-hydroxypregenenolone into DHEA and 17α-hydroxyprogesterone into androstenedione.
Results: zCyp17a genes were expressed from fecundation and in the adult interrenal, gonad and brain. Both zCyp17a enzymes converted progesterone 6 times more efficiently than hCYP17A1. zCyp17a2 hydroxylated pregnenolone 2.5 and 20 times more efficiently than zCyp17a1 and hCYP17A1, respectively. Unlike hCYP17A1, zCyp17a1 efficiently synthesised DHEA and androstenedione. zCyp17a2 lacked 17,20-lyase activity. In silico analysis using two newly developed three-dimensional zCyp17a models and the hCYP17A1 model suggest that our in vitro findings could be due to residue divergence within the substrate and redox interaction domains.
Conclusions: Herein, we prove that the zCyp17a enzymes are more efficient than hCYP17A1, showing a preference for the Ä5-pathway.
Furthermore, we describe a zCyp17a enzyme naturally lacking 17,20-lyase activity. Expression data indicate a role of both zCyp17a genes during early embryogenesis. Importantly, our data provides novel insights into CYP17A1 structure-function relationships. Furthermore, it will help to establish zebrafish as a comprehensive model to further the understanding of human pathology.

P1-d1-181 Autoimmune Endocrine Disease 1

Immunological synapses between lymphocytes and target cells in autoimmune thyroid diseases

Iwona Ben-Skowronek[1]; Leszek Szewczyk[1]; Maria Klatka[1]; Elzbieta Korobowicz[2]
[1]Medical University, Dept. Paediatric Endocrinology and Diabetology, Lublin, Poland; [2]Medical University, Chair Pathomorphology, Lublin, Poland

Background: The immune synapse (IS) is the interface between a lymphocyte and antigen presenting cell or target cell.
Objective and hypotheses: The aim of the study is presentation of the differences in ultrastructure of immune synapses in vivo in Graves'disease and Hashimoto's thyroiditis.
Methods: The study involved thyroids from 30 children with Graves' disease, 30 - with Hashimoto's thyroiditis and 30 healthy children - as a control group (during resection of thyroglossal cysts). The specimens from each patients

were routinely estimated and investigated under the transmission electron microscope.
Results: In Graves' disease, the non-polarized T- cells formed IS with active thyrocytes. The synapses between plasma cells and thyrocytes were formed with zone adherents and the space between cell membranes in which immunoglobulins were secreted. In Hashimoto's thyroiditis, T-cells and plasma cells were polarized – the centrioles, mitochondria, Golgi complex, and secretory vesicles were present in the part connected with the damaged thyrocytes. In control group the immune synapses weren't observed.
Conclusions: - The immune synapses occurred in autoimmune thyroid diseases. In Graves' disease, there exist non-polarized IS activating thyrocytes. In Hashimoto's thyroiditis, the lymphocytes forming the cytotoxic synapse were characterized by polarisation of the microtubule-organizing centre.

P1-d1-182 Autoimmune Endocrine Disease 1

Cellular mechanisms of B cell pathogenesis in AIRE deficient patients

Antonella Meloni[1]; Alessandra Magnani[2]; Michela Atzeni[1]; Marco Gattorno[2]; Alberto Martini[3]; Elisabetta Traggiai[2]
[1]II Clinica Pediatrica, Ospedale Microcitemico, Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Cagliari (Sardinia), Italy; [2]Laboratory of Immunology of Rheumatic Diseases, Pediatria II, G. Gaslini Institute, Genoa, Italy; [3]Laboratory of Immunology of Rheumatic Diseases, Pediatria II, G. Gaslini Institute; University of Genoa, Genoa, Italy

Background: Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a rare AR disorder caused by mutations in AIRE, characterized by mucocutaneous candidiasis, multiple endocrinopathies and/or ectodermal dystrophy. Circulating tissue-specific autoantibodies are a hallmark of APECED, whose generation has been mainly related to the escape of autoreactive T cells from tolerance mechanisms. Recent data suggest a T cell independent BAFF mediated mechanism implicated in altered peripheral B cell selection and its possible contribution to the pathogenesis of autoimmune disease. However, it is not still clear how B lymphocytes and the autoantibodies they produce are related to immunodeficiency and to the autoimmune process. This question is of practical importance given the advances in immunotherapy targeting B cells in a growing number of autoimmune diseases.
Objective and hypotheses: To characterize the B cell subsets distribution in peripheral blood in 12 APECED patients, and evaluate the presence of B cell related cytokines, i.e. IL-21 and BAFF, in order to better understand whether an intrinsic B cell defect was present.
Population and methods: Flow cytometric analysis of B cell subsets was performed in 12 Sardinian APECED patients (Image 1), compared to age-matched healthy donors. The following B cell subsets were analysed: transitional, naïve, IgM and switched memory, and plasmacells. ELISA assay was performed for cytokines determination in sera.

Number of patients	12	6 adults, 6 pediatrics (<18 yr)	
Origin	Sardinia		
Female/Male	7/5		
Age at diagnosis (mean/range)	6.6 yrs	0,5 - 11 yrs	
Age at sampling (mean/range)	23.5 yrs	0,5 - 46 yrs	
AIRE genotype → R139X/R139X → R139X/967-979del	11/12 patients 1/12 patients		
Prevalent disease componetns		No	%
	Chronic mucocutaneous candidiasis	12	100
	Addison's disease	8	66
	Hypoparathyroidism	7	58
	Ectodermal distrophy	9	75
	Alopecia	5	41
	Autoimmune hepatitis	4	33
	Intestinal disfunction	6	50
	Pernicious anemia	6	50
	Growth hormone deficiency	3	25
	Ovarian failure	4	57

Image 1: General characteristics of APECED patients

Results: A significant deregulation of B cells subsets was found in APECED patients, affecting principally two major subsets: 1) immature transitional B cells and 2) switch memory compartment. Concomitantly, we observed an increased in serum BAFF levels, which could be related to an exaggerated activation of IFN-γ pathway in DCs (Image 2).
Conclusions: Our hypothesis is that autoantibodies generation in APECED patients is mediated by an altered peripheral B cell selection not entirely dependent from T cells. We propose a BAFF dependent mechanisms acting on the immature transitional B cell compartment.

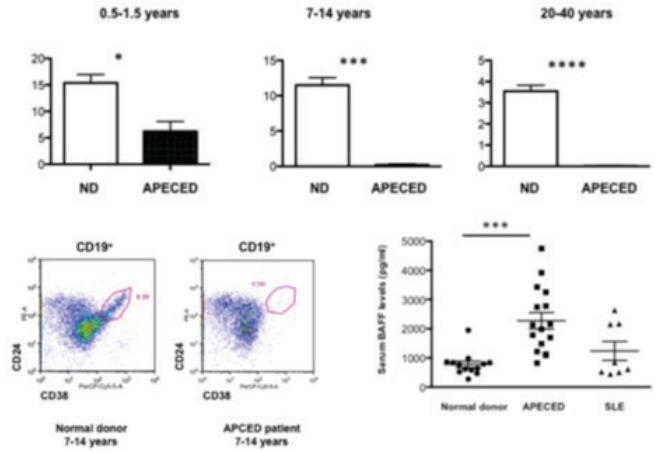


*Image 2: Percentage of transitional-immature peripheral B cells CD19*CD24^{hi}CD38^{hi} in perpheral blood of APECED patients compared to age matched healthy controls. Shown dot plot of normal donor*

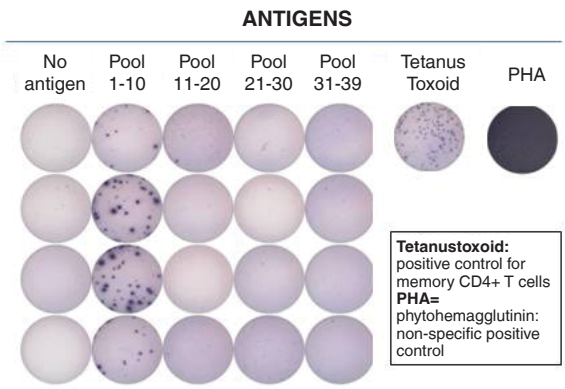
P1-d1-183 Autoimmune Endocrine Disease 1

Graves' disease: relationship between T cell reactivity to thyrotropin receptor peptides and clinical parameters

Audrey Dionne[1]; Françoise Le Deist[2]; Coralie Leblicq[3]; Diane Rottembourg[1]
[1]University of Sherbrooke, Pediatrics Department-Endocrinology service, Sherbrooke, Canada; [2]CHU Sainte-Justine, Department of Microbiology and Immunology, Montreal, Canada; [3]CHU Sainte-Justine, Department of Pediatrics, Montreal, Canada

Background: While thyrotropin receptor (TSHR) is recognized as the main autoantigen in Graves' disease (GD), the actual antigen specificity of T cells which infiltrate the thyroid and the orbit is unknown. The knowledge of immunodominant antigenic peptides is useful to depict pathogenesis as well as to design immunotherapeutical tools targeting T cells in GD.
Objective: The aim of this study is to identify T cell reactivity to TSHR peptides in GD patients. This study would be the first to map the entire extracellular domain sequence of the TSHR protein and use the enzyme-linked immunospot (ELISPOT) assay.
Methods: Adult and pediatric patients with GD were recruited. Peripheral blood T cells were exposed *in vitro* to 39 overlapping TSHR peptides. Cytokine secretion of activated autoreactive T cells was measured by ELISPOT assay. The relationship between T cell responses to TSHR peptides and clinical parameters was evaluated.
Results: Interferon gamma production of T cells in presence of pools of ten overlapping TSHR peptides were quantified: T cells from 11/39 (28%) GD patients and 2/29 (0.06%) healthy controls reacted to at least one peptide pool ($p=0.03$). Mean time since diagnosis was 3.5 years in responder patients and 5.5 years in non-responders ($p=0.14$). In one patient, T cell reactivity was not observed at diagnosis but only shortly after radioactive iodine treatment and not thereafter. In the responder GD patients, 5/11 were children vs 14/28 non-responders. Serum TSHR antibody status and presence/absence of ophthalmopathy were identical in responders vs non-responders.
Conclusions: Our findings demonstrate that the ELISPOT assay is effective to test T cell reactivity in GD patients and that GD patients have significantly more cytokine responses towards TSHR peptides than controls. The data suggest that screening for T cell responses in GD patients might be more efficient in recent-onset disease or after radioactive iodine treatment.

Interferon gamma ELISPOT assay: picture of wells illustrating a positive response to one thyrotropin receptor peptide pool in a patient with Graves' disease.



P1-d1-184 Autoimmune Endocrine Disease 1

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

Valeria Calcaterra[1]; Claudia Caramagna[2]; Mara De Amici[2]; Miryam Martinetti[3]; Anna Chiara Malvezzi[2]; Antonio Ricci[2]; Daniela Larizza[1]

[1]University of Pavia and IRCCS Policlinico S. Matteo Foundation, Dept. of Internal Medicine and Dept. of Pediatrics, Pavia, Italy; [2]IRCCS Policlinico S. Matteo Foundation, Dept. of Pediatrics, Pavia, Italy; [3]IRCCS Policlinico S. Matteo Foundation, Immunogenetics Laboratory, Immunohematology and Transfusion Centre, Pavia, Italy

Background: Type 1 diabetes mellitus (T1D), autoimmune thyroid disease (ATD) and celiac disease (CD) are autoimmune conditions relatively common in paediatric age and frequently occur in association in the same subject. Autoantibodies to the islet-specific zinc transporter isoform 8 (ZnT8Abs) are detected in the majority of T1D patients prior to and at clinical diagnosis. The presence of ZnT8Abs in other autoimmune diseases has not been investigated.
Objective and hypotheses: The aim of this study was to determine the prevalence and role of antibodies to ZnT8 in young patients with ATD and/or CD.
Methods: We analyzed ZnT8Abs, insulinoma-associated antigen-2 (IA-2) and glutamic acid decarboxylase 65 (GAD65) antibodies by radioimmunoassay in 77 patients (mean age 19.0±7.2; 59 F/18M); 28 patients had ATD, 37 CD, and 12 CD and ATD in association. The presence of one or more diabetogenic DQ molecules was investigated in all patients.
Results: ZnT8 were positive in 3 patients (3.8%), GAD65 in 20 (25.9%) and IA-2 in 21 (27.2%) patients. In the table, autoantibodies positivity according to autoimmune diseases was reported.

Autoantibodies	Autoimmune disease		
	ATD (n=28)	CD (n=37)	ATD+CD (n=12)
IA-2	6 (21.4%)	2 (5.4%)	3 (25%)
GAD65	6 (21.4%)	1 (2.4%)	3 (25%)
IA2+GAD65	0 (0%)	4 (10.8%)	6 (50%)
ZnT8+GAD65+IA2	1 (2.7%)	0 (0%)	2 (16.6%)

All patients with ZnT8Abs positivity, presented also very high titer of GAD65 and IA2 autoantibodies; 4 heterodimers and 2 heterodimers of susceptibility to T1D were found in 2 and 1 patients, respectively.
Conclusions: Our results showed a low prevalence of ZnT8 autoantibodies, respect to the others, in patients with ATD and/or CD. No patients presented positivity only for ZnT8Abs. Determination of ZnT8Abs does not seem useful in the screening of the risk of T1D in patients with ATD and/or CD.

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

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

Huamei Ma[1]; Sinian Pan[2]; Qiuli Chen[1]; Zhe Su[1]; Yanhong Li[1]; Hongshan Chen[1]; Minlian Du[1]

[1]The first Affiliated Hospital of Sun Yat-Sen University, Pediatric department, Guangzhou, China; [2]The third Affiliated Hospital of Sun Yat-Sen University, Pediatric department, Guangzhou, China

Background: The mechanism of linear growth reduction during GnRHa treatment in central precocious puberty has not been elucidated.
Objective: To investigate the pattern of serum amino-terminal proC-type natriuretic peptide (NT proCNP) in healthy girls throughout puberty, and the changes of serum NT proCNP in girls with idiopathic central precocious puberty (ICPP) before and during gonadotropin-releasing hormone analog(GnRHa) therapy.
Methods: Serum levels of E2, NT proCNP, insulin-like growth factor 1(IGF-1), N-MID Osteocalcin(OC) and carboxy-terminal cross-linking telopeptide of type I collagen (β-CrossLaps) were measured in healthy 57 girls at different pubertal stages, and in 13 girls with ICPP at the beginning and the end of 6th-month and 12th-month of GnRHa treatment. Height velocities of the 13 ICPP girls in each 6 months before and after GnRHa treatment was calculated.
Results: Serum NT proCNP level increases as the progress of pubertal development and peaks at the late puberty ($P<0.01$), paralleling with serum E2 and IGF-1 levels, like with the pattern of height velocity. All of serum NT proCNP, Osteocalcin and β-CrossLaps level decrease significantly in ICPP girls at the end of 6th-months of GnRHa therapy($P<0.01$ or $P<0.05$), and remain the same low level at the end of 12th-month of GnRHa. Different from the aboved markers, serum IGF-1 level remains high before and during GnRHa treatment despite growth deceleration.
Conclusions: Linear growth reduction in girls with ICPP treated with GnRHa is due at least in part to decreased CNP-mediated long bone growth after estrogen inhibition. Serum NT proCNP can be used as a biological marker of long bone growth indicating the activity of epiphyseal growth plate.

P1-d1-186 Bone, Growth Plate and Mineral Metabolism 1

Younger age at hematopoietic cell transplantation is associated with greater bone mineral deficits

Anna Petryk[1]; Lynda Polgreen[1]; Lei Zhang[2]; James Hodges[3]; Scott Baker[4]

[1]University of Minnesota, Division of Pediatric Endocrinology, Minneapolis, United States; [2]University of Minnesota, Division of Epidemiology and Community Health, Minneapolis, United States; [3]University of Minnesota, Division of Biostatistics, Minneapolis, United States; [4]Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, United States

Background: Low bone mineral density (BMD) has been reported in adults after hematopoietic cell transplantation (HCT). Little is known about the effect of HCT on BMD in children.
Objective and hypotheses: This study's goals were to determine 1) if children treated with HCT have lower BMD compared to healthy sibling controls, and 2) if BMD is associated with age at HCT or time since HCT. We hypothesized: 1) mean BMD Z-score is lower in HCT recipients than controls; 2) younger age at HCT is associated with higher BMD Z-score; 3) BMD Z-score increases with time since HCT.
Methods: The study included 151 patients (87 males), mean age at time of study 24.7±8.6 yr treated with HCT for acute leukemia (74%) or lymphoma at age 10.9±6.4 years, and 92 healthy siblings (49 males), mean age 22.3±8 yr. BMD Z-scores for total body (TBMD) and lumbar spine (LBMD) were measured by DXA. Statistical analyses used generalized estimating equations.
Results: Mean TBMD Z-score was mildly lower in cases than controls (0.0±1.0 vs 0.4±1.0, p=0.0011) and a larger proportion of cases than controls had TBMD Z-score < -1 (14% vs. 7%, p=0.033). However, mean LBMD Z-score and proportion of cases with LBMD Z-score < -1 did not differ significantly between cases and controls. Both TBMD (p=0.0001) and LBMD (p=0.0008) Z-scores tended to be higher with older age at HCT after adjusting for sex and time since HCT. Both TBMD (p=0.012) and LBMD (p=0.0003) tended to be higher with longer time since HCT after adjusting for sex and age at HCT.

Conclusions: In this cohort of patients mostly (84%) younger than 18 yr at HCT, BMD deficits were mild on average. However, age at HCT and time since HCT were significantly associated with BMD deficit. Younger age and shorter interval since HCT were associated with lower BMD Z-scores.

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

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

Rimm Huh[1]; Sung Yoon Cho[1]; Chang-Seok Ki[2]; Yoon La Choi[3]; Young Bae Sohn[4]; Su Jin Kim[5]; Dong-Kyu Jin[1]

[1]Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pediatrics, Seoul, Republic of Korea; [2]Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Laboratory Medicine & Genetics, Seoul, Republic of Korea; [3]Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pathology, Seoul, Republic of Korea; [4]Ajou University Hospital, Department of Medical Genetics, Seoul, Republic of Korea; [5]National Cancer Center, Center for Pediatric Oncology, Goyang, Republic of Korea

Background: Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by bone fragility, frequent fractures and low bone mass.
Objective and hypotheses: Dominant COL1A1 or COL1A2 mutations appeared to be causative in the majority of OI types but rare recessive genes have also been reported including CRTAP, P3H1, FKBP10, LEPRE1, PLOD2, PPIB, SERPINH1, and SP7.
Method: Given that many genes are involved in OI and COL1A1 and COL1A2 genes are difficult to be analyzed by conventional Sanger sequencing, we applied whole exome sequencing to identify mutations in a Korean OI patient who showed an initially mild and then progressively worsening form OI with severe deformities of the long bones.
Results: After strategic filtering, two deleterious variants (c.77dupC and c.421dupC) in the SERPINF1 gene were identified as the candidate mutations. Interestingly, during analysis of the exome data, the SERPINF1 has been reported as causative gene in German patients with autosomal-recessive OI.
Conclusions: The present study shows that whole exome sequencing may be an excellent tool for the identification of mutations in patients with OI and also support the notion that SERPINF1 mutations are genetic causes of recessive OI regardless of ethnicities.

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

Yuan Xiao; Wei Wang; Junqi Wang; Zhiya Dong; Xiumin Wang; Jihong Ni; Fengsheng Chen; Defen Wang

Shanghai Ruijin Hospital,Shanghai Jiao Tong University, School of Medicine, Pediatric Department, Shanghai, China

Background: Estrogen is a key regulator of growth plate development.It is found that c-type natriuretic peptide (CNP) regulated cartilage homeostasis and enchondral bone growth.
Objective and hypotheses: We observed the impression of different concentration estrogens on CNP and its receptor NPR-B levels and mRNA expression in rat growth plate chondrocyte.
Methods: Chondrocytes were isolated from growth plates in tibia of prepubertal rats and primarily cultured. After cell density was adjusted, 17β-estradiol in different concentrations (10^{-4}mol/L, 10^{-6}mol/L, 10^{-8}mol/L, 10^{-10}mol/L, and 10^{-12}mol/L) was added to the culture solution. And a control without 17β-estradiol was set up. After 48-hour cultured, chondrocyte viability was measured by MTT assay. CNP, NPR-B and IGF-1 mRNA expression was detected by real-time PCR. Finally, concentration of CNP in culture solution was measured by ELISA.
Results: It was found that 17β-estradiol impacted on chondrocyte viability when concentration was within 10^{-4}-10^{-12}mol/L. 17β-estradiol stimulated proliferation of chondrocytes at 10^{-8} and 10^{-10}mol/L, but had an inhibited effect at 10^{-4}mol/L. Consistent to these findings, CNP level in culture solution was highest (0.49±0.02 ng/ml, $P<0.05$) when 17β-estradiol was at 10^{-10}mol/L. Meanwhile, levels of CNP and NPR-B mRNA expression were significantly higher in 10^{-10}mol/L group than any others. $2^{-\Delta\Delta Ct}$ values of CNP and NPR-B mRNA were 73.18±11.35 and 19.20±8.56 respectively ($P<0.05$). Chondrocytes which were cultured with 10^{-8}mol/L expressed much more IGF-1 mRNA when compared to other groups. But level of IGF-1 mRNA expression declined significantly when 17β-estradiol was at 10^{-10}mol/L, its level was even lower than at 10^{-4}mol/L($P<0.05$).
Conclusions: 17β-estradiol in different concentrations could modulate differentiation and proliferation of chondrocyte from growth plate of prepubertal rats through CNP/NPR-B pathway. But cell viability was not accordant with paracrine/autocrine activities of IGF-1 in chondrocyte when stimulated with 17β-estradiol in different levels.

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

Laurent Maimoun[1]; Olivier Coste[2]; Pascal Philibert[1]; Karine Briot[3]; Thibault Mura[4]; Florence Galtier[5]; Denis Mariano-Goulart[6]; Françoise Paris[7]; Charles Sultan[8]

[1]Hopital Lapeyronie, CHU Montpellier and UMI, Hormonologie, Montpellier, France; [2]CHU and UMI and Direction Régionale de la Jeunesse, des Sports et Cohésion Sociale, Hormonologie, Montpellier, France; [3]Hôpital Cochin, APHP Paris, Rhumatologie, Paris, France; [4]CHU and UMI, Centre d'Investigation Clinique et Département d'information Médicale, Montpellier, France; [5]CHU and UMI, CIC 1001, INSERM and Centre d'Investigation Clinique et Département d'Information Médicale, Montpellier, France; [6]Hopital Lapeyronie, CHU Montpelllier and UMI, Médecine Nucléaire, Montpellier, France; [7]Hopital Lapeyronie, CHU Montpellier and UMI, Hormonologie and Unité d'Endocrinologie Pédiatrique, Montpellier, French Southern Territories; [8]CHU Montpellier and UMI, Hormonologie and Unité d'Endocrinologie Pédiatrique, Montpellier, France

Background: Intensive physical training in peripuberty may have a sport-dependent effect on bone mass acquisition.
Objective and hypotheses: The aim of this study was to compare the effects of sports that generate different mechanical loads, in terms of impact and intensity, on the bone mass acquisition in young girls over the peripubertal period.
Methods: Eighty peripubertal girls from 10.7 to 18.0 years old (mean 13.83±1.97) were recruited: 20 artistic gymnasts (AG; high-impact activity, mean hours training per week: 20.3 ± 4.2), 20 rhythmic gymnasts (RG; medium-impact activity; 21.1 ± 4.4 hr/wk), 20 swimmers (SW; no-impact activity; 14.5 ± 5.9 hr/wk), and 20 age-matched controls (CON; leisure physical activity <3h/wk). Areal bone mineral density (aBMD) was determined at whole body, lumbar spine, femoral region and radius using DEXA. Hip structural analysis software applied at the femur evaluated cross-sectional area (CSA, cm2), section modulus (Z, cm3), and buckling ratio. Bone turnover markers were analyzed.
Results: AG had higher aBMD than CON and SW at all bone sites and higher values than RG in the lumbar spine and radius. RG had higher aBMD than CON and SW only in the femoral region. CSA and mean cortical thickness were significantly higher and the buckling ratio was significantly lower in both gymnast groups compared with CON and SW. In RG only, endocortical diameter and width were reduced, while Z was only increased in AG compared with CON and SW. Reduced bone remodeling was observed in RG compared with AG only when groups were subdivided according to menarchal status.
Conclusions: High-impact activity clearly had a favorable effect on aBMD and bone geometry, although the bone health benefits appeared more marked after menarche.

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Hyperandrogenism in elite adolescent swimmers does not modify bone mass acquisition

Olivier Coste[1]; Laurent Maimoun[2]; Pascal Philibert[2]; Karine Briot[3]; Thibault Mura[4]; Florence Galtier[5]; Barbara Crastres-De-Paulet[2]; Denis Mariano-Goulart[6]; Charles Sultan[7]; Françoise Paris[7]

[1]CHU and UMI and Direction Régionale de la Jeunesse, des Sports et Cohésion Sociale, Hormonologie, Montpellier, France; [2]Hopital Lapeyronie, CHU Montpellier and UMI, Hormonologie, Montpellier, France; [3]Hôpital Cochin, APHP Paris, Rhumatologie, Paris, France; [4]Hopital St Elois, CHU Montpellier, CIC 1001, INSERM and Centre d'Investigation Clinique et Département d'Information Médicale, Montpellier, France; [5]Hopital St Elois, CHU Montpellier, CIC 1001, INSERM and Centre d'Investigation Clinique et Département des Maladies Endocriniennes, Montpellier, France; [6]Hopital Lapeyronie, CHU Montpellier and UMI, Médecine Nucléaire, Montpellier, France; [7]Hopital Lapeyronie, CHU Montpellier and UMI, Hormonologie and Unité d'Endocrinologie Pédiatrique, Montpellier, France

Background: Hyperandrogenism has been frequently reported in elite athletes. However, the effect of high testosterone levels on bone mass acquisition in this population remains largely unknown.
Objective and hypotheses: To investigate whether hyperandrogenism affects areal bone mineral density (aBMD), bone geometry and bone remodeling in young elite swimmers (SW).
Methods: Twenty-five SW (mean age: 14.9 ± 1.0 yr, training 15.2 ± 4.4 hours per week) and 21 controls (CON; mean age: 15.6 ± 1.6 yr). All participants had breast stage IV or V and a gynaecological age of 18 months. The areal bone mineral density (aBMD) at whole body, total proximal femur, lumbar spine and mid-radius was determined using dual-X-ray absorptiometry. Hip structural analysis (HSA software) was applied at the femur to evaluate bone geometry. Bone remodeling was evaluated by specific markers of bone formation and resorption.
Results: Two groups of SW were constituted on the basis of the total testosterone (T) level. Hyperandrogenic SW (HSW; n=15) presented higher T than SW with normal T (NSW; n=10) and CON (0.63±0.17; 0.36±0.07 and 0.38±0.14 ng/ml, respectively). The SHBG level (62.1±18.7 vs. 43.3±19.8 nmol/l) and the LH/FSH ratio (1.7±1.1 vs. 0.9±0.5) were higher and menstrual disorders (60% vs. 23.8%, $p<0.03$) were more frequent in HSW than CON. In contrast, no difference was observed between the three groups for the age of menarche (12.5±1.3, 12.9±1.3, 12.3±1.4 yr for HSW, NSW and CON, respectively), other sex hormones (free T, E2, etc.) and IGF-1 or IGFBP-3. Areal BMD was only modestly increased at the upper limbs in the SW groups, but no other bone-specific difference (aBMD, bone geometry, bone turnover markers) was demonstrated between SW and CON.
Conclusions: Hyperandrogenism has no detectable effect on bone mass and bone geometry in SW during the period of peak bone mass acquisition.

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Two children with short stature, pseudohypoparathyroidism, hypothyroidism and skeletal dysplasia of different origin

Jennifer Krueger[1]; Caroline Silve[2]; Alma Küchler[3]; Ute Groß[4]; Heinrich Maria Schulte[5]; Annette Richter-Unruh[1]

[1]Endokrinologikum Ruhr, Pediatric Endocrinology and Diabetology, Bochum, Germany; [2]Hospital Bicêtre, Service d´endocrinologie pédiatrique, Le Kremlin Bicêtre, France; [3]University hospital Essen, Human genetics, Essen, Germany; [4]Endokrinologikum Hamburg, Molecular Genetics, Hamburg, Germany; [5]Endokrinologikum Hamburg, Endocrinology, Hamburg, Germany

Background: Short stature with skeletal dysplasia and pseudohypoparathyroidism (PHP) suggests the diagnosis of Albright's hereditary osteodystrophy (AHO) which is caused by mutations in the gene of the α-stimulatory subunit (Gsa) of the G-protein (GNAS). However some patients with clinical signs of PHP do not have mutations in GNAS.
Objective and hypotheses: Alterations in the signal transduction cascade of the G-protein-coupled-receptor via Gsa, cAMP and proteinkinase A (GPCR-Gsa-cAMP-PKA pathway) can lead to resistance to parathormone (PTH) and other hormones sharing this pathway. We looked for variations in this pathway causing PHP.
Method: We report on two children with short stature, PHP, hypothyroidism and skeletal dysplasia. The first patient is a 12-year old girl who has short and plump hands and feet and suffers also from mental retardation, subcutaneous calcification, obesity and insulin resistance. The second patient is a 15-year old boy who was born small for gestational age (SGA). He has remarkable short and plump hands and feet. The big toes are broader and longer than the other toes. He has a big cranium and a hyperlordosis. Otherwise his body seems to be proportioned. He has no mental retardation.
Results: Molecular genetic testing in the girl revealed a heterozygous deletion in the promoter region of GNAS. This led to the diagnosis of AHO. In the boy we ruled out mutations in the fibroblast growth factor receptor 3 (FGFR-3), the short stature homeobox (SHOX) and the GNAS gene. Finally a mutation in the cyclic AMP-dependent regulatory subunit of protein kinase A gene (PRKAR1A) was detected and the boy was diagnosed with acrodysostosis. Mutations in the PRKAR1A-gene lead to an impaired proteinkinase A activity and so to PHP or to Carney complex (myxomas of the heart, lentiginosis and endocrine overactivity).
Conclusions: We recommend consideration of other infrequent genetic mutations of the GPCR-Gsa-cAMP-PKA pathway in patients with clinical signs of AHO / PHP who are negative for GNAS mutations.

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

Oliver Semler[1]; PV Asharani[2]; Katharina Keupp[3]; Yun Li[3]; Holger Thiele[4]; Esther Pohl[3]; Christian Netzer[3]; Matthias Hammerschmidt[5]; Peter Nuernberg[4]; Bernd Wollnik[3]; Thomas J. Carney[2]; Eckhard Schoenau[1]

[1]Childrens Hospital, Endicrinology/Osteology, Cologne, Germany; [2]Proteos, Institute of Molecular and Cell Biology, Singapore, Singapore; [3]University Hospital Cologne, Institute of Human Genetics, Cologne, Germany; [4]University Hospital Cologne, Cologne Center for Genomics, Cologne, Germany; [5]University Hospital Cologne, Institute of Developmental Biolog, Cologne, Germany

Background: Osteogenesis imperfecta (OI) is a hereditary disease with high variability of clinical symptoms, formerly associated with mutations in COL1A1/A2. Recently a widening range of causative genes has been discovered including Bmp1.
Objective and hypotheses: In a consanguine family two sons showed symptoms of OI with increased fracture rate, high bone mass and high osteoclastic activity. They presented at the age of 5.0/1.9 years with normal height (+0.1/-0.7SD) and BMI (+1.8/-0.4SD). They showed no typical signs of a pathologic collagen production (dentinogenesis imperfecta, hypermobility of joints, hearing loss or discoloured sclera). First fractures occurred with 23/14 months. Serum calcium and alkaline phosphatase levels were normal. Deoxypyridinoline/creatinine were elevated.
Methods: Using whole-exome sequencing we identified a homozygous missense mutation in BMP1 in our patients. We implemented this mutation in bmp1a into zebrafish. The phenotypes were compared. Additional functional analyses were performed as described [1]. Genetic, biochemical and histological analysis of this mutant were performed.
Results: Tab.: Clinical Features of patients and zebrafish

Findings	patient 1	patient 2	zebrafish
Birth length and birth weight	normal	normal	normal
Vertebral fractures	+	+	+
Deformities of bones/fins	+	+	+
Deformities of extremities /fins	+	+	+
Vertebral bone mineral density DEXA (z-Score)	+ (3.4SD)	+ (2.1SD)	+

Phenotypes of patients and zebrafish were comparable. Zebrafish showed a reduced secretion of procollagen from the ER and a reduced posttranslational glycolisation. A strong expression of bmp1a in larval zebrafish osteoblasts was observed.
Precise conclusions: We identified siblings with symptoms of OI with increased bone density despite increased osteoclastic activity with a mutation in Bmp1 and transferred this mutation into zebrafish. First time functional analysis demonstrated conservation of BMP1 function in osteogenesis across species. [1] Attenuated BMP1 function compromises osteogenesis leading to bone fragility in human and zebrafish; Asharani et al (AJHG, in press).

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Bone mineral status and prevalence of fractures in girls with Turner syndrome (TS); usefulness of phalangeal quantitative ultrasound (QUS) in addition to dual energy X-ray absorptiometry (DXA)

Francesco Vierucci[1]; Paola Erba[2]; Giovanni Federico[1]; Giuseppe Saggese[1]
[1]"S. Chiara" University-Hospital, Department of Pediatrics, Pisa, Italy; [2]"S. Chiara" University-Hospital, Department of Nuclear Medicine, Pisa, Italy

Background: TS guidelines do not recommend performing a densitometric evaluation during paediatric age despite increased risk of osteoporosis later in life.
Objective and hypotheses: To assess bone mineral status and prevalence of fractures in girls with TS.
Methods: 24 girls with TS were assessed at 13.4±3.0 yrs (21 were treated with GH from 4.7±3.0 yrs and 13 received HRT from 2.2±1.3 yrs) and at 17.1±3.1 yrs (all treated with GH from 6.9±2.8 yrs, 21 received HRT from 4.6±1.9 yrs).
We evaluated areal and volumetric bone mineral density (aBMD and vBMD) at lumbar-spine (L) and femoral neck (F) by DXA and amplitude-dependent speed of sound (AD-SoS) and bone transmission time (BTT) by phalangeal QUS. Prevalence of fractures was assessed in 50 girls as controls.
Results: At baseline L-aBMD, F-aBMD and F-vBMD Z-score were reduced in TS (-1.1±0.7, p<0.0001; -1.3±0.7, p<0.0001; -0.8±0.6, p<0.0001 respectively), while L-vBMD Z-score was normal. AD-SoS and BTT Z-scores were reduced (-1.6±1.7, p=0.0001 and -1.9±1.6, p<0.0001, respectively). By QUS we identified a higher percentage of girls with reduced bone mineral status than by DXA (58.3% vs 8.4%; BTT vs L-DXA p=0.003, BTT vs F-DXA p=0.0013). At the end of follow-up L-aBMD, F-aBMD and vBMD Z-score persisted reduced while L-vBMD Z-score remained normal. AD-SoS and BTT Z-scores were reduced (-1.4±1.2, p<0.0001 and -1.7±1.0, p<0.0001, respectively). The prevalence of fractures was higher in TS (0.6±1.0 vs 0.2±0.5, p=0.0242). AD-SoS and BTT were lower in TS who reported almost a fracture than fracture-free patients (p=0.0211 and p=0.0385, respectively). Duration of GH therapy and HRT did not correlate with bone status.
Conclusions: TS girls have a defect of bone mineralization, especially in skeletal sites where cortical bone is more represented. QUS compared to DXA may identify a greater number of patients with pathological bone mineral status and at increased risk of fractures.

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Subcutaneous ossifications in Albright hereditary osteodystrophy (AHO): is it possible to draw a genotype correlation?

Luisa de Sanctis[1]; Barbara Ceoloni[1]; M. Francesca Fissore[1]; Francesca M Elli[2]; Giovanna Mantovani[2]
[1]University of Torino, Dept. of Pediatric Sciences, Torino, Italy; [2]University of Milan, Fondazione IRCCS Ca' Granda Policlinico, Endocrinology Unit, Dept. of Medical Sciences, Milan, Italy

Background: Subcutaneous ossifications (SO) represent a typical even if not constant feature of Albright Hereditary Osteodystrophy (AHO), a complex phenotype that also includes brachydactyly, small stature, obesity with rounded face and mental retardation, caused by dominant mutations in exons 1-13 of the GNAS gene, that encodes for Gs-alpha protein, located within the GNAS locus.
Objective and hypotheses: Data about prevalence and a possible genotype/phenotype correlation of SO in AHO are still lacking.
Methods: Among a series of 62 GNAS mutated AHO subjects, we first investigated the prevalence of SO and then we searched for a possible genotype correlation by evaluating their clinical manifestation (age of onset, number of lesions and their progression).
Results: Thirty out the 62 GNAS mutated subjects showed SO; of the 30 identified GNAS mutations, 27 were located in exons 1, 5 and 7 (17, 4 and 6 mutations, respectively). It is noteworthy that all the subjects harbouring exon 1 nonsense mutations displayed SO; conversely, patients with recurrent mutations at exon 5 and 7 (codons 115 and 189, respectively) not always displayed this sign. No significant differences in terms of time of presentation, number, localization or progression of these lesions seemed to occur for the differently located gene alterations.
Conclusions: Although a definite genotype correlation cannot be drawn as for the severity of SO in AHO, exon 1 nonsense mutations seem to be associated with the presence of this sign, in line with recent evidence in animal models that heterozygous inactivation of Gs-alpha-specific exon 1 is able to promote osteoblast differentiation in wild-type adipose stromal cells, suggesting that Gs-alpha-specific mutations are sufficient to induce ectopic ossification in AHO patients and that the potential mechanism might be the accelerated osteoblast differentiation in adipose cells.

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Use of recombinant PTH in hyperparathyroidism resistant to conventional therapy

Elif Ozsu[1]; Filiz Mine Cizmecioglu[1]; Elif Yesiltepe Mutlu[1]; Nail Yologlu[2]; Metin Aydogan[2]; Sukru Hatun[1]
[1]Kocaeli Universty Medical School, Pediatric Endocrinology, Kocaeli, Turkey; [2]Kocaeli Universty Medical School, Pediatric Allergy, Kocaeli, Turkey

Background: In childhood, we use calcium and vitamin D analogues instead of hormone replacement for the traditional treatment of hypoparathyroidism. However, this treatment carries some risks and challenges. We used short term recombinant PTH in a subject with hypoparathyroidism resistant to conventional therapy and with recurrent severe hypocalcemia.
Case: A one-month old preeclamptic and diabetic mother's baby presented with sucking difficulty and vomiting. He had a history of convulsions. Serum Ca level was 6.8 mg/dl and P was 8.6 mg/dl whereas PTH level was <0.1 ng/dl. The patient with dismorphic features, tetralogy of fallot, thymic aplasia, vertebral abnormalities was diagnosed to have Di-George syndrome and commenced on calcium and calcitriol therapy. In the follow-up period, he presented with frequent severe hypocalcemic episodes and skin rash. Cellular immunodeficiency was detected. Although the dose was increased, sucking and swallowing difficulties due to laryngeal defect led to inefficient treatment. The calcium dose was titrated to 400 mg/kg/d, calcitriol dose to 400 ng/kg/d but serum Ca levels fluactuated from 4.6 to 10 mg/dl and P levels from 11 to 4.6 mg/dl (Table). The patient showed insufficient response to conventional therapy despite high doses and we commenced recombinant PTH analogue (teriparatide) therapy. Serum Ca leves rapidly returned to normal range. Despite the termination convansionel therapy on 10th day of the treatment, the patient maintained normocalcemic levels only single dose of teriparatide daily.
Conclusion: In some hypoparathyroid subjects, the management of hypocalcemia may take a long time and/or may fail with conventional therapy. Moreover, intravenous calcium therapy may lead to catheter complications and high dose calcitriol therapy to hypercalciuria and nephrocalcinosis risk. Recombinant PTH analogues may improve serum Ca levels more rapidly. Recently, its use was reported to be safe with low doses in childhood. However, controlled randomized trials are warranted for long term therapy.

Age (day)	Serum Calcium (mg/dl)	Serum Phosphorus (mg/dl)	Calsitriol (ng/kg/d)	oral/i.v Ca (mg/kg/d)	rPTH (mcg/kg/dose)
30	7,4	8,4	40	100	
45	5,5	11,5	75	300	
57	10	4,6	100	140	
64	7,7	5,6	100	120	
75	9,1	8	200	100	
83	4,6	14,8	300	200	
133	5,6	10,6	300	250	
138	5	11	400	280	
145	8,2	7,5	400	150	
152	6,3	5	400	400	1x1
153	7,6	4,4	200	160	2x1
154	9,3	3,9	140	140	2x1
157	9,5	5,6	70	70	
161	8,6	4,7	70	150	2x1
162	9,9	4,9	stop	stop	2x1
170	9,5	4,6			1x1

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Metacarpal width, length, medullary diameter and cortical thickness in hypophosphatemic rickets

Signe Sparre Beck-Nielsen[1]; Kim Brixen[2]; Jeppe Gram[3]; Mette Ramsdal Poulsen[4]
[1]Hospital of Southwest Denmark, Dept. of Paediatrics, Esbjerg, Denmark; [2]Odense University Hospital, Dept. of Endocrinology, Odense, Denmark; [3]Hospital of Southwest Denmark, Dept. of Endocrinology, Esbjerg, Denmark; [4]Odense University Hospital, Dept. of Clinical Radiology, Odense, Denmark

Background: Hypophosphatemic rickets (HR) is a group of rare, inheritable disorders caused by excessive renal phosphate wasting. We have previously reported a significant difference in bone mineral apparent density (BMAD) between the hip and spine (Z-scores 1.0 and 2.1, respectively) in HR children.
Objective and hypotheses: To evaluate the discordance in BMAD by measuring the metacarpal medullary diameter and cortical thickness from hand X-rays.
Methods: A total of 17 children were recruited from a larger study on HR and had hand X-rays performed. Two patients were excluded due to poor image quality, thus X-rays from 15 patients and DXA of the spine and hip were available in 11 patients. All but one patient had a *PHEX* mutation or proven X-linked disease (XLHR). The remaining patient had a *DMP1* mutation. BoneXpert (Visiana) was used to calculate the following parameters of the metacarpal bones: length (L), width (W), cortical thickness (T) and medullary diameter (M). HR patients were compared with age-matched healthy children from Switzerland.
Results: Since the measurements from the patient with a *DMP1* mutation differed significantly from the group of XLHR, his values are reported separately in brackets. All values are reported as median [range]. XLHR patients had significantly broader metacarpal bones W=1.6 SD [0.4-3.9], $p<0.001$, (5.5 SD), due to a wider marrow diameter M=2.2 SD [0.5-3.6], $p<0.001$, (4.8 SD). Their cortical thickness was significantly reduced T=-1.5 SD [-2.2 – 0.8], p=0.001, (1.3 SD). The length was not different compared with controls L=0.1 SD [-1.7 – 0.9], p=0.5, (1.3 SD). Cortical thickness was negatively correlated with the paired differences between Z BMAD spine and Z BMAD hip (p=0.028).
Conclusions: Children with HR have broader metacarpal bones with significantly thinner cortex compared to reference. Cortical thickness correlated with the discordant BMAD at the spine and the hip. HR caused by a *DMP1* mutation may affect bone size more severely than XLHR.

P1-d1-197 Bone, Growth Plate and Mineral Metabolism 1

Efficacy of bisphosphonates in the treatment of vitamin D intoxication

Kara Cengiz[1]; Gündüz Suzan[2]; Çetinkaya Semra[3]; Günindi Figen[1]
[1]Ondokuz Mayis University, Pediatric Endocrinology, Samsun, Turkey; [2]Kozluk State Hospital, Pediatrics, Batman, Turkey; [3]Dr. Sami Ulus Women's and Children's Hospital, Pediatric Endocrinology, Ankara, Turkey

Background: Traditional treatment tools of hypercalcemia caused by vitamin D overdose have been hydration, furosemide and glucocorticoids. In recent years, bisphosphonates have been used in treatment of vitamin D intoxication. Although there have been some anecdotal reports, the efficacy of bisphosphonates in the treatment of vitamin D intoxication has not been evaluated in large case series.
Objective and hypotheses: We aimed to determine the effectiveness and safety of this new treatment approach by reporting the outcome of 18 children with vitamin D intoxication treated with bisphosphonates.
Methods: The medical records of vitamin D intoxicated patients treated with bisphosphonates for the past 10 yrs were reviewed. The study group contained 18 children aged 1.6±1.1 yrs. All patients were treated with intravenous hydration and furosemide. Bisphosphonates were administered in 5 patients after unsuccessful prednisolone treatment and in 13 patients from baseline.
Results: Initial serum levels of calcium and 25(OH)D3 were 16.2±2.0 mg/dl and 531±214 ng/ml, respectively. In 3 of 5 patients treated with prednisolone, normocalcemia was achieved within median 17 (ranged 12-26) days. However, hypercalcemia recurred in 3 to 5 days after discontinuation of therapy and pamidronate was given. In other two patients, prednisolone switched to pamidronate on 6th and 8th days of treatment. Of 18 patients treated with pamidronate, 11 (61%) became normocalcemic within 3 days and 15 (83%) within 7 days. In three patients, alendronate was added to treatment because pamidronate did not achieve normocalcemia. The mean time to reach normocalcemia with bisphosphonates was 4.7±6.6 (2-12) days. During pamidronate infusions, body temperature increased to until 39.5 °C in 16 of 18 patients. Except fever, no side effect attributable to bisphosphonates was observed.
Conclusions: Bisphosphonates are effective and safe agents for treatment of hypercalcemia caused by vitamin D overdose. Instead of glucocorticoids, they should be considered as the first line therapy for vitamin D intoxication.

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Vitamin D intoxication associated with fish oil supplement in young children

Kara Cengiz; Günindi Figen; Üstyol Ala; Aydin Murat
Ondokuz Mayis University, Pediatric Endocrinology, Samsun, Turkey

Background: Vitamin D poisoning has been associated with contamination of cooking oil, adulteration of table sugar, over-fortification of milk, erroneous production of vitamin D supplements and incorrect use of vitamin D-containing preparations. However, there is no report on vitamin D intoxication associated with fish oil supplements.
Objective: We report here the unusual event of seven young children with vitamin D intoxication that appear to have been resulted from excessively vitamin D-fortified fish oil.
Subjects and methods: Three young children initially presented with unexplained vitamin D intoxication were meticulously inquired about dietary supplements. It was realized that they had been taking a fish oil supplement that was recently produced by a local manufacturer. Although the manufacturer immediately recalled the product, four additional patients taking same fish oil applied to our clinic during one-month period. After the patients were successfully treated with intravenous hydration, furosemide and pamidronate infusions, their medical records were reviewed.
Results: All seven patients whose ages ranged from 9 to 50 months had taken one or two bottles of same fish oil for 15 to 60 days. All had severe hypercalcemia (serum calcium, 16.2±1.8 mg/dl), hypercalciuria (urinary calcium/creatinine ratio, 1.6±1.0 mg/mg) suppressed levels of parathyroid hormone (6.9±4.0 pg/ml), and elevated serum 25(OH)D3 concentrations [636±222 (340-962) ng/ml]. Two patients developed nephrocalcinosis. After three- to four-month follow-up period, serum 25(OH)D3 levels returned to normal limits (30-80 ng/ml), with a mean value of 63±15 ng/ml.
Conclusions: Vitamin D intoxication may be caused by errors in the manufacturing of fish oil supplements for children. Physicians should be aware that their patients may be taking such dietary supplements. To prevent the occurrence of such unintentional incidents, the manufacturers should rigorously monitor levels of ingredient of dietary supplements. Also, they should be tightly controlled by the authorities of government.

P1-d1-199 Diabetes and Insulin 1

The effect of insulin intensification on glycaemic control and lipid levels in children and young persons with type 1 diabetes differs in relation to ethnic group

Renuka Dias[1]; Freya Brown[2]; Claire Wyatt[2]; Sharanjit Cheema[2]; Jeremy Allgrove[2]; Rakesh Amin[3]
[1]School of Clinical and Experimental Medicine, Genes, Reproduction and Development Group, Birmingham, United Kingdom; [2]Royal London Hospital, Paediatric Endocrinology and Diabetes, London, United Kingdom; [3]Great Ormond Street Hospital, Paediatric Endocrinology and Diabetes, London, United Kingdom

Background: Previous studies identify non-White ethnicity as predictive of poor diabetes related outcomes. However, many of these reports originate from the United States and may, in part, reflect complex interactions between ethnicity, healthcare inequality and social deprivation.
Objective and hypotheses: We aimed to prospectively determine the effect of insulin intensification on glycaemic control and lipid levels in relation to ethnicity in a UK cohort of children and young persons (CYP) with Type 1 diabetes (T1D).
Methods: Data were collected prospectively between 2008 and 2011 in CYP from a single paediatric diabetes centre (n=222; 40% White, 28% South

Asian, 32% Black). By 2009 nearly all CYP were treated with multiple daily injections or insulin pump therapy. Deprivation scores were derived from the UK 2010 Index of Multiple Deprivation. We used linear mixed level modelling to identify longitudinal differences between ethnic groups.
Results: At study end; Black CYP had higher HbA1c levels (9.4 (standard deviation 2.4) v South Asian 8.4 (1.9) v White 8.6 (1.7)%, P-value for ANCOVA=0.007) but South Asians had lower HDL-cholesterol (1.4 (0.4) v White 2.0 (1.2) v Black 1.6 (0.4)mmol/L, P-value=0.03) and higher triglyceride levels (1.8 (1.1) v 0.9 (0.4) v 1.0 (0.5)mmol/L, P-value=0.001). In linear mixed models, after adjustment for socio-economic deprivation and other predictors; (a) Black ethnicity associated with poorer glycaemic control (P<0.001) and (b) South Asian ethnicity associated with higher triglyceride levels (P<0.001), independent of HbA1c.
Conclusions: The effect of insulin intensification on glycaemic control and markers of future cardiovascular disease risk in CYP with T1D differs in relation to ethnic group. Ethnic specific thresholds for intervention should be considered during childhood.

P1-d1-200 Diabetes and Insulin 1

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

Wei Wu; Shan Huang; Yan Liang; Xiaoping Luo
Huazhong University of Science and Technology, Pediatrics, Wuhan, China

Background: ER-stress induced apoptosis of beta cells is an important mechanism of type 2 DM. PERK can be activated by the overactivation of ER-stress which will induce beta cells apoptosis.
Objective and hypotheses: To reveal the role of ER-stress, GSK-3 and the potential signal pathway during beta cells apoptosis.
Methods: The alterations of ER-stress related signal factors and kinases induced by OA are assessed by western blot, and the changes of PERK and AMPK are analyzed by ELISA meanwhile. The phosphorylated GSK-3β and total GSK-3 are detected by western blot, while PERK are inhibited by the transfection of P58IPK plasmid and AMPK are inhibited by the inhibitor Compound C. Finally, the interaction between PERK and GSK-3 are identified by co-immunoprecipitation and direct immuno- fluorescence.
Results: 1.Activation of ER-stress related signal factors and kinases in INS-1 cells. The expression of GRP78,ATF6,XBP1,PERK and AMPK significant increased in the presence of 0.4mM OA(P<0.01). 2. ELISA measurment of PERK and AMPK activity. Activity of PERK and AMPK significantly augmented in the presence of OA (P<0.01). 3. Detections of the alterations of GSK-3 after inhibiting the activity of PERK and AMPK. (1) GSK-3β was activated when treated with OA; (2) the phosphorylation of GSK-3β obviously increased after the inhibition of PERK by transfection of P58IPK plasmid(P<0.01); (3) the activity of GSK-3 had no change after the inhibition of AMPK by Compound C.(P>0.05). 4. Identification of interaction between PERK and GSK-3 co-immunoprecipitation. Co-immunoprecipitation of PERK and GSK-3 verified the association of PERK and GSK-3. 5. Co-localization of PERK and GSK-3 in INS-1 cells. Obvious co-localization was observed after merging FITC-conjugated PERK and Rhodamine-conjugated GSK-3β.
Conclusions: Activation of GSK-3 can induce beta cells apoptosis by ER-stress which is caused after long-time exposure to FFAs. PERK will activate GSK-3 directly. Collectively, PERK-GSK-3 signal pathway will play an important role during beta cell apoptosis in type 2 DM.

P1-d1-201 Diabetes and Insulin 1

Silent diabetic cochleopathy in type 1 diabetes mellitus

Nancy Elbarbary[1]; Rasha El-Kabarity[2]
[1]Ain Shams University, Department of Pediatrics, Pediatric Endocrinology and Diabetes Unit, Cairo, Egypt; [2]Ain Shams University, Department of ENT, Audiology Unit, Cairo, Egypt

Objective: To detect early asymptomatic hearing affection whether at the level of outer hair cells (OHC), inner hair cells (IHCs) and olivo-cochlear bundle and the relationship between these abnormalities and other variables such as diabetes duration, metabolic control, or presence of microvascular complications.
Methods: Seventy five adolescents with T1DM and 33 healthy controls participated in the study. Duration of DM, HbA1c levels, microvascular complications were analyzed All underwent basic audiological assessment to ensure normal middle ear function and hearing. Other tests comprised: transiently evoked otoacoustic emissions (TEOAEs) testing OHCs, TEOAEs with contralateral suppression testing the integrity of olivo-cochlear bundle and threshold equalizing noise (TEN) testing IHCs as evidenced by dead regions within the cochlea.
Results: Early asymptomatic OHCs affection as reflected by partial pass was detected in 33.75% of cases with diminished suppression compared to 9.1% in control . Eleven patients showed positive TEN Test reflecting resistance of IHCs to hyperglycemic injury . Patients had higher amplitude of TEOAE noise suppression when compared to controls (p=0.002). Mean difference in amplitude of TEOAE before and after suppression was significantly higher in diabetics with microvascular complications when compared to diabetics without complications at all frequencies (p<0.001 for all). Duration of diabetes and microvascular complications (nephropathy, peripheral and autonomic neuropathy were not associated with the TEOAE suppression except for retinopathy(P=0.02). In contrast, poor metabolic control was associated with TEOAE noise suppression(r=-0.443 P=0.001).
Conclusions: Cochleopathy can be detected in a relatively high proportion of subjects with Type 1 diabetes in spite of a normal audiometric hearing threshold. It should be considered as early central manifestations of diabetic neuropathy which is related to the degree of metabolic control and retinopathy independent of other microvascular complications.

P1-d1-202 Diabetes and Insulin 1

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

Romy Kursawe[1]; Deepak Narayan[2]; Ebe D'Adamo[1]; Cosimo Giannini[1]; Melissa Shaw[1]; Bridget Pierpont[1]; Samuel W. Cushman[3]; Gerald I. Shulman[4]; Sonia Caprio[1]
[1]Yale University, Pediatrics, New Haven, CT, United States; [2]Yale University, Plastic Surgery, New Haven, CT, United States; [3]NIDDK/NIH, Diabetes Branch, Bethesda, MD, United States; [4]Yale University, Internal Medicine, Cellular & Molecular Physiology, Howard Hughes Medical Institute, New Haven, CT, United States

Background: Insulin resistance associated with altered fat partitioning in liver and adipose tissues is a prediabetic condition in obese adolescents.
Objective and hypotheses: To examine the molecular mechanisms that link altered fat partitioning to insulin resistance, we measured the expression of key lipogenic genes in the abdominal subcutaneous adipose and liver tissues across the spectrum of glucose tolerance in equally obese adolescents. Imaging and clamp techniques were performed to best characterize their metabolic profiles.
Methods: Fifty-three obese adolescents underwent a subcutaneous periumbilical adipose tissue biopsy, OGTT, euglycemic-hyperinsulinemic clamp, MRI and DEXA scans. According to their 2h glucose, they were divided into three groups: #1 <120 mg/dl (n=27), #2 120-140 mg/dl (n=16), #3 >140 mg/dl (IGT/T2D; n=10). Liver biopsy was done in 8 subjects with persistent elevation in ALT.
Results: Insulin resistance, hepatic steatosis, abdominal VAT/SAT ratio, and subcutaneous adipocyte diameter significantly increased with increasing 2h glucose, while the fraction of large cells decreased. The expression of ChREBP, SREBP1c, FASN, ACC, LPL and GLUT4 was significantly lower in the subcutaneous adipose tissue of IGT/T2D vs NGT. In contrast, liver expression of ChREBP, SREBP1c, FASN and ACC was significantly higher in IGT/T2D. Adipose ChREBP expression and fraclarge had a significant negative correlation with 2h glucose. Repeated subcutaneous adipose tissue biopsy in 4 IGT patients after converting to NGT showed an increase in the expression of ChREBP, SREBP1c, FASN, ACC, LPL and GLUT4, as well as in the fraclarge. In vitro analysis of de novo lipogenesis in adipose tissue showed a reduced stimulation of lipogenesis in IGT/T2D.
Conclusions: Our data indicate that de novo lipogenesis is oppositely regulated in adipose tissue and liver from obese adolescents with early T2D. The resulting decreased ability to store fat in subcutaneous adipose tissue is likely to be an important contributor to the development of liver steatosis and insulin resistance.

P1-d1-203 Diabetes and Insulin 1

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

Carla Bizzarri[1]; Danila Benevento[1]; Ippolita Patrizia Patera[1]; Lucilla Ravà[2]; Riccardo Schiaffini[1]; Paolo Ciampalini[1]; Stefano Cianfarani[3]; Marco Cappa[1]

[1]Bambino Gesù Children's Hospital, Unit of Endocrinology and Diabetes, Rome, Italy; [2]Bambino Gesù Children's Hospital, Epidemiology Unit, Rome, Italy; [3]Tor Vergata University, Bambino Gesù Children's Hospital, Molecular Endocrinology Unit, Rome, Italy

Background: High birth weight has been related to an increased risk of type 1 diabetes (T1D), while low birth weight has been related to insulin resistance, metabolic syndrome and type 2 diabetes (T2D). Insulin resistance, related to poor metabolic control, has been described also in T1D patients.
Objective and hypotheses: Aims of the study were: 1) to analyze the distribution of birth size for gestational age in a large group of T1D patients 2) to investigate the impact of birth weight on clinical phenotype and metabolic control.
Methods: The clinical records of 602 Caucasian T1D patients were evaluated. Small for gestational age (SGA) and large of gestational age (LGA) were defined as birth weight <3rd percentile or >97th percentile for gestational age, respectively. Birth weights between 3rd and 97th were defined as appropriate for gestational age (AGA). The clinical characteristics of SGA, AGA and LGA were compared. Multivariable linear regression models were fitted in order to evaluate the independent effect of birth weight and the other covariates (age at T1D onset, gender, T1D duration) on different clinical outcomes (BMI, HbA1c%, daily insulin requirement, HDL cholesterol, triglycerides).
Results: The proportion of SGA subjects was slightly decreased in comparison with the percentage theoretically expected in the general population (13 subjects - 2.15% versus 3%), while the percentage of LGA subjects was slightly increased (39 subjects - 6.47% versus 3%). Daily insulin requirement (U/kg/day) was significantly higher in SGA (SGA: 1.0±0.2, AGA: 0.8±0.2, LGA: 0.7±0.2 - p: 0.0009). In contrast, LGA showed higher BMI (SGA: 22.3±5.1, AGA: 22.2±3.7, LGA: 24.7±6.3 – p: 0.024) and increased HbA1c% levels (SGA: 7.6±1.5, AGA: 7.7±1.1, LGA: 8.4±1.8). Multivariable linear regression showed a significant negative impact of birth weight on daily insulin requirement ($p < 0.0001$).
Conclusions: Suboptimal birth weight (both high and low) in T1D patients seems to be associated with clinical characteristics suggestive of insulin resistance.

P1-d1-204 Diabetes and Insulin 1

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

Rosa Nugnes[1]; Enza Mozzillo[2]; Adriana Franzese[3]; Carla Cerrato[3]; Dario Bruzzese[4]; Marianna Santopaolo[1]; Mario Galgani[5]; Giuseppe Matarese[5]

[1]"Federico II" University, Cellular and Molecular Pathology "L. Califano", Naples, Italy; [2]"Partenope" University, School of Movement Science (DiSiST), Naples, Italy; [3]"Federico II" University, Pediatrics, Naples, Italy; [4]"Federico II" University, Preventive Medical Sciences, Naples, Italy; [5]Consiglio Nazionale Ricerche (CNR), Istituto di Endocrinologia ed Oncologia Sperimentale (IEOS), Naples, Italy

Background: Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells. T1D development involves a complex interaction between pancreatic β-cells and both innate and adaptive immunity. In literature, there is a surprisingly lack of markers able to predict residual β-cell function and pancreas failure severity.
Objective and hypotheses: To elaborate a simple tool, based on immune/metabolic parameters measured at disease onset, able to predict residual pancreatic function over time.
Methods: We studied 114 T1D patients (66 M, 48 F; age 2-17.5), at onset and after 12 months. We performed an immunophenotypic analysis of peripheral blood cells, including Myeloid (mDCs) and Plasmacytoid Dendritic Cells (pDCs), and we measured serum levels of several immune/metabolic/inflammatory molecules, including Leptin (Lep) and soluble Leptin Receptor (sLepR). Patients were dichotomized in 2 groups, severe or mild, according to C-Peptide (C-Pep) levels (≤ or > 0.5 ng/ml, respectively). A multivariate logistic regression analysis was performed to identify, among the parameters measured at onset, those able to discriminate patients with good or worse pancreatic function twelve months later.
Results: We identified two immune cell subsets, not previously associated to pancreatic function, whose number and percentage, measured at T1D onset, emerged as independent predictors of C-Pep secretion after 12 months. We defined a predictive model, based on the counting at disease onset of these two immune cell populations, able to predict pancreatic residual C-pep secretion, a surrogates measure of β-cell mass, one year after disease onset.
Conclusions: This study provides a simple decision rule that predicts residual β-cell function since disease onset. Our approach could be a valuable tool to evaluate disease severity and delineate the basis for selecting potential candidates for innovative immune-based therapeutic approaches able to prevent complete loss of β-cell mass.

P1-d1-205 Diabetes and Insulin 1

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

Axel Dost[1]; Esther Molz[2]; Andreas Krebs[3]; Susanne Bechthold-Dalla Pozza[4]; Thomas M. Kapellen[5]; Tilman Rohrer[6]; Klemens Raile[7]; Maria Fritsch[8]; Karl O. Schwab[3]; Reinhard W. Holl[2]

[1]University Hospital Jena, Dept. of Pediatrics, Jena, Germany; [2]University of Ulm, Institute of Epidemiology, Ulm, Germany; [3]University Hospital Freiburg, Dept. of Pediatrics, Freiburg, Germany; [4]University Hospital Munich, Dept. of Pediatrics, Munich, Germany; [5]University Hospital Leipzig, Dept. of Pediatrics, Leipzig, Germany; [6]University Hospital Homburg, Dept. of Pediatrics, Homburg/Saar, Germany; [7]University Hospital, Charité-Universitätsmedizin Berlin, Dept. of Pediatrics, Berlin, Germany; [8]University Hospital Vienna, Dept. of Pediatrics, Vienna, Austria

Background: Impaired blood pressure regulation contributes to the development of diabetic cardiovascular complications. The influence of systolic (SBP) and diastolic blood pressure (DBP) is still controversial. Peripheral pulse pressure (PP), the difference between SBP and DBP, is an indicator for arterial stiffness. T1DM causes increased arterial stiffening and advanced vascular aging in adult patients. However, little data are available for PP in children. Therefore, we studied PP regulation in type 1 diabetic children and adolescents.
Methods: Blood pressure measurements of 47153 patients with T1DM <20 years are documented in the DPV database. The average blood pressure of the most recent year was calculated and patients with antihypertensive medication were excluded. Blood pressure values of the diabetic patients were compared with the control populations of the "4th report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents (4th report)" and the German KIGGStudy.
Results: Pulse pressure levels are significantly elevated in diabetic children (PP T1DM 49.13±11.1 vs. 4th report 45.38±3 and KIGGS 44.58±4.6 mmHg (all $p<0.0001$, Wilcoxon test). PP is increased in 63% (4th Report) or 67% (KIGGS) of the patients, respectively. Absolute PP is elevated independently of the control population and increases with age in both sexes. The rate of increased PP remains stable between 59 and 68%, irrespective of sex, age and the control population. Age, male sex, diabetes duration, insulin dose, BMI, and height are independent factors contributing to elevated PP levels and to a higher rate of increased PP. HbA1c is only related to increased PP levels (multiple linear regression).
Conclusions: Increased PP in type 1 diabetes is a marker for accelerated arterial stiffness and aging and should be considered as an additional risk factor in the treatment of diabetic children. The elevated PP values in children and adolescents with type 1 diabetes may contribute to their markedly high risk for early development of atherosclerosis.

P1-d1-206 Diabetes and Insulin 1

Use of meglitinides (glinides) in adolescent patients with HNF1A-MODY (MODY3)

Marianne Becker; Angela Galler; Klemens Raile
Charite University Children's Hospital, Department of Paediatric Endocrinology, Berlin, Germany

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

	Case 1	Case 2	Case 3
Mutation Nucleotide (protein)	c.872dupC (p.Gly292ArgfsX25)	c.162T>C (p.Leu54Pro)	c.1192C>T (p.Gln398Stop)
Age at diabetes onset [years]	12	14	11
Age at HNF1A diagnosis [years]	12	15	13
Gender	Female	Male	Female
HbA1c at diabetes onset	7.4%	7.0%	10.1%
Mean HbA1c with insulin therapy (range)†	-	8.5% (5.2-8.6%)	8.9% (7.8% - 9.8%)
Mean HbA1c with meglitinides (range)†	5.5% (5.2 – 6.0%)	6.2% (6.0-6.4%)	8.3% (7.7% - 9.2%)
Hypoglycaemic episodes (blood glucose < 60 mg/dl) with su	-	> 5/weeks, predominantly at night	-
Hypoglycaemic episodes with meglitinides	None	None	0-2/month (no severe)
BMI (SDS) before use of meglitinides	23.7 kg/m2 (+ 1.5)	19.2 kg/m2 (- 0.8)	28.8 kg/m2 (+ 2.2)
BMI (SDS) with meglitinides	23.7 kg/m2 (+ 1.4)	19.6 kg/m2 (- 0.8)	28.4 kg/m2 (+ 2.0)
Medication	Repaglinide 3 x 0.5 mg	Nateglinide 4 x 180 mg	Repaglinide 1.5 – 0.5 – 2 mg, NPH insulin 10 – 0 – 11 U

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

Case 2 was initially transferred on glibenclamide after molecular HFN1A diagnosis. After experiencing several nocturnal hypoglycaemic episodes treatment was changed to nateglinide. With nateglinide he did not have any hypoglycaemic episode.
Case 3 is an obese patient and we could not achieve a satisfying glucose control with repaglinide alone and so added NPH insulin (0.27 U/kg). Her compliance is fluctuant and attaining a good glucose control is difficult.
Conclusions: Meglitinides are effective in the treatment of adolescent patients with HNF1A-MODY. Because of the extremely low rate of hypoglycaemic episodes we recommend meglitinides as oral treatment of choice in all cases or at least in those with recurrent hypoglycaemic episodes under sulphonylureas.

P1-d1-207 Diabetes and Insulin 1

Two novel GATA6 mutations cause early-onset diabetes mellitus, pancreatic hypoplasia, and associated heart, liver, and CNS

Maolian Gong[1]; Deimante Simaite[1]; Peter Kühnen[2]; Michael Heldmann[3]; Francesca Spagnoli[4]; Oliver Blankenstein[5]; Norbert Hübner[6]; Khalid Hussain[7]; Klemens Raile[1]
[1]Charité Medical Faculty, Experimental and Clinical Research Center, Berlin, Germany; [2]Charité Medical Faculty, Institute for Experimental Paediatric Endocrinology, Berlin, Germany; [3]Helios Clinic, Clinic for Paediatrics, Wuppertal, Germany; [4]Max-Delbrück-Center for Molecular Medicine, stem cell, Berlin, Germany; [5]Charité Medical Faculty, Developmental Endocrinology Research Group, CMG Unit, Berlin, Germany; [6]Max-Delbrück-Center for Molecular Medicine, Cardiovascular genetics, Berlin, Germany; [7]Great Ormond Street Hospital, Developmental Endocrinology Research Group, CMG Unit, London, United Kingdom

Objective: GATA6 mutations cause pancreatic agenesis and diabetes in human sporadic cases. We report two novel GATA6 mutations in a cohort of eight children with pancreas dysplasia and diabetes.
Methodology: We investigated GATA6 in eight children with inborn pancreas dysplasia and diabetes in whom other known candidate genes for monogenic diabetes and pancreas dysplasia had been excluded.
Results: We found two novel heterozygous GATA6 mutations (c.951_954dup and c.754_904del) in two patients with sporadic pancreas dysplasia, diabetes, and severe cardiac defects (common arterial truncus and tetralogy of Fallot), but not in the remaining six patients. GATA6 mutations in carriers exhibited dysplastic pancreas with absent head in one patient and a hypoplastic pancreas in another patient.
Conclusion: Our findings add two novel cases with GATA6 mutations, who suffer not only from pancreatic dysplasia but also from progressive, postnatal endocrine and exocrine pancreatic disease. Our findings contribute to the understanding of GATA6 function in human pancreatic and islet-cell (patho) physiological function. They could help promote regenerative treatment strategies for diabetes and expand the phenotype of patients reported with GATA6 mutations.

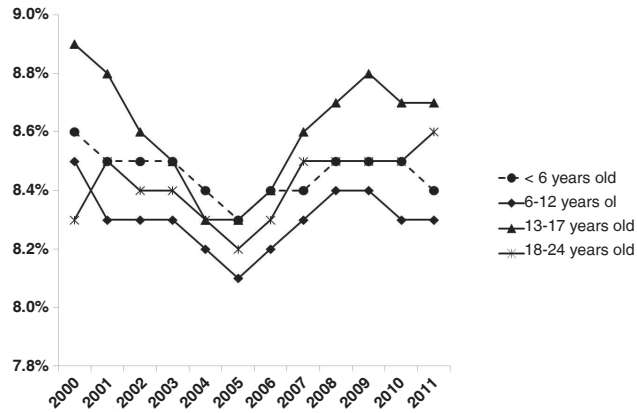
P1-d1-208 Diabetes and Insulin 1

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

Georgeanna Klingensmith[1]; Catherine Pihoker[2]; Stephanie DuBose[3]
[1]University of Colorado School of Medicine, Barbara Davis Center for Childhood Diabetes, Aurora, CO, United States; [2]University of Washington, Department of Pediatrics, Seattle, Washington, United States; [3]Jaeb Center for Health Research, Epidemiology Department, Tampa, FL, United States

Background: The Diabetes Control and Complications Trial demonstrated that lower Hemoglobin A1c (HbA1c) was associated with lower risk of long-term complications. This resulted in recommendations for intensive therapy for patients of all ages with type 1 diabetes (T1D).
Objective and hypothesis: To assess glycemic control over time in a cohort of children and young adults with T1D. The authors hypothesized that with newer insulins and greater focus on intensified management, HbA1c would have declined steadily over the past decade.
Population and methods: The T1D Exchange Clinic Registry is a cohort of >20,000 individuals with T1D (mean age = 22.7 years) from 67 centers throughout the U.S. This analysis included 13,660 participants <25 years of age, with ≥ 1 year T1D duration. The cohort is 80% non-Hispanic white, 5% non-Hispanic black, 10% Hispanic, and 5% other. Data (including demographics, insulin regimens, and frequency of glucose testing) was collected at enrollment from medical records and participant surveys. HbA1c values were entered, for up to the past ten years. Mean HbA1c for each year was compared by age group, and those on CSII were compared to other regimens.
Results: There was a statistically significant decline in HbA1c from 2000 to 2005 for all age groups (p values range from <0.001 to 0.02) except 18-24 years (p=0.55). There was a significant increase from 2005 to 2011 in all age groups (p<0.001) except <6 y/o (p=0.74) (Fig). CSII therapy use has

increased over time, from 2.5% in 2000 to 15% in 2005 to 51% use in 2011. In all age groups, those using CSII at the time of the HbA1c test had lower HbA1c than those not using CSII.
Conclusions: The expected decline in HbA1c seen from 2000-05 did not persist. The reason for HbA1c rise after 2005 cannot be explained by less intensive therapy.
Figure. Changes in A1c Over Time.



*Age groups indicate age at time of HbA1c test

P1-d1-209 Diabetes and Insulin 1

Prestarium pharmacogenetic efficacy in predicting diabetic nephropathy in children and adolescents

Aqida Sadykova; Gulnara Rakhimova
Center for the Scientific and Clinical Study of Endocrinology, Children endocrinology, Tashkent, Uzbekistan

Aim: The work was initiated to study efficacy of prestarium in normoalbuminuric patients with type 1 diabetes mellitus by ACE genotype upon primary diabetic nephropathy (DN) prevention.
Materials and methods: We examined normoalbuminuric 22 patients with type 1 diabetes mellitus aged from 12 to 17 years with DN duration ≥ 10 years divided into two groups by ACE polymorphism, that is, the one with II genotype (n=11) and the one with DD genotype (n=11). Activity of urinary neutral α-glucosidase was measured by rate of glucose production from maltose. PCR was performed by means of GenePackTM PCR Core reagent kit. All patients were prescribed with prestarium in the dose of 2.5 mg/day for 6 months.
Results and discussion: Systolic arterial pressure and diastolic arterial pressure were found reduced by 4.2% and 3.6%, respectively, heart rate decreasing by 6.4%. Pre- and post-therapy blood fibrinolytic activity was 15.3 ± 0.8% and 14.9 ± 0.6%, respectively, fibrinogen concentrations being respectively, 3.02 ±0.2 g/l and 3.08 ± 0.2 g/l. Total cholesterol was found decreased by 4.4%, HbA1cbeing reduced by 16.7%. There was 7.1% and 6.8% reduction in proteinuria in the 1st and 2nd groups of patients, respectively. Glomerular filtration rate and creatinine were found decreased by 9.2% and 4.6% in the 1st group, reducing by 2.2% and 1.2% in the 2nd one. Urinary neutral α-glucosidase in patients with II genotype was registered reduced by 63.6%, confident reduction by 10.5% being found in patients with DD genotype to be 8.5 times higher (P4=0.0001) than the parameter in the 1st group.
Conclusions: In normoalbuminuric diabetic patients with II genotype prestarium facilitated moderate reduction in systolic and diastolic arterial pressure as well as in heart rate. In these patients total cholesterol reduced by 4.4% versus 0.8% in patients with DD genotype. There was 16.7% and 4.7% reduction in HbA1c level, respectively.

P1-d1-210 Diabetes and Insulin 1

Possible influence of bone and adipose tissue on glucose metabolism in children and adolescents with type 1 diabetes mellitus

Anna Wedrychowicz; Jerzy Starzyk
Jagiellonian University in Cracow, Medical College, Polish-American Pediatric Institute, Department of Pediatric and Adolescent Endocrinology, Cracow, Poland

Background: Recent studies have shown a new link between skeleton, fat tissue, and insulin action. However, clinical data are still limited, especially in children.
Objective and hypotheses: The aim of the presented study was to investigate the relationship between bone-derived osteocalcin (OC), osteoprotegerin (OPG), and Receptor Activator of Nuclear Factor NF-κB ligand (RANKL), and fat tissue-derived leptin and adiponectin, with results of treatment of type 1 diabetes mellitus (T1DM) in children and adolescent.
Methods: Seventy eight patients, 43 girls and 35 boys, mean 11.5±4.3 year old with T1DM were included into the study. Blood samples were drawn at 8.00 a.m., after 8 hours fast. All above-mentioned parameters were measured by ELISA. HbA1c was measured by standardized ISCC method. Patients were divided into three groups according to HbA1c level, I - below 7%, II - between 7-9% and III - above 9%. In statistical analysis ANOVA, and multiple regression analysis were used.
Results: The mean data ± SDS are presented in the Table 1.

Group	N	OC [ng/ml]	Leptin [ng/ml]	Adiponectin [g/ml]	OPG [pmol/l]	RANKL [pmol/l]	OPG/ RANKL	HbA1c [%]	Age [year]	BMI [kg/m2]
I	24	37.5±17.9	2.1±1.9	11.1±3.5	1.7±1.4	0.2±0.2	53±79	6.4±0.4	10.5±4.2	17.6±2.9
II	28	33.0±18.3	1.8±1.7	12.3±4.9	1.8±1.3	0.2±0.1	68±99	7.8±0.6	12.7±4.6	18.5±3.1
III	26	28.8±12.9	1.0±0.6	13.8±6.4	2.3±1.5	0.2±0.2	111±133	11.2±1.8	11.2±3.9	16.6±2.9
p	NS	0.18	0.04	0.45	0.31	0.60	0.14	0.000001	0.19	0.08

Multiple regression analysis adjusted for age showed that serum OC and leptin negatively correlated with HbA1c (r= -0.22, p=0.004 and r= -0.27, p=0.0001 respectively). In opposite, serum OPG correlated positively with HbA1c (r=0.26, p=0.02) as well as with adiponectin (r=0.26, p=0.02) and RANKL (r=0.27, p=0.02). Moreover leptin correlated positively (r=0.47, p=0.002), and adiponectin (r=-0.29, p=0.0001) and RANKL (r=-0.24, p=0.001) negatively with BMI.
Conclusions: Our data suggest significant relationships between bone, fat tissue and glucose metabolism in pediatric patients with T1DM. They could give a background to use leptin as an additional therapeutic agent in T1DM.

P1-d1-211 Diabetes and Insulin 1

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

Maria Louraki[1]; Charalambos Tsentidis[1]; Marina Katsaloul[2]; Christina Kanaka-Gantenbein[3]; Nikolitsa Kafassi[4]; Asteroula Papathanasiou[5]; Kyriaki Karavanaki[1]
[1]University of Athens, "P&A Kyriakou" Children's Hospital, Second Pediatric Department, Diabetic Clinic, Athens, Greece; [2]"Aghia Sophia" Children's Hospital, Neurologic Clinic, Athens, Greece; [3]University of Athens, "Aghia Sophia" Children's Hospital, First Pediatric Department, Diabetes Center, Division of Endocrinology, Diabetes and Metabolism, Athens, Greece; [4]'Laiko' General Hospital, Department of Immunology, Athens, Greece; [5]"P&A Kyriakou" Children's Hospital, Department of Pediatric Endocrinology, Athens, Greece

Background: VST has been proven to accurately identify patients at risk to develop peripheral diabetic neuropathy (DN), including those with early neuropathic deficits. However in T1DM children and adolescents, there are no previous studies on the reproducibility of VST and associated factors.
Objective and hypotheses: To study in T1DM children and adolescents the reproducibility of VST and the factors affecting it.
Methods: 118 T1DM children and adolescents (mean±SD age:13.5±3.4 years, diabetes duration: 5.7±3.5yrs, HbA1c:7.9±1.4%) and 79 normal controls (aged 12.0±3.07 yrs), were evaluated by a single examiner. VST was measured twice on upper and lower limbs, using a biothesiometer. Concor-

dance between the two VST measurements was estimated using the Cohen's Weighted Kappa statistic (Kappa: 0.41-0.60:moderate concordance, Kappa=0.61-0.80:good concordance).
Results: Overall, there was a good concordance of VST values (Kappa:0.66-0.72), with tibia and thumb exhibiting higher reproducibility. T1DM children had significantly higher VSTs than controls in all sites (p=0.001), but with lower Kappa values (0.64-0.70). When dividing the diabetic population into 3 groups according to HbA1c (a.<8%, b.8-9.5%, c.>9.5%), or T1DM duration (a.<5 years, b.5.1-10, c.>10 years), VST values increased in parallel with HbA1c (left toe VST: 2.75 vs 3.09 vs 3.23 volts, p=0.12) and with increased T1DM duration (VST:3.10 vs 3.57 vs 3.75 volts, p=0.04). However, Kappa values were lower in the groups with poor control (HbA1c>9.5%) (Kappa=0.54-0.76) or the longest T1DM duration>10 years (Kappa:0.49-0.71). No association was observed between VST values, pubertal status, age and sex.
Conclusions: T1DM children and adolescents, although asymptomatic, had higher VSTs than controls. VSTs increased in parallel with HbA1c and T1DM duration. However in the groups with poor control or long duration, VST reproducibility was low. These findings suggest that the reproducibility of VSTs is low in the high-risk group for early subclinical DN development that needs a regular follow-up.

P1-d1-212 Diabetes and Insulin 1

Wolfram syndrome: new mutations, different phenotype

Aloi Concetta[1]; Salina Alessandro[1]; Pasquali Lorenzo[1]; Lugani Francesca[1]; Tallone Ramona[1]; Marchi Marta[1]; Ghiggeri Gian Marco[2]; Lorini Renata[1]; d'Annunzio Giuseppe[1]
[1]IRCCS G. Gaslini Institute, Pediatric Clinic, University of Genoa, Genoa, Italy; [2]IRCCS G. Gaslini Institute, Laboratory on Pathophysiology of Uremia and Department of Nephrology, Genoa, Italy

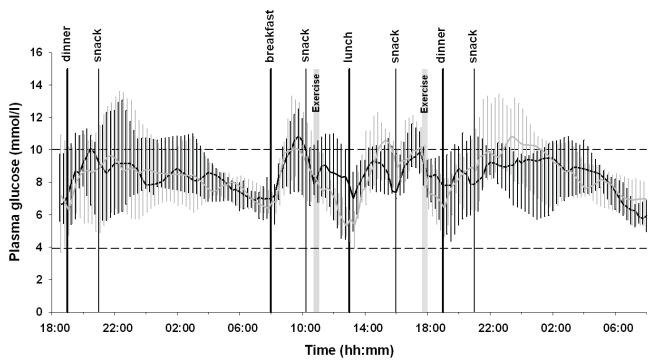
Background: Wolfram Syndrome (WS) is an autosomal recessive neurodegenerative disorder characterized by non autoimmune diabetes mellitus, optic atrophy, diabetes insipidus and deafness. The WS gene, WFS1, encodes a transmembrane protein called Wolframin which may serve as a novel endoplasmic reticulum calcium channel in pancreatic β-cells and neurons. WS is a rare disease, with an prevalence of 1/550.000 children, with a carrier frequency of 1/354.
Objective and hypotheses: The aim was to determine the genotype of WS patients in order to establish a genotype/phenotype correlation.
Methods: We clinically evaluated 12 children from 12 unrelated families (6 males, 6 females). Basic criteria for WS clinical diagnosis were coexistence of insulin-treated diabetes mellitus and optic atrophy occurring before 15 years of age. Genetic analysis for WFS1 was performed by direct sequencing.
Results: 15 heterozygous compound and 4 homozygous mutations were revealed. All of them were located in exon 8, except two in exon 4 and 5 respectively. Three new variants (c.2663 C>A; p.S888X, c.1381A>C; p.T461P e c.605A>G; pE202G) were found. The male patient carrying the compound mutation [c.1060_1062delTTC]+[c.2663 C>A] died at the age of 13 years (dead in bed). While two other patients carrying the 605A>G in homozygous state and the compound mutation [c.409_424dup16]+[c.1381 A>C] respectively showed a less severe phenotype (diabetes mellitus and optic atrophy).
Conclusions: Our study increases the spectrum of WFS1 mutations with three novel variants. The age at onset of diabetes mellitus was chosen as an indicator of disease severity. Missense mutations and 3 bp deletions, resulting in a deletion of one amino acid, were considered non-inactivating mutations. Nonsense, frameshift mutations, deletions and insertions of more than 3 bp were considered inactivating mutations. In our study we observed that patients with non-inactivating mutations showed less early age of onset of diabetes mellitus than patients with inactivating mutations.

P1-d1-213 Diabetes and Insulin 1

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

Daniela Elleri[1]; Janet M Allen[1]; Martina Biagioni[1]; Kavita Kumareswaran[1]; Lalantha Leelarathna[1]; Karen Caldwell[1]; Marianna Nodale[1]; Malgorzata E Wilinska[1]; Peter Calhoun[2]; Craig Kollman[2]; Carlo L Acerini[1]; David B Dunger[1]; Roman Hovorka[1]
[1]University of Cambridge, Institute of Metabolic Science and Department of Paediatrics, Cambridge, United Kingdom; [2]The Jaeb Center for Health Research, JCHR, Tampa, FL, United States

Background: Hybrid closed-loop insulin delivery (CL), coupling automated glucose-responsive between-meal insulin delivery with manual pre-meal boluses, may lead to post-prandial hypoglycaemia when meal boluses are overestimated.
Objective and hypotheses: We evaluated CL with reduced meal insulin boluses in comparison with CL with standard meal insulin boluses in adolescents with type 1 diabetes (T1D).
Methods: Eight adolescents with T1D [M 3; age 15.9±1.5yrs; A1C 8.9±1.6%; mean±SD; total daily dose 0.9(0.7, 1.1)U/kg/d; median (IQR)] studied at a research centre for 36h on two occasions in a cross-over design. Subjects were randomised to CL with either standard insulin boluses calculated using subjects' pump bolus calculator or CL with boluses reduced by 25%. Boluses were given before main meals (50-80gCHO) but not with snacks (15-30gCHO). On both occasions, between-meal insulin pump delivery was manually adjusted every 15min as per advice of a model-predictive-control algorithm informed by a real-time continuous glucose monitor. Subjects undertook moderate-intensity exercise on a stationary bicycle at 140bpm heart-rate for 20min (morning and afternoon).
Results: Overall insulin delivery was lower with reduced prandial insulin boluses [61.9 (55.2, 75.0) vs 72.5 (63.6, 80.3) U/36h, p=0.01] and was confirmed by lower plasma insulin concentration [186 (171, 260) vs 252 (198, 336) pmol/l, p=0.002]. Plasma glucose was identical 8.4±0.9mmol/l on the two occasions (p = 0.97). Time spent in target glucose 3.9-10mmol/l was also comparable [74(66, 84)% vs 80(65, 96)%; p=0.87]. Time above 10mmol/l was 21.8(16.3, 33.5)% vs 18.0(4.1, 34.2)% (p=0.87) and time below 3.9mmol/l was 0(0, 1.5)% vs 0(0, 1.8)% (p=0.88). Hypoglycaemia occurred once within 1.5h post-meal during CL with standard bolus.
Conclusions: In conclusion, closed-loop insulin delivery combined with 25% reduction of meal boluses may be beneficial as it decreases overall insulin exposure while maintaining comparable glucose control in adolescents with T1D.



Plasma glucose [median (IQR)] during closed-loop with standard meal boluses (black line) and reduced meal boluses (grey line).

P1-d1-214 Diabetes and Insulin 1

Assessment of the relative contribution of insulin resistance (IR) and beta cell (β-cell) dysfunction in the aetiology of impaired glucose tolerance (IGT) in young adult survivors of childhood leukaemia treated with bone marrow transplantation and total body irradiation (BMT/TBI)

Christina Wei[1]; Linda Hunt[2]; Rachel Cox[3]; Karin Bradley[4]; Ruth Elson[3]; Michael Stevens[5]; Elizabeth Crowne[1]

[1]Bristol Royal Hospital for Children, Paediatric Endocrinology & Diabetes, Bristol, United Kingdom; [2]University of Bristol, Paediatric Endocrinology & Diabetes, Bristol, United Kingdom; [3]Bristol Royal Hospital for Children, Paediatric Oncology, Bristol, United Kingdom; [4]Bristol Royal Infirmary, Endocrinology and Diabetes, Bristol, United Kingdom; [5]Institute of Child Life and Health, University of Bristol, Paediatric Oncology, Bristol, United Kingdom

Background: IGT and Diabetes Mellitus(DM) are increasingly recognised in adult childhood leukaemia survivors treated with BMT/TBI. The mechanism is unclear.
Objective: To investigate IR and β-cell function in BMT/TBI survivors.
Method: Leukaemia survivors treated with(group1) and without(group2) BMT/TBI were compared with obese subjects(group3). IR was represented by composite-insulin-sensitivity index(ISIcomp) derived from Oral glucose tolerance tests(OGTT), and β-cell function by acute-insulin-response after arginine(AIRarg) and glucose(AIRg) stimulation from Arginine intravenous glucose tolerance tests(aIVGTT). Body composition was assessed by Dual-emission X-ray absorptiometry(DEXA). Comparison was made by ANOVA with post hoc Scheffe at 5% significance.
Results: Groups 1(n=21),2(n=31) and 3(n=30) were aged 21.1(16.1-26.2), 21.5(16.1-26.0) and 17.8(16.1-24.8)years respectively. All BMT/TBI survivors received 10-14.4Gy TBI aged 9.3(1.0-10.8)years. Abnormal OGTTs were reported in groups 1(DM=2, IGT=7) and 3(IGT=1). There were no group differences in β-cell function but groups 1&3 were more IR than group 2. Groups 1&2 have lower fat and lean masses than group 3. Android/Gynoid fat-mass-ratios were higher in groups 1&3 than 2.

	Group					
	Mean(SD) or Geometric Mean(range)*			p-values		
	1	2	3	1vs2	1vs3	2vs3
AIRarg(mU/l)	81.1 (20.9-244)	64.1 (21.8-209.6)	84.3 (28.7-244.7)	0.40	0.97	0.22
AIRIn(mU/l)	68.9 (14.9-287.5)	55.0 (13.0-344.8)	58.0 (12.4-133.6)	0.56	0.71	0.96
ISIcomp	1.5 (0.4-9.7)	4.8 (0.8-9.6)	2.2 (0.8-7.5)	<0.001	0.60	0.001
Fat-Mass-Index (kg/m2)	7.3 (3.9)	8.9 (4.3)	16.4 (3.3)	0.28	<0.001	<0.001
Lean-Mass-Index (kg/m2)	13.0 (2.3)	14.4 (2.3)	16.4 (2.1)	0.12	<0.001	0.002
Android/Gynoid-Fat	1.1 (0.2)	0.9 (0.2)	1.1 (0.1)	0.02	0.78	0.003

*If not normally distributed.

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

P1-d1-215 Diabetes and Insulin 1

Induction of ER stress by variants of the prohormone convertase 1(PCSK1)-gene

Susanne Behrendt; Dennis Loeffler; Roy Tauscher; Wieland Kiess; Antje Koerner

University Leipzig, University hospital for children and adolescents, Leipzig, Germany

Background: Prohormone convertase 1 encoded by the *PCSK1* gene processes multiple prohormones such as proinsulin. Some variants within this gene were associated with polygenic obesity and rare mutations cause childhood obesity and abnormal glucose homeostasis. The *PCSK1* variants could lead to falsely folded proteins, accumulating in the ER and causing the unfolded protein response (UPR), a protective mechanism, alleviating the load of misfolded proteins in the ER. However, a continuous activated UPR leads to apoptosis.
Objective and hypotheses: We hypothesized that variants of *PCSK1* can lead to falsely folded proteins and subsequently harm β-cells by inducing ER-stress and apoptosis. This could link *PCSK1* variants to diabetes, the most common comorbidity of obesity.
Methods: We applied Hek293 cells and the insulinoma cells Ins1E and βTC3 to investigate the influence of the PCSK1 variants S24C, ΔEx8, R80Q, N221D and S307L on maturation and secretion by western blot. Enzyme activity of the newly identified variants S24C and ΔEx8 was determined by an enzyme assay. We evaluated ER stress and UPR activation by western blot and PCR of ER-stress mediators. Finally, we assessed the effect of overexpressed variants on proliferation versus apoptosis by FACS. Further, we explored the influence on insulin expression in the insulinoma cells by western blot.
Results: The S24C, R80Q and N221D variants were expressed and secreted like the wild type; no secretion was detectable for ΔEx8. We found a tendentially reduced maturation and secretion of S307L. The enzyme activity of S24C was not impaired, whereas ΔEx8 is an unfunctional variant. Further effects on UPR, apoptosis and insulin expression are currently investigated.
Conclusions: Our preliminary results indicate that S24C, R80Q and N221D do not disturb the maturation and secretion of PC1; S307L seems to have a slightly decreased secretion. The disturbed expression and secretion of ΔEx8 indicate that this variant possibly induces ER-stress.

P1-d1-216 Diabetes and Insulin 1

Successful hepato-pancreatic transplant in a case of Martínez-Frías syndrome caused by the RFX6 mutation p.R181Q

Ana Coral Barreda-Bonis[1]; Fernando Santos-Simarro[2]; Julio Guerrero-Fernández[1]; Blanca Soler[3]; Ana Gómez-Núñez[3]; Gerardo Prieto-Bozano[4]; Mercedes López-Santamaría[5]; Juan Antonio Tovar-Larrucea[5]; Isabel González-Casado[1]; Angel Campos-Barros[2]

[1]Hospital Universitario La Paz, Pediatric Endocrinology, Madrid, Spain; [2]IdiPAZ, UAM, Hospital Univ. La Paz & CIBERER, U753, ISCIII, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; [3]IdiPAZ, UAM, Hospital Universitario La Paz, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; [4]Hospital Universitario La Paz, Gastroenterology, Madrid, Spain; [5]Hospital Universitario La Paz, Pediatric Surgery, Madrid, Spain

Background: The Martinez-Frias syndrome (MFS) is a rare autosomal recessive syndrome characterized by intrauterine growth retardation, pancreatic hypoplasia associated with neonatal diabetes, duodenal atresia, gallbladder aplasia or hypoplasia, and neonatal hemochromatosis. Mutations in *RFX6*, a transcription factor involved in exocrine pancreas embryogenesis, have been recently described in six MFS patients who presented with a complete deficit of pancreatic endocrine hormones (Smith et al. 2010).
Clinical case: A female newborn, born from consanguineous parents, who presented intrauterine growth retardation (BW: 1.220 g, -3.27 SDS; BL: 41 cm, -2.56 SDS, CP: 31 cm, -0.76S DS), duodenal atresia and malrotation, pancreatic hypoplasia, absence of gallbladder, cholestasis, liver failure, hemochromatosis and hyperglycaemia (>200mg/dl). C-peptide and insulin were undetectable and autoimmunity tests were negative. Insulin treatment was started during the first 12 hours of life.
Methods and results: Mutation screening of *RFX6* identified an homozygous missense mutation, c.541C>T that alters a highly conserved residue, p.R181Q, located in the DNA binding domain. Both parents were non affected hetero-

zygous carriers. The same mutation had been previously described in a male MFS newborn who died at the age of three months (Smith et al 2010).
Follow-up: Over the following 20 months she had poor weight gain, refractory diarrhea, multiple sepsis events, malabsorption and a highly unstable glycaemic control, in spite of parenteral nutrition, pancreatic enzyme supplementation and insulin therapy. Recently, at the age of 20 months, she successfully underwent a hepatopancreatic transplant, which resolved the diabetes (HbA1c: 5.5%).
Conclusions: 1) Mutation screening of *RFX6* should be considered in patients with neonatal diabetes and pancreatic hypoplasia. 2) Although hepatopancreatic transplant initially successfully resolved diabetes, it is too early to know whether it can fully compensate the pancreatic endocrine hormones deficit in the long term.

P1-d1-217 Diabetes and Insulin 1

Variable phenotype in 5 patients with Wolcott-Rallison syndrome due to the same EIF2KA3 (c.1259delA) mutation

Manal Al-Shawi[1]; Angham Al-mutair[2]; Sian Ellard[3]; Abdelhadi M Habeb[4]
[1]Maternity and Children Hospital, Paediatrics, Al-Hassa, Saudi Arabia; [2]King Abdulaziz Medical City, Paediatrics, Riyadh, Saudi Arabia; [3]Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, Molecular genetics, Exeter, United Kingdom; [4]Maternity and Children Hospital, Paediatrics, Al-Madinah, Saudi Arabia

Background: Wolcott Rallison Syndrome (WRS) is a rare condition caused by mutations in EIF2KA3 gene. Patients typically have early onset diabetes, skeletal dysplasia and recurrent hepatic dysfunction. Other features including central hypothyroidism have been reported.
Objective: We aimed to compare the phenotype of two families WRS due to the same EIF2AK3 (c.1259delA) mutation
Patients and methods: Five patients from two unrelated consanguineous families with WRS who had regular assessment since diagnosis were studied. Direct sequencing of EIF2AK3 gene was performed in affected patients and their parents.
Results: All 5 patients presented with permanent neonatal diabetes (PND) and were homozygous for a frame shift mutation deletion (c.1259delA) of the EIF2AK3 gene which has been reported only in these families. Recurrent episodes of hepatic dysfunction encountered in 4 patients and 2 of them died with acute fulminant hepatic failure. Two children developed skeletal dysplasia and another 2 had transient central hypothyroidism, associated with viral illnesses, One family has a discordant phenotype with typical WRS features in the younger child compared to her elder sister with isolated PND. There was no evidence of neutropenia, developmental delay, renal dysfunction or exocrine pancreatic dysfunction with this genotype so far.
Conclusions: EIF2AK3 (c.1259delA) mutation has variable phenotype ranging from typical picture of WRS to isolated PNDM. In WRS thyroid dysfunction is a transient phenomenon reflecting euthyroid sickness status rather than a feature of the syndrome.

P1-d2-218 Diabetes and Insulin 2

Are GAD65 antibodies at delivery in mother and cord a risk or protection for the development of Type I diabetes (TID) in the offspring

Zvi Laron[1]; Christiane Hampe[2]; Shoshana Israel[3]; Fariba Vaziri-Sani[2]; Keren Miller[3]; Boris Kaplan[4]; Yuri Perepliotchikov[5]; Avi Ben-Haroush[6]; Lester Shulman[5]
[1]Schneider Children's Medical Center, Endocrine and Diabetes Research Unit, Petach Tikva, Israel; [2]University of Washington, Immunology, Seattle, United States; [3]Hadassah Medical Center, Tissue Typing Laboratory, Jerusalem, Israel; [4]Women's Hospital Rabin Campus, Women's Health, Petach Tikva, Israel; [5]Sheba Medical Center, Virology, Tel-Hashomer, Israel; [6]Women's Hospital Rabin Medical Center, Women's Health, Petach Tikva, Israel

Background: Studies by our group suggested that TID can start "in utero" during a viral epidemic.
Subjects and methods: Sera titers to beta cell auto-antibodies (GAD65, ZnT8, and IA2) were compared with anti-viral antibodies to Rota (SA11) and Enterovirus (coxB3) in maternal blood from 105 healthy women at birth and cord blood during 2 successive winter viral seasons. Auto-antibodies titers were determined by radio-ligand binding assays, anti-viral antibodies by ELISA, and newborn HLA DRB1 and DQB1 allele frequencies by PCR.
Results: As shown in the Table sera from 10% of chord and 8% of maternal serum samples were positive for GAD65Ab. Of these, 20% and 25% had high Ab titers to rotavirus and 50% and 50% had high Ab titers to enterovirus, respectively. One mother and child had antibodies to ZnT8, while no sample had antibodies to IA2. Class II HLA allele frequencies in individuals with high auto-antibodies and/or antiviral Abs were similar to the frequencies in all enrolled newborns.
Conclusion: The presence of significant GAD65Ab titers in 10 newborns and 8 of their mothers may indicate autoimmune damage to their â cells "in utero" caused by the transmission of a viral infection from the mother. Follow-up will determine whether this early trigger event will progress to development of T1D or that GAD65 transmission from mother to fetus with induce tolerance.

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

	Cord Blood Antibodies*	Cord Blood Antibodies*	Cord Blood Antibodies*	Postpartum Maternal Antibodies*	Postpartum Maternal Antibodies*	Postpartum Maternal Antibodies*
	GAD65	Rotavirus	Enterovirus	GAD65	Rotavirus	Enterovirus
GAD65	10(9.5%)	2/10(20.0%)	5/10(50.0%)	8(7.6%)	2/8(25.0%)	4/8(50.0%)
Rotavirus	2/22(9.0%)	22(20.9%)	11/22(50.0%)	2/12(16.7%)	12(11.4%)	3/12(25.0%)
Enterovirus	5/48(10.4%)	11/48(22.9%)	48(45.7%)	4/34(11.8%)	3/34(8.8%)	34(32.4%)

P1-d2-219 Diabetes and Insulin 2

Prevalence of increased liver enzymes in children and adolescents with type 1 diabetes mellitus in the DPV cohort

Maria Fritsch[1]; Edith Schober[1]; Jürgen Grulich-Henn[2]; Meissner[3]; Thomas Kapellen[4]; Desiree Dunstheimer[5]; Beyer Peter[6]; Vogel Christian[7]; Molz Esther[8]; Reinhard Holl[8]
[1]Medical University of Vienna, Department of Paediatrics and Adolescent Medicine, Vienna, Austria; [2]Heidelberg University Hospital, Center for Child and Adolescent Medicine, Vienna, Austria; [3]University's Children Hospital of Duesseldorf, Department of General Paediatrics and Neonatology, Duesseldorf, Germany; [4]Hospital for Children and Adolescents, Department for Women and Child Health, Leipzig, Germany; [5]University of Augsburg, Clinic for Children and Juveniles, Augsburg, Germany; [6]Evangelic Hospital Oberhausen, Department of Pediatrics and Adolescent Medicine, Oberhausen, Germany; [7]Klinikum Chemnitz, Clinic for Paediatrics and Adolescent Medicine, Chemnitz, Germany; [8]University of Ulm, Institute for Epidemiology and Medical Biometry, Ulm, Germany

Background: A persistent elevation of liver enzymes may be the first indicator of the underlying condition of Non alcoholic fatty liver disease (NAFLD). The prevalence of elevated liver enzymes in adult patients with type 1 diabetes (T1DM) ranges between 4.3 and 12%. Data about the prevalence of NAFLD in childhood T1DM is rare.
Objective and hypotheses: The aim of the study was to determine the prevalence of elevated aminotransferases and investigate possible relations to cardiovascular risk factors in a large cohort of children and adolescents with T1DM.
Methods: Data of 15232 patients (47.2% female) with T1DM from the German-Austrian DPV (Diabetes Patienten Verlaufsbeobachtung) were included into analyses. The observation period contains January 1995 until September 2011. Inclusion criteria were an age < 20 years and at least one measurement of amino transferases (TA) in the most recent year of treatment. Elevated aminotransferases were defined as ALT and/or AST > 50 U/l. Patients with celiac disease were excluded from the analysis.
Results: Median age of the patients was 14.45 (10.8-17.2) years with a median HbA1c 7.94% (7.11-9.09) and median insulin dose of 0.84 (0.66-1.04) IE/kg. 95.39% (n=14530) of the patients had normal, 3.98% (n=606) 1x increased and 0.63% (n =96) ≥ 2x increased aminotransferases. In a linear regression model, increase of aminotransferases was positively related to age (p<0.001), HbA1C (p<0.001), insulin dose (p=0.01), dyslipidemia (p=<0.001) and arterial hypertension (p=0.048) and negatively related to heigth (<0.001). No

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

P1-d2-220 Diabetes and Insulin 2

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

Christos Shammas[1]; Vassos Neocleous[1]; Marie Phelan[2]; Maria Pieri[3]; Nicos Skordis[3]; Leonidas A Phelactou[1]
[1]The Cyprus Institute of Neurology and Genetics, Molecular Genetics Function and Therapy, Nicosia, Cyprus; [2]University of Liverpool,Institute of Integrative Biology, NMR Centre for Structural Biology, Liverpool, United Kingdom; [3]Makarios III Hospital, Pediatric Endocrine Unit, Department of Pediatrics, Nicosia, Cyprus

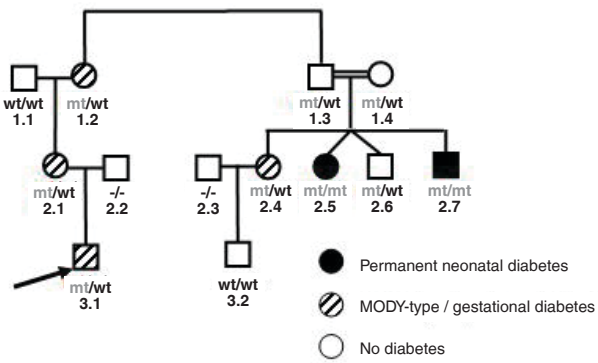
Background: Glucokinase with GLUT2 acts as a glucose sensor and stimulates the release of insulin from pancreatic beta cells. Mutations of the glucokinase gene (GCK) can lead to different forms of diabetes, such as GCK-monogenic diabetes of youth type2 (MODY2), permanent neonatal diabetes and congenital hyperinsulinism.
Objective and hypotheses: The present study was design to identify how glucokinase components that are required for glucose homeostasis are affected in patients with MODY2.
Methods: Two patients who fulfilled the clinical and biochemical criteria for MODY2 were genetically tested by DNA sequencing of the GCK gene. The identified mutations were further studied by in silico analysis using appropriate computational tools.
Results: Full mutation analysis of the patients' GCK gene revealed the two novel mutations: the nonsense p.E440stop and the missense p.R447P. In silico analysis demonstrated that p.R447P causes structural conformational changes and destabilize the functional properties of the protein leading to reduction in glucose and MgATP2- affinity.
The amino acid change at position 447, from arginine to proline, disrupts severely the helical protein structure due to distortion of the phi and psi torsion angles and as a result changes the inter-ionic interactions altering the orientation of helix á13 relative to the proximal helix á5. The novel p.E440stop mutation inactivates the cytoplasmic enzymatic activity of the protein. p.E440stop is responsible for the loss of the C-terminal end of the GCK peptide that includes vital residues essential for the release of á13 helix during glucose binding.
Conclusions: This study presents two novel MODY2/GCK mutations and their conformation changes have been analyzed in silico. These mutations disturb the functional role of glucokinase, by understanding the role of such essential components during glucose homeostasis and the determination of a glucokinase allosteric site can serve as potential drug target sites that aim to treat type2 diabetes.

P1-d2-221 Diabetes and Insulin 2

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

Paulina Aleksander[1]; Peter Kühnen[1]; Oliver Blankenstein[1]; Kathrin Griffig[2]; Klemens Raile[3]
[1]Charite Berlin, Institute of Experimental Paediatric Endocrinology, Berlin, Germany; [2]Charite Berlin, Interdisciplinary Social Paediatric Center, Berlin, Germany; [3]Charité, Experimental and Clinical Research Center, Berlin, Germany

Background: Glucokinase (GCK) is the key enzyme in pancreatic beta cells regulating glucose-dependent insulin secretion. Heterozygous inactivating mutations of the GCK gene result in maturity onset diabetes of the young type 2 (GCK-MODY), characterized by an autosomal dominant mode of inheritance and mild hyperglycaemia. Homozygous inactivating mutations are a rare cause of permanent neonatal diabetes mellitus (GCK-PND) with almost complete inability to secrete insulin from the first hours of life.
Objective and hypotheses: We report on a large family of polish origin with two members of consanguineous parents having PND and 4 other members with MODY type diabetes.
Methods: Samples from overall 11 family members, including all members with diabetes were collected as dried blood spot or EDTA blood. The 8 Exons and neighbouring intronic regions of human GCK were Saenger-sequenced in both directions, primer sequences and PCR conditions are available on request.
Results: Our index case with MODY type diabetes presented with increased fasting glucose (116 mg/dl) and HbA1c of 6.1% (N < 5.8%). Sequencing of GCK revealed a heterozygous D278E mutation, as it was finally also identified in 6 other family members. The 2 cases with PND were found to have the same mutation in the homozygous state. Heterozygous mutation carriers were characterized by MODY type diabetes (n=4) the female patients have been identified for the first time with gestational diabetes (n=3).The remaining 3 patients with the heterozygous mutation had no obvious symptoms of diabetes.
Conclusions: We report two cases with permanent, neonatal diabetes caused by the homozygous D278E mutation and 7 cases with a heterozygous D278E mutation and a mild phenotype with gestational diabetes or GCK-MODY. The homozygous D278E mutation has been reported earlier in one case with GCK-PND. This family demonstrates phenotype variability and needs for a systematic family screening preventing most importantly gestational diabetes in young women.



P1-d2-222 Diabetes and Insulin 2

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

Christian Denzer[1]; Martin Wabitsch[1]; Josef A. Vogt[2]
[1]University Medical Center Ulm, Department of Pediatrics and Adolescent Medicine, Ulm, Germany; [2]University Medical Center Ulm, Department of Anaesthesiology, Ulm, Germany

Background: Although mathematical models of glucose homeostasis like the oral minimal model are valuable research tools for the identification of early alterations in glucose metabolism, these models are only infrequently used due to high computational effort.
Objective and hypotheses: To prove diagnostic feasibility of a novel, readily accessible implementation of the oral minimal model.
Methods: The oral minimal model was paired with a "marginal likelihood" approach, which does not depend on parameters for the uptake except for one parameter ensuring automatic tuning of the uptake curve. The approach allows unsupervised evaluation of parameters of glucose homeostasis. Dynamic insulin sensitivity (S_I) and non-insulin dependent glucose disposal (SG) were calculated in a sample of n=38 obese adolescents with suspected non-alcoholic fatty liver disease (Øage 14.7 ±2.1 years, ØBMI z-score 2.79 ±0.48, ØALT 40.1 ±41.2 U/l) from 3h oral glucose tolerance test data.
Results: Validation of the approach demonstrated a low bias <5% in the determination of S_I. Mean S_I in the study population was 6.6 ±4.4. Regrouping the population in quartiles for S_I revealed mean values for S_I ranging from 2.7 ±0.7 in the lowest quartile to 12.4 ±4.6 in the highest, most insulin-sensitive quartile. Mean S_G was 1.26 ±0.35 and ranged from 0.77 ±0.12 in the lowest quartile to 1.65 ±0.10 in the highest quartile. S_I decreased with increasing

ALT level (Spearmans's Rank r -0.4, $p<0.05$), with a mean S_i of 10.8 ±7.1 in the lowest ALT-quartile to a mean S_i of 4.2 ±2.5 in the highest ALT quartile ($p<0.05$).
Conclusions: Our newly developed implementation of the oral minimal model with automatic adjustment of the uptake curve reliably identified a wide range of dynamic insulin sensitivity in obese adolescents. In our cohort, increasing ALT was associated with significantly reduced insulin-dependent glucose disposal and impaired inhibition of glucose production, demonstrating the feasibility of the approach in detecting early alterations in glucose metabolism in a clinical research setting.

P1-d2-223 Diabetes and Insulin 2

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

Ceren Cetin; Firdevs Bas; Banu Aydin Kucukemre; Ahmet Ucar; Ruveyde Bundak; Nurcin Saka; Feyza Darendeliler
Istanbul University,Istanbul Faculty of Medicine, Department of Pediatrics, Pediatric Endocrinology Unit, Istanbul, Turkey

Background: In recent years, various studies showed increased risk of insulin resistance in large for gestational age (LGA) born children during prepubertal ages.
Objective and hypotheses: We aimed to evaluate metabolic syndrom risk and the relationship between insulin sensitivity and anthropometric and metabolic parameters in LGA born prepubertal children.
Methods: Forty (19 female,21 male) LGA born prepubertal children (mean age 6.1±2.5 years) were evaluated with respect to glucose, insulin, IGFBP-1, IGF-1, leptin, adiponectin levels. Their data were compared to that of prepubertal 49 (25 female, 24 male) appropriate for gestational age(AGA) children (mean age 5.4±1.8 years).
Results: LGA children were taller, heavier than AGA children but had similar BMI SDS , waist/hip circumference ratio as AGA born children. There were no significant differences in glucose, insulin, HOMA-IR and IGFBP-1 levels between children born LGA and AGA. Adiponectin levels (p=0.009) and IGF-1SDS (p=0.01) were significantly lower in children born LGA . Leptin levels were higher in LGA born children(p=0.000). Univariate variance analysis revealed that being born LGA and having a higher HOMA-IR(higher than 2.5) had significant interaction and was associated with a higher leptin level. The analysis revealed that birth weight SDS, BMI SDS, HOMA-IR were each independent predictors of leptin levels .
Conclusions: The finding of high leptin and low adiponectin levels in LGA born children in prepubertal ages in the absence of obesity show that metabolic derangements can start early in childhood. Adipocytokine levels can be used as early signs of insulin resistance.

P1-d2-224 Diabetes and Insulin 2

Williams-Beuren syndrome as a genetic disorder with impaired glucose tolerance and diabetes in childhood and adolescence

Elisabetta Lapi[1]; Stefano Stagi[2]; Cecilia Cecchi[3]; Silvia Guarducci[1]; Francesco Chiarelli[4]; Maurizio de Martino[3]; Salvatore Seminara[3]
[1]Anna Meyer Children's University Hospital, Medical Genetics Unit, Florence, Italy; [2]Mugello's Hospital, Paediatric Unit, Florence, Italy; [3]Anna Meyer Children's University Hospital, Department of Paediatrics, Florence, Italy; [4]University of Chieti, Department of Paediatrics, Chieti, Italy

Background: In William-Beuren syndrome (WBS) adults, a far common endocrine abnormality is represented by diabetes mellitus or impaired glucose tolerance. However, sporadic data are available in children and young WBS subjects.
Aim: To evaluate the frequency of impaired glucose tolerance and/or diabetes mellitus in a cohort of children and young WBS patients.
Patients and methods: We longitudinally evaluated 12 WBS patients (4 males, 8 females, median age at the onset of the study: 13.8 years). The median follow-up was 3.6 years. In all subjects variables of IR and β-cell function were evaluated using OGTT. The Homeostasis Model Assessment (HOMA) of IR and the quantitative insulin-sensitivity check index (QUICKI) were calculated. Forty-five age- and sex-matched healthy subjects, and fifty-one age-, sex-, and Body Mass Index-matched subjects were recruited as two control groups.
Results: Considering nutritional status, 25% were obese, 33.3% overweight and 41.6% normal. No WBS patient had acanthosis nigricans. Glucose tolerance was abnormal in 25% of WBS patients and diabetes was diagnosed in 8.3%. WBS patients showed higher insulin values than healthy controls ($p < 0.001$), but similar values than BMI-matched control group (p = NS). However, WBS patients also showed a significantly higher values of glycemia and HOMA than healthy control group ($p < 0.001$), and BMI-matched control group ($p < 0.05$). Finally, a higher frequency of subjects with abnormality of glucose metabolism was found in WBS group than in healthy controls ($p< 0.0001$) and BMI-matched control group ($p < 0.0005$).
Conclusion: An impaired glucose tolerance and/or diabetes due to insulin-resistance may be frequently discovered in children, adolescents and young adults with WBS. These preliminary data might suggest that in WBS insulin resistance is partially independent of BMI, supposing a protective role of a gene in WBS region in the development of insulin resistance. This aspect still needs to be elucidated.

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Phenotypic characteristics of genetic subtypes of neonatal diabetes mellitus from 126 patients of the French NDM study group

Kanetee Busiah[1]; Séverine Drunat[2]; Amélie Bonnefond[3]; Albane Simon[4]; Nathalie Pouvreau[2]; Isabelle Flechtner[5]; Ravital Nimri[6]; Moshe Phillip[6]; Nadia Tubiana-Rufi[7]; Anne-Marie Bertrand[8]; Sylvie Nivot-Adamiak[9]; Marc De Kerdanet[9]; Chantal Stuckens[10]; Farida Jennane[11]; Véronique Sulmont[12]; Claire Letallec[13]; Nicole Ser[13]; Raphael Scharfmann[1]; Philippe Froguel[14]; Martine Vaxillaire[3]; Hélène Cave[2]; Michel Polak[15]
[1]INSERM U845, Centre de Recherche Croissance et Signalisation, Université Paris Descartes, Sorbonne Paris Cité, Paris, France; [2]Genetic Biochemistry, Robert-Debré Hospital, APHP, Paris, France; [3]CNRS-UMR8199, Lille Pasteur Institute, Lille Nord de France University, Lille, France; [4]Department of Paediatrics, Versailles hospital, Le Chesnay, France; [5]Pediatric Endocrine Unit, Necker Enfants-Malades Hospital, APHP, Paris, France; [6]The Jesse Z and Sara Lea Shafer Institute of Endocrinology and Diabetes, The National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; [7]Department of Paediatric Endocrinology and diabetology, Robert Debré Hospital, APHP, Paris, France; [8]Department of Paediatrics, Besançon hospital, Besançon, France; [9]Department of Paediatrics, Rennes Hospital, Rennes, France; [10]Department of Paediatrics, Jeanne de Flandre Hospital, Lille, France; [11]Department of Paediatric Endocrinology and diabetology, University children's hospital A.Harouchi, Casablanca, Morocco; [12]Department of Paediatric, American Memorial Hospital, Reims, France; [13]Department of Paediatric, Hôpital des enfants, Toulouse, France; [14]CNRS-UMR8199, Lille Pasteur Institute, Lille Nord de France University, 14 Department of Genomics of Common Disease, School of Public Health, Imperial College London, Hammersmith Hospital, London, United Kingdom; [15]Department of Paediatric Endocrinology and diabetology, Necker Enfants-Malades Hospital, APHP, INSERM U845, Centre de Recherche Croissance et Signalisation, Université Paris Descartes, Sorbonne Paris Cité, Paris, France

Background: Neonatal diabetes mellitus (NDM) is a rare genetically and clinically heterogeneous group of non-autoimmune diabetes mellitus leading to pancreatic β-cell dysfunctions that are diagnosed in neonates or early infancy.
Objective and hypotheses: 1) To extensively describe the clinical features of patients with NDM according to known genetic causes. 2) To find predictive clinical factors to guide the identification of molecular aetiologies.
Methods: A total of 176 patients (95 males/80 females) from the French NDM Study Group that had diabetes in the 1st year of life, without β-cell autoimmunity and no pancreas malformation and needed insulin treatment have been sequenced for *ABCC8*, *KCNJ11* and *INS* genes and screened for 6q24 abnormalities.
Results: We identified a genetic cause in 126 subjects (72%), 50/79 (63%) of permanent form, 73/89 (82%) of transient form and 3 patients too young to be classified. They had 6q24 abnormalities (n=40) and *KCNJ11*-Kir6.2 (n=43), *ABCC8*-SUR1 (n=31), *INS*-insulin (n=12) gene mutation.

Characteristic of patients with NDM				
	6q24 abnormalities	KCNJ11	ABCC8	Ins
N	40	43	31	12
Birth Weight (g)	1810 [720-2820]	2710 [880-3290]] *§	2800 [1400-3570] *§	1840 [1400- 3200]
Birth Height (cm)	45 [38-50]	48.5 [34-52] *§	49 [42-50] *§	42 [42-45]
Birth Weight<3rd percentile	30/37	16/41**	8/28*§	7/11
Age at diagnosis (d)	5 [1-120]	47 [1-237] *§	41 [1-278] *§	43 [1-256]
Age at remission (w)	14.4 [1-156.4]	33.4 [11.1-156.4]*	48.1 [1.3–230.6] *	14.1 [6.9-21.4]
Congenital heart disease	4/34	0/39*	0/26	0/10
Macroglossia	12/25	0/41*§	0/28*§	0/11
Comparison with 6q24 abnormalities: *<0.01 ; **<0.001 ; *§<0.0001				

As positive predictive values, age at diabetes diagnosis after 1 month and age at remission >12 months give 96% and 93% of chance to find a mutation in *ABCC8* or *KCNJ11* genes.
Conclusions: Clinical characteristics help in the prediction of the genetic aetiologies of NDM cases but overlap according to genotype. However, genetic studies are mandatory to elucidate each case and to define optimal treatment.

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

Christina Wei[1]; Linda Hunt[2]; Rachel Cox[3]; Karin Bradley[4]; Ruth Elson[3]; Michael Stevens[2]; Elizabeth Crowne[1]
[1]Bristol Royal Hospital for Children, Paediatric Endocrinology & Diabetes, Bristol, United Kingdom; [2]University of Bristol, Institute of Child Life and Health, Bristol, United Kingdom; [3]Bristol Royal Hospital for Children, Paediatric Oncology, Bristol, United Kingdom; [4]Bristol Royal Infirmary, Endocrinology and Diabetes, Bristol, United Kingdom

Background: Insulin resistance(IR) and abnormal body composition(BC) are common in adult childhood leukaemia survivors treated with BMT/TB, and glucose intolerance increasingly recognised.
Objective: To investigate the relationship between BC and IR in BMT/TBI subjects.
Method: Leukaemia survivors treated with (group1)(n=21) and without (group2)(n=31) BMT/TBI (TBI 10-14.4Gy) were compared with obese subjects(group3)(n=30). IR represented by composite-insulin-sensitivity index (ISIcomp) from oral glucose tolerance tests were compared with BC data from Dual-emission X-ray absorptiometry. Comparisons between groups were made by ANOVA with post hoc Scheffe and relationships between IR and BC explored by Pearson's correlation with significance at 5%.
Results: Median(range) age was 21.1(16.1-26.2), 21.5(16.1-26.0) and 17.8(16.1-24.8)years respectively. IR was greater in groups 1&3 than 2. Total and trunk fat mass and fat-mass-index(FMI) correlated with IR in groups 1&2 but not 3. Although group 3 has higher total and central fat masses and FMI than group 1, IR (ISIcomp>2.5) became evident at lower degree of central adiposity(trunk fat=4.1vs24.8kg) in group 1. There was no correlation between IR and lean mass in any groups.

	Mean(SD) or Geometric Mean(range)*			p-value			Correlations with ISIcomp		
GROUP	1	2	3	1 vs 2	1 vs 3	2 vs 3	1	2	3
Total fat mass(kg)	19.2 (10.8)	25.4 (11.2)	46.2 (8.5)	NS	<0.001	<0.001	-0.49*	-0.47*	NS
Trunk fat mass(kg)	10.8 (6.4)	12.9 (6.0)	25.1 (4.6)	NS	<0.001	<0.001	-0.54*	-0.49*	NS
Trunk/ total fat	0.55 (0.05)	0.50 (0.04)	0.52(0.05)	0.001	NS	NS	-0.63*	NS	-0.46*
FMI(kg/m2)	7.3 (3.9)	8.9 (4.2)	16.4(3.3)	NS	<0.001	<0.001	-0.58*	-0.44*	NS
LMI(kg/m2)	13.0 (2.3)	14.4 (2.3)	16.4(2.1)	NS	<0.001	0.01	NS	NS	NS
ISI(comp)	1.5 (0.4-9.7)	4.8 (0.8-9.6)	2.2(0.8-7.3)	<0.001	NS	0.001	NA	NA	NA

NA= not applicable, NS=not significance, *= p < 0.05

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

P1-d2-227 Diabetes and Insulin 2

Treg function as a marker to define the susceptibility to T1DM clinical onset in a healthy subject with antibodies positivity?

Gianluca Musolino[1]; Alessandra Tedeschi[2]; Alessandro Salvatoni[3]; Luigi Nespoli[3]; Roberto Sergio Accolla[2]
[1]University of Insubria Medical School, Pediatric Endocrinology Unit, Varese, Italy; [2]University of Insubria Medical School, Laboratory of Immunology and General Pathology, Varese, Italy; [3]University of Insubria Medical School, Pediatrics, Varese, Italy

Background: T regulatory cells are crucial elements in modulating immune responses. Their activity and/or number were shown to be increased in autoimmune diseases.
Objective and hypotheses: To investigate the phenotype and functional status of T regulatory cells in T1DM patients and 10 healthy controls.
Methods: We studied a 20-year-old T1 diabetic male (disease duration:14 years) and his healthy 14-year-old brother, who presented high-titer diabetes antibodies (IAA, ICA, IA2-A, GADA) for 3 years, though maintaining high first-phase insulin response (90th centile). CD4+CD25highCD127low Tregs in both subjects and in 10 healthy controls were assessed by FACS, sorted and used for functional analysis. Cells were isolated and cultured with autologous CD4+CD25-responder T cells (Teff) and stimulated with a pan-T stimulus (anti-CD2, anti-CD3 and anti-CD28 monoclonal antibodies-loaded beads). The suppressive capacity of Treg towards Teff cells in co-culture was expressed as the ratio between the percentage of cells proliferating in presence or in absence of Tregs according to the formula [100 x (1- % CFSE low CD4+CD25-T cells in co-culture / % CFSE low CD4+CD25-T cells alone)].
Results: The results of flow cytometric analysis showed similar Treg frequency in the diabetic patient and healthy controls. Treg function (see table) was significantly reduced in diabetic patient as compared to healthy brother and controls.

	Percentage of inhibition (1:0)	Percentage of inhibition (1:0,5)	Percentage of inhibition (1:1)
Patient	14,02%	42,05%	68,01%
Healthy brother	20,27%	66,25%	74,74%
Controls	14,4±2,1%	55,2±6,2%	68,2±6,6%

Conclusions: Results suggest that overt diabetes is associated not only with the presence of anti-self component antibodies but also with the functional status of Treg subpopulation. Absence of clinical symptoms in healthy brother can result from the present normal Treg function that may evolve towards a functional impairment of them or may not. Therefore careful follow-up of this population will be a crucial parameter to define the susceptibility to clinical onset of the disease in this as well as in similar subjects.

P1-d2-228 Diabetes and Insulin 2

Clinic appointment reminders and their effect on 'Did not attend' (DNA) rates & HbA1C, in a paediatric diabetes clinic

Pooja Sachdev[1]; Elaine Gunn[2]; Katie Harron[3]; Anuja Natarajan[2]
[1]University of Sheffield, Department of Human Metabolism, Sheffield, United Kingdom; [2]Doncaster and Bassetlaw NHS Foundation Trust, Department of Paediatrics, Doncaster, United Kingdom; [3]Institute of Child Health, University College of London, Paediatric Epidemiology and Biostatistics, London, United Kingdom

Background: Non attendance in outpatient clinics results in economic loss and poor patient care. Mobile phone intervention has been shown to be effective in improving attendance rates in chronic disease follow up. Furthermore, a recent meta-analysis which included different mobile phone interventions for diabetes self management including prompts for attendance showed a reduction in HBA1c by a mean of 0.5%. A pilot study conducted over 9 months in our diabetes clinic showed improved attendance (significant when patient was spoken to) following phone calls and text messages sent to carers/young

people prior to their clinic appointment.
Aims: 1) To analyse DNA rates when carers/patients were telephoned or texted prior to their clinic appointment over a 2-year period. 2) Did the change in attendance result in improved HBA1c's?
Methods: Prospective 2 year study with the 1st 8 months serving as control (routine hospital appointments made by patient) followed by an 8 month period of calling carers/ patients to remind them of their appointment and the third period of 8 months of text messaging reminders. Paired t testing was used to compare DNA rates in the control and intervention period. The overall control of the clinic as reflected by average HBA1c and HBA1c at end of each period (control/phone/text messaging) was also compared.
Results: Data for 104 patients available. The proportion of missed clinic appointments decreased over the time period, though not statistically significant. ($p<0.45$) Multi-level linear regression shows that children who attend more clinics have better control: for each additional clinic attended, HbA1c decreased by an estimated average of 0.3 ($p=0.004$).Those who were spoken to every time a call was made had significantly better attendance.Those who replied to all texts had better attendance ($p<0.001$), fewer DNA's ($p<0.001$), and better control.
Conclusion: Clinic appointment reminders particularly text messaging can be used to increase attendance rates in a paediatric diabetes clinic and this is reflected in better glycaemic control.

P1-d2-229 Diabetes and Insulin 2

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

Jesper Johannesen[1]; Siri Fredheim[2]; Lene Lyngsøe[3]; Andersen Marie-Louise[2]; Lauridsen Mette[4]; Hertz Birgitte[4]; Birkebæk Niels[5]; Olsen Birthe[2]; Svensson Jannet[2]
[1]Herlev University Hospital, Department of Paediatrics, Herlev, Denmark; [2]Herlev University Hospital, Paediatrics, Herlev, Denmark; [3]Hillerød Hospital, Paediatrics, Hillerød, Denmark; [4]Viborg, Paediatrics, Viborg, Denmark; [5]Skejby University Hospital, Paediatrics, Aarhus, Denmark

Background: Since 1996 The Danish Childhood Diabetes Register has collected data from all Danish paediatric diabetes centres treating type 1 diabetic patients aged 0-15 years. All newly diagnosed type 1 diabetic patients < 15 years have been enrolled since 1996.
Objective and hypotheses: (i) To evaluate the frequency and severity of DKA at onset and (ii) its association to future metabolic control.
Methods: DKA status was defined as: (i) none: $HCO3 > 15$ or $pH > 7.3$; (ii) mild: $HCO3 < 15$ or $pH < 7.3$; (iii) moderate: $HCO3 < 10$ or $pH < 7.2$ and (iv) severe: $HCO3 < 5$ or $pH < 7.1$ from blood gas analyses. Central HbA1c determination from all participants were analysed by means of multiple regression using age, gender, ethnicity, diabetes duration and DKA status as explanatory variables in a compound symmetric repeated measurement model.
Results: A total of 2939 recordings (1414 girls (48.1%) and 1525 boys (51.9%)) in 3364 persons were included in the analysis as 425 individuals did not have complete DKA data sets. 2422 individuals (82.4%) presented without DKA, whereas 237 (8.1%), 233 (7.9%) and 47 (1.6%) presented in mild, moderate and severe DKA, respectively. The multiple regression analysis revealed association of higher HbA1c levels over time (average diabetes duration 4.49 yrs) of 0.10%, 0.21% and 0.47% ($p=0.002$) for mild, moderate and severe DKA presentations compared to no DKA at onset. The HbA1c levels increased significantly with age ($p<0.001$) and was higher in the immigrant population. There was no effect of gender.
Conclusions: Presentation of T1D in DKA associates to higher HbA1c years ahead. Possible explanatory factors e.g. residual beta-cell function or adherence to treatment needs further exploration.

P1-d1-230 Endocrine Oncology 1

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

Patricia Papendieck[1]; Laura Gruñeiro-Papendieck[1]; Monica Sala[2]; Patricia Arce[2]; Oscar Acha[3]; Ana Chiesa[1]
[1]CEDIE, Ricardo Gutiérrez Children´s Hospital, Buenos Aires, Argentina, Endocrinology Division, Buenos Aires, Argentina; [2]Hospital de Clínicas Jose de San Martin, Buenos Aires, Argentina, Endocrinology Unit, Buenos Aires, Argentina; [3]Ricardo Gutierrez Children´s Hospital, Surgery Dept., Buenos Aires, Argentina

Background: Thyroglossal duct cysts (TGDC) are the most common thyroid developmental anomalies accounting for 75% of midline neck tumors in children and 7% in adults. Carcinoma of the TGDC has been reported in less than 1% in adults.
Objective and hypotheses: Describe the case of a pediatric papillary carcinoma of the TGDC.
Methods: Case report
Results: A 12 year old girl presented with an asymptomatic fast growing neck mass noticed 7 months previous to consultation. Her past medical history was unremarkable. US revealed a cystic-solid mass of 21 x13 mm with microcalcifications with a normal thyroid gland. With a presumptive diagnosis of TGDC a Sistrunk procedure was performed. Histological evaluation showed a papillary carcinoma of 12x6mm in the wall of a 35x25x25mm TGDC. The patient was referred to our Unit for follow up. Physical examination revealed an euthyroid pubertal girl with a non palpable thyroid gland without palpable cervical nodes. Neck US showed a normal eutopic thyroid gland without suspicious adenopathies. Neck and chest CT scan were normal. Histologic examination after total thyroidectomy revealed no tumor. Postoperatively, an ablative 131 I dosis of 50 mCi was administered. WBS performed on day 5 revealed focal radioiodine uptake confined to the inferior cervical region. Cervical US showed a right yugular adenopathy of 15x7mm with an heterogeneous vascularized rounded area. FNAB cytology was positive for papillary carcinoma with positive thyroglobulin in the needle wash-out .Surgical excision was performed with histologic diagnosis of papillary metastatic infiltration.
Conclusions: Although exceptional in pediatrics ,rapid growth of a thyroglossal duct cyst excluding infection and/or US signs suggestive of malignancy should alert of the possibility of TGDC carcinoma. The lack of thyroid involvement does not rule out the presence of metastasis and follow up should be the same as for differentiated thyroid cancer .

P1-d1-231 Endocrine Oncology 1

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

Mahalakshmi Gopalakrishnamoorthy[1]; Samuel Mindell[1]; Dawn Saunders[2]; Mark Gaze[3]; Helen Spoudeas[4]
[1]University College London Hospital, Paediatric Endocrinology, London, United Kingdom; [2]Great Ormond Street Hospital, Paediatric Radiology, London, United Kingdom; [3]University College London Hospital NHS trust, Radiology, London, United Kingdom; [4]University College London Hospital NHS trust, Paediatric Endocrinology, London, United Kingdom

Background: Second Primary Tumours (SPT), such as surgically resectable meningiomas and thyroid tumours, are known late effects of Posterior fossa Brain Tumours (PFBT) attributed to high dose radiation scatter at the field's edge. Concerns that their prevalence may be increased by GH therapy have been raised but adjuvant chemotherapy and genetic cancer predisposition may also contribute.
Methods and aims: In a long term (>10 years) evaluation of late outcomes, we performed surveillance MRI brain scans on 103 (65% Male) PFBT survivors (68 Primitive neuroectodermal tumours [PNET], 18 Astrocytomas, 17 Ependymomas) with a mean age of 21.8 (17-38) years transitioning to adult services. 54 (49%) had received childhood GH replacement, especially after PNET (80%). Non-CNS SPTs, prior oncological therapy and any known genetic cancer predisposition were noted.
Results: Over a median 14.4 (2.8-34.8) years, 20 SPTs occurred in 14 (29%M) neuraxially irradiated PFBT survivors, all but one (ependymoma), being PNET survivors. 11 had received prior GH therapy. Two patients each had 2 and 3 SPT's respectively, one of whom was found to harbour a FAP gene mutation. 10 (50%) SPTs occurred in the 8 PNET patients given che-

motherapy, these occurring earlier (12.3 vs 19 years) and tending to higher grade (sarcoma, leukaemia) than in the 5 not given chemotherapy. Three had never received GH.
Conclusion: PNET survivors are particularly susceptible to SPTs, 20% (1:5) developing at least one over an average time similar to that reported (13 vs15 years), but at a significantly greater (4%) prevalence than (2-3%) expected. Adjuvant chemotherapy, not GH, doubled the risk of, and halved the time to, SPT after PNET. These novel and preliminary data suggest that in PNET, the 10-15% increase in survival brought by adjuvant chemotherapy is offset by its potentiation of the number and severity of SPT's, in which GH replacement does not appear implicated.

P1-d1-232 Endocrine Oncology 1

Resveratrol induces cell death in human cancer cells and targets NAMPT

Susanne Schuster[1]; Stefanie Petzold- Quinque[1]; Theresa Gorski[1]; Antje Garten[1]; Melanie Penke[1]; Anja Barnikol-Oettler[1]; Sandy Laue[1]; Rolf Gebhardt[2]; Wieland Kiess[1]
[1]Center for Pediatric Research Leipzig (CPL), University Hospital Leipzig, Leipzig, Germany; [2]Institute of Biochemistry, Medical Faculty, University of Leipzig, Leipzig, Germany

Background: Nicotinamide phosphoribosyltransferase (NAMPT) is a key enzyme of NAD biosynthesis and regulates the activity of the NAD- dependent deacetylase sirtuin 1(SIRT1). SIRT1 is implicated in multiple aging-related diseases including cancer. Resveratrol (Resv) is a potential SIRT1 activator and has been proven to be an effective chemopreventive agent.
Objective and hypotheses: We hypothesized that NAMPT acts as key player in the Resv-mediated apoptosis of cancer cells through a reduced metabolisation of nicotinamide and consequently inhibition of SIRT1.
Methods: HepG2 cells, Jurkat cells, primary hepatocytes and PBMC were cultured. Induction of apoptosis was measured by Annexin/PI staining and Western Blotting of phosphorylated p53. NAMPT protein level and its enzymatic activity were quantified using a NAMPT ELISA and a radioactive filter disc assay. NAD-levels were measured by HPLC analysis.
Results: Resv reduced cell proliferation and caused an increase of cells in the S- and G2/M-phase of the cell cycle in HepG2 cells. Additionally, Resv induced apoptosis (68.7±10.7%), phosphorylation of p53 and an increased acetylation of p53. It also reduced the intracellular NAMPT protein by 48.1±15.4% and the NAMPT activity by 49.5±2.3%. In contrast, primary human hepatocytes did not show significant changes in NAMPT protein expression and cell viability. In human leukemia Jurkat cells we observed a reduction of cell viability after incubation with Resv. Treatment of leukemia cells with Resv and different chemotherapeutics induced cell death in an additive manner.
Conclusions: We demonstrated that Resv exerts opposite effects on cancer cells and normal, primary cells regarding cell viability, apoptosis, NAMPT protein amount and -activity. In leukemia cells Resv might act as a chemosensitizer. The apoptotic effects of Resv on cancer cells might be mediated through a reduced NAMPT activity and consequent inhibition of SIRT1 which leads to an increased p53 mediated apoptosis.

P1-d1-233 Fat Metabolism and Obesity 1

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

Raquel Corripio[1]; José Miguel González-Clemente[2]; Jacobo Pérez[1]; Silvia Näf[3]; Lluis Gallart[3]; Joan Vendrell[3]; Assumpta Caixàs[2]
[1]Sabadell Hospital, Parc Tauli Corporation, University Autonomous of Barcelona (UAB), Paediatric Endocrine Department, Sabadell, Spain; [2]Sabadell Hospital, Parc Tauli Corporation, University Autonomous of Barcelona (UAB), Diabetes, Endocrinology and Nutrition Department, Sabadell, Spain; [3]Hospital Universitari Joan XXIII de Tarragona, IISPV, Endocrinology and Diabetes Unit, Research Department, Tarragona, Spain

Context: BDNF (brain-derived neurotrophic factor) is a neurotrophin potentially involved in the pathophysiology of obesity and metabolic syndrome in adults. In children, it has scarcely been studied.
Objective: To analyze plasma BDNF and its relationship with metabolic syndrome components before and after two years of a lifestyle intervention programme in a prepubertal obese cohort.
Design and setting: Case-control study with a 2-year prospective follow-up in a referral paediatric endocrine outpatient centre. Patients and methods: Seventy-three prepubertal obese children, 8.03±1.08 years old and 47 age and gender-matched lean controls were studied. Anthropometric parameters, blood pressure, platelet count, oral glucose tolerance test, homeostatic model assessment for insulin resistance (HOMA-IR), lipid profile, BDNF, diet and physical activity were evaluated. Weight loss was considered if z-score body mass index (BMI) decreased at least 0.5 SD.
Results: At baseline, BDNF tended to be lower in prepubertal obese children compared to lean controls (p=0.076). BDNF did not correlate with any metabolic syndrome component. After two years, obese patients showed an increase in BDNF. Regression model analysis adjusted by age, sex, puberty, BMI, platelet count and HOMA-IR showed that BDNF increased in subjects who lost weight (p=0.036), practiced sports (p=0.008) and had an adequate carbohydrate intake (p=0.032).
Conclusions: Plasma BDNF tends to be lower in obese prepubertal children than in lean controls, is not related to any other metabolic syndrome component and increases after a lifestyle intervention programme.

P1-d1-234 Fat Metabolism and Obesity 1

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

Antti Saari[1]; Ulla Sankilampi[2]; Leo Dunkel[3]
[1]University of Eastern Finland, School of Medicine, Department of Pediatrics, Kuopio, Finland; [2]Kuopio University Hospital, Department of Pediatrics, Kuopio, Finland; [3]Queen Mary University of London, Centre for Endocrinology, William Harvey Research Institute, Barts and the London, London, United Kingdom

Background: BMI-for-age z-score (zBMI) is commonly used to screen children that are at risk for overweight or obesity. However, the sensitivity of zBMI in identifying these children is suboptimal. Adiposity rebound (AR) at young age correlates with excess weight gain, but has been insufficiently explored in screening of overweight and obesity in the general population.
Objective and hypotheses: To compare how the age at AR and zBMI in children aged 2-7 years predict development of overweight and obesity after 10 years of age.
Methods: Longitudinal growth data of full-term, appropriate-for gestational age (AGA) healthy girls (n= 2,606) and boys (n = 2,628) with 25,837 and 25,629 height and weight measurements from birth to over 10 years of age were included. Age at AR was defined as the nadir of the fitted curve through the observed BMI-for-age data points. The screening accuracy of the age at AR and zBMI (at different ages) for the subsequent overweight and obesity were evaluated using Receiver Operating Curve–analyses after 10 years of age.
Results: Overall, zBMI showed good to excellent accuracy in predicting overweight or obesity in girls (AUCs 0.83 and 0.94) and fair to good accuracy in boys (AUCs 0.79 and 0.86) at 2-7 years (Figure 1). The accuracy of prediction increased with age. At 7 years overweight or obesity were detected already relatively well (AUCs 0.91 and 0.97 for girls, 0.89 and 0.94 for boys, respectively). However, the age at AR was superior to zBMI at 7 years for overweight and obesity, with AUCs 0.96 and 0.98 for girls and 0.96 and 0.98 for boys, respectively.
Conclusions: In full-term AGA children the risk for excess weight gain leading to overweight and obesity is identified more accurately by the age at AR than by zBMI at any age.

P1-d1-235 Fat Metabolism and Obesity 1

Association of urinary bisphenol a with insulin resistance in obese children and effects on adiponectin and resistin expression

Anna Grandone[1]; Ciro Menale[2]; Grazia Cirillo[1]; Nadia Diano[2]; Carla Nicolucci[2]; Rosaria Gaeta[1]; Manuela Rinaldi[1]; Rosaria Marotta[1]; Francesca Clarizia[1]; Emanuele Miraglia Del Giudice[1]; Laura Perrone[1]
[1]Seconda Università degli Studi di Napoli, Pediatrics, Naples, Italy; [2]Seconda Università degli Studi di Napoli, Medicina sperimentale, Naples, Italy

Background: Bisphenol A (BPA) is used in polymerization reaction to produce plastics for food and water containers, baby bottles, lining of food and beverage metal cans and medical tubing. Small amount of BPA can migrate from the polymers to food and water upon heating. High urinary BPA concentrations have been associated in adults with cardiovascular diseases and with diabetes and, recently, with insulin resistance in elderly subjects.
Objective and hypotheses: We wanted to determine i) whether BPA associates with insulin resistance in obese children ii) the effect of BPA on adipokine expression in human mature adipocytes.
Population and methods: We enrolled 98 obese children (age 10±2.3 years, BMI-z-score 3.7±1.5). Blood pressure, waist circumference, lipids, insulin and glucose were measured. Insulin resistance was evaluated by HOMA. Urinary BPA was measured by HPLC. Preadipocytes, obtained from subcutaneous abdominal adipose tissue, were differentiated to mature adipocytes and were treated for 24 h with increasing doses of BPA (1nM, 10 nM, 100 nM). To evaluate the effect of BPA on adiponectin and resistin expressions, a Real-Time PCR was performed
Results: Mean urinary BPA was 1.1ng/ml (0.47-1.3). No correlations were found between BPA and BMI-z-score, waist, lipids and blood pressure. A GLM showed a significant positive correlation between BPA and HOMA (p: 0.03), adjusting for waist circumference, age and sex. Adiponectin mRNA levels were significantly inhibited (nearly 4 fold) in the adipocytes treated with BPA. Resistin mRNA, on the contrary, was not detectable in not treated cells but was present, in dipendent-dose manner, in BPA treated adipocytes.
Conclusions: Our results suggest that i) BPA may represent one of the factors able to modulate insulin resistance in childhood obesity and that ii) this action appears to be due to a combined effect of BPA on adipose tissue, consisting both in adiponectin expression inhibition and resistin expression stimulation.

P1-d1-236 Fat Metabolism and Obesity 1

Mechanography in childhood: new tests and new references

David Martin[1]; Rainer Rawer[2]; Roland Schweizer[1]; Gerhard Binder[1]
[1]Tubingen University Children's Hospital, Pediatric Endocrinology and Diabetology, Tubingen, Germany; [2]Novotec Medical GmbH, Ingeneering, Pforzheim, Germany

Background: Mechanography offers tests that reflect everyday motor function, but solid reference values for young children were missing.
Objective and hypotheses: Mechanography offers The aim of this study was to establish reference values for body mass related peak force (RPF), peak power (RPP) and force efficiency (FE) in children for counter movement jumps, one legged hopping and chair rising tests.
Methods: A total of 868 subjects (432 male, 436 female) aged 3 to 19 years were studied. In addition to auxological measurements and maximum isometric grip force (MIFG), the following movements were performed and assessed using the Leonardo Mechanograph®: Single Two Legs Jump (s2LJ), Multiple One Legged Hopping (m1LH) and Chair Rising Test (CRT).
Results: We present weight-adjusted age-, gender -related normative values for each of these tests and discuss their dynamics through childhood. Body weight related results are reported as multiples of earth's gravity (g). Maximum voluntary force (RPFm1LH) during hopping on one forefoot was found to be constant and independent of age and gender in the age group between 5 and 19 at 3.33 g (SD 0.31 g). Peak jumping power in relation to body mass during counter movement jump for maximum height (RPPs2LJ) was found to be almost linearly increasing with age for males age 5 to 19 and female age 5 to 11 at a rate of 4.6 W/kg per year. RPPs2LJ for females age 12 to 19 increased only slightly by less than 10%. CRT time per repetition was constant and independent of age and gender. Peak power per body mass during the rise phase (RPPCRT) showed similar but smaller age and gender relations as peak power during s2LJ.
Conclusions: This data from a healthy Caucasian middle class population hardly touched by the obesity and media exposure epidemics provide ideal reference values for these tests; they have been incorporated into Leonardo Mechanograph®.

P1-d1-237 Fat Metabolism and Obesity 1

Metabolic and haemodynamic aspects of cardiovascular risk in obese adolescents

Tetyana Chaychenko; Ganna Senatorova
Kharkiv National Medical University, Pediatrics 1 and Neonatology, Kharkiv, Ukraine

Background: Epidemiologic data shows an increasing tendency of obesity in childhood. Furthermore the exact nature of cardiovascular risk in a paediatric population remains obscure.
Hypothesis: Detrimental cardiovascular changes in obese adolescents are a result of metabolic-hemodynamic relationships.
Methods: 117 obese and 32 overweight adolescents were studied using detailed anthropometry, tissue doppler echocardiography, 24-hr blood pressure (BP) monitoring, carotid intima-media thickness (CIMT). Fasting lipids, carbs, leptin, adiponectin, TNF-α, uric acid concentrations were measured. Subjects were grouped according to BMI: +1-2SD (Gr.1); 2-3SD (Gr.2) and >3SD (Gr.3). Nonparametric statistic analysis and multiple regression technique were performed.
Results: There is a valid correlation between Left Ventricular Mass Indexed and increasing BMI, HOMA (p<0,004), FFA (p<0,004), waist to height ratio (p <0,0003), TNF-α (p<0,032). Established eccentric LV remodelling is a result of both increased systolic BP (p<0,026) and hypercholesterolemia (p<0,001). Systolic function (by the EF,%) significantly correlated with the total cholesterol (p<0,037), HDL(p<0,005) and % body fat (p<0,017). Diastolic LV function was dependent on HOMA (p<0,001) and revealed reduced diastolic filling time. The 24hr BP profile is characterized by both increased systolic and diastolic BP. Levels of systolic BP demonstrate a direct relationship with BMI (p<0,003), uric acid (p<0,002), HOMA (p<0,002) and diastolic BP - with leptin (p<0,012), HOMA (p< 0,016). Increasing levels of obesity are accompanied by increasing CIMT, vascular stiffness and maximum systolic flow velocity without significant changes in systolic-diastolic gradient. CIMT was associated with abdominal fat predisposition (p<0,038), diastolic BP (p<0,004), diastolic load (p< 0,001), uric acid (p<0,03) and TNF-α (p<0,05).
Conclusions: Myocardial and vascular remodelling in paediatric obesity may be stepwise contributed by excess abdominal adipose mass, insulin resistance, chronic subclinical inflammation and hypertension.

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Vitamin D status in obese children and its recovery after weight loss are influenced by the distribution of adipose tissue, adiponectin levels, ethnic background, pubertal stage and metabolic impairment

Gabriel Ángel Martos-Moreno[1]; Francisco Javier Caballero-Mora[1]; Vicente Barrios[1]; Sara Sirvent[2]; Julia Asensio[3]; Guillermo Martínez[4]; Federico Hawkins[4]; Jesús Argente[1]
[1]Hospital Infantil Universitario Niño Jesús, Endocrinology, Madrid, Spain; [2]Hospital Infantil Universitario Niño Jesús, Radiodiagnostics, Madrid, Spain; [3]Hospital Infantil Universitario Niño Jesús, Clinical analysis laboratory, Madrid, Spain; [4]Hospital Universitario 12 de Octubre, Endocrinology, Madrid, Spain

Background: Socioeconomic background, sex and puberty can influence 25-OH-vitamin D (vD) levels in obese children. Its anthropometrical, metabolic and hormonal determinants remain to be characterized.
Objective and hypotheses: To evaluate the relationship between vD status, body fat, bone mineral density (BMD), metabolic impairment and adiponectin levels in obese children before and after weight loss.
Methods: Obese children (n=150; 11.8±2.9 years; +4.1±1.4 BMI-SDS; 50%/sex;75 Latinos and 75 Caucasians) were enrolled. Serum glucose, insulin, lipid profile, body composition (DXA), abdominal magnetic resonance imaging, liver ultrasonography, vD (Chemoluminiscence, Liaison®) and total (RIA) and HMW-adiponectin (ELISA) levels (Millipore®, USA), were studied at baseline and after BMI reduction of over 1.5 SDS (n= 33).

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

Vitamin D	Deficient (<10ng/ml)	Insufficient (10-30ng/ml)	Sufficient (>30ng/ml)	
VLDL (mg/dl)	22.6±9.7	15.4±8.5	8.8±3.1	p<0.01
Triglycerides (mg/dl)	112.8±48.6	77.3±42.4	44.4±16.0	p<0.01
HOMA index	4.8±2.1	3.7±2.7	1.9±1.0	p<0.05

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

P1-d1-239 Fat Metabolism and Obesity 1

Can inflammatory marker levels help in detecting early complications of childhood obesity?

Patrizia Bruzzi; Lorenzo Iughetti; Chiara Cattelani; Viviana Dora Patianna; Giulia Vellani; Simona Filomena Madeo; Barbara Predieri
University of Modena and Reggio Emilia, Pediatrics, Modena, Italy

Background: Excess body weight may be associated with a state of chronic low-grade inflammation in childhood. There is increasing evidence that intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are biomarkers of endothelial dysfunction.
Objective and hypotheses: Aim of this study was to investigate the relationship of inflammation and endothelial activation in children with severe obesity and healthy controls. Data were also analyzed according to presence of insulin resistance (IR), metabolic syndrome (MS), and non-alcoholic fatty liver disease (NAFLD).
Methods: Thirty-seven (16 boys) obese children and adolescents (11.3±2.76 yr; BMI SDS 3.42±0.78) were examined and compared with 17 normal weight subjects matched for age and sex. Fasting levels of interleukin-6 (IL-6), ICAM-1, VCAM-1, and endothelin were determined. IR was assessed by the homeostasis method and MS was defined using Weiss criteria.
Results: Serum ICAM-1 concentrations resulted significantly higher in obese subjects respect to controls; IL-6, VCAM-1, and endothelin levels were non different. In obese patients VCAM-1 concentrations resulted positively correlated with both waist (r=0.33, p=0.048) and hip circumference (r=0.38, p=0.021). ICAM-1, despite high levels, was not correlated with measures of adiposity. Analyzing data according to presence/absence of IR, MS and NAFLD we did not find significant differences in inflammatory marker levels. Multivariate regression analysis did not identify each inflammatory marker as significant predictive factor for IR, MS, and NAFLD.

	Obesity	Controls	p
Age (yr.)	11.3±2.76	10.2±3.19	0.216
Height (SDS)	0.56±1.16	-0.12±1.88	0.164
BMI (SDS)	3.42±0.78	-0.92±3.39	0.000
IL-6 (pg/ml)	4.47±1.06	4.92±1.13	0.161
VCAM-1 (pg/ml)	13872.6±6295.3	12957.1±6030.6	0.617
ICAM-1 (ng/ml)	149.8±20.2	134.7±15.2	0.008
Endothelin (pg/ml)	7.12±7.65	9.34±13.4	0.459

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

P1-d1-240 Fat Metabolism and Obesity 1

Chronic leptin administration induces changes in circulating and adipose tissue inflammatory cytokines

Vicente Barrios[1]; Emma Burgos-Ramos[1]; Sandra Canelles[1]; Arancha Perianes-Cachero[2]; Eduardo Arilla-Ferreiro[2]; Jesús Argente[1]
[1]Hospital Infantil Universitario Niño Jesús, Endocrinology, Madrid, Spain; [2]Universidad de Alcalá, Biochemistry and Molecular Biology, Alcalá de Henares, Spain

Background: Obesity is characterized by hyperleptinemia, reduction of insulin sensitivity and an augment in circulating inflammatory factors synthetized by adipose tissue, among other tissues.
Hypothesis: Leptin promotes synthesis of cytokines in subcutaneous and visceral adipose tissue, generating a proinflammatory profile related with insulin resistance.
Methods: We studied 18 male Wistar rats divided into three groups; rats receiving saline icv (controls, C), treated icv for 14 days with a daily dose of 12 µg of leptin (L) and a pair-fed group (PF) that received the same food amount consumed by L. Serum leptin and insulin were measured by ELISA, mRNA levels of interferon-γ (IFN- γ), interleukins (IL) 2, 4, 6 and 10 and tumor necrosis factor-α (TNF-α) by real-time PCR and serum and subcutaneous and visceral adipose tissue levels of these cytokines by multiplexed bead immunoassay. Activation of signal transducer and activator of transcription 3 (STAT3) and protein kinase B (Akt) and levels of suppressor of cytokine signaling 3 (SOCS3) and insulin receptor in both fat depots were performed by Western blot.
Results: Serum leptin, IL-2, IL-4, IFN-γ and homeostasis model assessment of insulin resistance (HOMA-IR) index were increased in L and TNF-α was decreased in PF and L. Serum leptin, IL-2 and IL-4 levels correlate positively with HOMA-IR index. In L group, an increase in mRNA levels of IL-2 was found in both adipose depots and IFN-γ only in visceral tissue. Activation of leptin signaling, measured by the phosphorylation of STAT3, is increased in subcutaneous fat of L, and insulin signaling, determined by phosphorylation of Akt, is decreased, in subcutaneous adipose tissue of L group.
Conclusion: Leptin mediates the production of inflammatory cytokines by white adipose tissue independently of food intake reduction, generating a state of peripheral insulin resistance.

P1-d1-241 Fat Metabolism and Obesity 1

Differential effects of increased bodyweight due to neonatal over-nutrition and a sucrose-enriched diet on hypothalamic inflammation and glial markers

Esther Fuente-Martín[1]; Cristina García-Cáceres[1]; Miriam Granado[1]; Miguel A. Sánchez-Garrido[2]; Manuel Tena-Sempere[2]; Jesús Argente[1]; Julie A. Chowen[1]
[1]Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, CIBERobn, Endocrinology, Madrid, Spain; [2]University of Córdoba, Cell Biology, Physiology and Immunology, Córdoba, Spain

Background: Neonatal over-nutrition (NON) increases the propensity towards obesity in adulthood, with this being aggravated by a high fat diet. Recently hypothalamic inflammation and gliosis have been associated with obesity onset and exacerbation. However, current dietary habits also include increased carbohydrate consumption and little is known regarding its effects on hypothalamic inflammation.
Objective and hypotheses: Our aim was to determine how NON affects the response to a sucrose-enriched diet and if metabolic changes are associated with modifications in hypothalamic glial and inflammatory markers.
Methods: At birth, litters of Wistar rats were adjusted to 12 (control) or 4 (NON) pups. At weaning, half of each group received sucrose supplemented water (33% solution) ad libitum. Male rats were killed at 80 days of age (n=8-12/group) and serum and brains collected.
Results: NON rats weighed more than controls at weaning and sacrifice (ANOVA: p<0.0001). Sucrose intake decreased weight gain in spite of a higher Kcal intake, but increased fat mass and altered metabolic hormone levels (insulin: p<0.0001; leptin: p<0.0001; acylated ghrelin: p<0.01). Sucrose intake increased hypothalamic NPY mRNA levels in all rats (p<0.05), but increased AgRP and decreased LepR mRNA levels only in controls. NON increased the number of astrocytes in the arcuate nucleus (p<0.005), the num-

ber of processes/astrocyte ($p<0.03$) and vimentin levels, suggesting gliosis. The sucrose-enriched diet decreased hypothalamic levels of the astrocyte marker glial fibrillary acidic protein (GFAP; $p<0.0001$) and astrocyte projections ($p<0.02$) in all rats and the number of astrocytes in NON rats ($p<0.002$). High sucrose intake had no effect on hypothalamic IL6, IL1β or TNFα mRNA levels.
Conclusions: Although increased weight on a normal diet due to NON was associated with activation of glial markers, a sucrose-enriched diet reduced them. Thus, the relationship between hypothalamic inflammation/gliosis and obesity may differ depending on the dietary/metabolic status of the individual.

P1-d1-242 Fat Metabolism and Obesity 1

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

Eva Baquedano[1]; Yolanda Diz-Chaves[2]; Luis Miguel García-Segura[2]; Julie A. Chowen[1]; Jesús Argente[1]; Laura M. Frago[1]
[1]Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, CIBERobn, Endocrinology, Madrid, Spain; [2]Instituto Ramón y Cajal, CSIC, Functional and systemic neurobiology, Madrid, Spain

Background: Gestational stress affects fetal growth and has long-term effects on the response to later metabolic challenges. Increased sucrose consumption is suggested to be involved in the rise in obesity and some of the long-term effects of early stress differ between males and females.
Objective and hypotheses: Our aim was to investigate whether prenatal stress affects metabolic parameters in response to a sucrose enriched diet in adulthood and if this response is sexually dimorphic.
Methods: Pregnant C57BL/6 mice were subjected to restraint stress from gestational day 12 to delivery. After weaning, mice were allowed to eat normal chow ad libitum. At 2 months of age 8 mice of each group were given a solution of 33% sucrose instead of water for 2 weeks and then sacrificed. The experimental groups were: control+water (CtW), control+sucrose (CtS), prenatal stress+water (PSW) and prenatal stress+sucrose (PSS).
Results: Serum leptin levels increased in PSS males and in CtS and PSS females. Both prenatal stress and sucrose intake decreased insulin and IGF-I levels. CTS and PSS males had increased POMC and PSW increased NPY mRNA levels. POMC and NPY mRNA levels were decreased in CtS, PSW and PSS females (Table 1).

	MCTW	MCTS	MPSW	MPSS	FCTW	FCTS	FPSW	FPSS
Leptin	1048 ±116	1255 ±137	1136 ±190	1818 ±238*#$	727 ±69	999 ±143*#	488 ±73	855 ±200*#
Insulin	462 ±96	126 ±30*	92 ±14*	165 ±45*	330 ±52	199 ±43*	162 ±50*	21 ±5*#$
IGF-I	685 ±10	348 ±23*	295 ±15*	487 ±7*#$	740 ±10	375 ±51*	533 ±92*	528 ±45*
NPY	100 ±22	125 ±28	522 ±225*$	91 ±34#	100 ±28	35 ±17*	21 ±6*	12 ±3*
POMC	100 ±6	609 ±184*#	155 ±49	392 ±78*#	100 ±42	44 ±6*	20 ±12*	57 ±11*

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

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

P1-d1-243 Fat Metabolism and Obesity 1

Leptin-induced depletion of hippocampal somatostatinergic system promotes its anorexigenic effects

Vicente Barrios[1]; Arancha Perianes-Cachero[2]; Emma Burgos-Ramos[1]; Lilian Puebla-Jiménez[2]; Sandra Canelles[1]; Jesús Argente[1]; Eduardo Arilla-Ferreiro[2]
[1]Hospital Infantil Universitario Niño Jesús, Endocrinology, Madrid, Spain; [2]Universidad de Alcalá, Biochemistry and Molecular Biology, Alcalá de Henares, Spain

Background: Recent evidence indicates that the hippocampus, a brain area critical in learning and memory, is involved in the regulation of food intake and energy homeostasis. Leptin and somatostatin, as well as their receptors are widely distributed in this area and have opposite functions in energy regulation in other brain structures. In addition, several actions of these hormones are mediated by opposite changes in intracellular cAMP levels.
Hypothesis: Leptin-mediated suppression of food intake is related to changes in the hippocampal somatostatinergic system.
Methods: We studied 18 male Wistar rats which were divided into three groups; controls (C), rats treated icv for 14 days with 170 μg of leptin (L) and a pair-fed group (PF) that received the same food amount consumed by L. Somatostatin content was measured by RIA, somatostatin receptors by a binding assay and activity of adenylyl cyclase (AC) by a functional assay. The levels of the inhibitory G protein (Gi) subunits 1-3 and AC-I and AC-V/VI isoforms, as well as activation of signal transducer and activator of transcription 3 (STAT3), protein kinase B (Akt) and cyclic AMP response element binding protein (CREB) were determined by Western blot.
Results: The density of hippocampal somatostatin receptors is increased in PF and decreased in L, being due to changes in somatostatin receptor 2 protein levels. These changes in PF are concurrent with activation of hippocampal Akt and CREB. The inhibitory effect of somatostatin on AC activity, however, was lower in L group, coincident with lower G inhibitory α3 and higher AC-I isoform levels and STAT 3 activation.
Conclusions: These results suggest that activation of somatostatinergic system after food restriction may be a mechanism to potentiate the hippocampal orexigenic mechanisms in a situation of metabolic demand, whereas depletion of this inhibitory system after leptin infusion may represent a mechanism to potentiate anorexigenic effects of leptin.

P1-d1-244 Fat Metabolism and Obesity 1

The Val/Met-polymorphism and serum levels of BDNF in German children and adolescents: impact on metabolic parameters associated with obesity

Agnes Kalenda; Kathrin Landgraf; Dennis Löffler; Kathrin Dittrich; Madlen Neef; Wieland Kiess; Antje Körner
University of Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany

Background: The neurotrophin BDNF plays an important role in the development of neuronal structures and is found altered in clinical entities such as depression, anxiety and eating disorders. In recent years, a direct interaction with metabolic pathways has been discussed. However, rare data exist on a possible connection between BDNF and early-onset obesity.
Objective and hypotheses: The aim of our study was to investigate the association of both *BDNF* polymorphism rs6265 and BDNF serum levels with metabolic parameters in children.
Methods: We genotyped the Val/Met-polymorphism rs6265 of *BDNF* in 1171 lean and 1002 obese children and adolescents. Furthermore, we measured BDNF serum levels by implementing an ELISA in a subset of 371 children. Statistical analyses were performed in order to investigate an influence of BDNF on parameters of glucose and lipid metabolism.
Results: BMI-SDS was significantly lower in carriers of the minor Met-allele (r= -.071; p=0,001). Moreover, Met-allele carriers showed a reduced increase in levels of blood glucose following oral glucose challenge (AUC blood glucose: r= -.166; p=0,014) and reduced HbA1c levels (r= -.181; p=0,017), independent of BMI-SDS. This was only seen in the post-pubertal stage. An association of the polymorphism with fasting blood glucose or insulin parameters was not observed. BDNF serum levels were not changed in Met-allele carriers compared to wildtype. Furthermore, they were neither correlated with BMI-

SDS nor with parameters of glucose and lipid metabolism.
Conclusions: The Met-allele of rs6265 is associated with lower body weight in children and adolescents. A lower increase in blood glucose of Met-allele carriers following food intake might be a contributing factor in this context. Serum BDNF levels are not associated with obesity-related parameters in our cohort.

P1-d1-245 Fat Metabolism and Obesity 1

The zebrafish as a model for the characterization of MTCH2, NEGR1, and TMEM18 as potential regulators of adipogenesis in vivo

Kathrin Landgraf; Falk Bernhard; Wieland Kiess; Antje Körner
Centre for Pediatric Research, Hospital for Children and Adolescents, Dept. of Women and Child Health, University of Leipzig, Leipzig, Germany

Background: Genome-wide association studies have identified genes associated with obesity, but their biological function in the context of adipose tissue development has not been resolved. In previous studies, we have shown a role of *MTCH2*, *NEGR1* and *TMEM18* as potential regulators of human adipogenesis *in vitro*.
Objective and hypotheses: The aim of this study is to investigate a potential regulatory role of the obesity-associated genes *MTCH2*, *NEGR1* and *TMEM18* during adipose tissue development *in vivo* by applying the zebrafish (*Danio rerio*) as a model organism.
Methods: We identified orthologs of *MTCH2*, *NEGR1* and *TMEM18* in the zebrafish. We analysed their expression profile in sequential developmental stages from fertilization to adult organs by quantitative *real-time* PCR and *in situ* hybridization. In addition, we assessed the effect of high-fat diet and starvation on candidate gene expression.
Results: *In situ* hybridization analyses of early developmental stages (4 hpf-96 hpf) revealed that *mtch2* expression was mainly present in the developing brain, liver, and intestine, while *negr1* was restricted to neural tissues (eyes, brain, spinal cord). The expression of *mtch2*, *negr1* and *tmem18* was significantly up-regulated at 9 dpf. At this stage, we could also detect first visceral adipocytes by Nile red staining. Moreover, *mtch2*, *negr1* and *tmem18* were expressed in adipose tissue of adult zebrafish. Interestingly, both *mtch2* and *tmem18 mRNA* levels were down-regulated in adipose tissue after 7 days of high-fat diet. In contrast, *negr1* expression was not affected by high-fat diet but up-regulated after starvation.
Conclusions: In zebrafish, *mtch2*, *negr1* and *tmem18* are activated with the start of adipogenesis and regulated by nutrition indicating a potential role during adipogenesis *in vivo*.

P1-d1-246 Fat Metabolism and Obesity 1

Circadian clock gene expression in leukocytes of obese and lean subjects

Tobias Drechsler; Isabel Wagner; Daniela Friebe; Kathrin Dittrich; Dennis Löffler; Julia Gesing; Wieland Kiess; Kathrin Landgraf; Antje Körner
University of Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany

Background: Recent studies described associations of clock genes with glucose and fat metabolism. However, a potential association of clock gene expression with obesity in humans has not been assessed so far.
Objective and hypotheses: The aim of this study was to assess potential differences in clock gene expression levels in obese and lean subjects.
Methods: Based on previous publications, we selected three clock-genes (*CLOCK, CRY1,* and *PER2*) and assessed their expression in leukocytes isolated of lean and obese subjects. We analysed the expression profile of each gene during a 29h period including standardized meals, oral glucose tolerance test, resting period and a 30 minute sport unit, and assessed the response to nutrition, sport and oral glucose load.
Results: Measuring *CLOCK, CRY1,* and *PER2*, we could detect circadian rhythmicity. Moreover, clock gene expression of Per2 was lowered to 78.4% in a subject with BMI 30.8 and to 13.7% in a subject with BMI 49.7 compared to a lean control, which points to a correlation between clock gene expression and BMI. Additionally, we observed an increased expression after the meals and oral glucose load, indicating an effect of metabolic factors on clock gene expression levels.
Conclusions: Our data support an association between clock-gene expression and BMI, indicating a bigger role of clock genes in obesity and obesity related diseases.

P1-d1-247 Fat Metabolism and Obesity 1

GHR exon 3 polymorphism and metabolic parameters in obese children

Felix Schreiner[1]; Christian L Roth[2]; Bettina Gohlke[1]; Joachim Woelfle[1]
[1]Children's Hospital, University of Bonn, Pediatric Endocrinology, Bonn, Germany; [2]Seattle Children's Research Institute, Endocrinology, Seattle, WA, United States

Background: The growth hormone receptor exon 3 polymorphism (fl = full length / d3 = deletion of exon 3) has been associated with better growth response to rhGH in several study cohorts. We previously reported this variant to be associated with spontaneous catch-up growth and metabolic parameters such as HbA1c and fasting IGFBP1 levels in former extremely low birth weight preterm infants. Recently GHRd3 has also been linked to metabolic properties in obese children.
Objective: We analyzed auxological and metabolic parameters with respect to the GHR exon 3 polymorphism in a cohort of 97 obese children (mean age 10.8 yrs; range 3.9 -17.2 yrs).
Results: Genotype frequencies (fl/fl 54%; fl/d3 38%; d3/d3 8%) were comparable with those reported from non-obese children. Auxological parameters including BMI-SDS (fl/fl 2.74±0.56; d3-carrier 2.71±0.50) did not differ between genotype groups. We also did not find significant associations with IGF-I-SDS, IGFBP-3-SDS, or parameters of glucose metabolism such as fasting glucose, insulin, HOMA-IR, HbA1c, and IGFBP-1. Ghrelin levels, which significantly correlated with age (R -0.30), BMI-SDS (R -0.21), IGF-I (R -0.47), IGFBP3 (R -0.30), IGFBP1 (R 0.53), fasting insulin (R -0.27), and leptin (R -0.43), appeared to increase with every GHR d3-allele inherited (fl/fl 1074.6 ±329.3; fl/d3 1199.6 ±486.6; d3/d3 1325.5 ±599.4 pg/ml; p=0.049 after correction for age; p=0.030 when analyzing only prepubertal children (n=41)). Leptin levels did not differ between genotype groups.
Conclusions: In summary, we did not find significant effects of the GHR exon 3 genotype on body size or parameters of glucose metabolism. However, Ghrelin levels known to be decreased in obese as compared to non-obese children seem to be lowest in fl/fl-homozygous individuals. Given the still incomplete understanding of the role of GHRd3 in growth and metabolism, this finding warrants further investigation.

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Dysfunction of autonomic nervous system in overweight and obese children

Petra Baum[1]; David Petroff[2]; Joseph Classen[1]; Wieland Kiess[3]; Susann Blüher[3]
[1]University of Leipzig, Department of Neurology, Leipzig, Germany; [2]University of Leipzig, Clinical Trial Centre; IFB Adiposity Diseases, Leipzig, Germany; [3]University of Leipzig, Department of Women and Child Health, Hospital for Children and Adolescents; IFB Adiposity Diseases, Leipzig, Germany

Background: Autonomic nervous system (ANS) dysfunction has been implicated in childhood obesity, but data are scarce and conflicting.
Objective and hypotheses: We have assessed ANS dysfunction (sympathetic and parasympathetic) by three different methods in normal weight and obese, otherwise healthy children.
Methods: Parasympathetic and sympathetic ANS function was assessed in 159 children (mean age 12.1y; 98 overweight/obese (BMI>90.percentile), 50 boys; 61 normal weight, 34 boys) by analysis of heart rate variability (low frequency power ln(LF), high frequency power, ln(HF); ln(LF/HF) ratio and ratio of longest RR interval during expiration to shortest interval during inspiration (E/I ratio), sympathetic skin response (SSR), and quantitative pupillography (pupil diameter in darkness, light reflex amplitude, latency, constriction velocity, re-dilation velocity). A potential dependence of each quantity on BMI-SDS, age, gender and pubertal stage was assessed in a linear model using Akaike's information criterion. Additional analyses were performed by ANOVA with Bonferroni-Holm adjustment. Statistical significance was set at 5%.

Results: E/I ratio (p=0.007), ln(HF) (p=0.001), pupil diameter in darkness (p=0.007), and pupil re-dilation velocity (p=0.03) were negatively correlated with BMI-SDS. Gender, but not BMI-SDS, was significantly associated to SSR (lower limbs: p=0.01; upper limbs: p=0.02). Latency of pupillary constriction showed a significant dependence on age (p=0.02).
Conclusions: These findings demonstrate widespread ANS dysfunction in overweight and obese children and adolescents, involving several organ systems. Both parasympathetic and sympathetic activity is reduced. The pattern of ANS dysfunction resembles that observed in normal-weight diabetic children and adolescents.

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

Ebe D'Adamo; M. Loredana Marcovecchio; Tommaso de Giorgis; Chiara De Leonibus; Francesco Chiarelli; Angelika Mohn
University of Chieti, Department of Paediatrics, Chieti, Italy

Background: Liver steatosis is a frequent finding in obese children, and oxidative stress appears to be one of the main factors implicated in its pathogenesis. In obese adults, treatment with vitamin E has resulted in an improvement in liver histology, but there are no available data in children.
Objective and hypotheses: Our aim was to assess whether oral supplementation with Vitamin E might reduce oxidative stress in obese prepubertal children with steatosis.
Methods: 42 obese prepubertal children (16M/26F; BMI SDS: 3.2±0.5) affected by steatosis were randomised to treatment with vitamin E (n=21), at a dose of 600 mg/day, or placebo for 6 months. At baseline (T0) and after 6-month treatment (T6), BMI, oxidative stress (urinary isoprostanes (PGF-2α)), alanine aminotransferases (ALT), high sensitivity C-reactive protein (hsCRP), glucose and insulin were assessed. HOMA-IR was used as an index of insulin resistance.
Results: The obese and control groups were comparable for age (8.3±1.6 vs 8.4±1.3 yr), sex (8M/13F) and BMI SDS (3.3±0.5 vs 3.1±0.4). In addition, at the beginning of the study, PGF-2α, HOMA-IR, ALT and hsCRP were similar between the two groups (all p>0.05) (Table). After 6-month treatment, levels of PGF-2α significantly decreased in children treated with vitamin E (p<0.001). A significant reduction was also found in ALT (p=0.001) and HOMA-IR (p<0.001). In contrast, no significant change in any of these markers was detected in the placebo group (Table).

	Vitamin E group (T0)	Vitamin E group (T6)	P value	Placebo group (T0)	Placebo group (T6)	P value
BMI SDS	3.3±0.5	3.0±0.5	NS	3.1±0.4	3.0±0.4	NS
PGF-2α(ng/ml)	1.0±0.3	0.5±0.2	<0.001	1.2±0.6	1.2±0.4	NS
HOMA-IR	3.5±1.5	2.1±1.2	<0.001	2.7±1.6	2.4±1.5	NS
ALT (U/L)	39.9±11.6	32.2±10.6	0.001	35.4±8.8	36.5±10.8	NS
hs-CRP (mg/dl)	0.4±0.3	0.4±0.3	NS	0.5±0.4	0.4±0.2	NS

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

P1-d1-250 Fat Metabolism and Obesity 1

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

M. Loredana Marcovecchio; Ebe D'Adamo; Stefania De Marco; Chiara De Leonibus; Francesco Chiarelli; Angelika Mohn
University of Chieti, Department of Paediatrics, Chieti, Italy

Background: Subclinical cardiac abnormalities and impaired autonomic nervous function represent predisposing factors for cardiovascular disease in obese adults. However, there are scant data in obese youths.
Objective and hypotheses: To evaluate early cardiac abnormalities and autonomic nervous function in obese children and adolescents.
Methods: Doppler two-dimensional-echocardiographic studies and 24-hour ECG monitoring were performed in 30 obese (13M/17F; age: 11.3±2.2 years; BMI SDS: 2.1 ±0.6) and 13 age and gender matched normal-weight (9M/4F; age: 12.7±3.3years; BMI SDS 0.3 ± 1.1) children and adolescents. Left atrial (LA) and ventricular (LV) geometry were measured. LV diastolic function was assessed by mitral inflow velocities (peak early (E) and late (A) waves, the E/A ratio and E-wave deceleration time (DcT)). Myocardial flow velocities, including early and late diastolic mitral annular velocity (Em and Am) and their ratio (Em/Am), were also acquired. 24h ECG parameters included low- (LF) and high-frequency (HF) power, LF/HF ratio, and time-domain variables (SDNN, SDNNi, rMSSD, pNN50).
Results: In the obese group LA size was significantly increased compared to the control group, as indicated by higher maximal and minimal LA diameters and area (table). Obese children also showed diastolic filling abnormalities, as indicated by higher values of E, DcT, and Am and decreased Em/Am ratio.

	Obese group	Non-obese group	P
Maximal LA diameter (cm)	4.76 ± 0.47	4.23 ± 0.68	0.02
Minimal LA diameter (cm)	3.45 ± 0.51	2.98 ± 0.48	0.01
Left atrial area (cm2)	14.42 ± 2.57	10.76 ± 3.55	0.005
Left ventricular mass (g/m2)	69.77±13.58	61.48±11.62	NS
E (cm/sec)	96.8 ± 14.05	85.83 ± 12.4	0.02
A (cm/sec)	52.5 ± 11.88	50.83 ± 14.43	NS
E/A	1.92 ± 0.48	1.8 ± 0.54	NS
DcT (cm/sec)	193.15 ± 36.57	162.3± 25.96	0.02
Am (cm/sec)	9.18 ± 2.83	6.92 ± 1.83	0.01
Em (cm/sec)	20.14 ± 4.3	19.75 ± 2.09	NS
Am/Em	2.36 ± 0.78	3.07 ± 0.87	0.03

A significant and independent association with BMI SDS was found for all the above parameters (LA area: β=0.36; Am:β=0.34; Em/Am ratio: β-0.36, all p<0.05). 24-h ECG monitoring showed a decreased LH/HF ratio in obese children compared to controls (1.39±0.9 vs 2.12±0.94, p=0.006), whereas the other ECG parameters were similar between the two groups.
Conclusions: Obese children showed increased atrial size and impaired LV diastolic function associated with an increased parasympathetic tone, suggesting an increased cardiovascular risk associated with childhood obesity.

P1-d1-251 Fat Metabolism and Obesity 1

Apelinemia and the G212A APJ receptor gene polymorphism in obese children and adolescents

Eleni P Kotanidou[1]; Kalliroi Kalinderi[2]; Styliani Fidani[2]; Eleni Papakonstantinou[3]; Eufimia Papadopoulou-Alataki[4]; Assimina Galli-Tsinopoulou[4]
[1]Faculty of Medicine, 4th Department of Pediatrics, General Hospital Papageorgiou, Thessaloniki, Greece; [2]Faculty of Medicine, Aristotle University of Thessaloniki, Department of General Biology, Thessaloniki, Greece; [3]Faculty of Medicine, Aristotle University of Thessaloniki, 2nd Department of Pharmacology, Thessaloniki, Greece; [4]Faculty of Medicine, Aristotle University of Thessaloniki, 4th Department of Pediatrics, General Hospital Papageorgiou, Thessaloniki, Greece

Background: Data on serum apelin levels in obese children(OC) are few and controversial. No data are available concerning APJ gene mutations on childhood obesity.
Objective and hypotheses: To measure serum apelin levels in OC and adolescents(OA) and search for possible associations between them and the presence of the G212A APJ gene polymorphism.
Population and methods: Eighty-five obese individuals (43 children) and 45 lean matched for age/gender were enrolled in the study. All obese patients underwent oral glucose tolerance test (OGTT) and genomic DNA was extracted from peripheral blood amplified by PCR for genotyping G212A APJ gene polymorphism. Results are shown in Tables 1,2. Apelin levels were significantly lower in obese participants than in controls.
The G121A polymorphism genotype frequency distribution did not differ between obese (GG=54.1%, GA=38.8%, AA=7.1%) and the HapMap polymorphism frequency distribution of the Caucasians (GG=46.9%,GA=39.8%,AA =13.3%,p=0.232). The G212A genotype was associated with significantly different apelin levels (One-WayANOVA test: p=0.016). The Bonferroni post-hoc test revealed that GG and GA group had significantly lower apelin levels

compared to the AA group (p=0.013/p=0.029, respectively). Apelinemia did not differ between GG and GA group.

Table 1. Sample characteristics	Obese (n=85)	Lean (n=45)	p-value
Age (years)	11.01±2.8	10.75±3.72	0.690
BMI(kg/m2)	28.84±4.93	18.48±2.77	<0.001
Apelin(ng/ml)	1.62(0.67-3.85)	2.13(1.4-3.13)	<0.001
HOMA-IR	3.01(0.77-14.68)	1.67(0.49-2.55)	<0.001

Table 2. Apelin levels in G212A polymorphism genotype groups	GG	GA	AA	Total
Number	46 (54.1%)	33 (38.8%)	6 (7.1%)	85 (100%)
Apelin(ng/ml)	1.62±0.71	1.7±0.74	2.57±0.86	

Conclusions: Serum apelin levels are lower in OC and OA than in lean individuals. A correlation of the 212A allele with apelin levels might propose the APJ gene as a modifier in the apelinergic system.

P1-d1-252 Fat Metabolism and Obesity 1

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

Cosimo Giannini[1]; Maria Loredana Marcovecchio[1]; Ebe D'Adamo[1]; Tommaso de Giorgis[1]; Francesco Chiarelli[1]; Chris Kelnar[2]; Angelika Mohn[1]
[1]University of Chieti, Department of Pediatrics, Chieti, Italy; [2]University of Edinburgh, Department of Pediatric Endocrinology, Edinburgh, United Kingdom

Background: Asymmetric dimethylarginine (ADMA), a competitive inhibitor of nitric oxide synthase (NOS), is a risk marker for cardiovascular disease, a relevant issue in obesity. The aim of the present study was to evaluate ADMA and Nitric Oxide (NO) concentrations as well as oxidative stress (PGF-2α), and define their association with twenty-four hour ambulatory blood pressure (ABP) in obese adolescents.
Methods: A group of 87 obese adolescents were recruited and compared with 51 healthy age and gender matched peers. In all subjects, fasting blood samples were obtained for the evaluation of ADMA, NO, insulin, blood glucose and lipid profile, and urine samples, for urinary PGF2α measurement. Blood pressure was evaluated by a 24-hour ABP monitoring in all subjects.
Results: ADMA (0.711±0.166 vs 0.544±0.101 μmol/L, P<.001) and PGF-2α (21.61±14.25 vs 14.98±10.09 ng/ml, P=.015) values were significantly increased while NO significantly reduced (48.97±15.67 vs 90.38±47.89 mmol/L, P<.001) in obese adolescents compared to controls. 24-h systolic and diastolic blood pressure (SBP and DBP) as well as daytime and nighttime SBP and DBP were significantly higher in obese compared to lean subjects (all P<.009). After dividing the obese group by tertiles of ADMA, SBP, DBP and the percent of non-dipper subjects progressively and significantly increased across tertiles. Multiple regression analysis showed that ADMA concentrations were significantly associated with oxidative stress and BMI-SDS.
Conclusion: Obese youth present increased ADMA and decreased NO levels compared to healthy peers and these markers are associated to impaired blood pressure regulation. Adiposity and oxidative stress represent the major factors influencing ADMA levels.

P1-d2-253 Fat Metabolism and Obesity 2

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

Lenka Dusatkova[1]; Hana Zamrazilova[1]; Barbora Sedlackova[1]; Josef Vcelak[2]; Petr Hlavaty[1]; Bela Bendlova[2]; Marie Kunesova[1]; Vojtech Hainer[1]
[1]Institute of Endocrinology, Obesity Management Center, Prague, Czech Republic; [2]Institute of Endocrinology, Department of Molecular Endocrinology, Prague, Czech Republic

Background: Genome-wide association studies have identified several gene variants associated with body mass index (BMI) and obesity so far.
Objective and hypotheses: We performed a replication study of 9 previously reported variants in/near genes *PCSK1* (rs6232, rs6235), *BDNF* (rs925946, rs4923461), *SEC16B* (rs10913469), *TMEM18* (rs7561317), *SH2B1* (rs7498665), *KCTD15* (rs29941) and *FTO* (rs9939609) in the Czech adolescent population. We investigated their association with BMI status (underweight, overweight and obesity) and related anthropometric parameters.
Methods: Genotyping was performed in 1443 adolescents, including 670 overweight/obese (BMI ≥90th percentile), 713 normal weight (BMI 10—90th percentile) and 60 underweight (BMI <10th percentile) adolescents aged 13—18 years. Anthropometric parameters were assessed in all individuals.
Results: *FTO* rs9939609 was associated with overweight and obesity (OR = 1.28, 95%CI 1.02—1.60, p = 0.04 and OR = 1.47, 95%CI 1.24—1.73, p < 0.001, respectively). This variant was associated with body weight, BMI, abdominal and hip circumference, body and trunk fat (p < 0.001). The minor allele of *SEC16B* rs10913469 increased risk of overweight/obesity (OR = 1.24, 95%CI 1.02—1.51, p = 0.04) and values of body weight, BMI, abdominal circumference and body fat (p < 0.05). The remaining variants had not significant odds ratios and only slight impact of the increasing number of risk alleles on body weight, BMI, abdominal and hip circumference, body and trunk fat was observed (p < 0.05). Interestingly, the risk G-allele of *TMEM18* rs7561317 was negatively associated with underweight (OR = 0.56, 95%CI 0.36—0.88, p = 0.02).
Conclusions: In addition to widely replicated *FTO* gene variant we confirmed the association of the *SEC16B* variant with overweight/obesity and related traits in our population sample. The risk variant of *TMEM18* was not associated with obesity, but it seems to be protective against underweight.

P1-d2-254 Fat Metabolism and Obesity 2

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

Diego Yeste[1]; Claudia Marhuenda[2]; Maria Patricia Mesa[1]; Gabriela Guillem[2]; Vicente Martinez - Ibañez[2]; Antonio Carrascosa[1]
[1]Hospital Vall d'Hebron, Paediatric Endocrinology, Barcelona, Spain; [2]Hospital Vall d'Hebron, Paediatric Surgery, Barcelona, Spain

Introduction: The intragastric balloon (IGB) is a non-invasive and reversible procedure and may constitute a useful tool together with behaviour and life-style changes in the treatment of morbidly obese adolescents prior to the decision to perform bariatric surgery.
Aims: Evaluation of results obtained at 18-24 months following IGB withdrawal in a group of morbidly obese adolescents.
Patients and methods: Eight candidates (5 girls, 3 boys; age range: 13.9 -17.9 years) were selected. All had BMI > 40 with one or more severe comorbidities and met the internationally-accepted criteria for bariatric surgery. IGB (B.I.B. Bioenterics ®) were inserted endoscopically under general anaesthesia and left in place for six months. Patients followed a strict calorie-controlled diet.
Results: Patient clinical data, weight loss, BMI and BMI-z score at IGB removal at six months and at 18-24 months post-removal are shown in the table. Four patients maintained weight loss after IGB withdrawal (BMI loss: -11.0 ± 3.7). The remaining patients recovered or slightly gained their pre - IGB (+2.3 ± 3.7).

Age (yrs)	Sex (Female/ Male)	Weight (kg)	BMI (kg/m2)	BMI 6m post-IGB removal	Weight 18-24m post-IGB removal	IMC 18-24m post -IGB removal (kg/m2)	Δ BMI
13.9	F	129.5	49.2	39.8	92.0	35.0	-14.2
15.2	F	92.6	38.3	31.3	70.0	28.9	-9.4
14.9	F	135.0	54.4	41.4	100.0	40.3	-14.1
17.9	M	135.8	42.2	31.4	114.0	35.6	-6.6
15.8	F	116.5	42.7	38.5	118.0	43.3	+0.6
16.7	M	164.0	52.9	45.9	165.0	53.0	+0.1
15.2	F	109.0	40.5	31.9	116.0	43.1	+2.6
14.0	M	143.0	49.4	46.7	165.0	55.0	+5.6

Conclusions: In our experience IGB is a safe procedure with few complications. 50% of IGB patients presented significant weight loss at 18-24 months post - IGB withdrawal.

P1-d2-255 Fat Metabolism and Obesity 2

Relationship of thyroid function with body mass index, insulin-resistance and lipid status in nutritionally obese children and adolescents

Malgorzata Wasniewska; Tommaso Aversa; Mariella Valenzise; Giuseppina Salzano; Filippo De Luca
University of Messina, Department of Pediatrics, Messina, Italy

Background: In recent years, there has been an increasing attention to thyroid function in paediatric obese patients due to its possible role as metabolic and cardiovascular risk factor.
Objectives: a) to ascertain the association between thyroid function, lipid status and insulin resistance (IR) in nutritionally obese children and adolescents; 2) to evaluate the frequency of hyperthyrotropinemia in our cohort.
Design: Cross-sectional study.
Methods: We examined 311 obese children and adolescents (163 females), mean age 9.2 ± 2.8 yrs (62.7% prepubertal) from a iodine sufficient region. Anthropometric, metabolic and hormonal variables were determined when patients were referred to our Outpatients Clinic during the period 2005-2010. Patients with thyroid autoimmune disease (TAD) and/or other chronic diseases were excluded.
Results: Hyperthyrotropinemia (TSH >4.5 mIU/L) was diagnosed in only 9 cases (2.9%, 2 females). In the remaining 302 euthyroid subjects (161 females) we did not find any correlation between TSH levels and BMI-SDS, lipid panel and IR. Conversely, FT4 levels were correlated with both BMI-SDS (r=0.139, p=0.016) and HDL cholesterol levels (r=-0.150, p=0.018). There were no differences of FT4 levels, lipid status and HOMA-IR between patients with TSH <2.5 mIU/L and those with TSH 2.5 - 4.5 mIU/L. Among the 187 prepubertal children, HOMA-IR was significantly higher in the 130 subjects with TSH between 2.5 and 4.5 mIU/L compared with those with TSH <2.5 mIU/L (3.41 ± 2.87 vs 2.60 ± 1.92, p = 0.026). No differences were found in pubertal adolescents.
Conclusions: In our paediatric obese population: 1) the prevalence of hyperthyrotropinemia resulted only slightly higher than that reported in general paediatric population (2.9 vs 1.7%), probably due to the preliminary exclusion of subjects with TAD; 2) TSH levels were not correlated with the severity of the obesity; 3) among the euthyroid prepubertal obese children IR was significantly higher in the subgroup with TSH between 2.5 and 4.5 mIU/L.

P1-d2-256 Fat Metabolism and Obesity 2

Leptin upregulates expression of TNF-α in mouse alveolar macrophage through increased PLD activity

Se Min Lee[1]; So Hyun Park[2]
[1]College of Medicine, Hanyang University, Department of of Pediatrics, Seoul, Republic of Korea; [2]College of Medicine, The Catholic university of Korea,, Department of of Pediatrics, Seoul, Republic of Korea

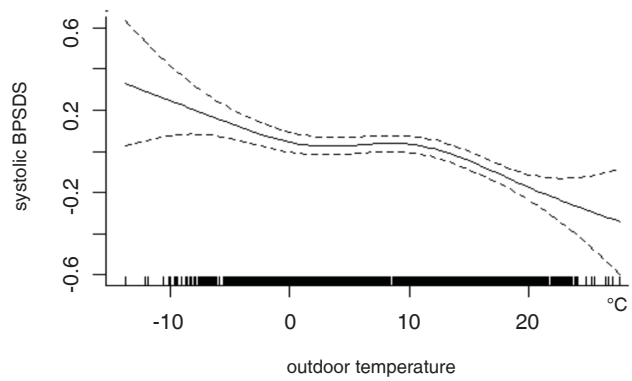
Background: Obesity is presumed to be associated with pathogenesis of asthma
Objective and hypotheses: The purpose of this study was to identify the role of phospholipase D1 (PLD1) in leptin-induced TNF-α production and to suggest molecular linkage between obesity and asthma.
Method: We investigated whether leptin, a classic adipocytokine, receptor take a role for expression and production of proinflammatory cytokine, TNF-α through increased PLD activity in mouse alveolar macrophage (Raw 264.7).
Results: Dominant negative PLD1 decreased leptin-induced TNF-α expression and production. Treatment of Leptin activated the phospholipase Cγ (PLCγ)/Src/mTor/JNK /p38 MAPK pathway. Leptin-induced PLD activation was attenuated by PLC γ inhibitors (PAO), Src inhibitor (PP2). These results indicate that PLCγ, Src act as upstream activators of PLD in leptin-treated Raw 264.7 cells. Furthermore, expression and production of TNF-α increased by leptin were also blocked by inhibition of PLCγ, Src, mTor, JNK, p38 MAPK. Taken together, PLD1 acts as an important regulator in leptin-induced expression and production of TNF-α in Raw 264.7 cells.
Conclusions: Thus, we suggest leptin may contribute to development of asthma through increase expression and production of TNF-α in Raw 264.7 cells. Our result support obesity might contribute to pathogenesis of asthma in molecular level.

P1-d2-257 Fat Metabolism and Obesity 2

Blood pressure measurements in children and adolescents: seasonal effects and tracking phenomena

André Miersch[1]; Mandy Vogel[2]; Ruth Gausche[2]; Wieland Kiess[3]
[1]University of Leipzig, Hospital for Children and Adolescents, Leipzig, Germany; [2]CrescNet, Leipzig, Germany; [3]University Hospital Leipzig, Hospital for Children and Adolescents, Leipzig, Germany

Background: High blood pressure even at a young age is considered a major risk factor for cardiovascular disease. Therefore, an understanding of variation and tracking of the blood pressure is necessary.
Objective and hypotheses: We have asked whether or not there is a seasonal variation, an influence of outdoor temperature and/or follow-up/tracking phenomena of blood pressure measurements in children and adolescents.
Methods: Blood pressure was routinely measured in healthy and sick children in outpatient clinics and clinical wards in a total of 6714 subjects (3497 boys and 3237 girls), age 3 to 21 years, median 10.6 years. Data were extracted from the CrescNet database. A generalized additive model was used to evaluate the variation of blood pressure measurements throughout the year. A subgroup of children was followed-up retrospectively for 2, 4 and 6 years.
Results: In a cross-sectional sub-analysis seasonal variation of systolic and diastolic blood pressure measurements in standard deviation score (SDS) with a range of about 0.395 SDS (systolic) was detected. The average outdoor temperatures correlated with the systolic blood pressure SDS (rho=-0.074 p=2.425e-09, confidence interval -0.0987 to -0.0500). The effect was most prominent at low temperatures (below 0° C/32° F) and at higher temperatures (above 10° C/50° F). After two years follow-up 46.6% of the children remained within their SDS track, after four years 35.1%, after six years 36.2%.



Conclusions: Blood pressure measurements in children are influenced by temperature and seasonal variation. The effect size caused by temperature is dependent on the climate and in temperate climate small compared to height or weight. Upon follow-up over six years about 65% of the children have left their blood pressure centiles (tracks). We conclude that blood pressure measurements are variable and sensitive to external influences. Repetitive measures and controlling for multiple co-variates is mandatory if one is to define blood pressure status.

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

Christian L Roth[1]; Clinton Elfers[2]; Andrew Hoofnagle[3]; Gregory Morton[3]; Matthew Yeh[3]; James Nelson[4]; Kris Kowdley[4]
[1]Pediatrics, University of Washington, Seattle Children's Research Institute, Seattle, United States; [2]Seattle Children's Research Institute, Center of Integrative Brain Research, Seattle, United States; [3]University of Washington, Medicine, Seattle, United States; [4]Virginia Mason Medical Center, Benaroya Research Institute, Seattle, United States

Background: Childhood obesity is associated with nonalcoholic fatty liver disease (NAFLD) and its more severe form, steatohepatitis (NASH). Recent studies have found associations between vitamin D deficiency (VDD), insulin resistance (IR) and NAFLD among overweight children.
Objective and hypotheses: To explore mechanisms and test whether VDD contributes to NASH progression.
Methods: We fed young (age 25d) Sprague-Dawley rats with a low-fat diet alone (LFD, group 1) or with vitamin D depletion (LFD+VDD, group 2). Additional rats were exposed to a Westernized diet (WD: high-fat/high-fructose corn syrup) that is more typically consumed by overweight children, and was either replete (WD, group 3) or deficient in vitamin D (WD+VDD, group 4). Liver histology was assessed using the NASH CRN scoring system and expression of genes involved in inflammatory pathways were measured in liver and visceral adipose tissue after 10 weeks.
Results: Weight gain, total caloric intake and Lee adiposity index were highest in the WD+VDD group. WD+VDD animals exhibited significantly greater hepatic steatosis compared to LFD groups. Lobular inflammation as well as NAFLD activity score (NAS) were higher in WD+VDD vs. the WD group (NAS: WD+VDD 3.2±0.47 vs. WD 1.50±0.48, $p<0.05$). In both LFD and WD animals, VDD stimulated inflammatory gene expression more in the liver than in adipose tissue. Hepatic mRNA levels of toll-like receptors TLR2, TLR4 and TLR9, as well as resistin, interleukins IL-1beta, IL-4 and IL-6, were higher in WD+VDD vs. WD animals ($p<0.05$). Logistic regression analyses showed significant associations between NAS and liver mRNA levels of endotoxin receptor CD14, TLRs 2,4, and 9.
Conclusions: VDD exacerbates NAFLD through TLR-activation possibly via endotoxin exposure and increased gut leakiness in a Westernized diet rat model. In addition it causes higher hepatic resistin gene expression, and up-regulation of hepatic inflammatory genes. These findings have implications for human NAFLD and also provide a novel model for experimental NASH.

P1-d2-259 Fat Metabolism and Obesity 2

Adipocyte Aquaglyceroporin 7 (AQP7) Expression in obese children: A morbidity marker of obesity

Eleni Oikonomou[1]; Alexia Karvela[1]; Eirini Matsigkou[1]; George Georgiou[2]; Bessie E. Spiliotis[1]
[1]University of Patras, Research Laboratory of the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, School of Medicine, Patras, Greece; [2]Karamandaneio Childrens Hospital, Department of Pediatric Surgery, Patras, Greece

Background: Childhood obesity predisposes to the development of metabolic disorders during adolescence. Adipose tissue is an endocrine organ which adapts to the body's energy requirements. The efflux of adipocyte glycerol, regulated by AQP7, influences lipid and glucose homeostasis. Impaired expression of AQP7 in adults leads to adipocyte hypertrophy. Three isoforms of AQP7 (41,37and 34kDa) have been identified in rodents.
Objective and hypotheses: To study AQP7 in lean and obese pre-pubertal children and adolescents to determine:(a)its physiological expression and (b) its involvement in childhood obesity.
Methods: Primary cultures of adipocytes were developed from surgical adipose tissue biopsies from 61 lean(BMI<85%) and 41 obese(BMI≥95%) prepubertal children (Groups A:2mos-7yrs and B:8-12 yrs) and adolescents (Group C:10-15yrs). mRNA expression of AQP7 was studied with RT-PCR and protein expression with Western Immunoblotting.
Results: AQP7 in the mature adipocytes showed: (1)that the 41 and 37kDa isoforms were significantly increased in the lean pubertal children of Group C vs. Group A and B respectively($p=0.001$,$p=0.009$), (2)significantly decreased AQP7 mRNA expression in the obese Group B($p=0.029$) and C($p=0.002$) children vs the obese of Group A,whereas the protein expression of the 41kDa isoform tended to decrease only in the obese Group C children and (3) a significant increase in the 41kDa isoform in the obese prepubertal Group A children($p=0.005$).
Conclusions: The increased protein expression of AQP7 in the lean adolescents possibly reflects the increased energy needs of puberty for glycerol. On the contrary, the obese pubertal children who have lower AQP7 mRNA expression and 41kDa isoform expression may possibly have impaired adipocyte glycerol efflux. The significant increase of the 41kDa isoform in the younger obese prepubertal children might suggest a protective mechanism against the development of adipocyte hypertrophy at this young age.

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The shape of the plasma glucose curve during an OGTT harbours information on whole body insulin sensitivity and β-cell function

Liene Bervoets[1]; Guy Massa[2]
[1]Hasselt University, Faculty of Medicine, Diepenbeek, Belgium; [2]Jessa Hospital, Department of Paediatrics, Hasselt, Belgium

Background: Up to now, little attention has been devoted to the possible information related to the shape of the oral glucose tolerance test (OGTT) curve.
Objective and hypotheses: We aimed to provide a classification and assessment of the prevalence of different shape types in postmenarcheal obese girls. It is hypothesized that the shape of the OGTT curve harbours valuable information on glucose metabolism.
Methods: We investigated the shape of the OGTT curve in 72 postmenarcheal obese girls (agemean: 14.5 ± 1.4 years; BMI SDSmean: 2.8 ± 0.6). Plasma glucose and insulin concentrations were measured. OGTT-derived indices were calculated. Subjects were classified into three shapes of OGTT-curve: monophasic, biphasic or triphasic. Subjects with a shape index (G90' – G120') > 0 were defined as monophasic; a shape index (G90' – G120') < 0 as biphasic; or a shape index (G60' – G90') < 0 as triphasic. The presence of the metabolic syndrome was assessed according to the IDF criteria.
Results: Most of the subjects showed a monophasic shape compared to bi- and triphasic shapes: 45.8, 37.5 and 16.6 %, respectively ($\chi^2 = 9.75$; $P = .008$). The shape index was positively associated with BMI SDS ($R^2 = 0.13$; $P = .002$); gAUC ($R^2 = 0.22$; $P = .000$); HOMA-%B ($R^2 = 0.05$; $P = .050$) and the number of components of the metabolic syndrome. The shape index was inversely related to whole body insulin sensitivity index ($R^2 = 0.06$; $P = .041$).
Conclusions: Subjects with a monophasic shape are at higher risk of having glucose abnormalities compared to subjects with a bi- or triphasic shape. A high shape index is indicative of increased pancreatic insulin secretion and low insulin sensitivity. Future and follow-up studies are needed to evaluate the usefulness of the shape of the OGTT curve to assess the risk for T2DM.

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Interleukin-1beta downregulates RBP4 secretion in human adipocytes

Primoz Kotnik; Michaela Keuper; Martin Wabitsch; Pamela Fischer-Posovszky
Ulm University Medical Center, Division of Pediatric Endocrinology and Diabetes, Ulm, Germany

Background: Obesity is a state of low-grade inflammation associated with an altered adipocyte secretion profile, causally leading to its metabolic complications. Retinol binding protein 4 (RBP4) is an adipokine implicated in these complications, especially insulin resistance.
Objective and hypotheses: To determine the effect of an inflammatory environment on RBP4 expression and secretion in human adipocytes.
Methods: Human adipocytes were cultured with conditioned media from human THP-1 macrophages (MacCM) or selected cytokines (TNFalpha, IL-1beta, IL-6 and IL-8). RBP4 production was studied by quantitative real-time PCR and ELISA. The correlation between RBP4 and IL-1 beta mRNA expression was studied in human adipose tissue explants.
Results: RBP4 mRNA expression and secretion was significantly reduced upon incubation with MacCM in SGBS (by ~70 % using 10 % MacCM for 48 h) as well as in human primary adipocytes. IL-1beta was identified as a new

and potent cytokine regulating RBP4, as it down-regulated RBP4 mRNA and secretion in a time- and dose-dependent manner (inhibition by ~50 % at 50 ng/ml after 48 h). Blocking IL-1beta signaling using a neutralizing IL-1R and a NFkB inhibitor (CAPE) abrogated the inhibitory effect of IL-1beta on RBP4 production. Most interestingly, RBP4 mRNA was negatively correlated with IL-1beta mRNA in subcutaneous adipose tissue obtained from 18 healthy female subjects ($R = -.535$; $p < 0.05$).
Conclusions: RBP4 expression and secretion was inhibited in an in vitro model of inflamed adipose tissue. IL-1beta was identified as a new and potent inhibitor of RBP4 production. Adipose tissue inflammation and increased RBP4 levels are associated with the development of insulin resistance. We found that inflammatory conditions lead to a downregulation of RBP4 in adipocytes suggesting that adipose inflammation and the increase in circulating RBP4 are two unrelated processes.

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

Shin Hye Kim; Park Mi Jung
Sanggye Paik Hospital, Inju University College of Medicine, Department of Pediatrics, Seoul, Republic of Korea

Background: Phthalates have documented biochemical activity as peroxisome proliferator-activated receptor activators and antiandrogens, which may contribute to the development of obesity and insulin resistance. Though a few studies have shown that concentrations of phthalates are associated with insulin resistance in adults, studies on association of phthalate concentrations with insulin resistance in children are limited.
Objective and hypotheses: We studied whether serum di(2-ethylhexyl) phthalate (DEHP) levels are associated with obesity and insulin resistance in Korean girls.
Methods: A total of 155 girls (53 obese/ 31 overweight cases and 71 controls; aged 6 to 13yr) were enrolled. Anthropometry, physical activity and nutrient intake were analyzed, and serum DEHP levels were measured by gas chromatography/mass spectrometry method.
Results: Geometric mean serum DEHP levels were higher in obese (191.3±272.2 ng/mL) and overweight (189.6±226.1 ng/mL) subjects than in controls (50.1±164.9 ng/mL, $P<0.0001$). According to the increased DEHP quartile, prevalence of overweight and obesity increased ($P<0.05$). Subjects in the top DEHP quartile had higher HOMA-IR(4.2±6.6 in Quartile 4 vs. 2.1±1.1 in Quartile 1, $P<0.05$) and fasting blood glucose levels ($P<0.05$) compared with the subjects in the lowest DEHP quartile. Serum DEHP levels showed positive correlation with fasting blood glucose ($r=0.30$, $P=0.001$), fasting insulin ($r=0.43$, $P<0.0001$) and HOMA-IR levels ($r=0.46$, $P<0.0001$), whereas it did not had significant correlation with serum ALT or lipid profiles. Subjects in the top DEHP quartile had an increased risk (Odds ratio = 4.05, 95% Confidence Intervals =1.11-14.74) for insulin resistance (defined as HOMA-IR $\geq$ 3.6) compared with the lowest quartile after adjusting for age, physical activity and total calorie intake.
Conclusions: Serum DEHP levels showed significant positive correlations with insulin resistance. Prospective studies are needed to determine potential causal links between DEHP exposure and insulin resistance in children.

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

Judit Bassols[1]; Prats-Puig Anna[2]; Gemma Carreras-Badosa[2]; Pilar Soriano-Rodríguez[3]; Mercè Montesinos-Costa[4]; Francis De Zegher[6]; Lourdes Ibáñez[6]; Abel López-Bermejo[2]
[1]Girona Institute for Biomedical Research, Pediatrics, Girona, Spain; [2]Biomedical Research Institute of Girona, Pediatrics, Girona, Spain; [3]Salut Empordà Foundation, Clinical Laboratory, Figueres, Spain; [4]Dr. Josep Trueta Hospital, Clinical Laboratory, Girona, Spain; [5]University of Leuven, Department of Woman & Child,, Leuven, Belgium; [6]Sant Joan de Déu Children's Hospital, Pediatric Endocrinology, Barcelona, Spain

Background: Chronic activation of the innate immunity is a key feature of the insulin resistance syndrome. Recent studies in mice suggest that B lymphocytes promote also insulin resistance by accumulating in adipose tissue and producing pathogenic IgG antibodies.
Objective and hypotheses: We aimed to study whether circulating IgG (seemingly including pathogenic antibodies produced by B cells) is associated with metabolic risk markers in asymtomatic prepubertal children.
Methods: Subjects were 177 school-age healthy prepubertal Caucasian children (93 girls and 84 boys) with normal height and weight distributions consecutively recruited among those seen within a setting of primary care. Circulating total IgG concentration, insulin resistance (HOMA-IR) and fasting lipids were assessed in all subjects. IgG levels were measured by nephelometry.
Results: Increasing IgG levels were associated with a less favorable metabolic phenotype, consisting of higher fasting insulin and HOMA-IR and lower HDL-cholesterol and HDL/TG ratio (all $p<0.005$ to $p<0.0001$). HOMA-IR increased by 60% (from 0.43 ± 0.1 to 0.69 ± 0.1) and the HDL/TG ratio decreased by 25% (from 1.60 ± 0.1 to 1.20 ± 0.1) from the lowest to the highest tertile of serum IgG. These associations were attenuated but remained significant after adjusting for confounding variables such as gender, age and BMI ($p<0.01$ to $p<0.001$).
Conclusions: A less favorable metabolic profile is observed in healthy prepubertal children with higher circulating IgG. These results suggest an association of adaptive immunity with energy metabolism in children.

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

Ceren Cetin; Firdevs Bas; Ruveyde Bundak; Ahmet Ucar; Banu Aydin Kucukemre; Nurcin Saka; Feyza Darendeliler
Istanbul University,Istanbul Faculty of Medicine, Department of Pediatrics, Pediatric Endocrinology Unit, Istanbul, Turkey

Background: Recent studies show that atherosclerotic process begins during childhood and clinical findings appear in adulthood. Being born large for gestational age (LGA) is a risk factor for metabolic disorders in advanced ages.
Objective and hypotheses: We aimed to investigate the risk of development of hypertension and dyslipidemia and to determine the plasma atherogenity index (AIP) and triglyceride/HDL cholesterol (C) ratio in in LGA born children in prepubertal ages.
Methods: Forty (19 femal,21male) LGA born prepubertal children (mean age 6.1±2.5 years) were evaluated with respect to total cholesterol, triglycerides, HDL-and LDL-C. Atherogenic index of plasma (AIP) was calculated as triglycerides/HDL-C ratio. To determine the cardiovascular risk, total cholesterol/HDL-C ratio and LDL-C /HDL-C ratio were calculated.Their data were compared to that of prepubertal 49 (25 female,24 male) appropriate for gestational age(AGA) children (mean age 5.4±1.8 year).
Results: LGA children were taller and heavier than AGA children but had similar BMISDS, waist/hip circumference ratio, skinfold thickness as AGA born children. Systolic blood pressure and diastolic blood pressure($p=0.030$,$p=0.009$,respectively) were higher in LGA children. There were no significant differences in triglycerides(TG) and VLDL-C levels between children born LGA and AGA. Total cholesterol and LDL-C ($p=0.004$,$p=0.000$,respectively) levels were higher in children born LGA than AGA. HDL-C levels were significantly lower in children born LGA than AGA($p=0.001$). AIP (TG/HDL-C) was higher in children born LGA than AGA($p=0.022$). Total.Cholesterol/HDL-C and LDL-C/HDL-C ratios were higher in children born LGA than AGA
Conclusions: Low HDL-C and high LDL-C levels and high AIP in LGA born prepubertal children with normal BMI SDS indicate that atherosclerotic changes start early in childhood ages even in the absence of obesity.

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Association of FTO, TCF7L2 gene single nucleotide polymorphisms with obesity and metabolic parameters in Chinese Han children and adolescents

Ruoqian Cheng[1]; Linfeng Cao[2]; Yi Yang[2]; Shui-xian Shen[1]; Feihong Luo[1]
[1]Children's Hospital of Fudan University, Department of Pediatric Endocrinology and Inborn Metabolic Diseases, Shanghai, China; [2]Children's Hospital of Fudan University, Pediatric Institute, Shanghai, China

Background: The prevalence of obesity in Chinese children and adolescents is ever increasing, but few studies focused on the genetic etiology.
Objective and hypotheses: To study the association of the SNP of FTO (rs9939609, rs1421085), TCF7L2(rs7903146) gene with the obese children and adolescents.
Methods: Subjects were divided into three groups: control, obese, and overweight group. Fasting Plasma Glucose (FPG), Triglyceride (TG), Total Cholesterol (TCH) and Fasting Insulin (Fins) were evaluated. Homeostasis Model of Assessment (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) were calculated to evaluate the insulin resistance. Taqman-MGB probe was used to detect genotypes.
Results: (1)The levels of FPG, FIns, TG and HOMA-IR were significantly higher in obesity /overweight group than that in normal control group. (2) FTO gene: The AA genotype frequency of rs9939609 w as 2.7% in obesity group and 0. 4% in overweight group and there existed significant difference compared with normal control group (genotype frequency 1.7%, P = 0.048, OR= 1.437). The CC genotype frequency o f rs1421085 was 2.7% in obesity group and 0.9% in overweight group and there existed significant difference compared with normal control group (genotype frequency 1.7%, P = 0.076, OR= 1.388). There existed significant difference in BMI between the rs1421085 TC + CC, rs9939609 TA + AA genotypes when compared with their wild TT genotypes(rs9939609: P = 0. 0003; rs1421085: P = 0. 0005). (3) TCF7L2 gene: There was no significant difference of allele C and T frequency of SNP rs7903146 between obese/overweight groups and control group (P>0.05). The level of HOMA-IR in CC genotype was significantly higher than that in CT genotype (P=0.046).
Conclusions: FTO gene rs9939609 and rs1421085 SNP are associated with obesity and BMI, and the SNP rs7903146 of TCF7L2 gene is associated with insulin resistance in Chinese children and adolescents. Both of the above SNPs are not correlated with metabolic parameters.

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Effects of an outpatient obesity treatment program for adolescents on health-related quality of life

Ingo Menrath; Angelika Gminder; Olaf Hiort; Ute Thyen
University of Lübeck, Department of Paediatrics, Lübeck, Germany

Background: Interventions in obesity in adolescents aim at the prevention of medical comorbidities as well improving health related quality of life (HRQoL). Studies showed that HRQoL in obese adolescents is impaired and treatment programs can help to enhance positive determinants of HRQoL.
Objective and hypotheses: In an on-going evaluation of the standardised patient education program "Active Kids" we analysed HRQoL data of 65 adolescents (mean age 13.6 years, 49% females). They and their parents took part in a structured ten month outpatient obesity program composed of medical, nutritional and psychological trainings. Our analyses focused on the HRQoL before and after treatment.
Methods: At the beginning and after completion of the treatment program all participants answered the generic and the disease-specific obesity module of the KINDL HRQoL-questionnaire. Effects of the program on HRQoL were tested for significance using ANOVA to account for age and gender effects. We also compared the HRQoL scores with norms of the general population (KiGGS study).
Results: The participants had a mean standardized BMI (zBMI) of 2.5. After treatment the zBMI was significantly reduced by 0.2 (P<0.000). Compared to German norms most of the HRQoL mean scores at baseline were low, the obesity specific scores were comparable to other studies of obese adolescents. Participation in the program led to a significant increase from 52.7 to 59.6 in the self-esteem scale (p=0.028) and from 62.2 to 70.0 in the disease specific HRQoL scale (p<0.000) independently of age and gender. After treatment the self-esteem score reached the level of the general population.
Conclusions: The results confirm that the Active Kids program is effective in both reducing weight and enhancing self-esteem and obesity specific HRQoL. HRQoL should be included as an important outcome in clinical studies on the efficiency of intervention programs.

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Physical activity in preschoolers: direct accelerometry in the course of the week and relation to weight status, TV consumption, and socioeconomic factors

Yvonne Vorwerg[1]; David Petroff[2]; Wieland Kiess[3]; Susann Blüher[4]
[1]University of Leipzig, Dept. of Women and Child Health; Hospital for Children and Adolescents, Leipzig, Germany; [2]University of Leipzig, Clinical Trial Centre and IFB AdiposityDiseases, Leipzig, Germany; [3]University of Leipzig, Dept. of Women and Child Health, Hospital for Children and Adolescents; IFB AdiposityDiseases, Leipzig, Germany; [4]University of Leipzig, IFB AdiposityDiseases; Dept. of Women and Child Health, Hospital for Children and Adolescents, Leipzig, Germany

Background: Physical inactivity is a risk factor towards the development of obesity. Data on objectively measured physical activity (PA) in preschool children are scarce.
Objective and hypotheses: We measured PA in preschoolers (direct accelerometry) and evaluated differences in PA patterns over the course of the week. PA data were analyzed with regard to gender, anthropometrics, lifestyle, and socioeconomic parameters.
Methods: PA was measured in 119 children 3-6 years by direct accelerometry and analyzed in the 92 (40 girls) that wore it for at least 4 days including one day of the weekend (median/mean measuring time 23.5 h/d/21.8 h/d). PA questionnaires were completed by 103 parents and 87 caregivers to collect anthropometric, lifestyle, and socioeconomic data.
Results: Median daily PA (Metabolic equivalent: MET>3) was 4.3 hours (mean: 4.4 hours). Boys spent an estimated 52 min/week more being very active (MET>6) than girls (95% confidence interval [6, 96] min/week, p=0.02). PA was lower during the weekend (3.7 h/d) compared to weekdays (4.5 h/d), p=3×10-6), where a 95% confidence interval for the difference is [0.5, 1.0] h/d. There was not a significant difference in PA levels between overweight/obese children (median 4.7 h/d) and normal-weight peers (median 4.2 h/d). Daily media consumption increased with decreasing social class, both on weekdays (p=0.05) and during the weekend (p=0.01), but was not related to the amount of daily PA.
Conclusions: The negative impact of obesity-promoting factors known to be relevant in older children is rather low for preschoolers, but there is evidently a gradient in PA between weekdays and weekends already in this age group.

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Inhibition of the phosphoinositide 3-kinase/mTOR pathway and influence on the viability of human PTEN-deficient lipoma cells

Franziska Käßner[1]; Franziska Wilhelm[1]; Gordian Lukas Schmid[1]; Jürgen Kratzsch[2]; Antje Körner[1]; Antje Garten[1]; Wieland Kiess[1]
[1]University Hospital for Children and Adolescents Leipzig, Center for Pediatric Research, Leipzig, Germany; [2]University Hospital Leipzig, Institute of Laboratory Medicine and Molecular Diagnostics, Leipzig, Germany

Background: We identified a child with PTEN Hamartoma Tumor Syndrome (PHTS) and massive lipomatosis caused by a deletion in the phosphatase and tensin homolog (*PTEN*) gene. Treatment with rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), was only partially and transiently successful in this patient.
Objective and hypotheses: We tested *in vitro* whether pharmacological inhibition of AKT, PI3-kinase or mTOR leads to reduced viability or induction of apoptosis in lipoma cells of the above mentioned patient. Furthermore, we evaluated IGF-binding protein (IGFBP)-2 as potential marker for therapy success.
Methods: Cells from a lipoma of the patient with PHTS were maintained in

long term culture. Viability and apoptosis were assessed using WST-1 and AnnexinV/PI assay. IGFBP2 production was detected by ELISA and quantitative PCR.

Results: Lipoma cells had a lifespan of 91 population doublings with a doubling time of 25h. PTEN mRNA and protein levels were decreased and AKT phosphorylation was increased compared to human SGBS preadipocytes. The mTORC1 inhibitor rapamycin decreased viability by 43.4±2% and adipocyte differentiation by 72.7±5% (100nM), but did not induce apoptosis. The mTORC1/2 inhibitor WYE-354 decreased viability by 75.7±2% (5µM), whereas the PI3-Kinase inhibitor LY294002 (500µM) decreased viability by 98.09±1% and induced 65.0±3% apoptosis. The AKT inhibitor perifosine (100µM) reduced cell viability by 96.4±1% and induced 84.5±1% apoptosis. IGFBP2 serum levels were significantly elevated (1224-165ng/ml; reference 277-640ng/ml) in our patient, but did not show a consistent change during rapamycin therapy. Lipoma cells were found to secrete IGFBP2 in amounts comparable to other adipocellular models.

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

P1-d2-269 Fat Metabolism and Obesity 2

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

Selim Kurtoglu[1]; Ahmet Ozturk[2]; Nihal Hatipoglu[1]; Betul Cicek[3]; Demet Unalan[4]; Vesile Senol[5]; Meral Bayat[6]; Mustafa Mumtaz Mazicioglu[7]; Ferhan Elmali[2]; Hasan Basri Ustunbas[7]

[1]Erciyes University, Medical Faculty, Pediatric Endocrinology, Kayseri, Turkey; [2]Erciyes University, Medical Faculty, Biostatistics, Kayseri, Turkey; [3]Erciyes University, Ataturk Health School, Nutrition and Dietetics, Kayseri, Turkey; [4]Erciyes University Halil Bayraktar Health Services Vocational College, Health Services, Kayseri, Turkey; [5]Erciyes Univesity, Halil Bayraktar Health Services Vocational College, Public Health, Kayseri, Turkey; [6]Erciyes University, Faculty of Health Science, Pediatric Nursing Departman, Kayseri, Turkey; [7]Erciyes University, Medical Faculty, Family Medicine, Kayseri, Turkey

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

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

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

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

This difference was less pronounced for girls than boys. FM and FFM gradually increased with age in each body part of both genders. The increase was more pronounced in the pubertal age (table 1, 2).

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

Table 1: The compositon of segmental body with BIA in girls

Age (years)	Fat%	fatmass (kg)	ffm (kg)	tfat%	tfatmass (kg)	tffm (kg)	armfat %	armfat-mass (kg)	armffm (kg)	Leg-fat%	legfat-mass (kg)	legffm (kg)
6	22.7 ±4.2	5.8 ±2.2	19.1 ±3	16.5 ±2.4	2.6 ±1.3	12.3 ±1.5	33.2 ±4.1	0.3 ±0.1	0.6 ±0.1	31.1 ±3.5	1.6 ±0.3	1.4 ±0.3
7	22.7 ±4.2	5.8 ±2.2	19.1 ±3	16.5 ±4.5	2.6 ±1.3	12.3 ±1.5	33.6 ±4.9	0.4 ±0.2	0.7 ±0.1	31.3 ±3.8	2 ±0.4	1.7 ±0.3
8	22.5 ±4.7	6.8 ±4.1	21.4 ±3.3	16 ±5	2.9 ±3.2	13.4 ±1.8	33.2 ±4.8	0.4 ±0.2	0.8 ±0.2	31.1 ±4.1	2.3 ±0.5	2 ±0.4
9	23.5 ±5	7.5 ±3.2	23.8 ±3.4	17.1 ±5.6	3.2 ±1.6	14.7 ±1.9	34.2 ±5.8	0.5 ±0.3	1 ±0.6	31.3 ±4.1	2.6 ±0.6	2.3 ±0.5
10	24.4 ±5.5	9.6 ±4.3	28.2 ±5	18 ±6.4	4 ±2.2	17 ±2.6	35.1 ±6.3	0.6 ±0.3	1.1 ±0.3	31.5 ±4.5	3.2 ±0.8	2.8 ±0.6
11	24.3 ±5.5	10.4 ±4.7	30.9 ±5.1	17.9 ±6.1	4.4 ±2.5	18.3 ±3.4	34.5 ±6	0.8 ±0.6	1.3 ±0.3	31.3 ±4.9	3.5 ±0.8	3.1 ±0.6
12	24.2 ±4.6	11.5 ±4.3	34.5 ±5.3	17.8 ±5.3	4.7 ±2.1	20.6 ±2.8	33.6 ±5.6	0.8 ±0.3	1.4 ±0.3	31.5 ±4.2	4 ±1.2	3.5 ±0.6
13	25.7 ±5.9	13.5 ±5.7	36.2 ±6	19.3 ±6	5.5 ±2.6	21.6 ±2.7	34.6 ±5.9	0.9 ±0.4	1.5 ±0.3	32.7 ±5.6	4.3 ±0.9	3.7 ±0.6
14	27.3 ±5.5	15.6 ±6	39.7 ±5.5	21.1 ±3.6	6.8 ±3.8	23.5 ±2.9	35.5 ±5.8	1.1 ±1.7	1.7 ±0.3	34.4 ±4.9	4.8 ±1	4 ±0.6
15	24.5 ±6.3	13.9 ±5.4	40.9 ±3.8	19.3 ±7.9	6.1 ±3.6	23.4 ±2.2	28.5 ±7.8	0.8 ±0.3	1.8 ±0.3	31 ±4.8	4.8 ±0.7	4.3 ±0.5
16	24.8 ±6.6	14.4 ±6	41.6 ±3.9	20.2 ±8.4	6.4 ±3.6	23.5 ±2.2	27.3 ±7.7	0.8 ±0.8	1.9 ±0.4	30.6 ±4.7	5 ±0.7	4.5 ±0.5
17	24.3 ±6.5	13.5 ±5	41.1 ±3.8	19.5 ±7.6	6 ±3	23.3 ±3.5	27.1 ±7.2	0.7 ±0.3	1.9 ±0.2	30 ±4.9	4.8 ±0.6	4.4 ±0.5

*:mean
±SD, ffm: fat free mass, tfat%: trunk fat percent, tfatmass: trunk fat mass, tffm:trunk fat free mass, armffm:arm fat free

Table 2: The compositon of segmental body with BIA in boys.

Age	Fat%	Fatmass (kg)	Ffm (kg)	tfat%	Tfatmass (kg)	Tffm (kg)	armfat %	Armfat-mass (kg)	Armffm (kg)	Leg-fat%	Legfat-mass (kg)	legffm
6	19.8 ±3.6	4.6 ±1.7	18.2 ±2.4	14.4 ±3.6	2.3 ±2	12.2 ±1	30 ±3.9	0.3 ±0.1	0.6 ±0.1	28.5 ±3.7	1.8 ±1	1.5 ±0.3
7	20.5 ±4.1	5.4 ±4.8	20.2 ±2.9	15 ±2.2	2.5 ±2.1	13 ±2	30.6 ±4.2	0.4 ±0.2	0.7 ±0.2	28.6 ±4.4	2 ±0.5	1.7 ±0.4
8	20.8 ±5.1	6.3 ±2.8	23 ±3.3	15.4 ±5.0	2.7 ±1.3	14.4 ±1.5	31 ±5.2	0.4 ±0.3	0.9 ±0.2	27.7 ±5.1	2.3 ±0.6	2.3 ±2.3
9	21.4 ±6.5	7.5 ±4.1	25.7 ±3.7	16.2 ±6.7	3.2 ±1.9	15.7 ±3.1	31.6 ±6.6	0.5 ±0.3	1.2 ±0.3	27.3 ±6.3	2.7 ±0.8	2.5 ±0.8
10	20.6 ±5.9	7.7 ±4	27.8 ±4.4	16.1 ±6.1	3.4 ±1.9	16.6 ±2.0	30.8 ±6.3	0.6 ±0.3	1.2 ±0.3	25.5 ±5.6	2.9 ±0.8	2.8 ±0.6
11	19.8 ±5.8	8.1 ±6.7	30.6 ±5.1	15.4 ±6	3.5 ±2.1	17.9 ±2.8	29.0 ±6	0.6 ±0.3	1.3 ±0.3	24.3 ±5.7	3.2 ±0.9	3.1 ±0.7
12	19.7 ±6.8	10.5 ±12	34.8 ±5.5	15.6 ±7.1	4 ±2.6	19.8 ±3	28.2 ±7	0.7 ±0.3	1.6 ±0.3	23.7 ±6.6	3.7 ±1	3.6 ±0.8
13	18.1 ±5.8	9.3 ±4.7	39.9 ±6.8	14.1 ±6	4 ±2.3	22.5 ±3.4	26.1 ±6	0.7 ±0.3	1.9 ±0.4	21.9 ±6.2	4.2 ±1.1	4.3 ±0.9
14	16.7 ±5.8	9.9 ±5.9	46.4 ±7.8	13.2 ±6	4.2 ±2.8	25.7 ±3.7	24 ±5.8	0.7 ±0.3	2.3 ±0.4	19.8 ±6	4.9 ±1.2	5.2 ±1
15	16.2 ±5.1	10.5 ±5.2	51.5 ±7.2	13 ±5.3	4.5 ±2.6	28.4 ±3.6	22.1 ±5.2	0.8 ±0.3	2.6 ±0.4	19.2 ±5.6	5.3 ±1.1	5.7 ±0.9
16	16.6 ±5.7	11.4 ±6.1	54 ±7.6	14 ±6	5.2 ±3.1	29.9 ±3.7	26 ±62.9	0.8 ±0.3	2.7 ±0.4	19 ±5.7	5.5 ±1.2	5.9 ±1
17	16.6 ±4.9	11.4 ±5.3	55.6 ±6.9	14.4 ±5.4	5.4 ±2.8	30.8 ±3.8	21.2 ±5.1	0.8 ±0.3	2.8 ±0.4	18.5 ±5.3	5.6 ±1	6.1 ±0.9

*:mean
±SD, ffm: fat free mass, tfat%: trunk fat percent, tfatmass: trunk fat mass, tffm:trunk fat free mass, armffm:arm fat free

P1-d2-270 Fat Metabolism and Obesity 2

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

Kathrin Dittrich[1]; Isabel Wagner[2]; Matthias Raschpichler[3]; Julia Gesing[2]; Maike vom Hove[2]; Freerk Prenzel[2]; Wieland Kiess[2]; Antje Körner[1]
[1]Centre for Pediatric Research, Hospital for Children and Adolescents, University of Leipzig, Deptartment of Women and Child Health, Leipzig, Germany; [2]University Hospital for Children and Adolescents, Leipzig, Germany, Deptartment of Women and Child Health, Leipzig, Germany; [3]Leipzig University Medical Center (IFB), AdiposityDiseases, Leipzig, Germany

Background: Respiratory function (RF) is supposed to be reduced in obese children, but findings are still heterogeneous. Some studies demonstrated negative effects of fat mass, particularly visceral fat, on RF in adults.
Objective and hypotheses: We aimed to evaluate, whether there is a difference in RF between lean and obese children in relation to body fat distribution and after maximal physical exercise.
Methods: A total of 77 children (40 girls, 37 boys) aged 14.6±2.7 years stratified into lean (n=37) and obese (n=40) children underwent spirometry before cycle ergometry and shortly after maximum effort was reached. Adipose tissue mass (AT) was estimated from Magnetic Resonance Imaging.
Results: For the z-score of the forced expiratory volume in 1 second (FEV1 z-score), the forced vital capacity (FVC z-score) and the maximum midexpiratory flow (MMEF z-score), we did not detect any significant differences between lean and obese children. However, we identified a significantly increased respiratory resistance (Rocc) in the obese subjects (0.60±0.25 vs. 0.46±0.15; p=0.004), and a positive correlation with BMI z-score (r=0.41; p=0.001). Neither in lean nor obese children, FEV1 and FVC z-score changed significantly after cycle ergometry. After adjustment for age, sex, pubertal stage and BMI z-score, FEV1 z-score was significantly negatively correlated with the percentage of subcutaneous AT (SAT%) (r=-0.36; p=0.014). Similarly, FVC z-score was significantly negatively correlated with the percentage of total AT (TAT%) (r=-0.52; p=0.000), SAT% (r=-0.56; p=0.000) and visceral AT (VAT%) (r=-0.30; p=0.039). For the MMEF z-score we found analogical results for TAT% (r=-0.60; p=0.000), SAT% (r=-0.50; p=0.000) and VAT% (r=-0.29; p=0.049).
Conclusions: We did not find major differences in RF between lean and obese children, except of an elevated Rocc in obese children. In addition, maximum physical exercise did not cause deterioration in RF. Nevertheless, the amount of adipose tissue seems to have negative implications on RF.

P1-d2-271 Fat Metabolism and Obesity 2

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

Gianpaolo De Filippo[1]; Domenico Rendina[2]; Angelo Campanozzi[3]; Pasquale Strazzullo[2]; Pierre Bougnères[1]
[1]Hôpital Bicêtre, Assistance Publique - Hôpitaux de Paris, Service d'Endocrinologie et Diabétologie Pédiatrique, Le Kremlin-Bicêtre, France; [2]University of Naples "Federico II", Department of Clinical and Experimental Medicine, Naples, Italy; [3]University of Foggia, Department of Paediatrics, Foggia, Italy

Background: The consumption of highly processed foods (HPF) is one of the markers of dietary habits leading to obesity. The total excretion of urinary Na (U(Na)) is a useful biomarker of the global intake of calories belonging to HPF, as more than 80% of total salt intake derives from this kind of foods. Furthermore, high salt intake has been associated with significantly increased risk of total cardiovascular disease.
Objective and hypotheses: To evaluate the salt consumption in a group of severe obese (i.e. z-score BMI ≥ 3) children and adolescent and its correlation with dietary habits and metabolic risk factors associated with obesity (i.e. insulin-resistance).
Methods: We considered dietary and urinary data from a total of 92 obese normotensive children and adolescents. Mean age was 12.55 ± 3.03 (9.1 – 17.9), BMI 30.35 ± 6.74, z-score of BMI 3.87 ± 0.97. 30 age-matched non obese children served as control group for urinary Na values. Dietary data were calculated on three-day dietary records before urine collection and insuline-resistence was evaluated by HOMA-IR.
Results: Mean U(Na) in obese patients was 135.25 ± 38.48 mmol/d, corresponding to 14.7 ± 4.2 g of salt intake, versus 44.6 ± 10.7 in controls (p <0.001). In 80 % of subjects the Na excretion exceeded the recommended upper limit of 100 mmol/d established for adults. There was a positive trend between age, BMI (absolute and z-score) and values of Na excretion, without reaching statistical significance. HOMA-IR was directly correlated with complex carbohydrate intake calculated on dietary data (p = 0.29) and inversely correlated with U(Na) (p = 0.33).
Conclusions: Salt consumption appears to be strictly related to dietary habits leading to development and persistence of obesity, even in children and young adolescents. A reduction in the consumption of salty food could therefore have a beneficial effect on body weight status.

P1-d2-272 GH and IGF Physiology 1

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

Eva-Maria Radermacher[1]; Jürgen Klammt[1]; Anja Barnikol-Oettler[1]; Janina Caliebe[2]; Monique Losekoot[3]; Michael Ranke[4]; Gudrun Rappold[5]; Marina Schlicke[1]; Heike Stobbe[1]; Jan M Wit[6]; Wieland Kiess[1]; Roland Pfäffle[1]
[1]University Hospital Leipzig, Hospital for Children and Adolescents, Leipzig, Germany; [2]SLK Hospitals, Children's Hospital, Heilbronn, Germany; [3]Leiden University Medical Center, Laboratory for Diagnostic Genome Analysis, Center for Human and Clinical Genetics, Leiden, Netherlands; [4]University Hospital Tübingen, University Children's Hospital, Tübingen, Germany; [5]Ruprecht-Karls-University, Department of Molecular Human Genetics, Heidelberg, Germany; [6]Leiden University Medical Center, Department of Pediatrics, Leiden, Netherlands

Background: Longitudinal growth is a trait regulated by a plethora of genes. Especially, the insulin-like growth factor 1 receptor (IGF1R) plays an essential role in growth regulation. For bone development the transcription factor short statured homeobox gene (SHOX) has been proposed to be of fundamental importance.
Objective: To investigate the underlying pathomechanism of growth failure of a patient, who carries a heterozygous exon 6 deletion in the *IGF1R* and a heterozygous point mutation in the *SHOX* gene (p.Met240Ile).
Methods: MLPA, long range PCR and DNA sequencing were used to identify the mutations and to define the deletion breakpoints. *IGF1R* mRNA stability was investigated by RT-PCR. We performed immunoblots to study IGF1R expression and IGF1 dependent receptor phosphorylation in patient's fibroblasts.
Results: The AGA-born patient showed growth retardation at the age of six years with a height of 102.5 cm (-3.3 SDS) and weight of 15.9 kg (-2.6 SDS). The mother bearing the IGF1R deletion had an adult height SDS of 1.1. Final height of the father harboring the SHOX mutation was normal (-0.9 SDS). Furthermore, patient's IGF1 levels were in low-normal range and the boy presented no additional abnormalities. In patient's fibroblasts we revealed that the 5.2 kb spanning *IGF1R* deletion results in nonsense-mediated mRNA decay leading to IGF1R haploinsufficiency. Accordingly, IGF1 stimulated phosphorylation studies showed a decrease in IGF1R autophosphorylation but, unexpectedly, AKT/PKB activation was not impaired.
Conclusions: In summary, we have identified the genetic cause of IGF1 resistance in a growth retarded boy and disclosed the underlying pathomechanism. Due to the unexpected activation pattern of the AKT/PKB signaling branch and a possible additive effect of the parental *IGF1R* and *SHOX* mutations, investigations to explore the interplay between both signaling pathways in cell models are in progress.

P1-d2-273 GH and IGF Physiology 1

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

Tsuyoshi Isojima[1]; Akira Shimatsu[2]; Susumu Yokoya[2]; Toshiaki Tanaka[2]; Kazuo Chihara[3]; Naomi Hizuka[3]; Akira Teramoto[3]; Keita Tatsumi[3]; Katsuhiko Tachibana[2]; Noriyuki Katsumata[2]; Kenji Fujieda[2]
[1]Graduate school of medicine, The University of Tokyo, Department of pediatrics, Tokyo, Japan; [2]The Foundation for Growth Science in Japan, GH and its Related Factors Study Committee and GH Treatment Study Committee, Tokyo, Japan; [3]The Ministry of Health, Labour and Welfare, The Research Study Group on the Hypothalamic-Pituitary Dysfunction, Tokyo, Japan

Background: Measurements of insulin-like growth factor-I (IGF-I) are useful not only for diagnosis and management of patients with growth hormone (GH) related disorders but also for assessing nutritional status. We have previously reported the population based references of serum IGF-I in 1996. However, they did not entirely reflect data in the transition period.
Objective and hypotheses: The aim of the present study was to re-establish age and gender specific national standard of normative data for IGF-I in Japanese population.
Methods: The study included 1,685 healthy Japanese subjects (845 males, 840 females) aged from 0 year to 83 years. The subjects suffering from diseases which may affect serum IGF-I levels were excluded. Obese or thin adult subjects were also excluded. Serum samples were obtained and IGF-I concentrations were determined by two kinds of commercially available immunoradiometric assays. The reference intervals were calculated using the LMS method. It is important to analyze the data covering from infants and elders by the LMS method since IGF-1 values had sharp peak around the late puberty.
Results: Median IGF-I levels were increased up to 310 ng/ml in boys at the age of 13-14 years and 349 ng/ml in girls at age of 13 years, respectively, which rapidly declined and reached 124 ng/ml and 103 ng/ml in males and females, respectively, at the age of 70 years. Pretreatment IGF-I measurements in patients with severe GH deficiency were obtained from the database of the Foundation for Growth Science, Japan (523 boys and 224 girls, age range: two month to 18 years) and from the clinical study for adult GH deficiency (34males and 33 females, age range: 18 to 24 years), and mean SD scores were -2.1±1.6 and -4.9±2.5, respectively.
Conclusions: The present study established age and gender specific national standard of normative data for IGF-I in Japanese population and showed the utility of this reference for screening the patients with severe GH deficiency before GH supplementation.

P1-d2-274 GH and IGF Physiology 1

A novel Leu409Phe IGFALS gene mutation results in complete ALS deficiency in a boy presenting short stature, pubertal delay and severe IGF-I and IGFBP-3 deficiencies

Horacio Domené[1]; Ignacio Bergadá[2]; Paula Scaglia[1]; Liliana Karabatas[1]; Débora Braslavsky[2]; Héctor Jasper[1]
[1]Centro de Investigaciones Endocrinológicas (CEDIE), CONICET, Buenos Aires, Argentina; [2]División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina

Background: GH insensitivity has been associated to *GHR*, *STAT5b*, *IGF1* and *IGFALS* gene defects. Despite similar degrees of IGF-I deficiency only *IGFALS* gene defects result in mild growth deficit. To date, at least 24 ALS-deficient patients have been characterized at the molecular level.
Objective and hypotheses: To characterize the molecular defect in a short boy presenting IGF-I and IGFBP-3 deficiencies.
Methods: The proband is a 13 10/12 year old prepubertal boy, born at term with 2800 g (father 160 cm, -1.88 SDS; mother 157 cm, -0.6 SDS). He is the fourth of nine siblings, weight 28.3 kg, height 134.7 cm (-2.65 SDS), BA 12 9/12 years for CA 13 5/12. Serum GH, IGF-I and IGFBP-3 levels were determined by ICMA, ALS by Western immunoblot (WIB) and *in vitro* ternary complex (TC) formation by size exclusion chromatography.
Results: Clinical and biochemical evaluation excluded blood, renal and thyroid disorders and celiac disease. He presented a normal GH response (22.0 ng/ml) to an arginine-clonidine test and low IGF-I (29 and 33 ng/ml; -3.29 and -2.90 SDS) and IGFBP-3 (<0.25 µg/ml) levels. ALS was undetectable by WIB and serum from the patient was unable to form TC, even after spiking with rhIGFBP-3. *IGFALS* gene sequencing revealed two homozygous novel missense mutations (c.1225C>T; p.Leu409Phe and c.1424C>T; p.Ala475Val). Whilst the p.Leu409Phe affects a highly conserved leucine residue located within consensus LRR β-strand motif (LxxLxLxxN/CxL) in the 15th LRR domain and is predicted to be probably damaging by *in silico* analysis (PolyPhen 2), the p.Ala475Val mutation changes a non conserved residue being probably a benign variant. These mutations were not found in 380 alleles from local controls.
Conclusions: As shown by this case, the association of severe IGF-I and IGFBP-3 deficiencies to mild growth retardation is suggestive of ALS deficiency, particularly when associated to pubertal delay. The increasing number of ALS deficient reported patients indicates that this condition may be more prevalent that previously suspected.

P1-d2-275 GH and IGF Physiology 1

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

Gabriela Guercio; Diana Monica Warman; Marta Ciaccio; Mariana Aziz; Carmen Riu; Milagros Aguiar; Matias Juanes; Roxana Marino; Esperanza Berensztein; Eduardo Chaler; Marco Aurelio Rivarola; Alicia Belgorosky
Hospital de Pediatria Garrahan, Endocrine Service, Buenos Aires, Argentina

Background: Several IGF1R gene mutations have been described as a cause of growth retardation due to IGF1 insensitivity.
Objective: To analyze the IGF1R gene for mutations in three children suspected to have IGF1 insensitivity. Population: Three children (2 boys, 1 girl) were evaluated. All patients were born small for gestational age (SGA) and presented microcephaly. The two boys were evaluated at 18 months (P1) and 2.8 years of age (P2). They showed a mild dysmorphic phenotype. External genitalia and scrotal testes were normal. A 3.2-year-old girl (P3) also presented a Klippel Feil malformation. P2 and P3 showed no postnatal catch-up growth, while P1 reached a normal Heigth SDS at 2 years of age without changes in head circumference. P1 and P3 also presented developmental delay.
Results: Basal and stimulated serum GH and basal serum IGF-1 and IGFBP3 levels were quite variable among them. No chromosome 15 anomalies were detected. Three novel heterozygous mutations, de novo R1256S (P1), N359Y (P2), and R1337C (P3) were detected in the IGF1R gene. The aminoacid substitutions were located at highly conserved aminoacid residues in the protein (location, P1 and P3 exon 21, P2 exon 4). These mutations were predicted to affect protein function using the sequence homology based SIFT tool, the structure-based PolyPhen approach and the Mutation Taster. The father and sister of P3, carrying the R1337C mutation, were phenotypically normal. P1 and P2 started high-dose rhGH treatment. After 5 months of therapy, P1 maintained his growth velocity (6.8 cm/years). P2 gained 0.73 Height SDS after 1 year of treatment.
Conclusions: Even thought IGF1R molecular studies should be considered in children with an undiagnosed history of SGA without postnatal catch-up growth and microcephaly, the clinical and biochemical picture and the response to rhGH, among subjects carrying IGF-IR haploinsufficiencies, are quite variable.

P1-d2-276 GH and IGF Physiology 1

Acid-labile subunit levels and puberty in short children born small for gestational age

Judith Renes[1]; Jaap van Doorn[2]; Petra Breukhoven[1]; Annemieke Lem[3]; Anita Hokken-Koelega[3]
[1]Erasmus MC - Sophia Children's Hospital, Paediatric Endocrinology, Rotterdam, Netherlands; [2]University MC Utrecht, Metabolic and Endocrine Diseases, Utrecht, Netherlands; [3]Dutch Growth Research Foundation / Erasmus MC - Sophia Children's Hospital, Paediatric Endocrinology, Rotterdam, Netherlands

Background: Acid Labile Subunit (ALS) forms a ternary complex with IGF-I and IGFBP-3. In the absence of ALS, IGF-I and IGFBP-3 levels are reduced. In children with an IGFALS mutation delayed puberty has been described.

Data on serum ALS levels in short children born SGA are unknown and associations with onset of puberty have not been established.
Objective and hypotheses: To determine serum ALS levels in short SGA children compared with those in controls and to assess the association between serum ALS levels and age at start of puberty.
Methods: Serum ALS levels were measured in 319 short SGA children (mean age 8.1 yr) at start of growth hormone treatment and in 516 subjects with normal stature (159 children, 357 adults). Age at start of puberty was available in 239 short SGA children.
Results: ALS SDS levels of the SGA group were lower compared with those of controls (P<0.001). Mean (SD) ALS levels in short SGA subjects was -0.4 (1.6) SDS. In 40 children (13%), ALS levels were ≤-2 SDS, which occurred more in girls than in boys (p<0.001). ALS levels ≥2 SDS were more common in boys (P<0.001). ALS SDS levels were significantly correlated with height SDS, weight SDS and BMI SDS. Also, strong positive correlations were observed with serum IGF-I and serum IGFBP-3 levels. There was no significant correlation between ALS levels and age at start puberty.
Conclusions: Short SGA children have lower serum ALS levels compared to controls, albeit less reduced than the IGF-I and IGFBP-3 levels. There is no correlation between serum ALS levels and age at start puberty.

Table 1. Clinical and laboratory characteristics

	n	mean (± SD)
Boys/girls	319	165/154
Gestational age (wk)	319	36.4 (3.8)
Birth length SDS	209	-3.0 (1.6)
Birth weight SDS	314	-2.2 (1.1)
Target height SDS	307	-0.6 (0.8)
At start of GH treatment		
CA (yr)	319	8.1 (3.1)
Height SDS	319	-2.9 (0.6)
Weight SDS	319	-2.8 (1.0)
BMI SDS	319	-1.3 (1.1)
IGF-I SDS	319	-1.2 (1.3)
IGFBP-3 SDS	319	-1.1 (1.0)
ALS SDS	319	-0.4 (1.6)

Table 2. Correlations between ALS SDS and clinical parameters

	n	R	p-value
Height SDS	319	0.22	<0.001
Weight SDS	319	0.28	<0.001
BMI SDS	319	0.19	0.001
IGF-I SDS	319	0.55	<0.001
IGFBP-3 SDS	319	0.68	<0.001
CA boys at start of puberty	131	0.06	0.50
CA girls at start of puberty	108	-0.10	0.32

P1-d2-277 GH and IGF Physiology 1

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

Paula Ocaranza; Fernanda Morales; Álvaro Matamala; Ximena Gaete; Germán Íñiguez; Fernando Cassorla
University of Chile, Faculty of Medicine, Institute of Maternal and Child Research, Santiago, Chile

Background: Growth hormone (GH) is required for normal postnatal growth, but it is unclear whether linear growth is dependent on GH during the newborn period, when GH circulating levels are relatively high, and serum IGF-I are relatively low. This suggests that GH sensitivity may be low during the newborn period.
Objective and hypotheses: To determine the intracellular activation of JAK2 and STAT5 after GH stimulation and the expression of *ALS* in skin fibroblasts obtained from normal newborn boys and prepubertal boys undergoing elective circumcision or surgery.
Methods: We studied the intracellular activation of JAK2 and STAT5 and the *ALS* expression in 8 newborn boys and in 9 prepubertal boys with normal stature. The activation of these proteins was studied by Western Blot in the non-nuclear fractions of fibroblast cultures obtained from a skin biopsy, both under basal conditions and following stimulation with rhGH (200 ng/mL) for 15-60 minutes. The expression of *ALS* was determined by RT-PCR after 16 h of stimulation in presence or absence of rhGH.
Results: Western blot analyses in the newborns revealed a lack of activation of JAK2 and STAT5 and in the expression of *ALS* after stimulation with rhGH when compared to the respective basal levels. Similar experiments conducted in prepubertal boys showed a rapid activation of JAK2 and STAT5, and in the expression of *ALS* after rhGH stimulation. The values are given as mean ± SEM.*P<0.05.

Clinical features of the subjects enrolled in the study

Subjects	Males	Age (mean ± SEM)	Height Z-score (mean ± SEM)	Weight Z-score (mean ± SEM)
Newborn	8	8.6 ± 0.8 days	-0.24 ± 0.20	-0.06 ± 0.33
Prepubertal	9	6.3 ± 0.4 years	-0.52 ± 0.15	-0.05 ± 0.18

Molecular features of the subjects enrolled in the study

Subjects	JAK2 activation "Basal"	JAK2 activation "15 min rhGH"	STAT5 activation „Basal"	STAT activation "30 min rhGH"	ALS expression „16 h Basal"	ALS expression "16 h rhGH"
Newborn	1.06 ± 0.02	1.05 ± 0.03	1.36 ± 0.19	1.43 ± 0.17	0.80 ± 0.03	0.82 ± 0.04
Pre-pubertal	0.97 ± 0.09	1.19 ± 0.08*	0.91 ± 0.24	2.01 ± 0.53*	0.83 ± 0.03	1.12 ± 0.09*

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

P1-d2-278 GH and IGF Physiology 1

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

Marie J.E. Walenkamp[1]; Dennis Cramer[1]; Boudewijn Bakker[2]; Edgar van Mil[3]; Dick Mul[4]; Roelof J. Odink[5]; Hermine A. van Duyvenvoorde[6]; Sitha A. Scheltinga[6]; Sarina G. Kant[7]; Willie Bakker-van Waarde[8]; Annemarie Verrijn Stuart[9]; Wilma Oostdijk[10]; Jan M. Wit[10]; Monique Losekoot[6]
[1]VU University Medical Center, Pediatrics, Amsterdam, Netherlands; [2]Reinier de Graaf Gasthuis, Pediatrics, Delft, Netherlands; [3]Jeroen Bosch Hospital, Pediatrics, Den Bosch, Netherlands; [4]Haga Ziekenhuis/Juliana's Children Hospital, Pediatrics, Den Haag, Netherlands; [5]Catharina Hospital, Pediatrics, Eindhoven, Netherlands; [6]Leiden University Medical Center, LDGA, Clinical Genetics, Leiden, Netherlands; [7]Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands; [8]University Medical Center Groningen, Pediatrics, Groningen, Netherlands; [9]University Medical Center Utrecht, Pediatrics, Utrecht, Netherlands; [10]Leiden University Medical Center, Pediatrics, Leiden, Netherlands

Background: The first reported patients with a heterozygous defect of the IGF-I receptor (IGF1R) showed a small birth size for gestational age (SGA), postnatal growth failure, low head circumference (HC) and IGF-I levels above the mean for age. However, later publications report a more variable phenotype. The effect of growth hormone (GH) therapy has only been described in individual cases.
Objective and hypotheses: To describe the spectrum of phenotypic characteristics and the effect of GH treatment in a large group of patients with a defect of *IGF1R*.
Methods: DNA of patients from 8 hospitals with a phenotype suggestive for an *IGF1R* defect was analyzed. Patients were classified in three subgroups: (a) terminal 15q deletion, including *IGF1R* (n=7), (b) pathogenic mutation (earlier described, predicted pathogenic or affected parent with short stature, n=6), (c) possible pathogenic defect (unclassified variant with unknown pathogenicity, n=4). Patient characteristics, including the growth response to GH, were collected from patient files.
Results: Patients had a mean birth weight of -2.1 ± 0.8 SD (a), -2.6 ± 0.4 SD (b), -1.2 ± 0.6 SD (c); birth length was -2.9 ± 1.1 SD (a), -2.7 ± 1.1 SD (b), -1.9 SD (n=1)(c); birth HC -2.0 ± 0.8 SD (a), -3.2 ± 0.9 SD (b). Delay in speech development, learning disabilities and ophthalmologic problems occurred more frequently in patients with a terminal 15 q deletion than in

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

	terminal 15q deletion (n=7)	pathogenic IGF1R mutation (n=4)
Height at start GH	-3.2 ± 0.6	-2.2 ± 0.4
HC at start GH	-2.0 ± 0.9	-3.7 ± 0.9
IGF-I at start GH	+1.7 ± 0.8	+1.9 ± 0.6
Age at start GH	5.5 ± 2.7	7.1 ± 4.3
Δ height after 1 year GH	+0.6 ± 0.3	+0.6 ± 0.4
Years of GH treatment (yr)	3.6 ± 2.7	3.2 ± 2.3
Δ height, last measurement	+1.1 ± 0.6	+0.7 ± 0.2
Δ HC, last measurement	+1.2 ± 1.3	+0.6 ± 0.3
Max IGF-I under GH	+4.2 ± 1.1	+3.0 ± 0.6

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

P1-d2-279 GH and IGF Physiology 1

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

Nikolaos Daskas[1]; Peta Sharples[1]; Nikki Davis[1]; Helen Miller[2]; Ute Leonards[3]; Wolf Woltersdorf[4]; Elizabeth Crowne[1]
[1]Bristol Royal Hospital for Children, Paediatric Endocrinology, Bristol, United Kingdom; [2]University of Bristol, Clinical Trials and Evaluation Unit, Bristol, United Kingdom; [3]Bristol University, School of Experimental Psychology, Bristol, United Kingdom; [4]Bristol Royal Infirmary, Clinical Chemistry, Bristol, United Kingdom

Background: Traumatic brain injury (TBI) in childhood can cause hypopituitarism. The reported prevalence of hypopituitarism varies and may be influenced by age, severity of trauma but also by timing and method of hormonal evaluation.
Objective and hypotheses: Aim was to assess the long-term impact of childhood TBI on the GH-IGF1 axis.
Methods: 18 participants with moderate (admission Glasgow Coma Score 9-12) or severe (GCS 3-8) TBI (time from injury 7-11 years) and 7 controls matched for age, BMI, sex and pubertal stage. All participants were clinically assessed and underwent an overnight 12 hour GH venous profile (15-20 min sampling) followed by an insulin tolerance test (ITT). GH was assayed with Cobas ECLIA and deconvolution software (pulse_XP) was used to assess both total GH secretion and pulsatile secretion (pulse frequency, duration, amplitude). Statistical analysis: Mann-Whitney U test.
Results: All participants were euthyroid and most were postpubertal (17/18 TBI and 6/7 controls). Four TBI subjects had abnormal GH responses in the ITT (GH peak <3mcg/l). Deconvolution analysis showed that total GH secretion, number of secretion events, mean secretion pulse mass/amplitude and inter-pulse intervals were statistically different between groups. IGF1 levels - although within the normal range - were significanlty lower in the TBI group.
Conclusions: TBI in childhood is associated with significant GH-IGF1 axis abnormalities 10 years post injury with evidence of both GH deficiency and neurosecretory dysfunction.

	TBI group	Control group
n	18	7
Age (years), m (SD)	20.0 (4.2)	17.9 (2.2)
Male, n (%)	15 (83%)	6 (86%)
BMI (SD)	22.4 (3.1)	21.7 (3.3)
GH-Area under curve (SD)	1492 (726)	2515 (1360)*
GH-Secretion events (SD)	7.18 (1.67)	5.14 (0.69)**
Mean secretion amplitude (SD)	0.30 (0.135)	0.56 (0.363)*
Mean secretion inter-pulse interval [minutes] (SD)	78.7 (26.6)	102.8 (14.8)**
Mean secretion pulse mass (SD)	5.9 (2.8)	14.1 (9.1)**
IGF-1 [nmol/l] (SD)	39.2 (9.1)	51.4 (15.4)*

*p<0.05, **p<0.01

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

Wolfgang Hogler[1]; David Martin[2]; Nicola Crabtree[3]; Timothy Barrett[1]; Jan Frystyk[4]; Jeremy Tomlinson[5]; Lou Metherell[6]; Ron Rosenfeld[7]; Vivian Hwa[7]; Stephen Rose[8]; Joanna Walker[9]; Nicholas Shaw[1]
[1]Birmingham Children's Hospital, Dept of Endocrinology & Diabetes, Birmingham, United Kingdom; [2]University Children's Hospital, Paediatric Endocrinology & Diabetology, Tuebingen, Germany; [3]Birmingham Children's Hospital, Dept of Nuclear Medicine, Birmingham, United Kingdom; [4]University Hospital Aarhus, Medical Research Labroatories, Aarhus, Denmark; [5]University of Birmingham, Centre of Endocrinology, Institute of Biomedical Research, Birmingham, United Kingdom; [6]Barts and the London School of Medicine, William Harvey Research Institute, London, United Kingdom; [7]Oregon Health Sciences University, Dept of Paediatrics, Portland, United States; [8]Heartlands Hospital, Dept of Paediatrics, Birmingham, United Kingdom; [9]Portsmouth Hospital, Dept of Paediatrics, Portsmouth, United Kingdom

Background: Acid-labile subunit (ALS) deficiency inhibits ternary complex formation leading to primary IGF-I deficiency and short stature. Potential metabolic consequences such as insulin resistance and low bone mass have not been studied in great detail.
Objective: This study aimed to measure insulin sensitivity, lipid profile and bone strength in members of 4 affected families, and explore possible gene-dose effects.
Methods: 4 patients (7-21years) with homozygous mutations in the IGFALS gene and 12 heterozygous carriers had intravenous glucose tolerance tests performed. Bone imaging included dual-energy x-ray absorptiometry (DXA) of spine, hip and total body, peripheral quantitative computer tomography of the radius as well as automated radiogrammetry of the hand.
Results: Height *z*-scores in patients (median -3.75 [range -4.25 to -2.62]) were significantly lower compared to carriers (-1.77 [-2.21 to +0.26], p<0.001). Glucose disappearance rate (k), HOMA-IR, fasting insulin, glucose and lipid levels were similar between the two groups. 3 carriers (age 29, 53 and 55y) had k rates below 1%, and elevated fasting glucose levels, indicating diabetes mellitus. Bone density measured by DXA was lower in patients (lumbar spine Z-score -1.6 [-2.6 to -0.9]) compared to carriers (-0.7 [-2.5 to 0.9],p=0.04), likely influenced by their short stature. One postmenopausal carrier fulfilled the criteria for osteoporosis. Radius total and trabecular densities were similar between the groups yet metacarpal bone width was significantly lower in patients (Z-score -2.67[-3.5 to -2.56]) than in carriers (-1.28 [-1.92 to -0.54], p=0.001). Percent body fat was normal and no subject had a history of low impact fractures.
Conclusions: 3/12 IGFALS gene carriers had type 2 diabetes and there was some evidence of insulin resistance in this cohort. ALS deficiency causes impairment in bone lengthening and widening but there is insufficient evidence for a reduction in bone density, with some evidence for a gene-dose effect.

P1-d2-281 GH and IGF Physiology 1

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

Renata Stawerska[1]; Malgorzata Szalapska[1]; Joanna Smyczyńska[1]; Elżbieta Czkwianianc[2]; Hanna Pisarek[3]; Maciej Hilczer[1]; Andrzej Lewiński[1]
[1]Polish Mother's Memorial Hospital – Research Institute, Department of Endocrinology and Metabolic Diseases, Lodz, Poland; [2]Polish Mother's Memorial Hospital – Research Institute, Department of Gastroenterology and Pediatrics, Lodz, Poland; [3]Chair of Endocrinology, Medical University of Lodz, Hormonal Diagnostic Laboratory, Lodz, Poland

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

served in children born small for gestational age (SGA), as well as in children with growth hormone deficiency and neurosecretory dysfunction.
Objective and hypotheses: The aim of the study was to compare the fasting ghrelin, leptin and IGF-I concentrations in healthy prepubertal SGA children to children with idiopathic short stature born appropriate for gestational age (AGA).
Methods: Sixty five SGA children (birth weight below -2.0 SD), aged 4.85-9.75 years (mean±SD: 6.81±1.32 years) and 33 AGA children with idiopathic short stature, aged 3.6-10.75 years (mean±SD: 7.66±2.2 years) were qualified into the study. The control group consisted of 8 healthy, prepubertal AGA children, aged from 5.53 to 10.2 years (mean ± SD: 8.39±2.26 years) with normal body height and normal body weight. In each child, fasting ghrelin, leptin and IGF-I concentrations were measured.
Results: BMI SDS values were not different among groups. Ghrelin concentrations in SGA (2537.63±1598.36 pg/mL) were significantly higher than in AGA ISS children (1500.98±796.47 pg/mL) and than in Controls (1188.84±351.83 pg/mL). Also leptin concentrations were higher in SGA children than in AGA ISS children (4.97±5.01 ng/ml vs 2.8±3.02 ng/ml, p=0.03). Moreover, IGF-I SDS were higher in SGA children than in AGA ISS children.
Conclusions: It is possible that increased secretions of ghrelin, leptin and IGF-I are adaptive mechanisms to achieve normal growth and weight gain in prepubertal SGA children.

P1-d3-282 GH and IGF Treatment 1

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

Gabriel Kalifa[1]; Christopher J Child[2]; Christine Jones[3]; Judith L Ross[4]; Gudrun A Rappold[5]; Charmian A Quigley[6]; Alan G Zimmermann[6]; Werner F Blum[3]
[1]Université Paris V René Descartes, Hôpital Cochin-Saint-Vincent de Paul, Paris, France; [2]Eli Lilly and Company, Lilly Research Laboratories, Windlesham, United Kingdom; [3]Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany; [4]Thomas Jefferson University, Department of Pediatrics, Philadelphia, United States; [5]Heidelberg University, Department of Molecular Human Genetics, Heidelberg, Germany; [6]Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, United States

Background: Pts with SHOX-D have variable degrees of skeletal anomaly, from none to pronounced mesomelic dysplasia.
Objective and hypotheses: To examine baseline differences & the effect of 2-yr GH treatment on radiological features in pts with SHOX-D & TS.
Methods: In a 2-yr controlled clinical trial, 51 pts with SHOX-D were randomized to GH (SHOX-GH; N=27; 0.35 mg/kg/wk) or non-treatment (SHOX-noGH; N=24). 26 GH-treated pts with TS were included for comparison. Entry criteria: genetically confirmed SHOX-D or TS; age ≥3yr; bone age (BA) <8yr (F), <10yr (M), <9yr (TS); prepubertal; height (ht) <3rd %ile (or <10th %ile & ht velocity <25th %ile). X-rays (hand/wrist, forearm, lower leg) were assessed at baseline & 2-yr for presence or severity of a variety of anomalies.
Results: At baseline, SHOX-noGH, SHOX-GH & TS pts had similar age, BA & ht SDS (Table). Tibial tuberosities & Kosowicz sign (hypertrophic internal femoral condyle) were less common in SHOX-GH vs TS. Prevalence of radial bowing & moderate/severe carpal wedging appeared higher in SHOX-D vs TS (but p>0.05). Baseline radial & ulnar lengths were not significantly different between SHOX-D & TS.
After 2-yr of study, no significant difference was observed between SHOX-GH & SHOX-noGH for prevalence or severity of any assessed anomaly. However, there were differences at 2-yr between SHOX-GH & TS (Table). Prevalence of radial bowing was significantly greater in SHOX-GH than TS, & despite no difference in ht gain, radial & ulnar lengths were significantly shorter in SHOX-GH vs TS. Carpal wedging was more severe in SHOX-GH vs TS.

Parameter	BL SHOX-noGH	BL SHOX-GH	BL TS	BL SHOX-GH vs TS p-value	2-yr SHOX-noGH	2-yr SHOX-GH	2-yr TS	2-yr SHOX-GH vs TS p-value
Age (yr, mean±SD)	7.5±2.7	7.3±2.1	7.5±1.9	0.914	-	-	-	-
Bone age (yrs, mean±SD)	6.6±2.8	6.5±2.0	6.7±1.6	0.928	8.9±2.9	9.2±2.1	9.1±1.7	Not available
Height SDS (mean±SD)	-3.3±1.0	-3.3±0.8	-3.7±0.9	0.111	-3.0±0.9	-2.1±1.0	-2.6±1.0	Not available
Tibial tuberosities (% of pts)	13.0	12.5	44.0	0.025	8.3	14.8	32.0	0.193
Kosowicz sign (% of pts)	8.7	12.5	56.0	0.002	16.7	14.8	36.0	0.112
Radial bowing (% of pts)	70.8	52.0	36.0	0.393	79.2	66.7	20.8	0.002
Moderate or severe carpal wedging (% of pts)	37.5	40	23.1	0.578	33.3	40.7	8.3	0.012
Radial length (cm, median)	12.8	12.6	13.3	0.074	14.5	14.6	16.1	0.032
Ulnar length (cm, median)	13.4	14.0	14.2	0.428	16.2	15.8	17.6	0.029

Table: Demographics and radiological findings at baseline (BL) and 2-yr.

Conclusions: Radiological findings of tibial tuberosities & Kosowicz sign are suggestive of TS. Forearm anomalies such as radial bowing & carpal wedging are suggestive of SHOX-D, but also occur in TS. After 2-yr, there were no significant differences between SHOX-GH & SHOX-noGH in any assessed radiological anomalies, indicating that 2-yr GH treatment had no significant effect on skeletal anomalies in SHOX-D.

P1-d3-283 GH and IGF Treatment 1

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

Charmian A Quigley[1]; Alan G Zimmermann[2]; Christopher J Child[3]; Ron G Rosenfeld[4]; Leslie L Robison[5]; Heike Jung[6]; Werner F Blum[6]
[1]Lilly Research Laboratories, Endocrinology, Indianapolis, United States; [2]Lilly Research Laboratories, Statistics, Indianapolis, United States; [3]Lilly Research Laboratories, Endocrinology, Windlesham, United Kingdom; [4]Oregon Health Sciences University, Pediatrics, Portland, United States; [5]St Jude Children's Research Hospital, Epidemiology, Memphis, United States; [6]Lilly Research Laboratories, Endocrinology, Bad Homburg, Germany

Background: Long-term follow up of the French SAGhE cohort (n=6928 adult pts with history of GHTx for childhood isolated idiopathic GH deficiency [IsIGHD], idiopathic short stature [ISS] or small for gestational age birth [SGA]) demonstrated a standardized mortality ratio (SMR) of 1.3 (95% CI, 1.08-1.64) vs French adult general population (Carel JC et al 2012;JCEM 97;416).
Objective: To assess mortality rates for GHTx pts in 2 prospective observational studies: n=11076 IGHD, ISS & SGA children in GeNeSIS; n=1428 adults with childhood-onset (CO) GHD in HypoCCS.
Methods: Death rates were calculated per 100000 person-years (PY) for IsIGHD, ISS, SGA in GeNeSIS (mean 3yr GHTx) & per 1000 PY for COGHD in HypoCCS (mean 4yr GHTx). SMRs were calculated using country, age, & sex-specific general population mortality data from CDC & WHO.
Results: Five deaths (age 4-20y) were reported among IsIGHD, ISS & SGA pts in GeNeSIS, for an overall death rate of 19.5/100000 PY; SMR 0.32 (95% CI, 0.10-0.75): IsIGHD,3 (diabetic ketoacidosis; respiratory failure; road accident); ISS,1 (septic meningitis); SGA,1 (disconnected V-P shunt). 14 deaths (age 19-53y) were reported in COGHD pts followed in HypoCCS, for an age/sex-standardized observed death rate of 3.2/1000 PY (vs 2007 CDC age/sex-adjusted US rate of 7.6/1000). All deaths were in pts with non-idiopathic GHD (due to craniopharyngioma,7; other brain tumor,2; empty sella,2; pituitary hemorrhage,1; unspecified,2). Causes of death were recurrent/2nd intracranial tumor,2; suicide,2; injury/accident,2; carotid artery stenosis, cerebral hemorrhage, pancreatitis, respiratory failure, sepsis 1 each; unspecified,3.

	Patient Number	Person-years of Follow-up	Deaths	Rate per 100,000 Person-years (95% CI)
GeNeSIS (Paediatric Patients)				
IsIGHD	7712	18715	3	16.0 (3.3-46.9)
ISS	2538	5060	1	19.8 (0.5-110.1)
SGA	826	1860	1	53.8 (1.4-299.6)
All	11076	25634	5	19.5 (6.3-45.5)
HypoCCS (Adults with Childhood-onset GHD)				Standardized Rate per 1,000 Person-years (95% CI)
Idiopathic	448	2026	0	0
Non-idiopathic	1080	4170	14	5.4 (0.2-10.6)
All	1528	6196	14	3.2 (0.2-6.2)

Conclusions: There was no evidence of increased mortality relative to general population rates for GHTx IGHD, ISS or SGA paediatric pts in GeNeSIS or adult COGHD pts in HypoCCS. However, this analysis reports data for pts during, not after, GHTx, so is not directly comparable with SAGhE. The analysis is also limited by use of general population data due to lack of untreated controls, and limited duration of follow-up.

P1-d3-284 GH and IGF Treatment 1

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

Sun Hee Heo[1]; Yoo-Mi Kim[2]; Young-Eun Seo[2]; Chang-Woo Jung[2]; Gu-Hwan Kim[3]; Beom Hee Lee[2]; Choong Ho Shin[4]; Jin-Ho Choi[2]; Han-Wook Yoo[2]

[1]Asan Medical Center, Genome Research Center for Birth defects and Genetic Diseases, Seoul, Republic of Korea; [2]Asan Medical Center, Pediatrics, Seoul, Republic of Korea; [3]Asan Medical Center, Medical Genetics Center, Seoul, Republic of Korea; [4]Seoul National University College of Medicine, Pediatrics, Seoul, Republic of Korea

Background: Recombinant human growth hormone (rhGH) treatment is recommended for children with idiopathic short stature (ISS). Discovery of biomarkers predicting the responsiveness to rhGH has tremendous clinical implications. Two-dimensional difference gel electrophoresis (2-D DIGE) that separate proteins from gels using different fluorescent dyes between specimens, is used to discover new biomarkers.
Objective and hypotheses: This study was undertaken to identify new serum biomarkers differentially expressed by short-term rhGH therapy in patients with ISS.
Methods: The study included 11 children (nine males and two females) with ISS. They were treated with rhGH at a dose of 0.31±0.078 mg/kg/week for three months. To examine the differences in serum levels of specific proteins associated with growth response, immunodepletion of six highly-abundant serum proteins followed by 2-D DIGE analysis, and subsequent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, was carried out in order to generate a panel of proteins differentially expressed after short-term rhGH therapy.
Results: Fourteen spots were differentially expressed after rhGH treatment. Among them, apo E and apo L-1 expression were consistently enhanced, while SAA was reduced after rhGH therapy. The differential expressions of these proteins were subsequently verified by western blot analysis using sera of the before and after rhGH treatment. This study found that only the serum apo L-1 levels were significantly elevated after rhGH therapy ($P<0.01$).
Conclusions: 2-D DIGE is a powerful strategy for the discovery of predictive biomarkers. This study suggests that rhGH therapy influences lipid metabolism and that apo L-1 protein can be used as a predictive marker of the short-term effects of rhGH therapy in ISS patients.

P1-d3-285 GH and IGF Treatment 1

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

Eduardo Adrian Chaler[1]; Juan Manuel Lazzati[1]; Maria Gabriela Ballerini[2]; Gabriela Ropelato[2]; Mercedes Maceiras[1]; Mauro Frusti[2]; Ignacio Bergada[2]; Marco Aurelio Rivarola[1]; Alicia Belgorosky[1]

[1]Hospital de Pediatria Prof. Dr. Juan P. Garrahan, Endocrinology, Buenos Aires, Argentina; [2]Hospital de Niños Ricardo Gutierrez, Endocrinology, Buenos Aires, Argentina

Background: Different cut-off values, from 3 to10 ng/ml, depending on the assays, have been defined for pharmacological test (PhT) of growth hormone (GH) secretion, based on biochemical and clinical studies. Recently, a 22K recombinant GH isoform IRP IS 98/574 have been commercialized. We had previously defined a 6.1 ng/ml value, in terms of ICMA IRP IS 80/505 as serum GH cut-off limit in PhT of GH secretion.
Objective and hypotheses: Our aim was to assess which is the GH deficiency (GHD) diagnostic cut-off limit of serum GH in PhT of GH secretion for ICMA immunoassay with the IRP IS 98/574.
Methods: We analyzed serum GH concentration in 138 serum samples, using ICMA calibrated with both IS 80/505 and IS 98/574. Blood samples (n=138), from 92 different individuals, (103 males and 35 female; age range, 2.5 15.0 years) were used. All samples were selected from arginine and clonidine PhT.
Results: We found high significant linearity ($y = 0.7307x + 0.2768$, $p<0.001$) between ICMA IS 98/574 (y) and ICMA IS 80/505 (x). The y value for fixed x value of 6.1 ng/ml in the ICMA IS 80/505 was calculated, and a diagnostic cut-off limit for ICMA IS 98/574 was estimated as 4.7 ng/ml. ICMA IS 98/574 / ICMA IS 80/505 ratio plot and 95% prediction interval was 0.79±0.17. Linearity and Bias were reconfirmed by Passing Bablock analyses and the Wilcoxon test respectively.
Conclusions: High significant linearity and differences among the assay with different IS were found. To suspect the diagnosis of GHD, we propose 4.7 ng/ml as the serum GH cut-off limit value in PhT for ICMA immunoassay with the IRP IS 98/574as. Finally for each GH assay, an appropriate cut-off value for serum GH maximum peak response to PhT should be strongly recommended .

P1-d3-286 GH and IGF Treatment 1

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

Ralph Decker[1]; Kerstin Albertsson-Wikland[1]; Berit Kriström[2]; Jovanna Dahlgren[1]

[1]Institute of Clinical Sciences, The Queen Silvia Children's Hospital Gothenburg, Department of Pediatrics, Gothenburg, Sweden; [2]Institute of Clinical Sciences, Umeå University, Department of Pediatrics, Umeå, Sweden

Background: Few studies have evaluated the metabolic outcomes of growth hormone (GH) treatment in prepubertal short children during different growth phases. We have studied individualized GH treatment in the maintenance growth phase and have previously found that IGF-I lost its importance as one of the main growth factors after reaching near mid-parental height (MPH). This was shown by lacking correlations between IGF-I and anabolic GH effects, i.e. Δ height, Δ lean soft tissue and Δ bone mineral content.
Objective and hypotheses: This study was to construct two groups - one with half the original dose, reduced individualized dose (RID, 17–50 µg/kg/day) and the other to remain at the original dose, unchanged individualized dose (UID, 17–100 µg/kg/day). A third group remained on fixed standard dose (FIX) constituted the control group (43 µg/kg/day). All three groups were indistinguishable at the randomization. In total, 98 prepubertal children, who were initially short due to either isolated GHD or ISS participated in this 2-year randomized trial.
Methods: We focused on the metabolic outcome of children growing channel-parallel within ± 0.3 heightSDS after two years of maintenance growth who had fulfilled their catch-up growth from previous treatment.
Results: We observed Δ leptin to be highly correlated with the anabolic GH effects in 18 children growing closest to the height SDS mean (Figure 1). They included 10 children receiving RID, 4 receiving the UID, and 4 in the fixed dose group (FIX).

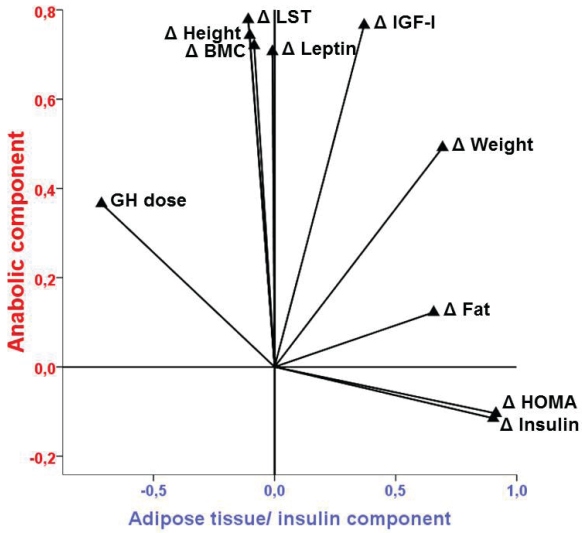


Figure 1 Two-dimensional principal component analysis (PCA) shows vectors with close mutual correlations form vector bundles with angles pointing in the same direction. Changes (Δ) between start and two years of maintenance treatment are included in the analysis. LST (lean soft tissue mass, kg), BMC (bone mineral content, kg), Fat (fat mass, kg).
Conclusions: Leptin gains importance as a growth factor, promoting both longitudinal bone growth, muscle growth and bone mass growth together with IGF-I during the maintenance period of GH treatment.

P1-d3-287 GH and IGF Treatment 1

Characterization and prevalence of primary and severe IGF-I deficiency in a large cohort of French children with short stature

Raphael Teissier[1]; Isabelle Flechtner[1]; Ana Colmenares[1]; Karen Lambot-Juhan[2]; Geneviève Baujat[3]; Christian Pauwels[1]; Dinane Samara-Boustani[1]; Jacques Beltrand[1]; Albane Simon[1]; Caroline Thalassinos[1]; Hélène Crosnier[4]; Hanane Latrech[5]; Graziella Pinto[1]; Martine Le Merrer[3]; Valérie Cormier-Daire[6]; Jean-Claude Souberbielle[7]; Michel Polak[8]
[1]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades, Pediatric Endocrinology and Centre des Maladies Endocriniennes Rares de la Croissance, Paris, France; [2]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades, Radiology, Paris, France; [3]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades, Genetic Department and Rare Bone Dysplasia Center, Paris, France; [4]Centre Hospitalier Intercommunal de Poissy-Saint-Germain-en-Laye, Hôpital de Saint-Germain-en-Laye, Pediatric Unit, Saint-Germain-en-Laye, France; [5]Oujda University hospital, Pediatric Unit, Oujda, Morocco; [6]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades - Université Paris Descartes, Genetic Department and Rare Bone Dysplasia Center, Paris, France; [7]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades, Hormonal Biochemistry, Paris, France; [8]Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Necker Enfants Malades - Université Paris Descartes, Pediatric Endocrinology and Centre des Maladies Endocriniennes Rares de la Croissance, Paris, France

Background: Primary and severe IGF-1 deficiency (IGFD) is defined by height < -3 Standard Deviation score (SD), IGF-I < 2.5th percentile, GH sufficiency and exclusion of secondary forms of IGFD. The prevalence of primary and severe IGFD is not known so precisely, despite different studies. However, the patients with IGFD must be identified as a new recombinant human Insulin-like Growth Factor-I (rhIGF-I) is available since 2007 in Europe.
Objective: The aim of this work was 1) to assess the prevalence of the primary and severe IGFD in a large French cohort from a tertiary Pediatric Endocrinology Unit and 2) to characterize those.
Population and methods: We included all the patients referred for a suspicion of growth disorder from January 2004 to December 2009. The patients were classified in 8 etiological categories. Low IGF1 levels were defined by persistent circulating levels below the 2.5th percentile of the distribution for age and, when appropriate, pubertal stage.
Results: 2546 patients were included in this study. Among the patients who were classified Small for Gestational Age or Idiopathic Short Stature, we identified 30 patients with criteria of IGFD corresponding to a prevalence of 1.2%. We analyzed IGF-I generation test response, IGF-I levels evolution, and the efficiency of growth hormone replacement therapy when introduced (12 out of 15). Finally, only 4 children out of 30 are candidates for the rhIGF-I replacement therapy.
Conclusion: We showed that the prevalence of primary and severe IGFD based on criteria of rhIGF-I treatment is 1.2 % in our cohort. This results in a small number of children eligible for such treatment.

P1-d3-288 GH and IGF Treatment 1

Analysis of metabolic risk parameters in small for gestational age (SGA) patients and its relationship with growth hormone treatment

Amaya Rodriguez Estevez[1]; Concepcion Fernandez Ramos[2]; Lorea Martinez-Indart[3]; Isabel Rios Orbañanos[1]; Beatriz Pacho del Castaño[1]; Itxaso Rica Etxebarria[1]; Amaya Vela de Sojo[1]; Gema Grau Bolado[1]; Francisco Javier Nuñez Rodriguez[2]; Ignacio Diez Lopez[4]; Ainhoa Sarasua Miranda[4]; Elena Artola Aizalde[5]; V Cancela Muñiz[5]; E. Blarduni Cardon[6]; Andere Eguireun Rodriguez[7]
[1]Hospital Universitario de Cruces, Paediatric Endocrinology, Barakaldo, Spain; [2]Hospital Universitario de Basurto, Paediatric Endocrinology, Bilbao, Spain; [3]Hospital Universitario de Cruces, Epidemiology, Barakaldo, Spain; [4]Hospital Universitario de Alava, Paediatric Endocrinology, Vitoria, Spain; [5]Hospital Universitario de Donosti, Paediatric Endocrinology, Donosti, Spain; [6]Hospital Universitario de Zumárraga, Paediatric Endocrinology, Zumárraga, Spain; [7]Hospital Universitario de Mendaro, Paediatric Endocrinology, Mendaro, Spain

Background: Short children born SGA are at increased risk for diabetes and cardiovascular disease. Growth Hormone (GH) treatment can also increase this metabolic risk.
Objective: Analyze the metabolic risk parameters in SGA patients at baseline and during treatment with GH in our region.
Population and methods: We performed a retrospective review of SGA children in treatment with GH in our region, using as data sources the GH Committee Register. We established 2 groups according to HOMA-RI (≤or > 3.16). Other metabolic risk parameters were evaluated: HDL-C, triglycerides (TG), systolic (SBP) and diastolic (DBP) blood pressures and GH dosage. Description of categorical (%) and continuous (mean±SD) variables. Comparative study of different parameters with Kruskal-Wallis non-parametric test.
Results: 76 patients (36 M, 40 F). Birth height 42.9±4.3cm (-2.8±1.2SD) and weight 2067.9±680g (-2.1±0.7SD). 31% were premature. HOMA-IR>3.16 group duplicated during first year with GH treatment.

HOMA-IR	% Initial n=74	% Fisrt year n=76	% Fourth year n=16
≤3.16	89.2	75	75
>3.16	10.8	25	25

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

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

p=0.022* p=0.040**

The percentage of SGA who started puberty grew 5.7%, 14.8% and 58.8% at baseline, 1 year and 4 years of GH treatment. There were no statistically significant differences between HOMA-IR at 1st year and 4th year in relation with other parameters.
Conclusions: HOMA-IR >3.16 group doubled during first year with GH treatment. This is the only metabolic risk parameter which has changed. This group also showed higher IGF-1 values than HOMA-IR ≤ 3.16 group.

P1-d3-289 GH and IGF Treatment 1

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

Elbrich Siemensma[1]; Roderick Tummers-de Lind v Wijngaarden[1]; Dederieke Festen[1]; Zyrhea Troeman[1]; Janielle van Alfen-van der Velden[2]; Barto Otten[2]; Anita Hokken-Koelega[1]

[1]Dutch Growth and Research Foundation, Paediatric Endocrinology, Rotterdam, Netherlands; [2]UMC St Radboud, Paediatric Endocrinology, Nijmegen, Netherlands

Background: Knowledge about the effects of GH-treatment on cognitive functioning in children with PWS is limited.
Objective and hypotheses: To evaluate the effect of GH-treatment on cognitive functioning in children with PWS.
Methods: Fifty pre-pubertal children, aged 3.5 to 14 years were studied in a randomized controlled GH trial during 2 years, followed by a longitudinal study during 4 years of GH-treatment. Cognitive functioning was measured biennially by short forms of the WPPSI-R or WISC-R, depending on age. Total IQ (TIQ) score was estimated based on 2 subtest scores.
Results: During the RCT, mean SD-scores of all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, while in untreated controls mean subtest SD-scores and mean TIQ score decreased and became lower compared to baseline. This decline was significant for the Similarities (p=0.04) and Vocabulary (p=0.03) subtests. After 4 years of GH-treatment, mean SD-scores on the Similarities and Block design subtests were significantly higher than at baseline (p=0.01 and p=0.03, respectively) and scores on Vocabulary and TIQ scores remained similar compared to baseline. At baseline, children with a maternal uniparental disomy had a significantly lower score on the Block design subtest (p=0.01), but a larger increment on this subtest during 4 years of GH-treatment than children with a deletion. Lower baseline scores correlated significantly with higher increase in Similarities (p=0.04) and Block design (p<0.0001) SD-scores.
Conclusions: Our study shows that GH treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract reasoning and visuospational skills, during four years of GH-treatment. Furthermore, children with a greater deficit had more benefit from GH-treatment.

P1-d3-290 GH and IGF Treatment 1

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

Reiko Horikawa[1]; Toshiaki Tanaka[2]; Susumu Yokayo[3]; Yoshiki Seino[4]; Yoshihisa Ogawa[5]; Fumiaki Kiyomi[6]; Anne-Marie Kappelgaard[7]

[1]National Center for Child Health and Development, Division of Endocrinology and Metabolism, Tokyo, Japan; [2]Tanaka Growth Clinic, Department of Pediatrics, Tokyo, Japan; [3]National Center for Child Health and Development, Department of Medical Subspecialities, Tokyo, Japan; [4]Osaka Kosei Nenkin Hospital, Department of Pediatrics, Osaka, Japan; [5]Novo Nordisk Pharma Ltd, Development Division, Tokyo, Japan; [6]Novo Nordisk Pharma Ltd, Clinical Statistics, Tokyo, Japan; [7]Novo Nordisk A/S, Medical Affairs, Global Marketing GHT, Søborg, Denmark

Background: In children with short stature born small for gestational age (SGA), growth hormone (GH) treatment is associated with an acceleration of growth allowing most to achieve an adult height adequate for their target height. The pathophysiology of growth failure may arise from anomalies in the GH–IGF axis, including deficits in GH/IGF-I concentrations and/or GH/IGF-I sensitivity.
Objective and hypotheses: There is limited data on long-term GH therapy in Japanese children with SGA. This report investigates the relationship between GH dose, Δheight SDS (HSDS) and ΔIGF-I SDS.
Methods: Data were analysed from a 156-week extension of a 104-week multi-centre, randomised, double-blind, parallel group trial investigating the efficacy and safety of GH. Sixty-five children with SGA (age 3–<8 years) received GH at 0.033mg/kg/day (n=31, 64.5% male, mean age 5.34 years) or 0.067mg/kg/day (n=34, 58.8% male, mean age 5.27 years). Change from baseline in HSDS, IGF-I SDS, and bone age (BA) were recorded.
Results: After 260 weeks, ΔHSDS for chronological age (CA) was significantly positively correlated with ΔIGF-I SDS (n=57; r=0.664; p<0.0001). A greater increase in ΔHSDS and ΔIGF-I SDS was observed in the 0.067 than 0.033mg/kg/day group; correlation between ΔHSDS and ΔIGF-I SDS was shown in both (0.067mg/kg/day: r=0.579; p=0.0010; 0.033mg/kg/day: r=0.715; p<0.0001). ΔHSDS was positively correlated with ΔIGF-I SDS in male (n=36; r=0.707; p<0.0001) and female (n=21; r=0.685; p=0.0006) patients. Mean bone age (BA) was behind CA at baseline (BA/CA ratio <1) but increased during GH treatment, reaching 1.01 in the 0.033mg/kg/day group and 1.09 in the 0.067mg/kg/day group at 260 weeks.
Conclusions: In short Japanese children born SGA long-term GH therapy was associated with a dose-dependent increase in IGF-I that positively correlates with changes in HSDS. No acceleration of BA was noted in the low dose group but a minor acceleration was seen in the high dose group.

P1-d3-291 GH and IGF Treatment 1

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

Michel Polak[1]; Joachim Woelfle[2]; Peter Bang[3]; Pascal Maisonobe[4]; Pascale Dutailly[4]

[1]Hôpital Necker Enfants Malades, Endocrinologie diabétologie pediatrique, Paris, France; [2]Children's hospital, Paediatric Endocrinology Division, Bonn, Germany; [3]Linköping University, Division of Pediatrics, Linköping, Sweden; [4]Ipsen Innovation, Research and Development, Les Ulis, France

Background: The post-authorization registry, European Increlex® (Mecasermin [rDNA Origin] Injection) Growth Forum Database (EU-IGFD) was initiated in Dec 2008 to monitor the safety and effectiveness of Increlex® in children with growth failure.
Objective: Report 2-yr data from the registry, particularly for naive pre-pubertal patients (pts).
Methods: Multicenter, open-label observational study.
Results: As of Oct 2011, 128 pts were enrolled in 9 countries: 34% female, 81% pre-pubertal, 60% growth treatment-naïve, 81% with severe primary IGF-1 deficiency. Mean (SD) age at first injection was 10.2 (3.9) yrs. Median treatment duration was 480 days (i.e. 183 pt-yrs). Median dose was 40, 110 and 113 µg/kg BID at start of treatment, yr 1 and yr 2 respectively. A total of 88 targeted adverse events (AEs) were reported for 43 pts (37%), the most frequent being hypoglycemia (18 pts, 16%) serious for 4 pts (3%). New treatment-related serious AEs since last report were papilloedema, thyroid nodule, splenic infarction, toxoplasmosis (1 pt each). Effectiveness data are summarised by mean (SD) of height SDS, Height Velocity (HV).

	N*	Height [SDS]	N*	HV [cm/yr]
ALL				
Baseline	107	-3.7 (1.5)	61	4.7 (1.6)
Yr 1	87	-3.2 (1.4)	86	6.7 (2.2)
Yr 2	40	-3.3 (1.6)	40	6.3 (3.9)
NAIVE PRE-PUBERTAL				
Baseline	57	- 3.4 (1.5)	26	4.8 (1.8)
Yr 1	33	- 2.9 (1.3)	33	7.4 (1.9)
Yr 2	11	- 2.6 (1.4)	11	5.6 (2.2)

* N data available at the timepoint

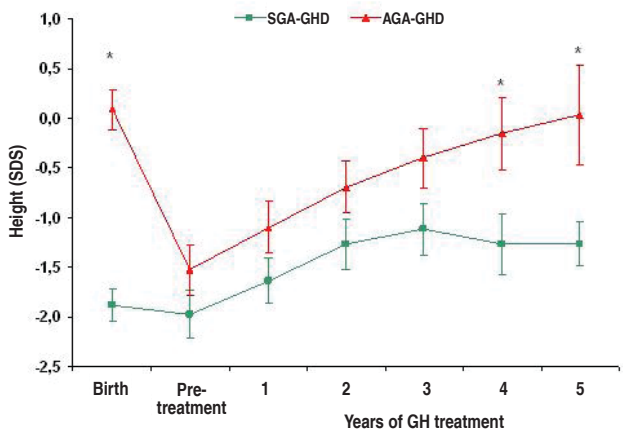
Conclusions: These 2-yr interim results from EU-IGFD registry did not show any new safety signals. However, we recommend special care for patients with complex syndrome, particularly if related to proliferative conditions. On average, HV increased after 1 yr of treatment and results of 2nd yr HV should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

P1-d3-292 GH and IGF Treatment 1

Different growth pattern in response to GH therapy in SGA and AGA GH-deficient prepubertal children

Cristina Meazza[1]; Carmine Tinelli[2]; Sara Pagani[1]; Kamilia Laarej[1]; Benedetta Pietra[1]; Giada Biddeci[1]; Mauro Bozzola[1]
[1]University of Pavia, Internal Medicine and Therapeutics, Pavia, Italy; [2]IRCCS San Matteo Foundation, Clinical Epidemiology and Biometrics Unit, Pavia, Italy

Background: Birth weight may influence the first year response to growth hormone (GH) therapy in GH-deficient (GHD) children, while its role during the following years of treatment is not fully elucidated.
Objective: We examined the growth of small for gestational age GHD children (SGA-GHD) and appropriate for gestational age GHD (AGA-GHD) children during the first five years of GH therapy.
Methods: Twenty-three SGA-GHD (birth weight and/or length < -2 SDS for sex and gestational age; 11 F and 12 M; age: 1.3-12.3 years) and 26 AGA-GHD (11 F and 15 M, age: 3-12 years) were randomly enrolled and followed for five years after the start of GH treatment, yearly collecting height, weight height velocity and body mass index (BMI). Both SGA-GHD and AGA-GHD patients received rhGH at the recommended weekly dose of 0.25 mg/kg subcutaneously, in six daily doses.
Results: Although SGA-GHD subjects had significantly reduced weight and length at birth, at time of GHD diagnosis the height-SDS and BMI-SDS were comparable between the two groups. During the first years of treatment height-SDS was still not different between SGA-GHD and AGA-GHD, although the values were always slightly lower in SGA-GHD; only from the fourth year of treatment AGA-GHD showed significantly increased height-SDS (figure). Pre-treatment height velocity SDS was similar between SGA-GHD and AGA-GHD (SGA-GHD: -1.82±0.30 SDS, AGA-GHD: -2.33±0.21 SDS), but it became significantly higher (SGA-GHD: 1.72±0.30 SDS, AGA-GHD: 2.67±0.21 SDS; p=0.039) in AGA-GHD during the first year of treatment. However, in the following years height velocity SDS was still comparable between the two groups.
Conclusions: In conclusion, height-SDS is comparable between SGA-GHD and AGA-GHD in the first years of GH treatment, but it becomes significantly reduced in SGA-GHD children from the fourth year on. Further studies are needed for confirming if this "waning effect" persists also in the following years affecting the final height of these subjects.



P1-d3-293 GH and IGF Treatment 1

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

Paul Saenger[1]; Vaman Khadilkar[2]; Klavdia A. Radjuk[3]; Elena Bolshova[4]; Rajesh Khadgawat[5]; Mohamed El Kholy[6]; Meena Desai[7]; Valentina Peterkova[8]; Veronica Mericq[9]; Hyi-Jeong Ji[10]; Yoon Ju Bae[10]; E. Christine Siepl[11]; Dieter Martin[11]
[1]Albert Einstein College of Medicine, Department of Pediatrics, New York, United States; [2]Jehangir Hospital, Department of Paediatric Endocrinology, Pune, India; [3]LPU 2nd Children's Hospital, Department of Endocrinology, Minsk, Belarus; [4]Academy of Medical Sciences of Ukraine, Institute of Endocrinology and Metabolism, Kiev, Ukraine; [5]All India Institute of Medical Sciences, Department of Endocrinology, New Delhi, India; [6]Ain Shams University of Cairo, Department of Pediatrics, Cairo, Egypt; [7]Sir Hurkisondas Nurrotumdas Hospital & Research Centre, Department of Endocrinology, Mumbai, India; [8]Endocrinological Scientific Center, Russian Academy of Medical Science, Children´s Department, Moscow, Russian Federation; [9]Institute of Maternal and Child Research, Department of Endocrinology, Santiago de Chile, Chile; [10]LG Life Sciences Ltd, hGH Development, Seoul, Republic of Korea; [11]Biopartners GmbH, Development, Baar, Switzerland

Background: Growth hormone has profound effects on body composition and lipid metabolism in children as well and adults. We previously demonstrated in a randomized phase III multicentre study that once weekly LB03002 is comparable to daily rhGH regarding safety and efficacy and now present data on metabolic parameters.
Objective and hypotheses: The effect of LB03002 treatment on glucose metabolism and lipid parameters was assessed.
Methods: 167 previously untreated children with growth failure (HTSDS ≤ -2 unless organic GHD, HVSDS ≤ -1) due to idiopathic or organic GH deficiency (GH peak ≤ 7 ng/mL in two tests) were randomized to receive either once weekly LB03002 (0.5 mg/kg) or once daily rhGH (0.03 mg/kg) for 12 month. Patients treated with daily rhGH were switched to weekly LB03002 in the second year.
Results: Selected growth and metabolic parameters (mean±SD):

	First year/second year treatment	Weekly/weekly (N=87)	Daily/weekly (N=80)
HV (cm/yr)	Baseline	2.64 ± 1.11	2.87 ± 1.04
	1st year	11.72 ± 2.58	12.16 ± 3.09
	2nd year	8.33 ± 1.92	7.28 ± 2.34
Cholesterol (mmol/L)	Baseline	4.70 ± 1.60	4.51 ± 0.94
	Month 12	4.58 ± 1.49	4.43 ± 0.80
	Month 24	4.43 ± 1.33	4.22 ± 0.94
Glucose (mmol/L)	Baseline	4.01 ± 1.08	4.09 ± 0.90
	Month 12	4.40 ± 0.68	4.66 ± 0.55
	Month 24	4.56 ± 0.48	4.47 ± 0.66
HbA1c (%)	Baseline	5.07 ± 0.38	5.04 ± 0.33
	Month 12	5.13 ± 0.29	5.14 ± 0.32
	Month 24	5.35 ± 0.36	5.33 ± 0.36

Growth parameters were comparable for both groups. There were no relevant differences between treatment groups in metabolic parameters (glucose, insulin, haemoglobin A1c, cholesterol, and triglycerides). Cholesterol slightly decreased, while fasting glucose, insulin, and HbA1c increased over time but remained within the normal range. Changes from baseline of the assessed parameters were not clinically relevant. No patients developed diabetes.
Conclusions: The data show that weekly treatment with LB03002 has a comparable metabolic effect as treatment with daily rhGH.
*In cooperation with Biopartners' and LG Life Sciences' GH Study Group

P1-d3-294 GH and IGF Treatment 1

The relationship between baseline IGF-I levels, first year growth and metabolic outcomes in children born small for gestational age during high dose growth hormone therapy- North European SGA Study (NESGAS)

Rikke Beck Jensen[1]; Ajay Thankamony[2]; Susan M. O´Connell[3]; Burak Salgin[4]; Jeremy Kirk[5]; Malcolm Donaldson[6]; Sten-A. Ivarsson[7]; Olle Söder[8]; Edna Roche[3]; Hilary Hoey[3]; David Dunger[2]; Anders Juul[1]

[1]Rigshospitalet, Copenhagen University Hospital, Department of Growth and Reproduction, Copenhagen, Denmark; [2]University of Cambridge, Institute of Metabolic Science, Department of Pediatrics, Cambridge, United Kingdom; [3]The National Children's Hospital, University of Dublin, Trinity College, Department of Pediatrics, Dublin, Ireland; [4]University Children's Hospital Düsseldorf, Department of General Paediatrics and Neonatology, Düsseldorf, Germany; [5]Birmingham Children's Hospital, Department of Endocrinology, Birmingham, United Kingdom; [6]Royal Hospital for Sick Children, Department of Endocrinology, Glasgow, United Kingdom; [7]University of Lund, Department of Clinical Sciences, Endocrine and Diabetes Unit, Malmoe, Sweden; [8]Karolinska Institutet & University Hospital, Pediatric Endocrinology Unit, Department of Women's and Children's Health, Stockholm, Sweden

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

Methods: The North European Small for Gestational Age Study (NESGAS) is a multicenter study (n=110, 69 males) of GH therapy in prepubertal short SGA children. Patients received GH therapy at 67µg/kg/day for 1 year. Glucose metabolism was assessed by a short intravenous glucose tolerance test. Insulin sensitivity (IS) and insulin secretion were estimated from HOMA and acute insulin response (AIR), respectively. Disposition index (DI) provided a measure of insulin secretion adjusted for IS.

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

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

P1-d3-295 GH and IGF Treatment 1

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

Nienke Bakker[1]; Anita Hokken-Koelega[2]; Maithe Tauber[3]; Hartmut Wollmann[4]

[1]Dutch Growth and Research Foundation, Pediatric endocrinology, Rotterdam, Netherlands; [2]Dutch Growth and Research Foundation/ Erasmus MC-Sophia Children's Hospital, Pediatric endocrinology, Rotterdam, Netherlands; [3]Hôpital des Enfants, Dept of Pediatrics, Div of Endocrinology, Genetics, Gynaecology and Bone Diseases, Toulouse, France; [4]Pfizer, Endocrine Care, Surrey, United Kingdom

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

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

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

Results: Mean(SD) birth weight and birth length SD score (SDS) were -1.25 (1.1) and -0.23 (1.4), resp. Mean (SD) mid parental height SDS was -0.1 (1.1). Prior to start of GH, 128 children had a GH stimulation test with a mean (SD) GH peak of 8,1 (9,3) ug/l and mean (SD) serum IGF-I SDS -1.1 (1.5). All children remained prepubertal during the 3 years of GH treatment. Mean (SD) GH dose was 0.23 (0.06) mg/kg/wk. Table 1. Growth data during 3 years of GH treatment in 415 prepubertal children.

	Baseline	1 year	2 years	3 years
Age (yr)	4.5 (2.9)	5.5 (2.9)	6.5 (2.9)	7.5 (2.9)
Height SDS*	-2.1 (1.4)	-1.1 (1.4)	-0.7 (1.4)	-0.4 (1.4)
H-MPHSDS	-2.0 (1.6)	-1.0 (1.5)	-0.6 (1.5)	-0.3 (1.4)
HV (cm/yr)	6.6 (3.5)	11.1 (2.9)	8.3 (2.0)	7.3 (1.8)
Weight SDS	-0.2 (2.1)	0.3 (1.8)	0.7 (1.6)	1.0 (1.5)
Bone age (yr)	4.3 (2.9)	5.6 (3.4)	7.0 (3.6)	8.5 (3.5)

*Prader reference (Helv. Paed. Acta 1988)

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

P1-d3-296 GH and IGF Treatment 1

Once-weekly, CTP-modified hGH (MOD-4023) effectively maintains IGF-1 levels within the normal range in growth hormone deficient adults, supporting initiation of clinical development in children

Vera Popovic[1]; Miklos Goth[2]; Peter Vanuga[3]; Juraj Payer[4]; Marija Pfeifer[5]; Martin Bidlingmaier[6]; Ron Rosenfeld[7]; Eyal Fima[8]

[1]University Clinical Center, Clinic for Endocrinology, Belgrade, Serbia; [2]Military Hospital, 2nd Department of Internal Medicine, Budapest, Hungary; [3]National Institute of Endocrinology and Diabetology, Department of Endocrinology, Lubochna, Slovakia; [4]University Hospital, Comenius University, V th.Internal Department, Bratislava, Slovakia; [5]University Medical Centre Ljubljana, Department of Endocrinology, Ljubljana, Slovenia; [6]Medizinische Klinik Campus Innenstadt, Endocrine Research Laboratories, Munich, Germany; [7]Oregon Health & Science University, Department of Pediatrics, Portland, United States; [8]Prolor-Biotech, Ltd, Clinical Development, Nes Ziona, Israel

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

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

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

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

Conclusions: Once-weekly, repeated doses of long-acting MOD-4023 were shown to be safe and well tolerated in GHDA. IGF-I levels were maintained

within the normal range in most MOD-4023 treated patients for the entire 4 weeks. Based on the positive results of Stage I, the pediatric clinical development program was initiated and a phase II study in naïve, pre-pubertal GH Deficient children is currently ongoing.

P1-d3-297 GH and IGF Treatment 1

Response to growth hormone (GH) treatment in GH deficient survivors of bone marrow transplantation with total body irradiation (BMT/TBI) in childhood

Nikki Davis[1]; Ruth Elson[1]; Jacqueline Cornish[1]; Michael Stevens[2]; Elizabeth Crowne[1]
[1]University Hospitals Bristol NHS Foundation Trust, Paediatric Endocrinology and Diabetes, Bristol, United Kingdom; [2]University of Bristol, Institute of Child Health, Bristol, United Kingdom

Background: Childhood BMT/TBI survivors are often short adults, despite GH treatment for growth hormone deficiency(GHD). Final height is adversely affected by reduced spinal and pubertal growth but there are no data examining the GH response after TBI.
Objective and hypotheses: To assess the response to GH treatment and GH sensitivity using IGF-1 generation tests in GHD BMT/TBI survivors compared to controls with isolated GHD.
Methods: 41 subjects were investigated.13/22 BMT/TBI survivors were GHD(Group 1) and 6/19 controls had isolated GHD(Group2) in insulin tolerance tests. All GHD subjects had pre-treatment IGF-1 generation tests (IGF-1 day 1 and day 5 after 4 days of GH (33mcg/kg/day). Auxology and whole body DEXA for body composition were assessed before and after 12 months GH treatment. Fat free mass index (fat free mass /height2) was calculated. GH dose was the same in both groups (5mg/m2/week).
Results: Group1:6 post-pubertal, 7 pre-pubertal. Group 2:All 6 pre-pubertal. Height velocity increased from 3.4 to 9.0cm/yr ($p<0.001$,N=7) and from 4.0 to 10.7cm/yr ($p<0.001$,N=6) and height SDS improved from -1.24(1.60) to -0.75(1.80) (p=0.004) and -2.17(1.05) to -1.84 (0.89) (p=0.001), in groups 1 and 2 respectively. IGF-1 in generation tests increased from 23.0(13.0-64.1) to 55.0(31.7-81.6)mcg/l ($p<0.001$,N=13,group 1) and 24.5(9.8-53.8) to 47.0(16.1-80.2)mcg/l (p=0.002,N=6,group 2). Body fat decreased in both groups: from 34.9(11.7) to 32.0(12.5)% (p=0.017,N=13,group1), and 32.5(16.1) to 27.8(14.4)% (p=0.034,N=6,group 2). Fat free mass index increased in both groups: from 11.89(2.09) to 13.06(1.60)(p=0.002,N=13,group1) and 12.45(1.04) to 15.16(1.78)kg/m2 (p=0.005,N=6,group 2). The magnitudes of all these changes did not differ between the groups.
Conclusions: Paired data comparing growth and IGF-1 responses, and body composition changes before and after 12 months GH treatment in BMT/TBI survivors with GHD and non-BMT controls with isolated GHD showed similar responses to GH treatment. We found no evidence of GH resistance in BMT/TBI survivors.

P1-d3-298 GH and IGF Treatment 1

IGF-I in isolated GHD (IGHD) and non-GHD boys; results from two randomized clinical trials with GH treatment to adult height

Elena Lundberg[1]; Kerstin Albertsson-Wikland[2]; Björn Jonsson[3]; Berit Kriström[1]
[1]Umeå University, Institute of Clinical Science, Paediatrics, Umeå, Sweden; [2]Institute of Clinical Science, The Sahlgrenska Academy at University of Gothenburg, Department of Pediatrics, Göteborg Pediatric Growth Research Center (GP-GRC), Gothenburg, Sweden; [3]Uppsala University, Department of Women's and Childen's Health, Uppsala, Sweden

Background: GHD children are more responsive to GH than non-GHD children regarding growth. Knowledge regarding IGF-I responsiveness is scarce.
Objective and hypotheses: To compare changes and mean levels of IGF-I in IGHD vs non-GHD boys from two clinical trials on GH treatment to adult height (AH). The same GH dose would give IGF-I levels higher in a GHD vs a non-GHD group.
Methods: The IGHD boys (n=85) had ≥1 prepubertal yr on GH 33µg/kg/d (GH^{33}) and were at onset of puberty randomized to 67µg/kg/d (GH(GH^{67}), n=50) or GH^{33} (n=35). The non-GHD boys (n=58), were randomized to GH (GH^{67}) (n=35) or GH(GH^{33}) (n=23) at GH start and 36 boys had one prepubertal yr. IGF-I was measured at baseline, 3m and yearly and converted to SDS. Study levels of $IGF\text{-}I_{SDS}$ were defined as individual mean levels from 12m to study stop for the non-GHD group and for the GHD group from 12m from randomization close after onset of puberty to AH and the ΔIGF-I as change from start to mean level. Heights were converted to SDS vs the childhood reference and at AH vs the reference at 18 yrs.
Results: The prepubertal $\Delta IGF\text{-}I_{SDS}$ was significantly lower in the non-GHD GH^{33} group vs the GH^{67} (1.6±1.2 vs 2.3±0.9, p=0.046) and vs the GHD GH^{33} (2.7±1.3, p=0.020) and GH^{67} (2.5±1.9, NS vs GH^{33}). The non-GHD pubertal $IGF\text{-}I_{SDS}$ study levels differed in GH^{33} vs GH^{67} (0.6±0.8 vs 1.3±1.1, p=0.008) but not in relation to and within the other groups. As a single variable, baseline $IGF\text{-}I_{SDS}$ explained 13-14% of the variance in total & prepubertal $height_{SDS}$ gain. Using multivariate regression prepubertal and pubertal $\Delta IGF\text{-}I_{SDS}$ were significant predictors of total, prepubertal and pubertal $height_{SDS}$ gain.
Conclusions: The $\Delta IGF\text{-}I_{SDS}$ and pubertal level was found to be lower for the non-GHD GH^{33} group when IGHD and non-GHD boys randomized in clinical trials to GH^{33} or GH^{67} were compared. Explaining total height gain for the merged group, ΔIGF-I but not GH dose was informative; thus it is not the dose per se that is important, but the responsiveness.

P1-d3-299 GH and IGF Treatment 1

Final height after long-term growth hormone therapy in SHOC2 mutation-positive patients

Federica Tamburrino; Emanuela Scarano; Annamaria Perri; Benedetta Vestrucci; Michele Torella; Monica Guidetti; Laura Mazzanti
Rare Disease and Auxology Unit, Department of Pediatrics, S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Background: Abnormalities of the RAS-MAPK signaling pathway has been identified as the cause of Noonan syndrome and related disorders (RASopathies). A single missense mutation in SHOC2 gene determine a specific condition previously termed Noonan-like syndrome with loose anagen hair (NS/LAH) [1,2]. Typical features are NS facial phenotype, growth retardation frequently associated with proven growth hormone (GH) deficiency, developmental delay with ADHD disorder, hair anomalies, darkly pigmented skin with eczema or ichthyosis, hypernasal voice, cardiac defects.
Methods: In our group of NS/LAH patients, 3 of them (1 male and 2 female) reached the final height (FH) with GH (dose 7 mg/m2/week) for a mean period of 12.7±2.2 yrs. 2 patients presented SHOC2 mutation and a female showed SHOC2 and SOS1 mutation. All the patients had a severe GH deficiency (GH peak < 4 ng/ml after arginine and L-dopa test or clonidine test) and bone age and puberty were delayed. A female was treated with L-tyroxine for hypothyroidism. No patient showed pubertal growth spurt.
Results: FH was 161,3 cm in the male (<3rd centile for H and age - Target H (TH) <25th centile) with height gain of 0 SD after GH therapy; in females, 147,1 cm (<3rd centile - TH <25th centile) with height gain of +0.4 SD and 148,5 cm (<3rd centile and TH > 50th centile) with height gain of +1.79 SD.
Conclusions: Differently to PTPN11+ patients, two-thirds of SHOC2+ subjects show severe GH deficiency and short stature. This is the first study reporting FH data: the height gain after long-term GH-therapy was variable and no pubertal growth spurt was observed. This behaviour seems to confirm the hypothesis of an interconnection between SHOC2 and GH signal pathway that remain to be elucidated in NS/LAH subjects. Other studies with FH data in SHOC2+ patients will be mandatory to collect statistically significant groups of subjects.
1. Mazzanti et al., Am J Med Genet A 2006;118A:279–286
2. Cordeddu et al., Nat Genet 2009; 41(9):1022-1026

P1-d3-300 GH and IGF Treatment 1

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

Alessandro Salvatoni[1]; Jenny Berini[1]; Giuseppe Chiumello[2]; Antonino Crinò[3]; Stefania Di Candia[2]; Luigi Gargantini[4]; Graziano Grugni[5]; Lorenzo Iughetti[6]; Antonella Luce[1]; Valentina Milan[1]; Gianluca Musolino[1]; Anna Saporiti[1]; Paola Sogno Valin[2]; Valeria Spica Russotto[1]; Giuliana Trifirò[7]

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

Background: Following few cases of death reported in Prader-Willi syndrome (PWS) children during GH treatment, several international committees recommend serum IGF-1 monitoring, otorhynolaryngologist examination and polysomnography before and during GH treatment in PWS. However there is no general consensus on how to interpretate these evaluations for a safe GH therapy management.
Objective: To develop a practical decisional tool, based on current clinical evidence and international recommendations that provides useful and reliable criteria to modulate GH therapy in children with PWS.
Methods: We developed a 15-point score, which measures the respiratory risk on the basis of polisomnography parameters (respiratory disturbance index, and mean SpO2), otolaryngologist examination by flexible fibroendoscopy (Brodky and Wang criteria for assessment of tonsils and adenoid hypertrophy) and levels of IGF-1. This score was named "POI" (Polisomnography, Otolaryngologist, IGF1). We used it retrospectively, as preliminary validation, in 7 patients in order to compare the therapy modulations established by empirical criteria and those resulting by using the score.
Results: In five patients the modulation based on empirical criteria was the same of that suggested by our score, in one case POI resulted slightly more restrictive and in another case slightly more tolerant.
Conclusions: Our proposal of a score to be used in the modulation of GH therapy in Prader-Willi syndrome, before becoming a tool for standardization of this treatment, requires first to be tested on a wider sample of patients in order to obtain general consensus.

	Parameters	Values (points)	Values (points)	Values (points)	Values (points)	Score
PSG	Mean SpO2	>97% (0)	>95% (1)	< 95% (2)		
	RDI	<1(0)	1-3 (2)	>3-5 (3)	>5 (4)	
ENT	Tonsils	<25%(0)	25-50%(1)	50-75% (2)	>75% (3)	
	Adenoids	<1/3 (0)	1/3 (1)	2/3 (2)	3/3 (3)	
IGF1	Percentiles	< 25th (0)	25-50th (1)	50-75th (2)	>75th (3)	
						TOTAL:

Score	Before start of GH Tx	During GH treatment
0-3	Start at 0.03 mg/kg/day	mantain or increase the dosage
4-6	Start at 0.015 mg/kg/day	mantain the dosage
7-9	Start at 0.01 mg/kg/day	50% dosage reduction
≥10	Do not start treatment	discontinuation of the treatment

P1-d3-301 GH and IGF Treatment 1

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

Bradley S. Miller[1]; Ferah Aydin[2]; Frida Lundgren[2]; Mitchell Geffner[3]

[1]University Of Minnesota Amplatz Children's Hospital, Pediatric Endocrinology, Minneapolis, MN, United States; [2]Pfizer Health AB, KIGS Medical Outcomes, Sollentuna, Sweden; [3]Children's Hospital Los Angeles, Endocrinology, Diabetes, and Metabolism, Los Angeles, United States

Background: Stimulant use for the treatment of Attention Deficit Disorder (ADD) has been associated with growth failure. Prevalence of ADD has been reported to be 3-8% worldwide in school-aged children.
Objective and hypotheses: Identify the frequency of ADD and stimulant therapy in children treated with Genotropin.
Methods: Children enrolled in KIGS with idiopathic GH deficiency (IGHD), idiopathic short stature (ISS), and Turner Syndrome (TS) were evaluated for the associated diagnosis of ADD prior to initiation of Genotropin. Concomitant medication entries for stimulant medications were captured. Baseline auxologic information was also extracted.
Results: Out of 75,261 children enrolled in KIGS between 1990 and 2011, 1,748 children (2.3%) with ADD stimulant therapy were identified. When analyzed by country of origin, a significantly greater number of children was from the U.S. (1,092/14,794=7.4%) than from the rest of the world (656/60,467=1.1%) ($p<0.001$). In the U.S., the frequency of ADD by treatment group was: IGHD: 8.7%, ISS: 8.2%, and TS: 3.8%. IGHD, ISS and TS children with ADD treated with stimulants from whole cohort were taller ($p<0.0001$) and thinner ($p<0.0001$) than those in all 4 groups without ADD at the time of initiation of Genotropin therapy.
Conclusions: There is significant difference in the frequency of ADD stimulant use in U.S. vs non-U.S. children in KIGS. These differences in prevalence may reflect regional differences in diagnosis and treatment of ADD. The overall prevalence of ADD in children with IGHD, ISS, and SGA in KIGS was similar to worldwide prevalence estimates of ADD in the general population. That of TS, however, was surprisingly less given that the prevalence of ADD is reportedly higher in girls with TS. At the time of entry into KIGS, linear growth failure in children with IGHD +ADD and ISS +ADD is less severe and BMI lower than in non-ADD counterparts. We speculate that low BMI diagnosis of ADD stimulant use may lead to referral at heights that are less severely affected.

	IGHD ADD	IGHD Non-ADD	ISS ADD	ISS Non-ADD	TS ADD	TS Non-ADD
Ht SDS	-2.56	-2.88	-2.60	-2.84	-3.04	-3.21
BMI SDS	-0.46	-0.25	-0.64	-0.43	+0.22	+0.51

P1-d3-302 GH and IGF Treatment 1

IGF-I resistance due to a heterozygous complete *IGF1R* deletion: response to treatment with rhGH

Elena Gallego-Gómez[1]; Jaime Sánchez del Pozo[1]; Jaime Cruz-Rojo[1]; Ana Gómez-Núñez[2]; Ricardo Gracia-Bouthelier[3]; Karen E. Heath[4]; Angel Campos-Barros[4]

[1]Hospital Univ. 12 de Octubre, Pediatric Endocrinology, Madrid, Spain; [2]IdiPAZ, UAM, Hospital Universitario La Paz, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; [3]Hospital Universitario La Paz, Pediatric Endocrinology, Madrid, Spain; [4]IdiPAZ, UAM, Hospital Univ. La Paz, & CIBERER, U753, ISCIII., Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain

Background: IGF-I resistance syndrome (IGF1RS) is characterized by intrauterine and postnatal growth deficit with normal or supranormal IGF-I levels. Additional features may include intellectual deficit, microcephaly and dysmorphism. IGF1RS may be caused by genomic or genetic defects affecting the *IGF1R* locus (15q26.3).
Clinical case: A girl born SGA at term by cesarean delivery due to symmetrical intrauterine growth retardation whose main clinical characteristics are summarized in the table below. At 1.7 yrs she had mild craniofacial dysmorphism, bilateral clinodactyly (5th finger) and a IQ of 69. At 4.5 yrs she started rhGH treatment (0.035 mg/kg/d) which increased her growth rate (GR) with concurrent IGF-I and IGFBP3 increases.

Chron. age (yr)	Bone age (yr)	Length (cm) (SDS)	BW g (SDS)	CP (cm) (SDS)	IGF-I (ng/ml) (SDS)
birth	-	42 (-4.71)	1.850 (-3.84)	30 (-3.21)	-
1.7	1	70.5 (-4.17)	7.2 (-3.38)	42 (-4.54)	118 (-0.69)
4.5	3	89 (-4.24)	13.2 (-1.86)	-	279 (+1.82)

Methods: *Genetic analysis:* Karyotype, molecular analysis of *IGF1R* by HRM and MLPA in the proband and both parents.
Results: Normal karyotype. MLPA analysis detected a *de novo* heterozygous complete *IGF1R* deletion in the proband. *Follow up:* After 42 months of rhGH treatment her height improved to 114.4 cm (-2.5 SDS; GR 5.68 cm/yr) and her IGF-I and IGFBP3 levels were markedly increased: 1,066 ng/ml (+4.7

SDS) and 643 ug/ml (+2.71 SDS), respectively, even under a relatively lower rhGH dosage of 0.022 mg/kg/d. Due to the marked IGF-I increase and the concomitant incidence of an epithelioma of Malherbe, rhGH treatment was interrupted. During the first 6 months after rhGH withdrawal, GR remained at 7.2 cm/yr (IGF-I 367 ng/ml; +2 SDS; IGFBP3 643ug/ml, +1.8 SDS), decreasing to 3.2 cm/yr during the following year. At her last check up (height 121,5 cm; -2.46 SDS) she showed a striking increase of body fat and BMI (22.73).
Conclusions: IGF1R haploinsuficency due to heterozygous *IGF1R* deletion produces a clinical phenotype characteristic of IGF1RS. In our patient, low dose rhGH treatment quickly normalized the growth rate but dramatically increased circulating IGF-I. Hence, pro and cons of rhGH treatment in these patients should be carefully considered.

P1-d3-303 GH and IGF Treatment 1

Long-term efficacy of GH therapy in 37 short children with Silver-Russell syndrome

Gerhard Binder[1]; Melanie Liebl[1]; Joachim Woelfle[2]; Roland Schweizer[1]
[1]University Children's Hospital, Pediatric Endocrinology, Tuebingen, Germany; [2]University Children's Hospital, Pediatric Endocrinology, Bonn, Germany

Background: Long-term efficacy of GH in patients with Silver-Russell syndrome (SRS) is unknown.
Objective: We aimed to compare final heights of GH-treated and GH-untreated.
Individuals: 37 GH-treated patients (16 females) were diagnosed according to strict clinical criteria and participated in this retrospective study with a matched control group conducted at our hospital. Molecular analysis revealed IGF2-H19 epimutations in 12 and matUPD7 in 5 patients (4 not tested). At start of GH therapy mean age was 7.54 /6.73 y (males/females) and mean height was -3.02/-3.96 SDS (Prader). Mean target height was -0.13/+0.05 SDS. Mean duration of GH therapy was 5.53/5.65 y. Mean GH dose was 54+/-14 µg/kg*d. In 16 patients (9 males) puberty was blocked by GNRH-analogs for 2.4 y (mean). The untreated cohort comprised 13 individuals (5 females) with a mean height of -3.19 SDS, a mean age of 7.4 y and a mean target height of -0.53 SDS.
Methods: The primary endpoints were adult height SDS at a growth velocity < 1 cm/y or at an age > 18 y and the gain in adult height (cm) in comparison to untreated matched by an outcome-blinded statistician according to gender, height, target height and age at puberty start.
Results: GH treated (males/females) reached a mean final height of -1.84 SDS (+/-1.00)/ -2.49 SDS (+/-0.83) gaining +1.18 SDS/+1.47 SDS in comparison to the height SDS at GH start. Mean height SDS of the untreated cohort stayed unchanged (-3.12 SDS). The matched treated males were in mean 11.1 cm (+/-6.1) taller while the effect size in females was +4.0 cm (+/-12.7). Positive predictors of final height after GH were height at start and duration of GH therapy. Height gain was highest in the shortest.
Conclusion: GH therapy improves final height in SRS to the same extent as in non-syndromic SGA children.

P1-d2-304 Gonads and Gynaecology 1

Deficient expression of genes involved in the endogenous defense system against transposons in cryptorchid boys

Faruk Hadziselimovic[1]; Nils Omar Hadziselimovic[2]; Philippe Demougin[2]; Gunthild Krey[3]; Eduard Oakeley[4]
[1]Kindertagesklinik Liestal, Pediatrics, Liestal, Switzerland; [2]Biocentrum Basel, Molecular Genetics, Basel, Switzerland; [3]Institute for Andrology, Histology, Liestal, Switzerland; [4]Novartis Institutes for Biomedical Research, Molecular Biology, Basel, Switzerland

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

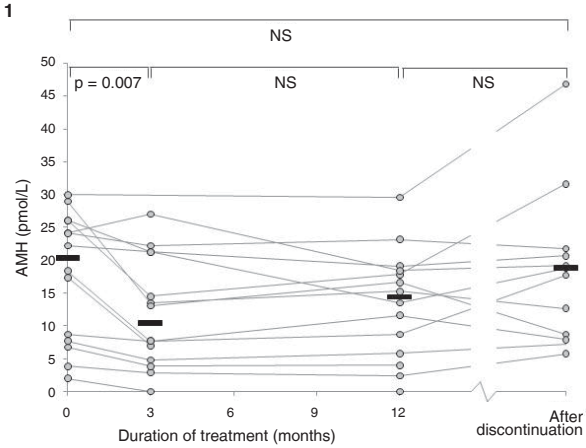
P1-d2-305 Gonads and Gynaecology 1

Serum levels of anti-Müllerian hormone in girls with central precocious puberty before, during and after gonadal suppression with GnRH agonist

Casper P Hagen; Sørensen Kaspar; Juul Anders
University of Copenhagen, Rigshospitalet, Dep. of Growth and Reproduction, Copenhagen, Denmark

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

Figure 1



Conclusions: Circulating AMH declined significantly following gonadal suppression with long-acting GnRHa, but recovered to pre-treatment levels after discontinuation of GnRHa. Thus, GnRHa treatment did not seem to affect the ovarian follicle pool permanently in pubertal girls.

P1-d2-306 Gonads and Gynaecology 1

Ethinyloestradiol-cyproteroneacetate versus low-dose pioglitazone-flutamide-metformin for adolescent girls with androgen excess: divergent effects over 1 year

Lourdes Ibáñez[1]; Marta Díaz[1]; Matilde R. Chacón[2]; López-Bermejo[3]; Elsa Maymó-Masip[2]; Cristina Salvador[4]; Joan Vendrell[2]; Francis de Zegher[5]

[1]Hospital Sant Joan de Déu, University of Barcelona & CIBERDEM, ISCIII, Endocrinology, Esplugues, Barcelona, Spain; [2]Hospital Universitari de Tarragona Joan XXIII, IISPV, Universitat Rovira i Virgili & CIBERDEM, ISCIII, Endocrinology, Tarragona, Spain; [3]Dr. Josep Trueta Hospital & Girona Institute for Biomedical Research, Pediatrics, Girona, Spain; [4]Hospital Sant Joan de Déu, University of Barcelona, Gynecology, Esplugues, Barcelona, Spain; [5]University Hospital Gasthuisberg, Pediatric Endocrinology, Leuven, Belgium

Background: There are essentially two strategies to treat adolescent girls with androgen excess. The classic approach is to silence the ovaries; an alternative approach is to normalize adipose-tissue metabolism, insulin sensitivity and ovarian function.
Objective: To compare the effects of the traditional therapy (an oral oestroprogestagen) to those of the novel treatment (a low-dose combination of generics) in adolescent girls with androgen excess.
Methods: Randomized, open-label trial over 1 year performed in adolescents (age, 16 yr; BMI, 23 Kg/m2) with hyperinsulinemic androgen excess and without risk of pregnancy. The girls were randomized to receive EthinylOestradiol-CyproteroneAcetate (EO-CA; Diane 35 Diario) (n=17) or a low-dose combination of pioglitazone 7.5 mg/d, flutamide 62.5 mg/d and metformin 850 mg/d (PioFluMet) (n=17). Main outcome measures: hirsutism and acne scores; androgen excess; circulating C-reactive protein (CRP) and high-molecular-weight (HMW) adiponectin; carotid intima media thickness (cIMT); body composition by absorptiometry; abdominal fat partitioning by MRI; gene expression in biopsies of subcutaneous adipose tissue at the abdominal level.
Results: EO-CA and low-dose PioFluMet reduced clinical and biochemical androgen excess comparably, but had divergent effects on CRP and HMW adiponectin; on cIMT; on lean mass; on abdominal and visceral fat; and on the expression of genes such as CD163, TNF-like weak inducer of apoptosis (TWEAK) receptor and angiopoietin-like protein 4 (ANGPTL4), respectively related to macrophage activation, inflammation and lipoprotein metabolism in adipose tissue. All these divergences pointed to a healthier condition on low-dose PioFluMet.
Conclusion: Over 1 year, PioFluMet compared favourably to EO-CA in adolescents with androgen excess.

P1-d2-307 Gonads and Gynaecology 1

Ovarian function and reproductive hormone levels in girls with Prader-Willi syndrome: a longitudinal study

Elbrich Siemensma[1]; Janielle van Alfen-van der Velden[2]; Barto Otten[2]; Joop Laven[3]; Anita Hokken-Koelega[1]

[1]Dutch Growth and Research Foundation, Paediatric Endocrinology, Rotterdam, Netherlands; [2]UMC St Radboud, Paediatric Endocrinology, Nijmegen, Netherlands; [3]Erasmus MC, Obstetrics & Gynaecology, Rotterdam, Netherlands

Background: The etiology of hypogonadism in girls with Prader-Willi Syndrome (PWS) remains uncertain.
Objective and hypotheses: To evaluate gonadal function longitudinally in girls and female adolescents with PWS.
Measurements: Longitudinal assessment of AMH, gonadotrophins, estradiol (E2), inhibin B and A and pubertal development in girls and female adolescents with PWS.
Patients: Sixty-one girls participating in the Dutch PWS Cohort study. Serum AMH, gonadotrophins, E2 and inhibin B and A levels were compared with reference values.
Results: AMH levels in girls and female adolescents with PWS were comparable to reference levels between 6 months and 22 years of age. From 10 years of age, FSH and LH levels increased to above the 5th percentile compared to reference levels. E2 and inhibin B levels were in the low normal range in the majority, and inhibin A levels were low, but detectable in almost half the female adolescents with PWS. The median age at puberty onset was comparable, but the median ages at attaining Tanner M3 ($p=0.05$) and M4 ($p<0.0001$) were significantly higher in girls with PWS than in healthy references.
Conclusions: Our study shows that the primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. We found no classical hypogonadotropic hypogonadism. However, maturation of follicles and progression of pubertal development are impaired. As these impairments are not absolute, ovulation and thus conception cannot be ruled out in individual female adolescents with PWS.

P1-d2-308 Gonads and Gynaecology 1

Serum kisspeptin levels in adolescent girls

Olga Gumenyuk[1]; Yuriy Chernenkov[2]; Natalya Zacharova[3]; Svetlana Kunina[2]; Anna Kunina[2]

[1]Saratov Medical University, Hospital #5, Polyclinic paediatric endocrinology, Saratov, Russian Federation; [2]Saratov Medical University, Hospital, polyclinic paediatrics and neonatology, Saratov, Russian Federation; [3]Saratov Medical University, Research laboratory, Saratov, Russian Federation

Background: The kisspeptin, the neuropeptide product of KiSS-1 gene has recently emerged as essential gatekeeper of pubertal activation of the reproductive axis. Mutations or targeted disruptions in the gene cause hypogonadism or premature sexual development and sterility.
Objective and hypotheses: The study was undertaken to research the kisspeptin levels in healthy adolescent girls and adolescents with endocrine and gynaecological disorders.
Methods: Serum kisspeptin levels were determined in 77 adolescent girls (aged 15-18 yrs): healthy (control group, n=22), girls with amenorrhea (n=23), adolescent girls with dyshormonal mammary dysplasia (n=12), patients with polycystic ovary syndrome (n=9), pregnant adolescent girls (n=11). The concentration of kisspeptin was measured using Kisspeptin-10 (Metastin) and competitive enzyme immunoassay. The SPSS 16.0 software package was used to perform statistical analyses. Results were analyzed using two-way ANOVA. Data are expressed as median, P value of < 0.05 were considered statistically significant.
Results: The investigation shows that healthy and pregnant adolescent girls had kisspeptin median levels 0.03 ng/ml. Among the girls with amenorrhea kisspeptin levels was lower 0.01 ng/ml ($p=0.014$). Girls with mastopathy (dyshormonal dysplasia) had kisspeptin levels 0.05 ng/ml ($p=0.033$). Serum kisspeptin levels were significantly higher in adolescent girls with polycystic ovary syndrome than in control group (0.3 vs 0.03 ng/ml, $p<0.01$).
Conclusions: Kisspeptin levels is various in healthy adolescent girls, girls with menstrual disorders, mastopathy, with polycystic ovary syndrome. In this study, we demonstrated that serum kisspeptin level was significantly lower in girls with amenorrhea and higher in girls with mastopathy and polycystic ovary syndrome. Serum kisspeptin may be used as a marker of sexual disorders, mammary diseases and polycystic ovary syndrome in girls.

P1-d2-309 Gonads and Gynaecology 1

Therapeutic trends in the treatment of paediatric and adolescent menstrual disorders

Orla Neylon[1]; Sonia Grover[2]; Margaret Zacharin[1]

[1]Royal Children's Hospital/Murdoch Children's Research Institute, Department of Endocrinology, Melbourne, Australia; [2]Royal Children's Hospital, Department of Gynaecology, Melbourne, Australia

Background: Medical care for girls with menstrual difficulties falls between general practitioners, endocrinologists, paediatricians and gynaecologists, among others.
Objective and hypotheses: To survey the practice of doctors treating young women for menstrual-related issues.
Methods: A questionnaire was developed and distributed to a wide range of

doctors involved in the treatment of young women.
Results: 306 questionnaires were returned/filled out electronically, 81% fully completed. Respondents comprised general paediatricians (50%), general practitioners (13.4%), paediatric endocrinologists (11.4%), and others (20%), with 46% of the cohort reporting >10 years experience. GPs were the most self-confident with 42.5% rating their competency as "very" or "extremely confident", followed by paediatric endocrinologists (40.6%). Overall, the oral contraceptive pill was the most frequently prescribed hormonal treatment, followed by the 'Implanon©', progesterone-only pills, an intrauterine device and depot progesterone injections. GPs and paediatric endocrinologists were much more likely ($p<0.01$ for all) to self-manage menstrual disorders Vs the other groups who referred patients preferably to their GP for management. Overall, doctors regarded potential for adherence as the most important factor when considering choice of therapy (34%), followed by side-effect profile (24%), patient preference (16%), interaction with concurrent medications (12%), and past (10%) or family history (4%). Doctors gave a treatment plan for 5 challenging scenarios. Community and general paediatricians were more likely to choose suboptimal treatment regimens than GPs or paediatric endocrinologists ($p<0.05$).
Conclusions: Despite quoting potential for adherence as a primary consideration, all doctor groups still prefer to prescribe the OCP as a first line. Some groups would benefit from education provision on this topic.

P1-d2-310 Gonads and Gynaecology 1

Screening for NR5A1 (SF-1) gene abnormalities in 26 cases of 46,XX primary ovarian insufficiency: an infrequent etiology

Pascal Philibert[1]; Besma Lakhal[2]; Françoise Paris[1]; Françoise Audran[3]; Philippe Bouchard[4]; Ali Saâd[2]; Sophie Christin-Maitre[4]; Charles Sultan[1]
[1]CHU Montpellier - Université Montpellier 1, Hormonologie, Montpellier, France; [2]Farhat Hached University Teaching Hospital, Cytogenetics and Reproductive Biology, Sousse, Tunisia; [3]CHU Montpellier, Hormonologie, Montpellier, France; [4]Hôpital Saint-Antoine - AP-HP, Endocrinology, Obstetrics & Gynecology, Paris, France

Background: Primary ovarian insufficiency (POI) is defined as an absence or cessation of normal ovarian function before 40 years of age. It accounts for about 10% of all female sterility. The genetic basis for POI was well established by reports of numerous familial cases and the detection of associated chromosomal abnormalities. Mutations in several genes like ATM, AIRE, FSH receptor, GALT1, BMP15, GDF9, FOXL2 and Inhibin A, as well as FMR1 premutation, were found to be associated with POI. The nuclear receptor steroidogenic factor 1 (NR5A1) plays a crucial role in the transcription of multiple target genes involved in adrenal and gonadal development, steroidogenesis and reproduction. Several works in man showed the very wide phenotypic spectrum of NR5A1 gene deficiency from 46,XY complete gonadal dysgenesis to male infertility.
Objective and hypotheses: Except for a report in 2000 of a p.Arg255Leu mutation in a 46,XX prepubertal girl with adrenal deficiency and apparently normal ovaries, it was only recently that NR5A1 abnormalities were formally identified as a cause of POI. We designed this work to evaluate the frequency of NR5A1 mutation through a homogeneous cohort of 26 young women of Maghrebian origin.
Results: The direct sequencing of the NR5A1 gene revealed a new unreported mutation c.763C>T (p.Arg255Cys) in one patient with primary amenorrhea and no visible abnormalities in the external genitalia. In vitro testing confirmed the functional impact of this variant. In addition, we identified the known variant c.437G>C (p.Gly146Ala) in exon 4 in 11 patients (44%) in heterozygous state and one patient in homozygous state (4%). The Gly/Ala polymorphism was more frequently identified in primary amenorrhea patients and its frequency was significantly higher in patients than in control females.
Conclusions: Although NR5A1 mutations are rarely identified in POI, the p.Gly146Ala variant is much more frequent in POI women than in controls.

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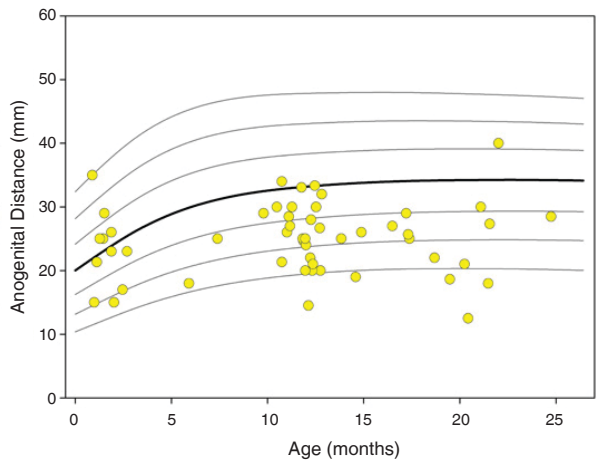
Anogenital distance and the masculinisation programming window: a clear association in isolated hypospadias

Ajay Thankamony[1]; Ken Ong[2]; David Dunger[1]; Daniel Carroll[3]; Martyn Williams[3]; Carlo Acerini[1]; Ieuan Hughes[1]
[1]University of Cambridge, University Department of Paediatrics, Cambridge, United Kingdom; [2]Institute of Metabolic Science, MRC Epidemiology Unit, Cambridge, United Kingdom; [3]Cambridge University Hospitals NHS Trust, Department of Paediatric Surgery, Cambridge, United Kingdom

Background: The concept of a masculinisation programming window (MPW) in the male fetus is established from rodent studies. Anogenital distance (AGD) is a reliable readout of factors acting during the MPW in animals.
Objective and hypotheses: To explore AGD in boys with hypospadias, a condition whose origin coincides with the MPW.
Methods: Boys with isolated hypospadias were recruited (n= 53, age 12.1±6.5 months) for a cross-sectional assessment of AGD, measured from the centre of the anus to the perineo-scrotal junction. AGD and penile length were compared to age-appropriate data from 485 normal full-term boys from the Cambridge Baby Growth Study (CBGS) with repeated measurements between 0-2 years. Standard deviation scores for AGD and penile length were calculated using the LMS method.
Results: Of the 41 boys with no prior surgery, the type of hypospadias was glandular, penile and perineal in 19, 17 and 5 patients respectively. Boys with hypospadias had lower weight (-0.33±1.54 vs 0.01±0.96 SDS, p=0.027) and length (-0.22±1.30 vs 0.29±0.93 SDS, $p<0.0001$) compared with normal boys; they also had markedly shorter AGD (-0.88±0.90 vs 0.03±0.77 SDS, $p<0.0001$) and penile length (-1.41±1.29 vs -0.02±0.82 SDS, $p<0.0001$), and these differences persisted after adjusting for weight, height and age.



Scatter plot of AGD in boys with hypospadias related to normative data (centiles 3-97th)

In boys with hypospadias, AGD was positively correlated with penile length (r=0.29, p=0.039), but did not differ by the type of hypospadias.
Conclusions: The aetiology of hypospadias is usually unknown. Nevertheless, an association of AGD with hypospadias points to the developmental stage in humans when disruption of organogenesis occurs. This observation and the reported links between AGD and sperm count, and testosterone in adults indicates that AGD is programmed long term. Our findings provide additional supporting evidence for the use of AGD as a biomarker of *in utero* exposure to endocrine disrupting agents which may affect the development of tests and male reproductive tract.

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

Polina Bogdanova[1]; Maria Kareva[1]; Irina Yarovaya[2]; Alexander Ilyin[3]; Olga Zlotnikova[4]

[1]Endocrinology Research Centre, Pediatric Endocrinology, moscow, Russian Federation; [2]Endocrinology Research Centre, Gynecological Department, moscow, Russian Federation; [3]Endocrinology Research Centre, Biochemical and Hormonal Laboratory, moscow, Russian Federation; [4]Endocrinology Research Centre, Fuctional Diagnostics Department, moscow, Russian Federation

Background: There are two main items that may be used to assess the dysfunction of ovarian tissue in hyperandrogenism (HA) – steriodogenesis and folliculogenesis. Women presenting with so called "typical" polycystic ovary syndrome (PCOS) seem to have higher stimulated 17-hydroxyprogesterone (17-OHP) level after LH-RH stimulation, suggesting impairment of steroidogenic pathways. Anti-Mullerian hormone (AMH) precisely reflects the number of small follicles in the ovary. Taken together LH-RH test and AMH might serve as a good way to define ovarian hyperandrogenism.
Objective and hypotheses: Adolescent girls with early pubarche in anamnesis (earlier than 10 yrs) are more likely to have adrenal HA, those who present with HA but underwent all the stages of puberty timely (telarche prior to pubarche) are more likely to have ovarian HA.
Methods: <25 girls matching PCOS Rotterdam criteria, all of them 2 yrs past menarche. 14 with early pubarche in case history (gr1) and 11 without (gr2). LH-RH test was performed during first 5-7 days of menstrual cycle with 17-OHP measurement 12 hours after LH-RH administration. AMH was measured by ELISA. All patients underwent pelvic ultrasound and hirsutism assessment (Ferriman-Gallway score).
Results: Adolescent girls with normal puberty were significantly different from those with early pubarche having bigger ovarian volume, less number of menses per year, less level of hirsutism. Stimulated 17-OHP in gr1 was 3.7[2.3:4.1]nmol/l vs. 6.2[3.6:7.0]nmol/l in gr2 (p=0.01). Same differences were found in the levels of AMH: gr1 – 3.2[1.5:4.5]ng/ml vs. gr2 – 8.9[5.3:11.9]ng/ml (p=0.006).

	Gr1 (n=14)	Gr2 (n=11)	p
Ovarian Volume, ml	8.4 [7.7:14.1]	14.0 [11.0:18.0]	0.05
Number of menstrual cycles per year	11.0 [7.0:12.0]	5.0 [3,0:11.0]	>0.05
Hirsutism (F.-Gallway score)	17.5 [13.0:23.0]	6.2 [3.5:9.0]	0.0002
AMH, ng/ml	3.2 [1.5:4.5]	8.9 [5.3:11.9]	0.006
17-OHP basal, nmol/l	2.8 [2.5:3.7]	2.4 [1.9:3.2]	>0.05
17-OHP after LH-RH, nmol/l	3.7 [2.3:4.1]	6.2 [3.6:7.0]	0.01

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

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Primary ovarian insufficiency in adolescent patients

Andrea Arcari[1]; María Eugenia Escobar[1]; Alejandra Ginaca[2]; Patricia Carabajal[2]; Violeta Chiauzzi[3]; Graciela Del Rey[1]; Rodolfo Rey[1]; Mirta Gryngarten[1]

[1]Hospital de Niños Ricardo Gutiérrez, Endocrinology, Buenos Aires, Argentina; [2]Hospital de Niños Ricardo Gutiérrez, Immunology, Buenos Aires, Argentina; [3]Instituto de biología y medicina experimental, Immunology, Buenos Aires, Argentina

Background: Primary ovarian insufficiency (POI) is diagnosed when a woman younger than 40 years old has amenorrhea and serum FSH levels in the menopausal range. In the absence of oophorectomy, chemotherapy, irradiation or chromosomopathies, POI is a heterogeneous condition whose etiology remains unknown in 90% of the cases. Scanty information exists about POI in adolescence.
Objective and hypotheses: To describe the phenotype and the prevalence of autoimmunity in an adolescent cohort with POI.
Methods: We performed a cross-sectional study of clinical, endocrine and ovarian ultrasound characteristics of 35 consecutive POI adolescents. We analyzed clinical records of girls with pubertal delay, primary or secondary amenorrhea and two FSH levels >30 mUI/ml. Patients with Turner syndrome, oophorectomy, chemotherapy and radiotherapy were excluded. FSH and LH were measured by IFMA, serum AMH by ELISA and estradiol by DELFIA. Karyotype was performed in all patients. Screening for organ and non organ specific autoantibodies by immunofluorescence, antiovarian antibodies by immunoblotting, antibodies against FSH-receptor by a radiometric assay and pelvic ultrasound were performed.
Results: Median age at diagnosis was 15.8 years. Familiar history of POI and of autoimmune disease were present in 31 % and 37% respectively. Seventy one percent presented with pubertal delay or primary amenorrhea, 29% had secondary amenorrhea. Pelvic ultrasound showed follicles in 37% of patients. In 15/32 girls various organ and non organ specific antibodies were found. Three of 5 patients (9%) with autoimmune diseases had antiovarian antibodies. AMH levels, evaluated in 13 girls, were low or undetectable. All patients had 46, XX karyotype.
Conclusions: In our cohort of adolescents 9% had autoimmune diseases plus antiovarian antibodies. Besides, 31% had different autoantibodies suggesting that an autoimmune mechanism could be involved. However the etiology of POI remains unknown in a high number of patients. More specific studies are needed to clarify this point.

P1-d2-314 Gonads and Gynaecology 1

The effect of short course treatment with raloxifen on pubertal gynecomastia

Iuliana Gherlan[1]; Cristina Patricia Dumitrescu[1]; Andreea Cristiana Brehar[1]; Andra Caragheorgheopol[2]; Camelia Procopiuc[1]

[1]"C.I.Parhon" National Institute of Endocrinology, Pediatric Endocrinology, Bucharest, Romania; [2]"C.I.Parhon" National Institute of Endocrinology, Research Laboratory, Bucharest, Romania

Background: Pubertal gynecomastia, affecting 50% of Tanner 3-4 pubertal boys may necessitate a surgical approach due to its dimensions/benign structural alterations; selective estrogen receptor modulators might be a convenient medical alternative in these cases.
Objective and hypotheses: To assess the efficacy of SERM - raloxifene in the medical management of persistent/important/cystic pubertal gynecomastia.
Methods: Prospective study over 15 pubertal boys with important/complicated pubertal gynecomastia (maximum diameter of breast tissue on ultrasound assessment over 3 cm, tenderness and/or cystic lesions/ductal dilatations); the patients received a 3 to 9 months course treatment with raloxifene 60 mg/day; clinical and US assessment were done every 3 months until the completion of treatment.
Results: Symptomatic amelioration was accomplished in all patients - reduced tenderness and breast swelling. All patients with cystic lesions/ductal dilatations had a minimum 50% reduction of these lesions at 3 months with their complete disappearance at 6 months of treatment. Excepting one patient that had a fibrous organization of the glandular tissue with only a slight reduction of its dimensions on US, all other patients had a reduction of breast tissue dimensions of minimum 50% after 3 months of treatment. The pubertal progression was normal; there was a slight increase of plasmatic estradiol values in 30% of cases after 3 months of treatment; all patients had normal statural growth and bone maturation. There were no side effects.
Conclusions: Raloxifene - a selective estrogen receptor modulator with blocking action on breast estrogen receptors seems to be an efficient and safe therapeutical option in important/complex pubertal gynecomastia.

P1-d2-315 Growth 1

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

Heike Hoyer-Kuhn[1]; Daniel Kowalski[2]; Werner F. Blum[3]; Eckhard Schoenau[1]
[1]University of Cologne, Children's Hospital, Pediatric Endocrinology, Cologne, Germany; [2]PSI Company Ltd., Biometric, Warsaw, Poland; [3]Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany

Background: Growth hormone (GH) treatment in children with SHOX deficiency or Turner syndrome is approved and widely used to increase height velocity (HV) and adult height. Prediction of the growth response continues to be a challenge. A comparatively accurate method is the "Cologne Prediction Model" developed in GH-treated children with GH deficiency.
Objective and hypotheses: The aim of this analysis was to investigate whether this model can also be applied in patients with SHOX deficiency or Turner syndrome to predict HV in the first year of GH treatment.
Methods: This analysis of a multinational trial included prepubertal patients with SHOX deficiency (N=49, 26 with Leri-Weill syndrome, 21 with idiopathic short stature, 2 with unspecified phenotype) or Turner syndrome (n=26), confirmed by chromosome/DNA analysis, who received somatropin treatment at a dose of 0.05 mg/kg/day. First year HV prediction by the Cologne Model uses the following variables: relative bone age retardation and IGF-I at baseline, urinary deoxypyridinoline cross-links at 4 weeks and HV at 3 months.
Results: HV and height SDS increased significantly during the first year of GH treatment in both SHOX deficiency and Turner syndrome groups. The Pearson correlation coefficient between Cologne predicted vs. observed first year HV was 0.63 (adjusted $r^2 = 0.39$) in patients with SHOX deficiency and 0.79 (adjusted $r^2 = 0.61$) in patients with Turner syndrome.

Variable	SHOX deficiency (N=49)	Turner syndrome (N=26)
Females/males (n)	26/23	26/0
Age (yr) at start	8.3±2.7	7.5±1.9
Bone age SDS at start	-0.9±0.9	-1.0±1.1
IGF-I SDS at start	-0.8±1.2	-1.1±1.2
Height SDS at start	-3.2±0.8	-3.7±0.9
Height SDS at 1 yr	-2.6±0.9	-2.9±1.0
Pre-treatment HV (cm/yr)	5.3±1.5	4.6±1.3
Observed first year HV (cm/yr)	8.4±1.7	8.9±2.0
Predicted first year HV (cm/yr)	8.1±1.1	9.3±1.6
Predicted first year HV – observed first year HV (cm/yr)	-0.32±1.29	0.42±1.25

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

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

P1-d2-316 Growth 1

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

Ana Canton[1]; Tatiane Rodrigues[2]; Ana Krepischi[3]; Ivo Arnhold[4]; Berenice Mendonca[4]; Carla Rosenberg[2]; Alexander Jorge[1]
[1]Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia Genetica LIM/25, Sao Paulo, Brazil; [2]Instituto de Biociencias da Universidade de Sao Paulo, Departamento de Genetica e Biologia Evolutiva, Sao Paulo, Brazil; [3]Hospital do Cancer AC Camargo, Centro Internacional de Pesquisa e Ensino Oncologico, Sao Paulo, Brazil; [4]Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular LIM/42, Sao Paulo, Brazil

Background: The etiology of prenatal onset growth retardation with postnatal persistence is heterogeneous, often encompassing complex genetic disorders of difficult diagnosis.
Objective: To analyze the frequency of submicroscopic chromosomal deletions and duplications in a group of patients born small for gestational age (SGA) without a known cause.
Methods: We evaluated 32 patients with pre and postnatal growth retardation associated to other physical or developmental defects (dysmorphic features or intellectual disability), but without criteria for the diagnosis of known syndromes. Array-based comparative genomic hibridization (aCGH) was performed with DNA from all patients. The array comprised 60.000 oligonucleotides, with a probe spacing of 50Kb over the whole genome. The results were compared with copy number variations (CNVs) already described in normal controls databases and 18 relatives were evaluated for familial segregation.
Results: We identified 15 CNVs (7 duplications and 8 deletions) in 13 of the 32 patients (40%). None of these imbalances has been reported in healthy individuals. Considering their sizes, their gene contents and their familial segregations, at least 8 CNVs, found in 6 patients (I to VI), were categorized as probably pathogenic. These imbalances and their sizes were as follows: I) a 1.6Mb dup(10)(q26.2;26.3) and a 4.5Mb del(10)(q26.3); II) a 2.5Mb del(22)(q11.21); III) a 2.5Mb dup(5)(q21.3); IV) a 0.4Mb del(20)(p13); V) a 25Mb dup(8)(q24.2;24.3) and a 1.5Mb del(15)(q26.3); VI) a 3.5Mb del(3)(q27.1;q27.3). All patients had normal G-banded karyotyping, except for the patient V whose result was reported as 46,XY,15q+.
Conclusions: The frequency of pathogenic CNVs in patients born SGA associated with dysmorphic features or intellectual disability was high (at least 18%), showing the importance of aCGH as a clinical genetic test to clarify the diagnosis of these patients and to identify new chromosomal regions implicated in this condition.

P1-d2-317 Growth 1

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

Renata Scalco[1]; Carlos Tonelli[2]; Patricia Pugliese-Pires[1]; Julio Cechinel[3]; Ivo Arnhold[4]; Alexander Jorge[1]
[1]Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia Genetica - LIM/25 - Disciplina de Endocrinologia, Sao Paulo - SP, Brazil; [2]Faculdade de Medicina da Universidade do Extremo Sul de Santa Catarina, Ambulatorio de Endocrinologia Pediatrica, Criciuma - SC, Brazil; [3]Laboratorio Pasteur, Geral, Criciuma - SC, Brazil; [4]Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular - LIM/42, Disciplina Endocrinologia, Sao Paulo - SP, Brazil

Background: STAT5b is a protein involved in the signaling pathway of growth hormone (GH) and interleukins. Homozygous inactivating mutations in *STAT5B* gene cause GH insensitivity with chronic pulmonary disease and/or other immune dysfunctions. The effect of heterozygous *STAT5B* mutations on phenotype is still poorly characterized.
Objective and hypotheses: To test the hypothesis that *STAT5B* haploinsufficiency could cause an intermediate phenotype between patients with homozygous mutations and controls.

Methods: Twenty-seven direct relatives of two patients homozygous for the p.L142fsX161 (c.424_427del) *STAT5B* mutation were evaluated. We compared clinical and laboratory characteristics between relatives heterozygous for this *STAT5B* mutation and non-carriers. In this way the differences related to different genetic backgrounds could be minimized.
Results:

	Homozygous (n=2)	Heterozygous (n=7)	Wild type (n=20)	p*
Height SDS	-6.4 / -4.7	-1.0 ± 0.4	0.1 ± 1	0.029
Basal GH (µg/L)	8.7 / 0.2	0.8 ± 1.1	1.5 ± 1.8	0.683
IGF-1 SDS	-3.6 / -3.5	-0.6 ± 0.9	0.3 ± 1.3	0.135
IGFBP-3 SDS	-4.6 / -4.0	-2 ± 1.9	-1.2 ± 1.4	0.212
Prolactin (ng/mL)	34 / 37	14 ± 6	11 ± 5	0.265
Fasting glucose (mmol/dL)	5.2 / 4.8	5.0 ± 1.0	5.0 ± 0.6	0.985
Insulin (µUI/mL)	17.3 / 10.7	11 ± 14	7.4 ± 4	0.428
C-peptide (ng/mL)	5.6 / 2.8	2.1 ± 1.1	1.6 ± 0.7	0.223
Respiratory allergy	100%	57%	33%	0.377
Lymphocytes (cells/mm3)	1000 / 1000	1656 ± 408	2107 ± 446	0.788
IgG (mg/dL)	759 / 1013	990 ± 143	1170 ± 535	0.587
IgA (mg/dL)	222 / 252	313 ± 191	331 ± 111	0.810
IgE (UI/mL)	NA / NA	335 ± 291	96 ± 132	0.041

* Statistical difference between individuals heterozygous for the *STAT5B* mutation and non-carriers. NA – not available.

Conclusions: Individuals heterozygous for the studied *STAT5B* mutation are shorter than their non-carriers relatives, although in the normal height range. In the same direction, IGF-1 and IGFBP-3 SDS were lower in heterozygous individuals, although without reaching significance. A tendency for more respiratory allergy and a significant increase in the IgE levels were also observed in carriers. These results suggest a mild phenotype in individuals heterozygous for *STAT5B* mutations, but it is necessary to expand the number of studied relatives in this and other families to confirm the present findings.

P1-d2-318 Growth 1

Effectiveness and cost effectiveness of automated growth monitoring in children in primary care: population based cohort study

Ulla Sankilampi[1]; Antti Saari[2]; Laura Valpio[2]; Tiina Laine[3]; Päivi J Miettinen[3]; Leo Dunkel[4]
[1]Kuopio University Hospital, Department of Pediatrics, Kuopio, Finland; [2]University of Eastern Finland, School of Medicine, Department of Pediatrics, Kuopio, Finland; [3]University of Helsinki and Helsinki University Central Hospital, Children's Hospital, Helsinki, Finland; [4]Queen Mary University of London, Centre for Endocrinology, William Harvey Research Institute, London, United Kingdom

Background: The assessment of linear growth in children is a well-established part of preventive health care. However, no evidence exists on how growth monitoring should be optimally organised. We developed a conceptually novel automated growth monitoring (AGM) strategy and implemented it into an electronic health record system in primary care.
Objective and hypotheses: We tested whether AGM, in comparison to standard growth monitoring (SGM), would improve referral rates, the appropriateness of referrals and the diagnostic yield of disorders that affect growth in a cost effective way.
Population and methods: A prospective, population-based cohort study with one-year AGM intervention including computerised screening algorithms and automated online consultation prior to referral was conducted in the child population (0.01-12 years) of a large municipality in Finland. Main outcome measures were the referral rate to secondary care for abnormal growth, diagnostic yield of growth disorders, diagnostic delay, and cost effectiveness of growth monitoring, all in comparison to the preceding year with SGM.
Results: The diagnostic yield increased from one per 4,090 children (eight new growth diagnoses in SGM year) to one per 675 children (48 in AGM year) among the 32,404 and 32,718 children screened, respectively. 24 of 48 children diagnosed during AGM had abnormal screening results during SGM but had not been referred leading to a median diagnostic delay of 2.0 years (0.1-10.3 years). AGM strategy was cost saving: price of one new growth diagnosis was 10,292€ versus 55,961€ during SGM.
Conclusions: An automated growth monitoring strategy in a previously screened child population in primary care resulted in over 6-fold rise (95% CI 2.87 to 12.80) in the number of newly diagnosed growth disorders. AGM increases diagnostic yield at the population level with acceptable costs, in comparison to SGM. Clinical and cost effectiveness of growth monitoring in primary care is improved by automation.

P1-d2-319 Growth 1

Mild GH deficiency due to two novel homozygous mutations in the gene encoding growth-hormone releasing hormone receptor (GHRHR) in a single family

Louise C. Gregory; Mark J. McCabe; Kyriaki S. Alatzoglou; Paul Letissier; Mehul T. Dattani
UCL Institute of Child Health, Developmental Endocrinology. Clinical and Molecular genetics Unit, London, United Kingdom

Background: Growth hormone-releasing hormone (GHRH) binds to its G-protein coupled receptor (GHRHR) leading to the synthesis/release of growth hormone (GH) from the somatotroph cells of the pituitary. Recessive GHRHR mutations are associated with severe isolated GH deficiency (IGHD Type 1B), with an untreated final height of 130cm±10cm and 114±0.7cm in males and females respectively. The phenotype is not one of classic IGHD, with minimal facial hypoplasia, no hypoglycaemia or microphallus and variable anterior pituitary hypoplasia (APH) on magnetic resonance imaging (MRI).
Objective and hypotheses: We hypothesised that a consanguineous Pakistani family with IGHD in 3 siblings would have mutations in GH1 or GHRHR.
Methods: PCR amplification and direct sequencing analysis were used to screen both genes.
Results: In all three affected siblings we identified two novel homozygous missense mutations (p.R4Q, p.P79L), absent from 200 Pakistani controls, in a conserved region of the extracellular domain of GHRHR. Both are predicted to affect protein conformation and ligand binding. The brothers were diagnosed with GHD at ages 9.8 and 6 years with a height SDS of -2.24 and -1.23 respectively. They had micropenis and bilateral undescended testes with peak GH concentrations to glucagon stimulation of 2.9ig/l and low IGF-1/IGFBP3. Their elder sister presented at 16 years with untreated IGHD (peak GH to insulin tolerance test <0.1µg/l, IGF-1<3.3mmol/L), abdominal fat deposition, a high-pitched voice and frontal bossing. Surprisingly, she attained an untreated final height of 144cm; the tallest untreated height in a patient with a GHRHR mutation. All 3 siblings had APH on MRI. No mutations were identified in GH1. Their asymptomatic mother was heterozygous for both GHRHR mutations.
Conclusions: We report the presence of two novel homozygous mutations in GHRHR in a single pedigree. The phenotype in this family appears to be relatively mild despite the presence of two mutations in the same gene.

P1-d2-320 Growth 1

Determinants of early growth response and final height gain to GH treatment in patients with SHOX deficiency: results of a multi-centre trial

Werner F Blum[1]; Judith L Ross[2]; Christopher J Child[3]; Alan G Zimmermann[4]; Daniel Kowalski[5]; Eckhard Schoenau[6]; Gudrun A Rappold[7]
[1]Eli Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany; [2]Thomas Jefferson University, Department of Pediatrics, Philadelphia, United States; [3]Eli Lilly and Company, Lilly Research Laboratories, Windlesham, United Kingdom; [4]Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, United States; [5]PSI Company Ltd., Biometric, Warsaw, Poland; [6]University of Cologne, Children's Hospital, Pediatric Endocrinology, Cologne, Germany; [7]Heidelberg University, Department of Molecular Human Genetics, Heidelberg, Germany

Background: Patients with mutations of the Short Stature Homeobox-containing (*SHOX*) gene have impaired growth, with or without a spectrum of skeletal anomalies consistent with mesomelic skeletal dysplasia. Growth hormone (GH) has been shown to improve the growth rate and final height.

Objective and hypotheses: The aim of this analysis was to identify determinants of the growth response to GH.
Methods: Prepubertal children (27/22 females/males [F/M]; 21 with a phenotype of idiopathic short stature (ISS), 26 with Leri-Weill syndrome (LWS), 2 with unspecified phenotype) with genetically confirmed SHOX deficiency received GH treatment (tx) at 0.05 mg/kg/d. Study entry criteria were: age ≥3yr; bone age <8yr (F), <10yr (M); height (Ht) <3rd %ile (or Ht <10th %ile and height velocity [HV] <25th %ile). At study closure, 28 patients had attained final height (FH; last HV <2cm/yr or last bone age ≥14yr [16yr] in F [M] or judgment by investigator). Multiple regression analyses were performed with 1st-yr HV, 1-yr delta HtSDS, 4-yr delta HtSDS and FH SDS gain (FH SDS - HtSDS at start of GH tx) as response variables and various auxological and biochemical explanatory variables.
Results: At start of GH tx mean±SD age was 8.3±2.7 yr, bone age SDS -0.8±0.9, and HtSDS -3.2±0.8. Duration of GH tx at FH was 6.2±2.0 yr. The response variables increased significantly during GH tx.
Table. Parameter estimates (P-values) of stepwise multiple regression models for various response variables.

Explanatory variable	1st-yr HV (cm/yr)	1-yr delta HtSDS	4-yr delta HtSDS	FH SDS gain
N	46	46	38	28
Gender (M=0, F=1)				0.76 (0.003)
ISS(0)/LWS(1)			-0.51 (0.013)	
Age at start (yr)	-0.28 (0.003)	-0.10 (<0.001)	-0.11 (0.007)	
Bone age SDS at start				-0.34 (0.022)
IGFBP-3 SDS		0.10 (0.039)		
Duration of GH tx (yr)				0.13 (0.053)

While young age at start predicted a better GH response during the first 4 yr, it was not a significant predictor of FH SDS gain. A good FH SDS gain was predicted by female sex and more pronounced bone age retardation. The following variables did not remain in any model: HtSDS at start, target HtSDS, IGF-I SDS.
Conclusions: The early growth response to GH tx in children with SHOX deficiency is significantly determined by the age at start of tx: the younger the better. In contrast, a good height gain to adult height can be expected, if bone age at start of GH tx is substantively retarded.

P1-d2-321 Growth 1

Body composition and circulating high-molecular-weight adiponectin and IGF-I in infants born small for gestational age: breast- versus formula-feeding

Giorgia Sebastiani[1]; Marta Díaz[1]; David Sanchez-Infantes[1]; Abel López-Bermejo[2]; Francis De Zegher[3]; Lourdes Ibañez[1]
[1]Sant Joan de Déu Hospital, Paediatric Endocrinology, Barcelona, Spain; [2]Hospital Dr. Josep Trueta & Institut de Recerca Biomèdica, Paediatric Endocrinology, Girona, Spain; [3]University of Leuven, Paediatric Endocrinology, Leuven, Belgium

Background: Small for gestational age children (SGA), especially those with early and rapid catch-up of weight, are at increased risk for developing insulin resistance and other features of the metabolic syndrome by late childhood. The mechanisms whereby neonatal nutrition may modulate such risks are poorly understood.
Objective and hypotheses: To assess the effects of nutrition (breast- versus formula-feeding; BRF vs FOF) on the weight partitioning and endocrine state (as judged by high-molecular-weight [HMW] adiponectin and IGF-I) of infants born SGA.
Methods: Body composition (measured by absorptiometry), circulating glucose, insulin in fasting state and circulating HMW-adiponectin and IGF-I were assessed at birth and 4 mo in BRF infants born appropriate for gestational age (AGA; N=72) and in SGA infants receiving BRF (N=46) or FOF (N=56), the latter being randomized to receive a standard (FOF1) or protein-rich formula (FOF2), during 4 months
Results: Compared to AGA-BRF infants, the catch-up growth of SGA infants was confined to lean mass, independently of nutrition. Compared to AGA-BRF infants, SGA-BRF infants had normal HMW-adiponectin and IGF-I levels at 4 mo, whereas SGA-FOF infants had elevated levels of HMW-adiponectin (particularly SGA-FOF1) and IGF-I (particularly SGA-FOF2).
Conclusions: Neonatal nutrition seems to influence the endocrinology more readily than the body composition of SGA infants. Follow-up will disclose whether the endocrine abnormalities in SGA-FOF infants can serve as early markers of an unfavourable metabolic course, and whether they may thus contribute to design early interventions that prevent subsequent disease including diabetes.

P1-d2-322 Growth 1

Absence of NEUROD4 mutations in patients with congenital isolated growth hormone deficiency

Fernanda A. Correa; Luciani R. Carvalho; Marcela M. Franca; Aline P. Otto; Everlayny F. Costalonga; Vinicius N. Brito; Ivo J. P. Arnhold; Berenice B. Mendonca
Hospital das Clinicas, Faculdade de Medicina da Universidade de Sao Paulo, Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular LIM/42, Sao Paulo, Brazil

Background: The incidence of Isolated Growth Hormone Deficiency (IGHD) is estimated to be 1:4000-1:10000. Mutations in GH1 and GHRHR are known causes of IGHD but a large number of patients remain without molecular diagnosis. The study of Neurod4 knockout mice showed that this gene is critical for maturation and expansion of somatrotropes by regulating the expression of GHRHR. We hypothesized that NEUROD4 loss-of-function mutations could underlie some cases of IGHD.
Objective: To screen NEUROD4 for mutations in patients with IGHD.
Methods: Mutations in GH1 and GHRHR were ruled out in all patients. All patients met the diagnostic criteria of the Brazilian centre: the patients had short stature (heights SD score < -2) and peak GH levels < 5ng/ml by immunoradiometric assays in two GH stimulation tests. The entire coding region of NEUROD4 was evaluated in 30 patients (17 males) by Sanger Method using automatic sequencing.
Results: Ten patients presented a heterozygous allelic variant HGVS NM_021191.2: c.31 C>T previously described as polymorphism rs2656804. This variant is a 3PRIME-UTR type and its position in the transcript is 1405. It is not conserved among species. The other 20 patients presented the most common allele C/C. Clinical and MRI findings are described in the table below: Table 1 – Clinical features and MRI findings of 30 patients with IGHD

Gender	Gender	Consanguinity	Familial cases	Anterior pituitary hypoplasia	*Abnormal posterior pituitary	**Abnormal stalk
Male	Female					
17	13	4	10	23	23	18

*Includes ectopic or non visualized posterior pituitary.
** Includes transection or absent stalk.

Conclusions: Despite the role that Neurod4 has in somatrotope development in mice, we found no loss-of-function mutations implicated in the aetiology of IGHD in a selected group of Brazilian patients.

P1-d2-323 Growth 1

Copy number variations in patients with idiopathic short stature

Hermine van Duyvenvoorde[1]; Sarina Kant[2]; Wilma Oostdijk[3]; Antoinet Gijsbers[2]; Marcel Karperien[4]; Marie-José Walenkamp[5]; Cees Noordam[6]; Paul Voorhoeve[7]; Sandy van Gool[8]; Monique Losekoot[2]; Claudia Ruivenkamp[2]; Jan-Maarten Wit[3]

[1]Leiden University Medical Center, Pediatrics, Endocrinology and Metabolic Diseases, and Center for Human and Clinical Genetics, Leiden, Netherlands; [2]Leiden University Medical Center, Center for Human and Clinical Genetics, Leiden, Netherlands; [3]Leiden University Medical Center, Pediatrics, Leiden, Netherlands; [4]University of Twente, Developmental BioEngineering, Enschede, Netherlands; [5]VU University Medical Center, Pediatrics, Amsterdam, Netherlands; [6]Radboud University Nijmegen Medical Center, Pediatrics, Nijmegen, Netherlands; [7]Canisius-Wilhelmina Hospital, Pediatrics, Nijmegen, Netherlands; [8]Haga Hospital/Juliana Children's Hospital, Pediatrics, Den Haag, Netherlands

Background: Height is a highly heritable and classic polygenic trait. Recent Genome-wide Association Studies have revealed that at least 180 genetic variants influence adult height. However, these variants explain only about 10% of the phenotypic variation in height. It is estimated that in approximately 80% of the short children presenting to a pediatrician the underlying cause cannot be identified, which classifies them as having idiopathic short stature (ISS).
Objective and hypotheses: To identify rare copy number variants (CNVs) that may influence height, using whole genome SNP array analysis in patients with ISS.
Methods: 180 patients (96 males) with ISS were included. To detect CNVs in these patients, whole genome SNP array analysis was performed, using either Affymetrix GeneChip Human Mapping 250K or Illumina HumanHap300 BeadChip arrays and their corresponding analysis software. The detected CNVs were classified into four different groups: I, known pathogenic; II, potentially pathogenic, not described in the Database of Genomic Variants (DGV); III, not described in the DGV, but not containing any protein-coding genes; and IV, polymorphic variant described in the DGV or observed in our in-house reference set. All type III and IV CNVs were excluded from further analysis. All type II CNVs were assessed with Ensembl and DECIPHER for gene content and similar cases, respectively. If parents were available, segregation analysis was performed by SNP array.
Results: In 4 cases (2%) type I CNVs were found and in 45 cases (25%) type II CNVs. The type I CNVs were located in the chromosomal regions containing *SHOX* and the *IGF1R*. The four most interesting type II CNVs were further analyzed, which led to 3 novel candidate genes.
Conclusions: Whole genome SNP array analysis identified known pathogenic CNVs in 2% and potentially pathogenic CNVs, which may influence the regulation of longitudinal growth, in 25% of 180 patients with ISS.

P1-d2-324 Growth 1

A quest for copy number variations causing extreme tall stature

Hermine van Duyvenvoorde[1]; Claudia Ruivenkamp[2]; Dick Mul[3]; Wilma Oostdijk[4]; Marcel Karperien[5]; Monique Losekoot[2]; Alberto Pereira[6]; Sarina Kant[2]; Jan-Maarten Wit[4]

[1]Leiden University Medical Center, Pediatrics, Endocrinology and Metabolic Diseases, and Center for Human and Clinical Genetics, Leiden, Netherlands; [2]Leiden University Medical Center, Center for Human and Clinical Genetics, Leiden, Netherlands; [3]Haga Hospital/Juliana Children's Hospital, Pediatrics, Den Haag, Netherlands; [4]Leiden University Medical Center, Pediatrics, Leiden, Netherlands; [5]University of Twente, Developmental BioEngineering, Enschede, Netherlands; [6]Leiden University Medical Center, Endocrinology and Metabolic Diseases, Leiden, Netherlands

Background: Height is a highly heritable and classic polygenic trait. In the majority of individuals with extreme tall stature (ETS) no genetic cause is known.
Objective and hypotheses: To identify new loci that may influence height by identifying rare copy number variants (CNVs) in people with ETS.
Methods: 18 adults with ETS (from +2.4 to +4.4 SDS) were studied. Clinical and family history was obtained. Whole genome SNP array analysis using Affymetrix 262K NspI arrays was performed to detect CNVs. All potentially pathogenic CNVs were assessed with Ensembl and DECIPHER for gene content and similar cases, respectively. If family members were available, segregation analysis was performed.
Results: In 4 (3 males) of the 18 cases potentially pathogenic CNVs were identified and further analysis was performed. Additional segregation studies suggested that the identified CNV was familial in 2 of the cases and probably is not an explanation for ETS. In a 204.2 cm (+2.9 SDS) tall male, a 1p34.1 duplication containing part of *MAST2* and *PIK3R3* (that binds the intracellular part of the IGF1R) was observed. His son and daughter with tall stature (+1.9 and +1.8 SDS) both carried the duplication. We recently identified a case with a similar duplication, but without tall stature, in our in-house reference set. In the fourth case, a male with a height of 201.9 cm (+2.5 SDS) 3 aberrations were detected; a duplication of 12q21.1 (also identified in his sister with a normal height), a 13q33.1 deletion (no genes) and an extra copy of the Y chromosome (47,XYY).
Conclusions: Whole genome SNP array analysis in 18 cases with ETS identified potentially pathogenic CNVs in 2 cases (11%). Additional research showed that the 1p34.1 duplication, although of interest because it contains part of *PIK3R3*, is probably not the cause of ETS. The observed extra Y chromosome in the other case is known to contribute to increased height, but the role of the 13q33.1 deletion still remains unclear.

P1-d2-325 Growth 1

Growth retardation, delayed mental development and mild dysmorphic features in a boy with a novel interstitial deletion of chromosome 8p21.1-p12

Gian Luigi Spadoni[1]; Annalisa Deodati[2]; Giuseppe Scirè[1]; Anna Maria Nardone[3]; Diana Postorivo[3]; Marta Bertoli[4]; Marco Cappa[5]; Stefano Cianfarani[2]

[1]Bambino Gesù Children's Hospital-University "Tor Vergata", Endocrinology Unit, Rome, Italy; [2]Bambino Gesù Children's Hospital-University "Tor Vergata", Molecular Endocrinology Unit, Rome, Italy; [3]Tor Vergata University, Lab. of Medical Genetics, Rome, Italy; [4]Ospedale San Pietro FBF, UOSD di Genetica Medica, Rome, Italy; [5]Bambino Gesù Children's Hospital, Endocrinology Unit, Rome, Italy

Background: Few cases with interstitial 8p deletions have been described in the literature with a rather heterogeneous phenotype. We report on a boy with in whom an array-CGH disclosed a novel interstitial 8p deletion.
Clinical report: At birth he weighted 2730g; he showed a delay in psychomotor development associated with strabismus and nistagmus. At 8-yrs he was referred to us for short stature. Screening for celiac disease and other chronic diseases resulted negative; peak GH responses to two stimulation tests were normal as were IGF1 levels. Since mild dysmorphic features were noted (epicantus and ptosis, strabismus, turricefaly, downward mouth angles) a karyotype was performed which resulted normal. A complete skeletal X-ray assessment and brain MRI scan revealed no anomalies. At 14 6/12-yrs-old, height was 138.5cm (-4.1 SDS), testicles were 6ml, PH2, G2. LH-RH test showed a peak FSH value of 18.92 and a peak LH value of 54.14 mUI/ml; testosterone was 266.5 ng/dl. Bone age was constantly delayed. Mother's and sister's age at menarche was 15 yrs.
Methods: Array-CGH analysis of lymphocytes was performed according to the manufacturer's protocol on a whole genome oligo-array 105K (BlueGnome, UK).
Results: Array-CGH analysis detected a microdeletion in the region 8p21.2p12, spanning 7,6 Mb, which was confirmed by FISH. Rearrangement is de novo.
Conclusions: The deletion harboured in our patient is slightly smaller than other previously described in the same region. The case confirms the heterogeneous phenotype of 8p interstitial deletions, showing some clinical features in common with previously described patients (growth retardation, psychomotor development delay, mild facial dysmorphic features) but not others expected according to the genes of the deleted portion: a hypogonadotropic hypogonadism, which could have been predicted in accordance with the presence of GNRH1 gene in the deleted region, is not present, up to now; the mild delay in puberty might be interpreted taking in account the familial data.

P1-d2-326 Growth 1

Effect of gonadotropin-releasing hormone analog combined with stanazolol on final height in girls with idiopathic central precocious puberty and apparent decrease of linear growth

Yan-hong li; Min-lian Du; Hua-mei Ma; Zhe Su; Hong-shan Chen; Qiu-li Chen; Yu-fen Gu
The First Affiliated Hospital of Sun Yat-Sen University, Pediatric, GuangZhou, China

Background: In girls with central precocious puberty(CPP) treated with gonadotropin-releasing hormone analog(GnRHa) for improvement of final adult height(FAH), the expected effects on FAH is not achieved if the linear growth decrease apparently because of the over-accelerated bone maturation.
Objective and hypotheses: The study was designed to evaluate the effect of combined use of stanazolol on the FAH in girls with idiopathic CPP and apparent decrease linear growth during GnRHa therapy.
Methods: 60 girls with idiopathic CPP and decreased growth velocity(GV) were separated into 3 groups (20 girls in each group) base on the following types of interventions: group1, GnRHa+stanazolol (25-30ug/kg.d, every 3-months followed by 3-months discontinuation, total duration: 12.2±3.6 months), group2, GnRHa+growth hormone(1-1.1u/kg.w for 11.4±5.7 months), group3, GnRHa alone.
Results: 1. GV increased significantly both in group1 (2.8±0.6 vs 6.4±2.1cm/yr, p<0.01) and in group2 (2.8±0.6 vs 6.3±2.2cm/yr, p<0.01) during combined therapy, but maintained at low levels in group3 (3.9±1.0 vs 3.3±0.9cm/yr, p>0.05) . 2. FAH was significantly higher than predicted AH before combined therapy, as well as than target height both in group1 (156.3±2.9 vs 150.8 ±3.7cm, p<0.01, 156.3±2.9 vs 153.9±2.6cm,p<0.01), and in group2 (157.3 ±4.7 vs 152.6±3.9cm, p<0.01, 157.3±4.7 vs 154.4±4.7cm, p=0.01), but was similar to predicted AH and target height in group3(154.1±5.5 vs 153.1±6.2cm, p=0.06, 154.1 ±5.5 vs 155.6±4.3cm, p=0.26). 3. Menarche occurred 13.2±1.4, 15.8±10.7, 10.2±4.4 months after discontinuation of therapy in group1, group2 and group 3 respectively. No hirsutism, clitorism or irregular menstruation was recorded in girls treated with stanazolol.
Conclusions: Combine use of stanazolol can improve FAH in girls with CPP and apparent decreased linear growth during GnRHa therapy.

P1-d2-327 Growth 1

Acromesomelic dysplasia type Maroteaux: broadening the spectrum of NPR2 mutations

Lidia Castro-Feijóo[1]; Daniel Torrecilla[2]; Jesús Barreiro[1]; Encarna Guillén-Navarro[3]; Hubert Journel[4]; Emma Wakeling[5]; Fernando Domínguez[6]; Paloma Cabanas[1]; Claudia Heredia[1]; Rosaura Leis[1]; Manuel Pombo[1]; Lourdes Loidi[2]
[1]Hospital Clínico y Universidad de Santiago de Compostela, Unit of Paediatric Endocrinology, Santiago de Compostela, Spain; [2]Fundación Pública Galega de Medicina Xenómica, Molecular Medicine, Santiago de Compostela, Spain; [3]Hospital Universitario Virgen de la Arrixaca, Clinical Genetics, Murcia, Spain; [4]Centre Hospitalier Bretagne Atlantique, Clinical Genetics, Vannes, France; [5]North West London Hospitals NHS Trust, Clinical Genetics, Harrow, United Kingdom; [6]Fundación Pública Galega de Medicina Xenómica & Universidad de Santiago de Compostela, Fisiology, Santiago de Compostela, Spain

Background: Acromesomelic dysplasia type Maroteaux (AMDM) (OMIM 602875) is a rare autosomal recessive skeletal dysplasia with an estimated prevalence of 1/1.000.000. It is characterized by severe dwarfism with shortening of the middle and distal segments of the limbs. Although the patients have normal length and weight at birth, a skeletal disorder can be suspected by one year of age. The skeletal organ system appears to be the only one affected in AMDM patients. The disorder is caused by mutations in the *NPR2* gene, which encodes for the transmembrane natriuretic peptide receptor B. To date, only 21 different *NPR2* mutations have been described.
Objective and hypotheses: The objective of this study was the genetic confirmation of AMDM and the identification of the causal mutations in *NPR2* gene.
Methods: Five individuals diagnosed of AMDM under clinical and radiological criteria, plus 14 relatives, were studied. The patients belonged to four unrelated families. The entire coding region as well as and intron-exon boundaries of the *NPR2* gene was analyzed by direct sequencing after PCR amplification.
Results: AMDM was genetically confirmed in all the patients. Five different mutations were found and all of them were novel. Four patients were homozygous and one was a compound heterozygous, see table. The parents of all the patients and some relatives were mutation carriers.

Family	Age at diagnosis	AMDM Phenotype	Mutation cDNA (NM_003995.3)	Mutation protein
1	34y	+	[c.494del]+[c.494del]	p.Arg165LeufsX80
1	35y	+	[c.494del]+[c.494del]	p.Arg165LeufsX80
2	6y3m	+	[c.1330del]+[c.1330del]	p.Asp444ThrfsX33
3	1y3m	+	[c.245T>C]+[c.2118C>A]	p.Leu82Pro+p.Asp706Glu
4	18y	+	[c.2548_2551del]+[c.2548_2551del]	p.Glu850LeufsX32

Conclusions: 1.-AMDM was genetically confirmed in all the cases and the mutations were identified. 2.-All the mutations were novel and represent almost 20% of *NPR2* reported mutations. 3.-These findings represent an important contribution to the spectrum of *NPR2* mutations.

P1-d2-328 Growth 1

Baseline gene expression associated with growth response to growth hormone (GH) in children with GH deficiency (GHD) and Turner Syndrome (TS): the PREDICT long-term follow-up study

Adam Stevens[1]; Fabio Buzi[2]; Dag Veimo[3]; Benoit Destenaves[4]; Pierre Chatelain[5]; Peter Clayton[6]
[1]University of Manchester, Medicine, Manchester, United Kingdom; [2]A.O. "C.Poma", S.C. Pediatria, Mantova, Italy; [3]Nordlandssykehuset, Barneavdelingen, Bodø, Norway; [4]Merck Serono S.A. - Geneva, Endocrinology, Geneva, Switzerland; [5]Université Claude Bernard, Department Pediatrie, Bron, France; [6]University of Manchester, Manchester Academic Health Sciences Centre, Manchester, United Kingdom

Background: The PREDICT long-term follow-up study investigates relationships between conventional biomarkers, genetic polymorphisms, gene expression and long-term auxological changes in GH treatment-naive prepubertal children with GHD or TS during GH therapy.
Objective: To study the association between baseline (BL) gene expression and Year 1 growth response to GH therapy in children with GHD and TS.
Methods: Height velocity (HV) after 1 year of GH therapy was grouped into quartiles and correlated with BL gene expression measured using Affymetrix Human Genome U133 Plus 2.0 Arrays. Gene ontology of associated changes and biological network inference were assessed using Ingenuity Pathway Analysis (IPA) software. Predictive modelling was performed using K-nearest neighbour classification, with 1-level cross validation.
Results: In BL GHD (n=71) and TS (n=41) samples, differences in expression of 526 and 90 probe sets, respectively, correlated with the lowest quartile of HV (analysis of variance, p<0.01). There was no overlap between probe sets. Associated gene ontology was enriched for growth-related processes including anti-oxidant activity (false discovery rate corrected p-value, q<0.05) and protein ubiquitination (q<0.05) in GHD, along with nitrogen metabolic pathways (q<0.1) and regulation of transcription (q<0.1) in TS. IPA identified multiple inferred pathways in both conditions associated with metabolic functions, cell cycle and pathways related to apoptosis and growth (q<0.05). Predictive modelling identified a group comprising 26 probe sets in GHD and 71 probe sets in TS associated with poor response to GH treatment, which had positive predictive values of 0.48 and 0.62, and negative predictive values of 0.86 and 0.89, respectively.
Conclusions: Using BL gene expression, candidate biological pathways associated with low response over the first year of GH therapy have been identified in prepubertal children with GHD and TS. BL gene-expression profiles may contribute to a predictive model for individualized treatment.

P1-d2-329 Growth 1

Single nucleotide polymorphisms (SNPs) associated with growth response over 3 years on growth hormone (GH) therapy and changes from baseline through the third year of GH therapy in children with GH deficiency (GHD) and Turner syndrome (TS)

Pierre Chatelain[1]; Roland Pfäffle[2]; Klaus Kapelari[3]; Jean-Pierre Salles[4]; Armand Valsesia[5]; Benoit Destenaves[6]; Peter Clayton[7]

[1]Université Claude Bernard, Hôpital Mère Enfant de Lyon, Department Pediatrie, Bron, France; [2]University of Leipzig, Department of Pediatrics, Div. of Pediatric Endocrinology, Leipzig, Germany; [3]Universitätsklinik für Kinder- und Jugendheilkunde, Department Pädiatrie 1, Innsbruck, Austria; [4]Hôpital d'Enfants, Service d'Endocrinologie pédiatrique, Toulouse, France; [5]Merck Serono SA, Biomarker Technologies - Bioinformatics, Geneva, Switzerland; [6]Merck Serono S.A. - Geneva, Endocrinology, Geneva, Switzerland; [7]University of Manchester, Manchester Academic Health Sciences Centre, Manchester, United Kingdom

Background: The PREDICT long-term follow-up study investigates the link between SNPs in growth- and metabolism-associated genes and long-term growth in children with GHD or TS receiving GH therapy.
Objective: Evaluate the association of SNPs with growth response (cm/yr) in years (Y) 1-3 of GH therapy in children with GHD or TS.
Methods: Treatment-naive children with classic idiopathic GHD or TS were prepubertal at start of GH therapy and Tanner stage 1-2 at Y3. Median GH doses were 35, 33 and 32µg/kg/day for GHD and 50, 49 and 50µg/kg/day for TS at Y1, Y2 and Y3, respectively. 1451 SNPs from 98 candidate genes were successfully genotyped (GHD: Y1, n=110, Y2, n=93, Y3, n=73; TS: Y1, n=60, Y2, n=42, Y3, n=56). Associations of SNPs with growth (cm) over Y3 and changes from baseline (BL) through Y3 were assessed in continuous analyses by Kruskal–Wallis non-parametric tests. Adjusted p-values less than 0.05 were considered significant.
Results: For GHD, SNPs in 5 genes were associated with growth response in Y3 (*AKT2, GAB1, PROP1, SLC2A1* and *-A4*) and 7 from BL to Y3 (*FOS, GRB2, JAK2, PI3KR1* and *-R2, SHC1, SLC2A4*). SNPs in both *FOS* and *GRB2* were associated with growth from both BL to Y2 and BL to Y3. None of these genes had been identified previously as associated with Y1 growth, although the genes associated with Y1 growth did include *GRB10*. For TS, SNPs in 4 genes were associated with growth response in Y3 (*FGF3, CYR61, PI3KCB, PIK3R2*) and 4 from BL to Y3 (*ARRB1, FGF3, PTPN1, SLC2A1*). SNPs in both *FGF3* and *PTPN1* were associated with growth from both BL to Y2 and BL to Y3. None of these genes were associated with Y1 growth.
Conclusion: Results suggest that in children with GHD or TS receiving GH therapy, genes associated with first year (catch-up) growth differ from those that associate with longer term response (over the first 2 or 3 years of treatment). Growth factor receptor-bound proteins are, however, involved in both phases in GHD. These data point to the diversity of genetic modulation of growth response to GH treatment.

P1-d2-330 Growth 1

Magnetic resonance imaging (MRI) of the cranium and functional findings in 15043 children with non-acquired growth hormone deficiency (GHD) from KIGS

Michael B. Ranke[1]; Anders Lindberg[2]; Maria Koltowska-Häggström[2]; Mohamad Maghnie[3]

[1]Eberhard Karls University, Tuebingen, University Children's Hospital, Tuebingen, Germany; [2]Pfizer Endocrine Care, KIGS/KIMS/ACROSTUDY, Sollentuna, Sweden; [3]University of Genova, Departments of Pediatrics, IRCCS G. Gaslini, Genova, Italy

Background: Neuro-imaging of the CNS by MRI has become an essential part of the diagnostic work-up in children with GHD.
Objective and hypotheses: The aim of the study was to document the frequency and diversity of neuro-anatomical anomalies in a very large cohort with GHD (max GH to provocation tests < 10 µg/L) and to associate the findings with patients characteristics.
Methods: In 15043 of 43725 children documented 1987-2011 within KIGS (Pfizer International Growth Database) with non-acquired GHD ([idiopathic] IGHD, Neurosecretory Dysfunction [NSD] or GHD of known congenital cause) results of cranial MRI (85% before GH treatment) were reported by the investigators. In cohorts of patients with normal MRI and abnormal pituitaries (pituitary hypoplasia, empty sella [ES], Hypoplastic anterior pituitary, Missing stalk, Ectopic posterior pituitary [HME]) characteristics before GH treatment were compared.
Results: In 4032 [26.8 %] children various abnormal MRI findings were documented within (N=2968) and outside the pituitary region. The most frequent were: pituitary hypoplasia (N=1178 [7.8%]), ES (N=449 [3.0 %]) and HME (N=1019 [6.8%]). In 2361 children IGHD or NSD were diagnosed before MRI was done, but MRI abnormalities were observed in subsequent MRIs [e.g. pituitary hypoplasia: N= 974; HME: N=459)]. The characteristics in patients with GHD were found related to the neuro-anatomical abnormality (see Table 1).

Table 1: Patients Characteristics

MRI Diagnosis		Normal	Pit. Hypoplasia	Empty Sella	HME
	N	10378	1178	449	1018
maxGH	µg/L	6.6	4.9*	3.1*	3.1*
MPHD	%	11.1	25.0*	52.1*	49.8*
Breech delivery	%	2.2	4.2*	3.8*	7.5*
Birth weight	SDS	-0.69	-0.54	-0.39*	-0.39*
Height - MPH	SDS	-1.55	-2.12*	-2.34*	-2.71*
Age - GH start	yrs	11.0	9.7	8.2*	6.2*

Median values; * = p< 0.01 vs. Normal MRI. Group comparison by Wilcoxon

Conclusions: In children with non-acquired GHD neuro-anatomical abnormalities are present in high frequency and great variety. There is an association between anatomical and functional abnormalities of the pituitary, which may have implications for the outcomes of treatment and the prognosis. Standardisation for the interpretation of MRI findings (e.g. pituitary size) is needed.

P1-d2-331 Growth 1

Abnormal growth in Noonan syndrome: correlation between growth parameters and genotype

Mariangela Cisternino[1]; Francesca Marabotto[1]; Alexandra Madè[1]; Giulia Rossetti[1]; Laura Losa[1]; Arianna Zaroli[1]; Paola Civallero[1]; Paola Riva[2]

[1]Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Department of Pediatrics, Pavia, Italy; [2]University of Milan, Department of Biology and Genetics for Medical Science, Milan, Italy

Background: Noonan Syndrome (NS) is an autosomal dominant disorder characterized by short stature, dysmorphic features, congenital heart defects and other anomalies. Familial or de novo mutations in PTPN11, RAF1, SOS1, KRAS, and NRAS are detected in 60-75% of cases.
Objective and hypotheses: The aim of this study was to find out a possible correlation between the linear growth, the GH secretion and the genotype in NS patients.
Methods: A cohort of 34 patients affected by NS diagnosed by Van der Burgt criteria was studied: 13 had a PTPN11 mutation, 9 SOS1 (in one case associated with RAF1) and 12 were mutation negative. All of these patients underwent a clinical and auxological evaluation, GH secretion was evaluated in 29 patients.
Results: Short stature was detected in 9/13 (69%) PTPN11+ patients, in 8/12 (67%) mutation negative and only in 2/9 (22%) of SOS1+ patients. The average H-SD was significantly higher in the SOS1+ group compared to PTPN11+ and to mutation negative groups, while no significant difference was found between the latter two groups. The average H-DS of PTPN11+ and of mutation negative groups was significantly lower (p<0,002) than their respective target height (TH) (-1,9±0,88 vs 0,43±1,26 and -2,18±1,4 vs -0,33±1,2, respectively) while it was close to TH in SOS1+ group (-0,63±0,49 vs -0,53±0,37). GH deficiency (GHD) was diagnosed in 4/9 (44%) PTPN11+, 2/8 (25%) SOS1+ and in 3/12 (25%) mutation negative. The final height reached from 5 patients SOS1+ was normal, while it was low in 2 cases PTPN11+.
Conclusions: In this study short stature was more frequently seen in PTPN11+ and mutation negative patients than in those SOS1+. Short stature was rarely observed in SOS1+ patients, and when present, it was secondary to GHD. The SOS1+ patients reached a normal final height, suggesting that this mutation seems to preserve the linear growth in patients with NS.

P1-d2-332 Growth 1

Acid-Labil Subunit (ALS) haploinsufficiency and phenotypic variability of *IGFALS* p.N276S mutation carriers

Elena Gallego-Gómez[1]; Jaime Sánchez del Pozo[1]; Jaime Cruz-Rojo[1]; Ana Gómez-Núñez[2]; Ricardo Gracia-Bouthelier[3]; Campos-Barros[4]

[1]Hospital Universitario 12 de Octubre, Pediatric Endocrinology, Madrid, Spain; [2]IdiPAZ, UAM, Hospital Universitario La Paz, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain; [3]Hospital Universitario La Paz, Pediatric Endocrinology, Madrid, Spain; [4]IdiPAZ, UAM, Hospital Univ. La Paz & CIBERER, U753, ISCIII, Institute of Medical & Molecular Genetics (INGEMM), Madrid, Spain

Background: Recent reports have indicated that ALS haploinsufficiency due to heterozygous inactivating *IGFALS* (16p13.3) mutations, may be a factor involved in the aetiology of moderate postnatal growth deficit associated with IGF-I deficiency. p.N276S is a founder *IGFALS* mutation in the Spanish population, which has been reported in 5 unrelated families.
Objective: To characterize the genotype/phenotype correlations of the p.N276S *IGFALS* mutation in a Spanish family with multiple p.N276S heterozygous carriers.
Clinical case: The index case is a male with postnatal growth deficit since the age 1.4 yrs, born at term from non consanguineous parents. Father height: 171.5 cm (-0.91 SDS); mother height: 149 cm (-2.51SDS). His main auxological characteristics at diagnosis and during the follow up period are summarized in table 1. His clinical history noted peculiar facial features, mild hypertelorism, craneo-facial dysmorphism, clinodactyly of the 5th finger, gastro-esophageal reflux and persistent eosinophilia since age 2.5 yrs. At age 10 yrs he was diagnosed with eosinophilic esophagitis.

Chronol. age (yrs)	Height (cm) (SDS)	BW (kg) (SDS)	IGF-I (ng/ml) (SDS)	IGFBP-3 (µg/ml) (SDS)
birth	51 (0.37)	3.37 (-0.07)	-	-
1.4	74 (-2.79)	8.25 (-2.66)	< 30 (-2.74)	-
8.4	125.5 (-1.79)	23 (-1.5)	78 (-1. 45)	2.7 (-0. 4)
12	134.3 (-2.37)	32 (-1.36)	81.5 (-3.09)	2.65 (-1.87)
14.9	149.5 (-1.95)	36 (-1.85)	351 (-0.92)	3.44 (-1.89

Follow-up: During the follow-up period his height remained between -1.79 and -2.47 SDS. Hormonal tests revealed consistently decreased IGF-I and IGFBP3 levels and a peak GH of 18.8 ng/ml (Propranolol). Serum ALS concentration was 55% of age-matched healthy controls. At his last control, age 14.9 yrs, his height was 149.5 cm (-1.95 SDS; Tanner III).
Methods and results: *Genetic analyses:* Mutation screening of *IGFALS* identified the mutation c.827A>G, p.N276S, in heterozygosis in the index case, mother and in 2/3 siblings, whose main characteristics are summarized in Table 2.

	FATHER	MOTHER	SISTER I	INDEX CASE	BROTHER I	SISTER II
IGFALS mutation	none	p.N276S	p.N276S	p.N276S	p.N276S	none
Birth weight (kg) (SDS)	NA	NA	2.82 (-1.32)	3.37 (-0.07)	3.7 (0.75)	3.4 (0.32)
Chron. age (yr)	44	44	15.7	13.8	11.4	5.2
Height (cm) (SDS)	171.5 (-0.91)	149 (-2.51)	153.8 (-1.33)	145 (-2.11)	146.8 (-0.12)	110.8 (-0.05)
IGF-I (ng/ml) (SDS)	102 (-1.74)	135 (-0.74)	410 (-0.2)	309 (-0.82)	248 (-0.15)	183 (+0.82)
IGFBP3 (µg/ml) (SDS)	3.99 (-0.99)	3.02 (-2.54)	4.06 (-1.37)	3.09 (-1.52)	3.56 (-0.76)	4.46 (+1.33)
ALS (mU/ml) (% of age matched controls)	1290.65 (80.4%)	770.93 (45.1%)	1387 (56.6%)	1135 (55.0%)	1200 (68%)	1342 (112%)

Conclusions: Although serum ALS levels were significantly decreased in all mutation carriers (45-68% of controls), the consequences of ALS haploinsufficiency on growth and final stature differs among the carriers, suggesting that ALS haploinsufficiency is a contributing risk factor but not the single determinant of the postnatal growth deficit observed in the index case.

P1-d2-333 Growth 1

Development of new Danish growth charts

Jeanette Tinggaard[1]; Lise Aksglaede[1]; Kaspar Soerensen[1]; Christine Wohlfahrt-Veje[1]; Annette Mouritsen[1]; Casper P Hagen[1]; Jørgen Holm Petersen[2]; Katharina M Main[1]; Anders Juul[1]

[1]University Hospital of Copenhagen, Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark; [2]University Hospital of Copenhagen, Rigshospitalet, Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark

Background: Current Danish growth charts are based on measurements of children born in 1954-1973. A recent Danish study of parentally reported heights from children aged 0-5 year suggested secular increases in height, weight and BMI compared to the current national references. Therefore, new Danish height curves based on contemporary children are needed.
Objective and hypotheses: To develop new Danish growth charts for children aged 0-20 years, and to compare them with historic Danish references and the WHO standards.
Methods: A combined cross sectional and longitudinal Danish population-based study on 4009 children born in 1983-2002 was conducted. We applied the following inclusion criteria: singleton births, GA ≥ 37 and GA <42, breast-feeding ≥ 3 months, no smoking during pregnancy, white Caucasian, BMI ≥ -2 SD and BMI ≤ +2 SD (based on old references). This resulted in 2057 and 2650 height measurements from 624 girls and 652 boys, respectively. Growth charts were made from the Generalized Additive Models for Location, Scale and Shape (GAMLSS). Medians and centiles were compared with national references and WHO standards.
Results: Overall, contemporary height curves differed significantly from historic charts. In boys, median height at 3-8 years was approximately 2 cm higher and this difference increased to 6-10 cm in 11-15 year olds. In boys, 1.1-1.5 % of measurements were below the 3rd percentile of historic height charts. Compared to WHO standards, approximately 1 % of height measurements were below the -2 SD line and 7-8 % were above the +2 SD line at all ages. In girls, similar patterns were observed.
Conclusions: New Danish growth standards for children aged 0-20 years differ from WHO growth standards with Danish children being significantly taller. A secular trend in height is observed compared to historic national references.

P1-d2-334 Growth 1

Analysis of data from the ANSWER Program® from pediatric patients treated with growth hormone who were prescribed aromatase inhibitor therapy

Peter Lee[1]; Judith Ross[2]; Robert Gut[3]; John Germak[3]

[1]Penn State College of Medicine, The Milton S. Hershey Medical Center, Pediatric Endocrinology, Hershey, PA, United States; [2]Thomas Jefferson University, DuPont Hospital for Children, Department of Pediatrics, Philadelphia PA, Wilmington, DE, United States; [3]Novo Nordisk Inc., Clinical Development, Medical and Regulatory Affairs, Princeton, NJ, United States

Background: The American Norditropin Studies: Web-enabled Research (ANSWER) Program®, a US-based registry, has collected data on patients treated with Norditropin® (somatropin rDNA origin, Novo Nordisk A/S).
Objective and hypotheses: To analyze baseline characteristics and longitudinal data in growth hormone (GH)-treated patients who were prescribed aromatase inhibitor therapy (AIT: anastrozole or letrozole) at the discretion of their physicians.
Methods: As of October 2011, 57 male GH-naive patients with GH deficiency (n=34), idiopathic short stature (n=17), or other growth disorders (n=6) were included in this analysis.
Results: For the overall population (n=57), mean (±SD) chronologic age (CA) at GH start and AIT start, respectively, were 12.2±3.3 y and 15.1±1.9 y. At start of AIT, the number of patients classified as Tanner stage II, III, IV, or V was 6, 15, 8, and 25, respectively. Mean height standard deviation score (HSDS) increased from baseline (—2.1 ±0.8) to start of AIT (—1.1±0.8). In a longitudinal population of 13 patients with data before and after AIT (Table 1), the mean duration of GHT before AIT was 3.8±2.6 y and mean duration for AIT was 0.93±0.45 y. HSDS increased from —2.3±0.4 at baseline, to —1.2±0.6 at start of AIT, and to —0.8±0.7 after AIT. Bone age (BA) increased from 9.4±3.9 y at baseline, to 13.1±0.9 y at start of AIT, to 14.1±1.5 y

after AIT. Mean BA/CA ratio increased from 0.81 at baseline to 0.96 at start of AIT, and remained at 0.96 after AIT.
Conclusions: AIT was initiated during GH treatment coincident with advanced age and Tanner stage, suggesting that physicians were concerned about a diminishing treatment window for optimizing growth due to skeletal maturation and impending epiphyseal fusion. In the longitudinal population, BA/CA ratio showed accelerated increase before AIT and remained unchanged after AIT, coincident with a continued increase in HSDS. These results are consistent with a potential effect of AIT in slowing bone maturation and prolonging the period during which GHT may increase growth potential.

Table 1. Patient characteristics before and after AIT

	Baseline		Start of AIT		After AIT	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Age (y)	13	10.18 (4.23)	13	13.95 (2.06)	13	14.88 (2.17)
HSDS	13	—2.29 (0.44)	13	—1.17 (0.64)	13	—0.83 (0.65)
BA (y)	9	9.44 (3.92)	13	13.12 (0.94)	13	14.10 (1.47)
BA/CA ratio	9	0.81 (0.17)	13	0.96 (0.14)	13	0.96 (0.13)

P1-d2-335 Growth 1

Auxiological characteristics of patients with constitutional delay of growth and puberty (CDGP)

Ulrich Fuchs[1]; Sandra Barth[1]; Astrid Dempfle[2]; Johannes Hebebrand[3]; Stefan A. Wudy[1]
[1]University of Giessen, Children's Hospital, Dept. of Paediatric Endocrinology and Diabetes, Giessen, Germany; [2]Philipps University of Marburg, Institute for Biometry and Epidemiology, Marburg, Germany; [3]University of Duisburg/Essen, Clinics for Psychiatry, Psychosomatics and Psychotherapy of Children and Adolescents, Duisburg/Essen, Germany

Background: CDGP is a common cause of idiopathic growth delay in childhood. It is not due to endocrine or any other known disease. Several auxiological characteristics have been reported to aid in the diagnosis before delay of puberty becomes evident.
Objective and hypotheses: Up to now, after endocrinological work-up, repeated clinical visits for auxiological re-evaluation are required for reliable diagnosis of CDGP. The present study aims at defining model parameters which might allow prediction of future growth before pubertal age is reached.
Methods: We studied a group of Caucasian children (Index 229, Siblings 126) with a positive family history of CDGP. We built a subgroup of 43 children (11 female, 32 male, age 5.4-18.7 years), well characterized with regard to CDGP. All of them had retarded bone-age and no evidence of other known causes of small stature (including familiar short stature). We analyzed height and weight from birth through early childhood (data of well-child visits) to find evidence of growth deceleration. These data as well as height, weight, sitting-height, subischial-leg-length and some ratios at first presentation were calculated as mean standard deviation scores (SDS).
Results: At birth, length and weight were –0.75 SDS. Growth deceleration became evident between age 2 and 4, with SDS at age 4 being -1.45 for height. This difference remained stable until presentation (mean age 12.2). At this time, normal results were obtained for BMI (-0.37). A SDS of -1.51 was found for sitting-height and -1.02 for subischial-leg-length. SDS-Difference of sitting-height and subischial-leg-length was significantly ($p<0.05$) different before (-0.96) and at the beginning of puberty (-0.33).
Conclusions: Our study indicates that the data of the well-child visits, together with endocrinological and radiological work-up before puberty can be used to predict the diagnosis CDGP. Early and reliable diagnosis of this normvariant of growth would save medical resources.

P1-d2-336 Growth 1

Establishment of hGH cut-off concentrations for stimulation tests in children and adolescents by the IDS-iSYS hGH assay

Juergen Kratzsch[1]; Anne Mueller[1]; Claudia Pätzold[1]; Ruth Gausche[2]; Eberhard Keller[2]; Antje Körner[2]; Joachim Thiery[1]; Wieland Kiess[2]; Roland Pfaeffle[2]
[1]University of Leipzig, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany; [2]University of Leipzig, Hospital for Children and Adolescents, Leipzig, Germany

Objective: Growth hormone deficiency (GHD) is confirmed if hGH peaks of two stimulation tests fail to exceed a cut-off in the range between 5-10 ng/mL. This analytical procedure requires a low variability, since otherwise the diagnosis GHD is severely biased and depends more on the quality of the laboratory method than on the presentation of the patient. We, therefore, compared hGH concentrations in serum samples applying commercially available hGH assays and established a new cut-off for interpretation of GH stimulation tests.
Methods: Samples for method comparison were obtained from 312 children with short stature suspected for GHD (8.00+3.44 years of age, 168 males, 144 females). Seven assays were used for GH measurement (iSYS, IDS; IMMULITE 2000, Siemens; Liaison, DiaSorin; UniCel DxI 800 Access, Beckman Coulter; Auto DELFIA, PerkinElmer; BC-IRMA, Beckman Coulter; ELISA, Mediagnost). Samples for cut-off determination were retrieved from stimulation tests of 46 paediatric patients who failed the suspected diagnosis GHD by normal growth data in later life.
Results: The IDS-iSYS method was used as the reference assay. The regression equation (correlation coefficient) between the methods was: iSYS = 0.902 x Immulite-0.040 (r = 0.964), iSYS = 1.025 x Liason + 0.401 (r = 0.919), iSYS = 1.124 x DxI + 0.889 (r = 0.880), iSYS = 0.846 x AutoDELFIA + 0.523 (r = 0.922), iSYS = 1.381 x BC-IRMA + 0.873 (r = 0.927), and iSYS = 1.114 x Mediagnost-ELISA + 0.924 (r = 0.869). The preliminary data deduced from stimulation tests of paediatric patients without GHD suggested a cut-off of 7.18 ng/mL with a diagnostic specificity of 97.5%.
Conclusions: There was a good or acceptable correlation between different hGH assays. Considering conversion factors of recombinant hGH preparations the established cut-off concentration was in agreement with results from last published data approximately 50 years ago.

P1-d2-337 Growth 1

De novo IGF1R gene deletion in an IUGR-SGA boy with high IGF1 levels and without catch-up growth

Antonio Ripepi[1]; Valentina Milan[1]; Gianluca Musolino[1]; Marzia Pollazzon[2]; Paola Granata[2]; Rosario Casalone[2]; Alessandro Salvatoni[1]
[1]Università degli Studi dell'Insubria, Pediatrics Unit, Varese, Italy; [2]Ospedale di Circolo Fondazione "Macchi", Genetics Unit, Varese, Italy

Background: Impaired foetal growth has been related to quantitative or functional IGF1 deficiency. Hemizygosity or mutations of IGF1 receptor gene (*IGF1R*), located in 15q26.3, has been reported in 2% of short stature IUGR subjects with or without mental retardation and dysmorphic feature.
Objective: To report a case of short stature investigated for alterations in *IGF1R* gene.
Patient and methods: A 2.3-year-old boy with mild psychomotor retardation, born SGA (bw 1,73 Kg; ga 36 w) has been evaluated for severe short stature (75.5 cm; -3.22 SD and -1.7 SD below his target height). Coeliac disease and hypothyroidism were excluded. Bone age was delayed of about 1 year. IGF1 levels were >97° centile for age (206 ng/ml). No significant dysmorphic signs were observed at a careful clinical examination. The clinical picture suggested an IGF1 resistance requiring genetic confirmation.
Results: CGH-Array was performed showing a *de novo* 15q26.2-26.3 deletion of 4.07 Mb. The deleted region encompasses 12 OMIM genes, including *IGF1R*.
Conclusions: In our patient the genetic results support the hypothesis that short stature and high IGF1 levels are due to IGF1 peripheral resistance. However the correlation between genotype and phenotype requires further investigations. Since some patients are described compound heterozygotes for mutations in *IGF1R*, we could consider to study the sequence of the entire gene, in order to discover a possible point mutation in the other allele. Furthermore, functional analysis could be useful to study the expression of the protein in

fibroblasts of the patient. In our case the IGF1 levels are particularly high suggesting severe resistance, while other cases of *IGF1R* deletion reported in the literature, responsive to GH treatment, have low or high-normal IGF1 levels. We wonder if this case, with high levels of IGF1, may or may not favourably respond to GH treatment.

P1-d2-338 Growth 1

Height and health-related quality of life: a nationwide population-based study

Jean-Claude Carel[1]; Jacques Pouchot[2]; Joel Coste[3]
[1]Assistance Publique-Hôpitaux de Paris, Robert Debre Hospital, Pediatric Endocrinology, Paris, France; [2]Assistance Publique-Hôpitaux de Paris, Hôpital européen Georges Pompidou, Department of Internal Medicine, Paris, France; [3]Assistance Publique-Hôpitaux de Paris, Hôpital Cochin, Biostatistics and Epidemiology Unit, Paris, France

Background: Short stature is increasingly viewed as an unfavorable health status and treatment for short stature in childhood is commonly recommended with the purpose of improving adult health-related quality of life (HRQoL). However, there is only limited data available concerning the consequences of body height for HRQoL in adulthood.
Objective and hypotheses: To investigate the relationship between body height and HRQoL in a national representative, cross-sectional household survey.
Methods: 8857 men and 9248 women, aged 18-50 years in 2003 from the national general non-institutionalized population were randomly sampleed. Scores on the eight subscales of the Medical Outcomes Study 36-item Short Form (SF-36) were the primary outcomes. Univariate and multivariate linear regression analyses were used to evaluate the effect of height on HRQoL while controlling for age and various socio-economic variables and pathological conditions.
Results: Height was found to be a very weak predictor of HRQoL both for men and women. Only heights lower than 149.2 cm and 136.0 cm, and higher than 203.6 cm and 188.7cm, in men and women respectively, were associated with a clinically significant reduction in physical functioning. The effects of body height on other (mental, social) dimensions of HRQoL were negligible or undetectable.
Conclusions: Height appears to have minimal consequences for physical functioning, and negligible effects on other dimensions of HRQoL. These results do not support the treatment of childhood short stature to increase adult HRQoL.

P1-d2-339 Hypoglycaemia 1

Prospective follow up of patients with diffuse congenital hyperinsulinism successfully treated with octreotide therapy

Senthil Senniappan; Ved Bhushan Arya; Khalid Hussain
Institute of Child Health, Great Ormond Street Hospital for Children, London Centre for Paediatric Endocrinology and Metabolism, London, United Kingdom

Background: Congenital Hyperinsulinism (CHI) is the most common cause of severe and persistent hypoglycemia in the neonatal period. The diffuse form of CHI, which is unresponsive to diazoxide, is managed with octreotide therapy or near total pancreatectomy. There are no prospective studies on patients with diffuse CHI managed with octreotide therapy.
Objectives: To prospectively follow up the children with diffuse CHI on octreotide therapy over a 7 years period.
Population and methods: Data was prospectively collected on 3 children (mean age 7.3 years) with genetically confirmed diffuse CHI (homozygous KCNJ11 or ABCC8 mutations) and managed with long term octreotide therapy.
Results: All 3 children were born at term (mean birth weight 4.2 kilograms) and presented with severe hyperinsulinaemic hypoglycemia soon after birth. Following lack of response to diazoxide, subcutaneous octreotide injections (20-30micrograms/kilogram/day) along with overnight continuous gastrostomy feeds and day time bolus feeds were commenced. All children were monitored at 6 monthly intervals with a 24 hour blood glucose profile, assessment of fasting tolerence, measurements of serum IGF-1/IGFBP3 levels, thyroid function, ultrasound of gall bladder and growth measurements. Over a period of 7 years, all children have been able to stop the octreotide treatment and gastrostomy feeds. The growth remained normal (mean height SDS 0.32 and mean weight SDS 0.81) with normal IGF1 (mean 107 ng/ml) and IGFBP3 levels (mean 2.5 mg/L). The ultrasound of the gall bladder did not reveal gall stones.
Conclusions: This is the first prospective study to report intense monitoring of children with diffuse CHI on octreotide therapy over a 7 years period. Our study has shown that a subgroup of children with diffuse CHI can be effectively and safely managed with low dose octreotide averting the need for surgery. These children will need to be followed up in the long-term to assess their glucose tolerance.

P1-d2-340 Hypoglycaemia 1

Activating mutations in the AKT2 gene cause a novel sydrome of hypoinsulinaemic hypoglycaemia and hemi-hypertrophy

Khalid Hussain[1]; B Challis[2]; N Rocha[2]; F Payne[3]; A Thompson[3]; A Daly[3]; C Scott[3]; J Harris[2]; BJL Smillie[2]; D Savage[2]; U Ramaswami[4]; P De Lonlay[5]; S O'Rahilly[2]; I Barroso[3]; R Semple[2]
[1]Institute of Child Health, Clinical and Molecular Genetics, London, United Kingdom; [2]Institute of Metabolic Science, University of Cambridge Metabolic Research Laboratories, Cambridge, United Kingdom; [3]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom; [4]University of cambridge, Department of Paediatric Endocrinology, Diabetes and Metabolism, Cambridge, United Kingdom; [5]Département de Pédiatrie,, Hôpital Necker–Enfants Malades, Paris, France

Background: Hypoglycaemia is a common problem in the newborn, infancy and childhood periods and can be due to many different causes. Hyperinsulinaemic hypoglycaemia is the most common cause of severe hypoglycaemia and is due to unregulated insulin secretion from pancreatic β-cells.
Objective and hypotheses: To understand the molecular basis of a unique phenotype in three patients charaterised by recurrent severe hypoinsulinaemic hypoglycaemia associated with macrosomia and left-sided hemi-hypertrophy.
Methods: Exome-wide sequencing was undertaken and resulting variants were filtered to exclude those that were common or not predicted to alter amino acid sequences.
Results: 326 rare mutations were found including the heterozygous c.49G>A mutation in the AKT2 gene, leading to substitution of glutamate 17 in the pleckstrin homology (PH) domain for lysine. Sanger sequencing confirmed the heterozygous mutation in lymphocytes and left and right sided dermal fibroblasts from all patients but not from parents consistent with a de novo germline mutation. Overexpression of AKT2 p.Glu17Lys in 3T3-L1 adipocytes produced non insulin dependent membrane localisation of the GLUT4 glucose transporter. Consistent with this the mutant AKT2 exhibited plasma membrane localization even in serum-starved HeLa cells and to produce tonic nuclear exclusion of FoxO1 in 3T3-L1 cells.
Conclusions: This is the first study to described the molecular basis of a novel disorder which leads to autonomous activation of the insulin signalling pathway and hemi-hypertrophy. AKT2 is an upstream positive regulator of the Mammalian Target of Rapamycin (mTOR) which is an atypical protein kinase that controls growth and metabolism in response to nutrients, growth factors and cellular energy levels. This study illustrates the key role played by AKT2 in regulating insulin action and has opended up new treatment options for these patients.

P1-d2-341 Hypoglycaemia 1

Assessment of pancreatic exocrine function in children following near-total pancreatectomy for diffuse congenital hyperinsulinism

Ved Bhushan Arya[1]; Senthil Seniappan[1]; Sarah Flanagan[2]; Sian Ellard[2]; Khalid Hussain[1]

[1]Great Ormond Street Hospital for Children NHS Trust and The Institute of Child Health, University College London, London Centre for Paediatric Endocrinology and Metabolism, London, United Kingdom; [2]Peninsula Medical School, Institute of Biomedical and Clinical Science, Exeter, United Kingdom

Background: Diffuse congenital hyperinsulinism (CHI) is a clinically heterogeneous disease, which if unresponsive to medical therapy (diazoxide, octreotide and continuous feeding) may require near-total pancreatectomy. There are very few studies describing long term pancreatic exocrine function in a large cohort of patients with diffuse CHI following near-total pancreatectomy.
Objective: To describe the clinical characteristics, genetic aetiology and pancreatic exocrine function in a large cohort of medically unresponsive diffuse CHI patients following near-total pancreatectomy.
Methods: Retrospective review of case notes of children treated with near-total pancreatectomy for diffuse CHI over a 10-year period.
Results: Twenty-three children (mean age 7.5±4.1 yr) had near-total pancreatectomy for diffuse CHI.Of these, 21(91%) had mutations (homozygous/compound heterozygous) in *ABCC8/KCNJ11* genes. All had faecal elastase measured within 3 months of pancreatectomy. Of these, 17(74%) had faecal elastase either undetectable (<15µg/g) or suggestive of severe pancreatic exocrine insufficiency (15-100µg/g).Of these, 7(41%) did not show clinical evidence of pancreatic exocrine insufficiency (pale stools, abdominal discomfort, flatulence, weight loss) over a mean follow up period of 6.1±3.5yr and are currently not on pancreatic enzyme supplementation. Mean weight and height SDS of these 7 children (mean age 7.6±5.0yr) is 0.59(±1.27) and 0.48(±1.43) respectively. The remaining 10(59%) with undetectable faecal elastase, developed clinical pancreatic exocrine insufficiency and needed pancreatic enzyme supplements.Mean weight and height SDS of these ten children (mean age 7.7±3.5yr) is 0.35(±0.77) and 0.19(±1.08) respectively.
Conclusions: In our large cohort of 23 children with near-total pancreatectomy for diffuse CHI, faecal elastase proved to be a poor marker for pancreatic exocrine function. No correlation was found between faecal elastase and clinical pancreatic exocrine insufficiency. Faecal elastase should be used with other markers to assess pancreatic exocrine function.

P1-d2-342 Hypoglycaemia 1

Medical treatment of congenital hyperinsulinism: side effects and response to treatment depending on the underlying mutation

Alena Braun[1]; Jan Marquard[1]; Burak Salgin[1]; Ertan Mayatepek[1]; Christian Lerch[2]; Thomas Meissner[1]

[1]University Children`s Hospital Duesseldorf, Department of General Pediatrics, Duesseldorf, Germany; [2]University Hospital Duesseldorf, Department of General Practice, Metabolic and Endocrine Disorders Group, Duesseldorf, Germany

Background: Congenital hyperinsulinism (CHI) is a heterogeneous genetic disorder leading to an unregulated secretion of insulin from pancreatic beta cells. Medical treatment of CHI still remains challenging and keenly depends on the clinicians experience. Knowledge about efficacy, dosage and safety of the medication in current use is limited. An optimisation of pharmacological treatment is particularly important for diffuse CHI as it may avoid subtotal pancreatectomy.
Objectives: Aim of this study was to review the literature on long-term conservative treatment in CHI. In addition, we evaluated the underlying disease-causing mutation (ABCC8, KCNJ11, GLUD1, GCK, HADH, HNF4A, UCP2) with respect to the clinical phenotype and response to pharmacological treatment.
Methods: We searched MEDLINE (from 1947) and EMBASE (from 1988) using the OVID interface. The last search was run in March 2011. In addition, we searched reference lists of retrieved articles and reviews. No language restrictions were applied.
Results: Data for more than 1000 patients with CHI were evaluated. 600 patients had received long-term conservative treatment. Common side-effects of treatment with diazoxide (DZX) were hypertrichosis, fluid retention and dyspeptic syndrome. Severe side effects such as encephalopathy and heart failure were rare. Typical side effects of octreotide included tachyphylaxis and abdominal discomfort. Gallstones, necrotizing enterocolitis and reduced growth velocity were less frequent. No side effects were described for treatment with calcium channel blockers. However, half of the patients treated with these agents required extra medication. About 70% of patients with a mutation in ABCC8 or KCNJ11 and about 14% of patients with a mutation in GCK were resistant to treatment with DZX. The majority of patients with a mutation in GLUD1, HADH, HNF4A or UCP2 were responsive to DZX.
Conclusions: This review provides the basis for a structured discussion with regard to optimal treatment strategies in children with the heterogeneous disorder CHI.

P1-d2-343 Hypoglycaemia 1

Surgery in non-focal congenital hyperinsulinism: less may be more

Winfried Barthlen[1]; Carsten Müller[1]; Emine Varol[1]; Martin Zenker[2]; Ilse Wieland[2]; Wolfgang Mohnike[3]; Silke Vogelgesang[4]; Klaus Mohnike[5]

[1]University Medicine Greifswald, Pediatric surgery, Greifswald, Germany; [2]Otto-von-Guericke-University, Institute of Human Genetics, Magdeburg, Germany; [3]Diagnostic-Therapeutical Center, Frankfurter Tor, Berlin, Germany; [4]University Medicine Greifswald, Institute of Pathology, Greifswald, Germany; [5]Otto-von-Guericke-University, Pediatrics, Magdeburg, Germany

Background: In children with non-focal congenital hyperinsulinism (CHI) who are resistant to medical therapy extensive pancreatic resections (95-98%) have been recommended. However, the incidence of diabetes until puberty has been reported in up to 90% after subtotal pancreatectomy.
Objective: We evaluated a less extensive, more selective minimal invasive surgical approach.
Patients and methods: 7 children (mean age 19 months) and one adult with severe non-focal congenital hyperinsulinism which was not responsive to medical therapy underwent laparoscopic biopsies under frozen section control. A laparoscopic pancreatic tail resection removing approx. 30-40% of the gland was performed in all patients. In one child additionally an open resection of the uncinate process was carried out. Today the follow-up-time is 5.4 months (2 - 11 months).
Results: In six patients no genetic mutation, in one a heterozygous mutation in ABCC8 and homozygous KCNJ11 mutations in another have been detected. Three children revealed the recently described segmental mosaic form of CHI, four children and the adult a classical diffuse CHI. After surgery, one child with segmental mosaic CHI can be estimated as cured. Five children are off any medication today and manage their glucose level by starch-enriched meals. The adult is euglycemic without further treatment. There were no complications except one hematoma .
Conclusions: In 8 patients with non-focal congenital hyperinsulinism, resistant to medical therapy (diazoxide or octreotide) a restrictive, frozen-section guided laparoscopic pancreatic tail resection led to considerable improvement in quality of life for children treated in the past by mutilating pancreatectomy. Further work is necessary to identify this subgroup within the molecular and clinical heterogenous diagnosis of CHI.

P1-d2-344 Hypoglycaemia 1

Genotype phenotype correlations in congenital hyperinsulinism

Klaus Mohnike[1]; Ilse Wieland[2]; Winfried Barthlen[3]; Susann Empting[1]; Martin Lindner[4]; Thomas Meissner[5]; Martin Zenker[2]

[1]OvG University Magdeburg, Department of Pediatrics, Magdeburg, Germany; [2]OvG University Magdeburg, Institute of Human Genetics, Magdeburg, Germany; [3]University Medicine Greifswald, Clinic Ped. Surgery, Greifswald, Germany; [4]University Heidelberg, Department of Pediatrics, Heidelberg, Germany; [5]University Duesseldorf, Department of Pediatrics, Duesseldorf, Germany

Background: Congenital hyperinsulinism (CHI) is characterized by clinical and genetic heterogeneity. Mutations in K-ATP channel are reported in nearly 50%, with null or reduced functional activity.

Objective and hypotheses: In order to use mutational data as biomarker for diagnostic and treatment decisions it is crucial to correlate them with valid clinical data.
Methods: 113 patients (incl. 7 families) referred between 2004 and 2011 with clinical diagnosis of CHI were screened for mutations in the genes ABCC8 and KCNJ11 by bidirectional sequencing of all coding exons and adjacent intronic regions. Segregation was tested for all families where parental samples were available.
Results: We detected 48 mutations in ABCC8 and 5 different mutations in KCNJ11 in 36 and 6 unrelated patients, respectively, including 28 novel mutations. In ABCC8, we identified 20 missense mutations, 7 nonsense mutations, 7 frame-shift mutations, 2 in-frame deletions, and 11 splice-site/splicing mutations. In KCNJ11, 5 different missense mutations were found and one in-frame deletion. Six mutations of ABCC8 and one KCNJ11 mutation were recurrent in more than one family. Molecular results suggested focal type CHI in 9 children (6 ABCC8 and 3 KCNJ11) and could be proven by Dopa-PET/CT and histology. 8 patients carried a heterozygous ABCC8 mutation affecting one of the nucleotide binding domains. In 5 of them family history or published data suggested that the respective mutations act in a dominant manner. Furthermore in 26 children a diffuse type CHI was confirmed by compound-heterozygosity or homozygosity for K-ATP channel mutations (23 ABCC8 and 3 KCNJ11).
Conclusions: In 36 of affected children mutations of ABCC8 (with 48 different alleles) and in 6 of KCNJ11 (with 5 different alleles) could be identified. In all 9 histological confirmed focal type CHI a paternally inherited mutation of ABCC8 or KCNJ11 was detected. In the majority of cases molecular findings in the family are good predictors of the phenotype.

P1-d2-345 Hypoglycaemia 1

Elevated postprandial increase in GLP-1 and GIP levels in atypical form of congenital hyperinsulinism

Yanqin Shi[1]; Mars Skae[2]; Bindu Avatapalle[2]; Lindsey Rigby[2]; Neil Hanley[3]; Peter Clayton[3]; Indi Banerjee[2]; Mark J Dunne[1]; Karen E Cosgrove[1]
[1]University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom; [2]Royal Manchester Children's Hospital, Paediatric Endocrinology, Manchester, United Kingdom; [3]University of Manchester, Faculty of Medical and Human Sciences, Manchester, United Kingdom

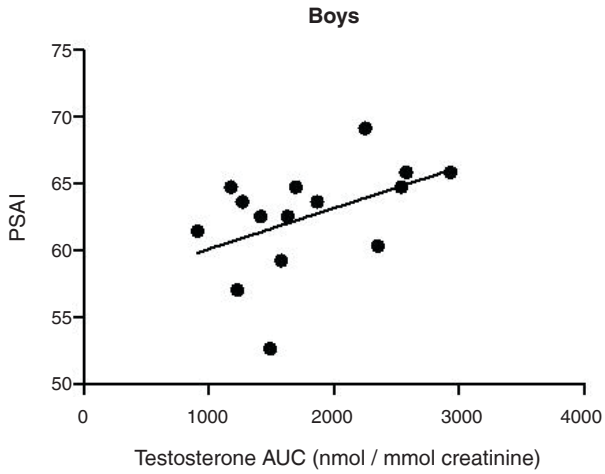
Background: Congenital hyperinsulinism (CHI) is characterised as inappropriate insulin release for the level of glycaemia and can present as either a transient condition or a persistent, more severe disorder. Persistent CHI is classified into two forms: diffuse-, and focal-CHI with some diffuse patients being sub-grouped as atypical disease for which the genetic cause of hyperinsulinism is not known.
Objective: Here we aimed to investigate postprandial increases of the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependant insulinotropic polypeptide (GIP) in CHI to assess whether these may be diagnostic of the different subtypes of the condition.
Methods: Enzyme-linked immunosorbent assays (ELISA) were used to examine the fasting and postprandial plasma active and total GLP-1 and total GIP levels from CHI patients, n=15. Eight patients with persistent CHI were defined as diffuse (n=5), focal (n=2) or atypical (n=1) on the basis of predictive genotyping and / or histological examination following surgery. Diffuse and focal patients were positive for gene defects in ABCC8/KCNJ11 whereas no gene defects were found to be associated with the atypical patient. Seven patients had transient hypoglycaemia and no identified genetic abnormalities in ABCC8/KCNJ11.
Results: Statistical analysis revealed no difference between GLP-1/GIP levels among diffuse, focal and transient CHI patients (one-way ANOVA, $p>0.05$). However, in the atypical patient postprandial plasma GLP-1 levels were increased by approximately 15-fold higher than the other patients and GIP levels were increased by approximately 3-fold more than the other patient groups.
Conclusions: Measurements of post-prandial GLP-1 and GIP levels did not distinguish transient CHI from diffuse and focal forms of CHI. However, marked increases in postprandial GLP-1 and GIP levels were observed in the atypical CHI patient suggesting that abnormal incretin values may prove to be diagnostic for atypical CHI.

P1-d2-346 Perinatal and Neonatal Endocrinology 1

Testosterone measured in infancy predicts subsequent sex-typed behaviour in boys and in girls

Ulla Sankilampi[1]; Annamarja Lamminmäki[2]; Tanja Kuiri-Hänninen[3]; Melissa Hines[4]; Leo Dunkel[5]
[1]Kuopio University Hospital, Department of Pediatrics, Kuopio, Finland; [2]Kuopio University Hospital, Department of Oncology, Kuopio, Finland; [3]University of Eastern Finland, Department of Pediatrics, Kuopio, Finland; [4]University of Cambridge, Department of Social and Developmental Psychology, Cambridge, United Kingdom; [5]William Harvey Research Institute, Barts and the London, Queen Mary University of London, London, United Kingdom

Background: The testes are active during gestation, as well as during early infancy. Testosterone elevation during fetal development has been shown to play a role in human neurobehavioral sexual differentiation. The role of early postnatal testicular activation in human psychosexual development is largely unknown, however.
Objective and hypotheses: In the present study, we examined the hypotheses that testosterone measured in urine samples during early infancy would relate to subsequent sex-typed behavior in girls and in boys.
Methods: We measured testosterone in 48 full term infants (22 boys, 26 girls) by monthly urinary sampling from day 7 postnatal to age 6 months, and related the area under the curve (AUC) for testosterone during the first 6 months postnatal to sex-typed behavior at the age of 14 months, using the Pre-School Activities Inventory (PSAI), and playroom observation of toy choices.
Results: In boys, testosterone AUC correlated significantly with PSAI scores (Spearman's rho = 0.54, $p = 0.04$) (Figure). In addition, play with a train and with a baby doll showed the anticipated sex differences, and play with the train correlated significantly and positively with testosterone AUC in girls (Spearman's rho = 0.43, $p = 0.05$), while play with the doll correlated significantly and negatively with testosterone AUC in boys (Spearman's rho = -0.48, $p < 0.03$).
Conclusions: These results support a role for testosterone during early infancy in human neurobehavioral sexual differentiation. Urinary sampling may provide a non-invasive method for reliable assessment of the early postnatal surge in testicular hormones and the relationship of this surge to human development.



P1-d2-347 Perinatal and Neonatal Endocrinology 1

Infants born with extremely low-birth weight have increased urinary steroid excretion at age of 8-11 yrs

Bettina Gohlke[1]; Stefan Wudy[2]; Sonja Stutte[1]; Peter Bartmann[3]; Martina Hartmann[2]; Joachim Woelfle[1]
[1]University Hospital, Pediatric Endocrinology, Bonn, Germany; [2]Justus Liebig University, Steroid Research and Mass Spectrometry Unit, Division of Paediatric Endocrinology & Diabetology, Giessen, Germany; [3]University Hospital, Neonatology, Bonn, Germany

Background: Adverse experiences in utero might result in persistent modulating consequences on metabolism and cardiovascular system (‚fetal programming'). This sensitive period has been extended as ‚developmental programming' to the early extrauterine life of premature children. The hypothalamic-pituitary-adrenal (HPA) axis is susceptible to programming and may be linked to risk of disease later in life.
Objective and hypotheses: In our study we hypothesized that premature infants born with extremely low birth weight (ELBW, birth weigh < 1000g) have higher levels of urinary steroid metabolites than healthy controls.
Patients: 27 ELBW-infants (17 girls, 10 boys, all prepubertal, age 8-11 yrs) were included. Control children were age and sex matched. There was no significant difference of height, weight or BMI between the groups. All results were adjusted according to body surface area.
Methods: Urinary steroids were extracted, enzymatically hydrolysed, derivatized and profiled by gas chromatrography-mass spectrometry using selected ion monitoring. 36 different steroid metabolites were quantified.
Results: All 36 measured steroid metabolites were higher in the ELBW boys (six significantly) in comparison to controls. In girls 33/36 steroid metabolites were higher (19 significantly). Sums for mineralocorticoid precursors, cortisol production, 11-β-HSD-activity, and the adrenal androgen production are shown in the table:

Sum of [Mean (µg/l)]	Female ELBW (n=17)	Female Control (n=17)	p-value	Male ELBW (n=10)	Male Control (n=10)	p-value
Mineralcorticoid precursors (THB + THA + Allo THB (=5a-THB))	1016	467	0.002	1725	472	0.02
Cortisol production (Tetrahydro-Cortisol+Allo-Tetrahydrocortisol+a-Cortol+β-Cortol+Tetrah.cortison+a-+β-Cortolon)	10,717	6200	0.007	6494	4131	0.2
11β-HSD activity (Tetrahydrocortisol + Allo-Tetra-Hydrocortisol / Tetrahydrocortison)	1.94	1.46	ns	1.48	0.23	ns
adrenal Androgenes (Androsteron+Etiocholanolon+5-Androsten-3β,17-diol+5-Androsten-3,17+DHEA+16Hydroxy-DHEA +Androstentriol)	1087	550	0.03	1145	502	0.04

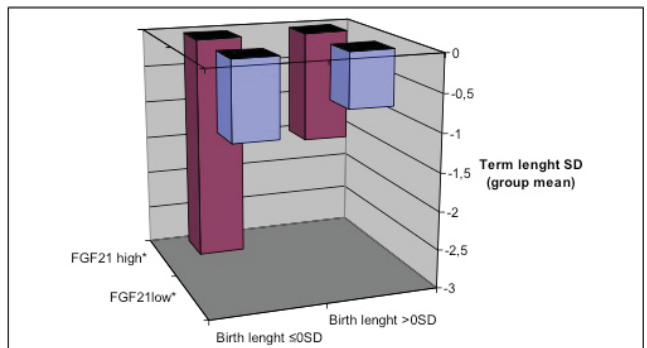
Conclusions: These findings suggest that the premature stressful extra-uterine environment has long-lasting augmenting effects on the function of the hypothalamo-pituitary-adrenal-axis. We found significantly increased urinary androgen excretion, and a significant higher cortisol production rate and higher mineralcorticoid precursors in the ELBW infants pointing to an overall increased activation of the adrenals in ELBWs. Global 11-βHSD-activity was not significantly different and does not seem to account for the changes in cortisol metabolism.

P1-d2-348 Perinatal and Neonatal Endocrinology 1

Elevated FGF21 attenuates postnatal growth in very preterm infants

Sanna Silvennoinen[1]; Mari Rehu[2]; Leo Dunkel[3]; Ulla Sankilampi[4]
[1]Kuopio University Hospital and University of Eastern Finland, Department of Pediatrics, Kuopio, Finland; [2]University of Eastern Finland and Eastern Finland Laborotory Centre (ISLAB), Kuopio, Finland, Department of Clinical Chemistry, Kuopio, Finland; [3]Queen Mary University of London, Centre for Endocrinology, William Harvey Research Institute, London, United Kingdom; [4]Kuopio University Hospital, Department of Pediatrics, Kuopio, Finland

Background: Fibroblast growth factor 21 (FGF21) is considered a key regulator in adaptation to fasting. Recently, a causative role of increased FGF21 in inhibition of skeletal growth during prolonged undernutrition was suggested in mice, mediated by antagonistic effect on GH action and reduced IgF1 synthesis (Kubicky et al, Endocrinology 2012). However, there are no studies on its role in chronic growth failure in man.
Objectives and hypotheses: We hypothesised that FGF21 is a mediator of extrauterine growth retardation in very preterm (PT) infants. We assessed FGF21 at birth and at 1, 3, and 5 weeks of age (w1-w5), and growth from birth to term in 32 very PT infants (56% boys, median gestational age 28,2 weeks, range 23,4-31,9; median birth weight (BW) 990g, range 480- 1910; median birth length (BL) 36,8cm, range 28,0-44,0).
Methods: Plasma FGF21 was measured with an ELISA kit. BW, BL, weight, and length at term (median postmenstrual age 40,0 weeks, range 37,0-41,6) were converted into SDS with population-based birth size reference. Lg-transformation was used for normality. Mixed model and linear regression were used for analyses.
Results: FGF21 was unmeasurably low at birth in 85% of infants. In mixed model adjusted for gestational age, sex, BW-SD and BL-SD, a significant increase from w1 to w5 was observed (p=.021). Increasing BW-SD had a significant negative effect on lgFGF21 (p=,003). Only 6,2 and 3,1% of infants had BW or BL <-2SD, whereas at term, 34.4% had both weight and length <-2.0SD. In linear regression model, w1-w3 FGF21 had a significant negative correlation (beta=-,455, p=,001) with length-SD at term, and BL-SD a positive correlation (beta= ,499, p=.001).



	Birth lenght ≤0SD	Birth lenght >0SD
FGF21low*	-1,01	-0,71
FGF21 high*	-2,91	-1,45

*FGF21 low indicates that mean w1-w5 level is below median level.
*FGF21 high indicates that mean w1-w5 level is at or above median level.

Conclusions: These results indicate that elevated FGF21 is independently associated with extrauterine growth failure in very PT infants. This supports the hypothesis that FGF21 inhibits skeletal growth in humans as well.

P1-d2-349 Perinatal and Neonatal Endocrinology 1

Association of fetal IGF-I, leptin, and adiponectin with fetal and early postnatal growth in NCCHD cohort study

Kengo Miyashita; Akiko Yamamoto; Satuki Nishigaki; Yusuke Mizuno; Masahiro Noda; Yasuhiro Naiki; Reiko Horikawa
National Center for Child Health and Development, Division of Endocrinology and Metabolism, Tokyo, Japan

Objective: To investigate contributing factors for fetal and early postnatal growth, we investigated correlation between birth weight, length, and weight gain at one month of age, maternal constitution and age background, and biochemical factors in cord blood.
Subjects and methods: Eight hundred and nine babies from mothers registered on the Cohort Study for Children and Mothers at NCCHD were involved in this study. One hundred eleven subjects were born small for gestational age (SGA). The economical background of participants was relatively homogenous. Cord blood samples were obtained at delivery, IGF-I, insulin, leptin, and adiponectin were measured by commercially available kits.
Results: Subjects' birth weight was 2903.9±493.3 g (mean±SD), birth length was 48.49±1.94 cm. Birth weight showed significant correlation with IGF-I (R2=0.093, P<0.0001), insulin (R2=0.023, P<0.0001), leptin(R2=0.1228,P<0.0001), and adiponectin(R2=0.08359,P<0.0001)in cord blood. Birth weight among subjects with full-term, AGA delivery significantly correlates with maternal weight before pregnancy but not with maternal age. In SGA children (n=111) among our subjects, birth weight strongly correlates with IGF-I and adiponectin (R2=0.249, P=0.0041 and R2=0.254, P=0.0004, respectively) and weakly correlates with leptin (R2=0.0157, P=0.0448), but not with insulin levels. IGF-I, leptin and adiponectin levels in cord blood were significantly higher in AGA than in SGA children (P<0.0001). SGA children showed significantly higher BMI increment than AGA children during one month after birth.
Conclusion: These results indicate the contribution of IGF-I on fetal growth. Leptin and adiponectin might contribute for fetal growth, however, it is more likely that the levels of these factors show only the results of fetal growth. The significant correlation of maternal weight before pregnancy and child's birth weight suggests the involvement of genetic factors. The factors that have influence on early postnatal growth are under investigation.

P1-d2-350 Perinatal and Neonatal Endocrinology 1

Serum levels of receptors for advanced glycation end products in normal-weight and obese children born small and large for gestational age

Valentina Chiavaroli; Tommaso de Giorgis; Stefania De Marco; Cosimo Giannini; Francesco Chiarelli; Angelika Mohn
University of Chieti, Department of Paediatrics, Chieti, Italy

Background: Children born small (SGA) and large (LGA) for gestational age are at increased risk of cardio-metabolic complications later in life. Interestingly, in recent years the soluble (sRAGE) and the endogenous secretory (esRAGE) receptors for advanced glycation end products have been proven to be involved in the pathogenesis of cardiovascular diseases.
Objective and hypotheses: Our aims were to evaluate sRAGE and esRAGE in normal-weight (NW) and obese (Ob) pre-pubertal SGA and LGA children compared to subjects born appropriate (AGA) for gestational age, and to explore the potential association of these markers with birth weight (BW), insulin resistance (IR) and obesity.
Methods: We categorized 130 pre-pubertal children into 6 groups according to BW and obesity. sRAGE, esRAGE and homeostasis model assessment of IR were evaluated.
Results: sRAGE and esRAGE were lower in Ob SGA and LGA children than Ob AGA subjects (all P<0.05), and in NW SGA and LGA children than NW AGA subjects (all P<0.05). In a multiple linear regression analysis with sRAGE as the dependent variable, SGA (β=-0.237, P=0.008) and LGA (β=-0.414, P=0.0001) BW categories as well as HOMA-IR (β=-0.275, P=0.01) were significantly related to sRAGE, independently of BMI-SDS, age and gender. In a second model, using esRAGE as the dependent variable, SGA (β=-0.307, P=0.001) and LGA (β=-0.400, P=0.0006) BW categories as well as HOMA-IR (β=-0.246, P=0.04) were significantly related to esRAGE, independently of BMI-SDS, age and gender.
Conclusions: sRAGE and esRAGE are decreased in pre-pubertal SGA and LGA subjects, particularly in those showing excess body weight during childhood. Furthermore, IR emerged as an independent determinant of reduced RAGE levels. Further longitudinal studies are needed to verify the cause-effect relationship between IR and RAGE in these children.

P1-d3-351 Pituitary 1

Timed-12h urinary gonadotropin during gonadotropin-releasing hormone analog stimulation testing for diagnosing the onset of hypothalamic-pituitary-gonadal axis in girls

Zhuangjian Xu; Yaping Ma; Shuyan Xie; Qing Wang; Tingting Zhang
The Fourth Affiliated Hospital of Soochow University, Pediatrics, Wuxi, China

Background: GnRH stimulation testing requires venipuncture and multiple blood sampling at short intervals, an accurate but at times not always comfortable method. Furthermore, GnRH is commercially limited.
Objective: To estimate usefulness of timed-12h urinary gonadotropin during GnRH analog (GnRHa) stimulation testing for diagnosing the onset of hypothalamic-pituitary-gonadal (HPG) axis.
Methods: Following injecting triptorelin (08:30 am, Decapeptyl, 0.1 mg, s.c.), consecutive double timed-12h urine samples were respectively collected in 42 girls who suffered from disorders of growth or pubertal development. Gonadotropin was assayed by immunochemiluminometric assays (ICMA).
Results: There were 34 girls with the onset of HPG axis and the remaining 8 girls in the situation of pre-puberty. The correlation coefficient between the serum peak LH (PLH) and the content (concentration × volume) of the first urinary LH (FULH) was 0.759, and for PLH and the second urinary LH (SULH) content 0.746. The means of FULH content and SULH content in girls with the onset of HPG axis were all more than girls who were in pre-puberty (P < 0.05). The areas under the receiver operator characteristic curves for diagnosing the onset of HPG axis for PLH, FULH content and SULH content were 0.982, 0.952 and 0.982, respectively. When PLH, FULH content and SULH content were respectively no less than 4.12 IU/L, 1.130 IU and 0.433 IU, the sensitivities were respectively 97.1%, 88.2% and 97.1%, and all three indexes were 100% specific.
Conclusions: It is concluded that timed-12h urinary LH content assayed by ICMA during GnRHa stimulation testing provides an effective, noninvasive methods for the diagnosis of onset of HPG axis in girls, and SULH content may be better than FULH content.

P1-d3-352 Pituitary 1

Initial hypothalamic involvement is the major risk factor for impaired prognosis and quality of life in childhood craniopharyngioma regardless of chosen treatment strategies – results of kraniopharyngeom 2000

Hermann L. Müller[1]; Ursel Gebhardt[1]; Monika Warmuth-Metz[2]; Rolf-Dieter Kortmann[3]; Andreas Faldum[4]; Torsten Pietsch[5]; Gabriele Calaminus[6]; Niels Sörensen[7]
[1]Klinikum Oldenburg, Department of Pediatrics, Oldenburg, Germany; [2]University of Würzburg, Department of Neuroradiology, Würzburg, Germany; [3]University of Leipzig, Department of Radiooncology, Leipzig, Germany; [4]University of Mainz, Institute for Medical Biostatistics, Epidemiology and informatics (IMBEI), Mainz, Germany; [5]University of Bonn, Institute of Neuropathology, Bonn, Germany; [6]University of Bonn, Department of Pediatric Hematology and Oncology, Münster, Germany; [7]Evangelisches Krankenhaus Oldenburg, Dep. of Neurosurgery, Oldenburg, Germany

Background: Hypothalamic obesity has major impact on prognosis and quality of life (QoL) in childhood craniopharyngioma (CP). The pathogenic relevance of initial hypothalamic involvement versus treatment-related hypothalamic lesions is a matter of controversy.
Methods: 120 patients were recruited prospectively (2001-2007) and evaluated after 3 yrs of follow-up. BMI and QoL at diagnosis (dgx) and 36 mo after dgx were analyzed based on reference assessment of tumour localization and post-surgical hypothalamic lesions using a novel grading system (no, anterior,

posterior involvement/lesion). Treatment was analyzed regarding neurosurgical strategy of 50 participating neurosurgical centres and the centre sizes. Based on patient load during the 6-year recruitment, centres were categorized as small (1 pt / 6 yrs), middle (2-5 pts /6yrs) or large-sized centres (>5 pts / 6 yrs).
Results: BMI SDS at diagnosis was similar in patients with or w/o hypothalamic involvement. Surgical lesions of anterior and posterior hypothalamic areas were associated with higher increases in BMI SDS during 36 mo post-diagnosis compared to patients without or only anterior lesion (+1.8 BMISD, p=0.033; +2.1 BMISD, p=0.011), negatively impacting QoL in patients with posterior lesions. Surgical strategies varied between the 50 neurosurgical centres. Patients treated in small centres presented with a higher rate of hypothalamic involvement compared to middle and large-sized centres. Treatment in large centres was less radical, the rates of complete resection and hypothalamic surgical lesions lower than those of middle-sized and small centres. However, multivariable analysis showed that pre-operative hypothalamic involvement was the only independent risk factor for severe obesity (p=0.002).
Conclusions: Radical strategies leading to posterior hypothalamic lesions are not recommended. Because our results show that initial hypothalamic involvement has an apriori effect on the clinical course, our recommendations are based on recognizing CP as a chronic disease.

P1-d3-353 Pituitary 1

Pituitary dysfunction following traumatic brain injury in children based on a large prospective cohort of 103 patients

Claire Personnier[1]; Philippe Meyer[2]; Mathilde Chevignard[3]; Isabelle Flechtner[1]; Magali Viaud[1]; Zeina Ajaltouni[1]; Dominique Renier[4]; Elisabeth Hoppe-Hirsch[4]; Caroline Mignot[4]; Gérard Friedlander[5]; Kathleen Laborde[6]; Nathalie Boddaert[7]; Hanna Touré[3]; Stéphanie Puget[8]; Christian Saint-Rose[8]; Michel Polak[9]
[1]Hôpital Necker-Enfants Malades, Pediatric Endocrinology Unit, Paris, France; [2]Hôpital Necker-Enfants Malades, Pediatric Anesthesiology Unit, Paris, France; [3]Hôpital National de Saint-Maurice, Department of Rehabilitation, Saint-Maurice, France; [4]Hôpital Necker-Enfants Malades, Pediatric Neurosurgery Unit, Paris, France; [5]Hôpital Necker-Enfants Malades, Université Paris Descartes, Functionnal Explorations Unit, Paris, France; [6]Hôpital Necker-Enfants Malades, Functionnal Explorations Unit, Paris, France; [7]Hôpital Necker-Enfants Malades, Université Paris Descartes, Radiology Unit, Paris, France; [8]Hôpital Necker-Enfants Malades, Université Paris Descartes, Pediatric Neurosurgery Unit, Paris, France; [9]Hôpital Necker-Enfants Malades, Université Paris Descartes, Pediatric Endocrinology Unit, Paris, France

Background: Traumatic brain injury (TBI) is common in childhood but endocrine consequences in this age group are yet to be documented by strong prospective data.
Objectives: To evaluate the frequency of pituitary deficiency in 2 different populations of patients: children with accidental TBI and infants with abusive head traumatism; to identify predictive factors of subsequent endocrine dysfunction.
Methods: A prospective study was conducted on children from 0 to 15 years old, with previous history of severe accidental TBI (Glasgow Coma Scale (GCS) ≤ 8) or unexplained subdural haematoma ("Shaken Baby Syndrome" SBS) admitted to an intensive neurosurgery care unit. Clinical evaluation and pituitary hormonal testing (basal levels and dynamic tests) were performed at least 6 months and until 18 months after brain traumatism.
Results: 103 children were included (81 TBI, 22 SBS). 86 underwent hormonal investigations (72 TBI, 14 SBS), 12 stopped the research and 5 were lost. Among the tested patients, 55 had normal pituitary function and 31 had hormonal dysfunction (26 TBI, 5 SBS): isolated growth hormone deficiency (GHD) in 5 (5,8%, 4 TBI, 1 SBS), thyreotropic and corticotropic deficiencies in 2 (2,3%) and 1 (1,2%) children with TBI respectively, insufficient GH stimulation tests with conserved growth velocity in 24 (28%, 20 TBI, 4 SBS): IGF1 level was normal in 22 and low in 2 (one of the two patients had associated thyreotropic deficiency).
Conclusions: Paediatric TBI is an important cause of pituitary deficiency found in 9,3% of our cohort. Moreover, almost 28% of patients have deficient stimulated GH response so far with normal growth velocity. To date, no considered factors related to mechanism of injury or to intensive care management were correlated to hormonal abnormalities. Brain imaging at the acute phase and association with subsequent endocrine disorders are currently under analysis. Supported by an educational grant from Pfizer SAS.

P1-d3-354 Pituitary 1

Novel KAL1 sequence variants associated with septo-optic dysplasia (SOD) in three female patients

Mark McCabe[1]; Youli Hu[2]; Louise Gregory[1]; Ajay Thankamony[3]; Ieuan Hughes[3]; Sharron Townshend[4]; Pierre-Marc Bouloux[2]; Mehul Dattani[1]
[1]UCL-Institute of Child Health, Developmental Endocrinology, Clinical and Molecular Genetics Unit, London, United Kingdom; [2]Royal Free Hospital and University College Medical School, Centre for Neuroendocrinology, London, United Kingdom; [3]University of Cambridge, Addenbrookes Hospital, Cambridge, United Kingdom; [4]Princess Margaret Hospital for Children, Clinical Genetics, Subiaco, Australia

Background and aims: *KAL1* is essential for GnRH neuronal migration and olfactory bulb development with mutations implicated in Kallmann syndrome (KS; hypogonadotrophic hypogonadism with anosmia). *KAL1* is located in the X-chromosome pseudoautosomal region, which may account for the recent report of female KS patients exhibiting *KAL1* variations. There is increasing evidence of overlapping genotypes/phenotypes between KS and congenital hypopituitarism including septo-optic dysplasia (SOD). Therefore, we aimed to screen 421 patients with the latter for mutations in *KAL1*.
Methods: The coding region of *KAL1* was assessed by direct sequencing. *In vitro* functional analyses of identified variants included immunocytochemistry and western blot analyses for protein secretion from Cos7 cells as well as a novel quantitative luciferase-reporter assay.
Results: Two variants [p.K185N (n=1) and novel p.P291T (n=2; sisters)], occurring at highly conserved residues and absent from 480 controls, were identified in three female patients with SOD. Each had optic nerve hypoplasia and GH deficiency, with the p.K185N variant also being associated with TSH deficiency and an ectopic posterior pituitary. A qualitative decrease in secretion of mutant p.P291T protein was shown by its retention in Cos7 cells and a 40% decrease in transcriptional activity ($p<0.001$). Secretion of p.K185N was unaffected but the variant was associated with a 21% decrease in transcriptional activity ($p<0.01$). This variant is located between protein-protein interaction domains and may affect the binding of the protein to FGFR1 and heparan sulfate. Both variants were inherited from the unaffected mothers and are suggestive of variable penetrance or digenicity in the affected individuals, none of whom exhibit variations in any of the other known KS genes.
Conclusions: We implicate *KAL1* in females with hypopituitarism/SOD for the first time to our knowledge, reflecting an overlap between KS and SOD that has also been observed with *FGF8, FGFR1* and *PROKR2* variants.

P1-d3-355 Pituitary 1

Screening of LHX2 in patients presenting growth retardation with posterior pituitary and ocular abnormalities

Marie-Laure Sobrier[1]; Christelle Pérez[1]; Florence Dastot-Le Moal[2]; Nathalie Collot[2]; Marie Legendre[2]; Isabelle Abadie[3]; Anne-Marie Bertrand[4]; Serge Amselem[1]
[1]Inserm, U933, Paris, France; [2]AP-HP Trousseau, Génétique et Embryologie Médicale, Paris, France; [3]Hopital Intercommunal, Service de Pédiatrie, Créteil, France; [4]Centre hospitalo-universitaire, Service de Pédiatrie, Besançon, France

Background: In humans, pituitary hormone deficiency may be part of a syndrome including extra-pituitary defects like ocular abnormalities. Very few genes have been linked to this particular phenotype. In the mouse, Lhx2, which encodes a member of the LIM class of homeodomain proteins, was shown to be expressed during early development in the posterior pituitary, eye and liver, and its expression persists in adulthood in the central nervous system. Lhx2-/- mice display absence of posterior pituitary and intermediate lobes, malformation of the anterior lobe, anophthalmia and they die from anemia.
Objective and hypotheses: We tested the implication of the LHX2 gene in patients presenting pituitary hormone deficiency associated with ectopic or non visible posterior pituitary and ocular defects.
Methods: A cohort of 76 patients, including two familial cases, was studied. Direct sequencing of the LHX2 coding sequence and intron/exon boundaries was performed. LHX2 transcriptional activity on pituitary promoters was tested in vitro.

Results: Seven heterozygous sequence variations were identified, among which two are novel missense (p.Ala203Thr and p.Val333Met). In vitro, LHX2 activates transcription of TSHβ, PRL and POU1F1 promoters in HEK293 cell line. A synergistic action of POU1F1 and LHX2 was also shown on these promoters. The two variations were tested and no significant difference was observed leading to the conclusion that there are not deleterious.
Conclusions: These results suggest that, if LHX2 is involved in pituitary hormone deficiency associated with posterior pituitary and ocular defects, it would be a rare cause of this disease condition.

P1-d3-356 Pituitary 1

Genetic screening in a cohort of 2030 patients with congenital hypopituitarism; current knowledge and future directions

Kyriaki S. Alatzoglou[1]; James PG. Turton[2]; Marc J. McCabe[1]; Louise C. Gregory[1]; Emma A. Webb[1]; David EG. McNay[1]; Kathryn S. Woods[1]; Ameeta Mehta[1]; Mehul T. Dattani[1]
[1]UCL Institute of Child Health, Developmental Endocrinology Research Group, London, United Kingdom; [2]National Institute for Medical Research, Division of Molecular Neuro-Endocrinology, London, United Kingdom

Background: Congenital hypopituitarism (CH) encompasses a spectrum of disorders: isolated or multiple pituitary hormone deficiencies with/without associated midline or ocular defects and/or structural pituitary abnormalities. Known genetic factors have explained a relatively small percentage with varying results depending on the cohort. We analysed the results of genetic screening in a large cohort of patients with CH performed in our centre.
Patients and methods: Over 15 years we screened 2366 individuals including 2032 patients and 334 unaffected family members. 540 had combined pituitary hormone deficiencies (CPHD), 522 had variable hypopituitarism, 540 septo-optic dysplasia (SOD) and its variants (26%), 368 isolated GH deficiency (IGHD) (18%) and 62 had midline clefts including holoprosencephaly (3%); 17.4% (n=362) had a positive family history. According to phenotype patients were screened for mutations in *HESX1, SOX2, SOX3, OTX2, LHX3/LHX4, SIX3, SHH, GLI2, CHD7, FGF8/FGFR1, KAL-1, PROK2/PROKR2, PROP1, POU1F1, GH1, GHRHR.*
Results: In patients with SOD *HESX1* mutations were identified in 1.3% (7/540); mutations in *SOX2*(n=13) and *OTX2*(n=4) were rare but accounted for almost 28% and 4.5% respectively of cases with severe eye phenotypes (13/45 and 4/45). Changes in *SOX3* dosage were uncommon (n=6). Variations in *FGF8* were identified in <1% whilst *PROKR2* variations were the commonest (2%) but their contribution to phenotype is yet to be established. In patients with CPHD *PROP1* mutations were identified in 3.3% (n=18) and *POU1F1* in 2.2% (n=12) with higher percentages in familial cases. Genetic changes (*GH-1, GHRHR*) were identified in 34 patients (9%) with IGHD.
Conclusions: Known genetic factors account for 5% of CH (n=106); this increases in familial cases and if specific phenotypes are taken into account. Next generation sequencing and careful phenotyping will be critical in the identification of other, yet unidentified, factors implicated in the etiology of CH.

P1-d3-357 Pituitary 1

Long term follow-up in congenital hypopituitarism: Bayesian analysis of clinical, hormonal and neuro-radiological relationships

Gerdi Tuli[1]; Patrizia Matarazzo[1]; Marialia Repici[1]; Daniele Tessaris[1]; Ludovica Fiore[1]; Francesca Verna[1]; Alessandro Mussa[1]; Paola Berchialla[2]; Alberto Borraccino[2]; Roberto Lala[1]
[1]University of Turin, Regina Margherita Children's Hospital, Pediatric Endocrinology and Diabetology, Turin, Italy; [2]University of Turin, Department of Public Health, Turin, Italy

Background: Congenital Hypopituitarism (CH) includes several rare diseases with variable clinical manifestations, anatomical defects, hormonal deficiency and outcome; cause may be genetic, environmental or infectious. Follow-up is needed to detect possible evolution of CH, which can be partly predicted by statistical analysis.
Objective and hypotheses: Analysis of possible relationships among clinical, anatomical, hormonal features and disabilities in a cohort of pediatric and adolescent patients affected by CH.
Methods: Study included 34 subjects affected by congenital hypopituitarism diagnosed between 1996 and 2010. Possible relationships among hypothalamic-hypophyseal defects, clinical manifestations, hormonal deficiencies and disabilities were searched by Bayesian analysis.
Results: Patients were divided in four classes, according to anatomical defects: Class 1, agenesis or hypoplastic or ectopic neurohypophysis; Class 2, hpypoplasia or hyperplasia of adenohypophysis or pituitary stalk hypoplasia; Class 3, classical triad (hypoplastic pituitary + pituitary stalk interruption + ectopic neurohypophysis); Class 4, septo-optic dysplasia. Using Bayesian analysis it is possible to combine anatomic defects (Classes 1-4) to various profiles of hormonal dysfunction and disabilities, being able to create various clinical scenarios and the probability for each of them to be realized. Among all possible combinations of MRI, hormonal deficiencies and disabilities, six risk profiles are reported, selected on the basis of clinical experience. Bayesian network analysis confirms, in this setting, the relevance of anatomical defects as the main variable interconnected both with hormonal picture and disabilities. Furthermore, the assessment of the relative prevalence and implication in terms of endocrine dysfunction of the four classes of hypothalamic-pituitary malformation provides a useful tool in orienting clinical action.
Conclusions: Congenital hypopituitarism is a chronic invalidating disease, with variable outcome.

P1-d3-358 Pituitary 1

Pituitary and ocular features of a novel OTX2 mutation in a family

Rebecca Bradford[1]; Cathy Williams[2]; Denize Atan[3]; Karen Low[4]; Sarah Smithson[4]; David Fitzpatrick[5]; Elizabeth Crowne[1]
[1]Univeristy Hosptials Bristol NHS Foundation Trust, Paediatric Endocrinology, Bristol, United Kingdom; [2]University of Bristol, School of Social and Community Medicine, Bristol, United Kingdom; [3]Univeristy Hosptials Bristol NHS Foundation Trust, Ophthalmology, Bristol, United Kingdom; [4]Univeristy Hosptials Bristol NHS Foundation Trust, Clinical Genetics, Bristol, United Kingdom; [5]Medical Research Council, Human Genetics Unit, Edinburgh, United Kingdom

Background: The orthodenticle homologue 2(OTX2) homeobox gene encodes a transcription factor important in ocular and pituitary development. More than 30 OTX2 heterozygous mutations have been described with varied phenotype expression.
Objective: We describe a novel OTX2 mutation with marked phenotypic variability within a family.
Methods: Review of clinical features of novel OTX2 mutation in family.
Results: Pheynotype: Case1 (father) had 4 children with the same partner: 3 affected (cases 2-4), 1 unaffected. One child from a previous relationship had hypermetropia and squint with no other information available. Table 1 provides a summary of the clinical features seen in this family case series. Table 1: Summary of ocular abnormalities, pituitary dysfunction, additional co-morbidities and MRI findings for each case.

Subject	Ocular abnormalities	Pituitary dysfunction	Additional co-morbidities	MRI findings
Case 1 (male)	Hypermetropia,L-sided amblyopia, normal optic discs & maculae	No	None	N/A
Case 2 (male)	Rotary nystagmus, L convergent squint & amblyopia, reduced vision in R eye, pale optic discs, mild bilateral ptosis	No	Transposition of the great arteries, developmental delay, autism, hearing loss	Thick corpus callosum, cerebellar hyperplasia
Case 3 (female)	See-saw nystagmus, hypermetropia, pale optic discs, corrected vision reduced in both eyes, no squint	Growth hormone deficiency	Developmental delay, high frequency hearing loss, poor coordination	Small pituitary fossa, ectopic posterior pituitary
Case 4 (male)	B/L microphthalmos, atrophic irides, retinal & optic nerve aplasia, squint, horizontal nystagmus, choroidal coloboma, ptosis,	Growth hormone deficiency	Severe developmental delay, R-sided congenital nasal cleft	Abnormal sella, ectopic posterior pituitary

Genotype: Direct DNA sequencing found a previously unreported heterozygous mutation c.249G>A in exon 2 of OTX2. Although this mutation is synonymous for p.Gln83, it is likely to be pathogenic as it alters the last nu-

cleotide of the exon and may disrupt normal splicing. It segregates with the phenotype in the family.
Conclusions: We describe a novel OTX2 mutation characterised by ocular pathology, abnormal neuro-imaging and GHD. This adds to our understanding of OTX2 mutations, and the extreme variability of their clinical manifestations.

P1-d3-359 Pituitary 1

PROKR2 Variants in multiple hypophyseal with pituitary stalk interruption

Rachel Reynaud[1]; Sujatha A. Jayakody[2]; Carine Monnier[3]; Alexandru Saveanu[4]; Jérome Bouligand[5]; Anne-Marie Guedj[6]; Pierre Lecomte[7]; Anne Barlier[6]; Philippe Rondard[3]; Juan Pedro Martinez-Barbera[2]; Anne Guiochon-Mantel[5]; Thierry Brue[1]
[1]Aix-Marseille Univ, CRN2M, Assistance Publique-Hôpitaux de Marseille (APHM), Centre de Référence des Maladies Rares DefHy, Hôpital de la Timone, Marseille, France; [2]UCL Institute of Child Health, Neural Development Unit, University College London, London, United Kingdom; [3]Universités de Montpellier 1 & 2, CNRS UMR-5203, Institut de Génomique Fonctionnelle, INSERM U661, Montpellier, France; [4]Aix-Marseille Univ (CRN2M), Assistance Publique-Hôpitaux de Marseille,, 5Laboratory of Biochemistry and Molecular Biology, Hôpital de la Conception, Marseille, France; [5]Univ Paris-Sud, Hôpital Bicêtre, AP-HP, Génétique moléculaire, Pharmacogénétique et Hormonologie, Le Kremlin Bicêtre, France; [6]Centre Hospitalier Universitaire de Nîmes, Service des maladies métaboliques et endocriniennes, Nîmes, France; [7]Centre Hospitalier Universitaire de Tours, Service d'Endocrinologie, Hôpital Bretonneau, Tours, France; [8]Aix-Marseille Univ, CRN2M, Assistance Publique-Hôpitaux de Marseille (APHM), Centre de Référence des Maladies Rares DefHy, Hôpital de la Conception, Marseille, France

Background: Pituitary stalk interruption represents a frequent feature of congenital hypopituitarism but only rare cases have been assigned to a known genetic cause.
Objective and hypotheses: Using a candidate gene approach we tested several genes as potential causes of hypopituitarism with pituitary stalk interruption. We hypothesized that ectopic posterior pituitary may be a consequence of defective neuronal axon projections along the pituitary stalk or defective angiogenesis of hypophyseal portal circulation. Considering the role of the prokineticin 2 pathway in angiogenesis and neuronal migration, we screened PROK2 and PROKR2 genes.
Methods: PROK2 and PROKR2, and all genes previously known to be involved in hypopituitarism with pituitary stalk interruption (LHX4, HESX1, OTX2, SOX3) were screened in 72 index cases with PSIS from the GENHYPOPIT database. In vitro studies were performed to assess the functional consequences of allelic variants.
Results: We identified two heterozygous PROKR2 mutations (p.Leu173Arg and p.Arg85His) previously reported in isolated hypogonadotroph hypogonadism, and a novel PROKR2 variant (p.Ala51Thr) that, in contrast with both other mutations, did not impair receptor signaling activity. Three allelic variants of HESX1 were identified: the heterozygous p.Phe156Ser and the homozygous p.Arg109X mutations were functionally deleterious whereas p.Ser67Thr was found as a rare allelic variant in association with p.Arg85His PROKR2 mutation in the same patient.
Conclusions: We report PROKR2 variants in congenital hypopituitarism with pituitary stalk interruption suggesting a potential role of the prokineticin pathway in pituitary development.

P1-d3-360 Pituitary 1

A balanced translocation disrupting a copy of the hominoid-specific TBC1D3 gene embedded in a CNV region in syndromic CPHD

Kalotina Machini[1]; Bénédicte Duriez[1]; Yardena Rakover[2]; Philippe Duquesnoy[1]; Florence Dastot-Le-Moal[1]; Nathalie Collot[3]; Serge Amselem[1]
[1]Inserm UMR_S933, UPMC Univ Paris 06 and AP-HP, Medical Genetics, Hôpital Trousseau, Paris, France; [2]Hamek Hospital, Pediatric Endocrinology, Afula, Israel; [3]AP-HP, Medical Genetics, Hôpital Trousseau, Paris, France

Background: The etiology of CPHD has been resolved only in a small proportion of patients. Over the last few years, accumulating sequencing data has illustrated the major role of copy number variation (CNV) regions in genome architecture and evolution. Such regions can be associated with sporadic, Mendelian or complex diseases. However, no example of chromosomal translocation interrupting these sequences has so far been reported.
Objective and hypotheses: The aim of this study was to identify the molecular basis of a syndromic form of CPHD in a young girl with a de novo translocation involving chromosomes 17 and 14. The patient had deficits in GH, TSH and ACTH, and anterior pituitary hypoplasia. She was also diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD).
Methods: Genomic DNA of the patient and her parents was studied by means of array-based SNP/CGH analysis. The patient's DNA was subjected to FISH analyses, followed by targeted sequencing. Transcripts were analyzed in lymphoblastoid cell lines.
Results: The array CGH data were in favor of a balanced translocation. We mapped the breakpoints of the translocation in a region of segmental duplication of chromosome 17, which has been shown to confer risk of developing autism spectrum disorders. The translocation disrupts one copy of the hominoid-specific gene TBC1D3, which is absent in lower species and present in multiple copies in the human genome and has been shown to regulate the highly conserved EGF pathway in humans. This chromosomal rearrangement does not affect the gene copy number of TBC1D3 and no chimeric transcripts were detected in the patient's lymphoblastoid cell line, raising the possibility that the translocation affects gene expression only in the pituitary and possibly in a specific developmental window.
Conclusions: Overall, these data, which unveil the pathogenic role of a translocation disrupting a CNV region, strongly suggest that the hominoid-specific gene TBC1D3 plays a key role in pituitary development.

P1-d3-361 Programming/Epigenetics 1

High incidence of deletion, subtle difference in phenotype — study from 31 patients with Prader-Willi Syndrome

Wei Lu
Children's Hospital of Fudan University, Endocrine and Inherited Metabolic Diseases, Shanghai, China

Background: Prader-Willi syndrome (PWS) is a congenital neurodevelopmental disorder resulted from the absent expression of paternal genes in the region 15q11-13 affecting multiple systems.
Objective and hypotheses: The clinical and genetic features of PWS is incomplete and scarcely of literature in Chinese patients.
Methods: 31 cases with or suspicious of PWS clinically (18 male, 13 females; age range 1 month-14 years) were confirmed by methylation-specific polymerase chain reaction (MS-PCR) analysis and followed by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) analysis to determine if a deletion was present. Microsatellite linkage analysis was performed to distinguish maternal uniparental disomy (UPD) from imprinting defect (ID). Clinical manifestations were discussed and compared between patients with deletion and UPD.
Results: Complete genetic analysis was performed in 31 patients with or suspicious of PWS clinically. Among them, deletion was present in 26 (83.9%), UPD in 5 (16.1%). Neonatal hypotonia, feeding problems in infancy and decreased fetal activity were present in all 31 patients. PWS with deletion was more likely than that with UPD to be characterized by speech articulation defects, small hands and/or feet, hyperphagia and excessive weight gain.
Conclusions: In contrast to most Western populations with a higher incidence of UPD, PWS in China shows a higher incidence of deletion, it is unclear

whether this discrepancy in the incidence of UPD arises from under-diagnosis or because of ethnic differences. Subtle phenotypic differences between the deletion and UPD genotypes may be related with the age of diagnosis and their clinical importance keeps unknown, a question worthy of further study.

P1-d3-362 Programming/Epigenetics 1

Metabolic syndrome and endothelial dysfunction in a population born small for gestational age

Antonio De Arriba Muños[1]; Mercedes Domínguez Cajal[1]; José Ignacio Labarta[2]; Beatriz Puga[3]; Esteban Mayayo[2]; Ángel Ferrández Longás[3]
[1]Hospital Miguel Servet. Hospital Obispo Polanco. Andrea Prader Center, Pediatric Endocrinology, Zaragoza, Spain; [2]Hospital Miguel Servet, Pediatric Endocrinology, Zaragoza, Spain; [3]Andrea Prader Center, Pediatric Endocrinology, Zaragoza, Spain

Background: Being born SGA is associated with metabolic syndrome (MS) and cardiovascular disease (CD).
Objective: To evaluate the presence of markers predictive of MS and CD in a population born SGA and to assess its relationship with spontaneous catch-up growth and rhGH treatment.
Population and method: Cross-sectional study: 181children born SGA (95 SGA with rhGH, 86 SGA with spontaneous catch-up growth) and a control population (n=88) born appropriate for gestational age. They were classified as prepubertal and pubertal according to Tanner stage. Parameters analyzed: perinatal auxology, physical examination, blood pressure (BP), carotid intima-media thickness (IMT-c) and analytic markers of MS. Longitudinal study of carbohydrate metabolism in 89 SGA rhGH treated.

		PREPUBERTAL			PUBERTAL		
	GROUP	N	MEAN	p	N	MEAN	p
AGE (years)	SGA with rhGH	54	7.37±2.01		41	13.02±1.44	
	SGA with catch-up	55	5.75±1.95	***	31	13.03±1.49	n.s.
	CONTROLS	58	5.57±2.01		30	12.87±1.43	
WEIGHT DS	SGA with rhGH	54	-1.46±0.39		41	-0.95±0.83	
	SGA with catch-up	55	-0.9±1.05	n.s.	31	0.6±1.44	***
	CONTROLS	58	-0.13±0.94		30	-0.33±0.82	
HEIGHT DS	SGA with rhGH	54	-2.00±0.83		41	-1.38±0.85	
	SGA with catch-up	55	-0.96±1.06	***	31	-0.15±0.93	***
	CONTROLS	58	-0.34±0.95		30	-0.18±1.04	
BMI DS	SGA with rhGH	54	-0.85±0.74		41	-0.50±0.87	
	SGA with catch-up	55	-0.51±1.03	n.s.	31	0.78±1.41	***
	CONTROLS	58	-0.31±0.93		30	-0.25±0.86	
SYSTOLIC BP	SGA with rhGH	54	93.5±9.04		41	106.4±10.98	
	SGA with catch-up	55	101.0±10.44	***	31	112.5±9.24	***
	CONTROLS	58	100.2±11.35		30	101.4±12.07	
DIASTOLIC BP	SGA with rhGH	54	54.5±7.21		41	61.8±7.06	
	SGA with catch-up	55	59.7±8.69	***	31	62.1±8.31	n.s.
	CONTROLS	58	57.6±6.31		30	58.1±9.54	
IMT-c	SGA with rhGH	54	0.34±0.05		41	0.34±0.06	
	SGA with catch-up	55	0.41±0.09	***	31	0.42±0.09	***
	CONTROLS	58	0.32±0.05		30	0.34±0.05	
GLUCOSE	SGA with rhGH	54	82.44±9.12	n.s.	41	84.65±8.50	n.s.
	SGA with catch-up	55	82.38±9.15		31	85.06±7.94	
INSULIN	SGA with rhGH	54	6.26±4.9	**	41	9.34±5.91	**
	SGA with catch-up	55	8.56±5.02		31	12.85±5.47	
INSULIN/ GLUCOSE	SGA with rhGH	54	0.07±0.06	**	41	0.10±0.06	*
	SGA with catch-up	55	0.11±0.06		31	0.15±0.06	
HOMA INDEX	SGA with rhGH	54	1.3±1.02	*	41	1.99±1.42	*
	SGA with catch-up	55	1.75±1.07		31	2.71±1.20	

Results: In the prepubertal group, patients born SGA with spontaneous catch-up growth showed values of systolic BP, diastolic BP, IMT-c, insulin, insulin/glucose ratio and HOMA index, statistically significantly higher compared to patients born SGA with rhGH and control group. In the pubertal group similar results (except for diastolic BP) were found and weight SDS and BMI SDS were significantly higher in SGA with spontaneous catch-up growth compared to rhGH-treated SGA and the control group (Table I). IMT-c correlated negatively with birth weight and length and positively with age, weight, BMI, systolic BP, diastolic BP, triglycerides, LDL-cholesterol, insulin and HOMA index. Longitudinal carbohydrate metabolism evaluated by HOMA index did not show significant changes.
Conclusions: Children born SGA with spontaneous catch-up growth show lower insulin sensitivity determined by insulin/glucose ratio and HOMA index than children born SGA with rhGH.Prepubertal children born SGA with spontaneous catch-up growth show signs of endothelial dysfunction, indicative of an increased cardiovascular risk that persists during puberty.In children born SGA without spontaneous catch-up growth, rhGH treatment did not induce modifications in any of the studied parameters.

P1-d3-363 Programming/Epigenetics 1

A high birth weight is associated with increased risk for type 2 diabetes and obesity

Inger Wahlström Johnsson; Bengt Haglund; Fredrik Ahlsson; Jan Gustafsson
Women´s and Children´s Health, Uppsala University, Uppsala, Sweden

Background: In parallel to increasing maternal weights the proportion of infants born large for gestational age has increased during recent decades. The association between low birth weight (BW) and adult disease is well known. Much interest is now focused on the effects of a high BW.
Objective and hypothesis: To investigate the hypothesis that a high BW increases the risk of adult metabolic disease.
Population and methods: This is a cohort study of term, single births in Sweden 1973-1982, n=759 999 (subjects born small for gestational age are excluded). The registers used are the Swedish Medical Birth Register, the Inpatient Care Register, the Drug Register and the Causes of Death Register. Hazard ratio (HR) was calculated in relation to BW for components of the metabolic syndrome. Follow-up time is January 1998 to July 2010.
Results: Males with a BW above 2 SDS had a 1.6 fold increased risk (HR 1.57, 95% CI 1.33-1.87) for obesity, whereas the risk for females was 1.3 fold increased (HR 1.34, 95% CI 1.21-1.47). For males and females with BWs above 3 SDS the risks for adult obesity were higher, 2.5 fold (HR 2.48, 95% CI 1.65-3.74) and 1.8 fold (HR 1.81, 95% CI 1.41-2.32) increased, respectively. Males with a BW above 2 SDS had a 2.3 fold increased risk (HR 2.27, 95% CI 1.57-3.27) for type 2 diabetes, whereas those with a BW above 3 SDS had a 5.4 fold increased risk (HR 5.42, 95% CI 2.68-10.94). No increased risk for type 2 diabetes in relation to BW was seen for females.
Conclusions: Being born with a high BW, particularly very high BW, increases the risk for type 2 diabetes in young adult males, but not in females. The risk for obesity increases with increasing BW for both genders.

P1-d3-364 Programming/Epigenetics 1

Childhood adiposity is associated with maternal long chain polyunsaturated fatty acid status in late pregnancy

Rebecca Moon[1]; Nicholas Harvey[1]; Sian Robinson[1]; Georgia Ntani[1]; Justin Davies[2]; Hazel Inskip[1]; Keith Godfrey[1]; Elaine Dennison[1]; Philip Calder[3]; Cyrus Cooper[1]; SWS Study Group[1]
[1]University of Southampton, MRC Lifecourse Epidemiology Unit, Southampton, United Kingdom; [2]University Hospital Southampton NHS Foundation Trust, Paediatric Endocrinology, Southampton, United Kingdom; [3]University of Southampton, Human Development and Health, Southampton, United Kingdom

Background: Studies in adults have shown an association between greater n-3 long chain polyunsaturated fatty acid(PUFA) status and lower risk of obesity. Maternal diet during pregnancy has been linked to offspring body composition. However, the specific nutrients and underlying mechanisms require characterisation and it is unclear whether maternal PUFA status during pregnancy influences offspring body composition.
Objective: To investigate the associations between maternal PUFA status during pregnancy and offspring body composition in childhood.
Methods: We evaluated the relationships between maternal plasma PUFA status (n-3 and n-6) in late pregnancy(34 weeks gestation) and proportionate body composition assessed by whole body DXA(Hologic Discovery) in their children at 4 and 6 years of age in a population-based prospective mother-offspring cohort. Linear regression methods were used to investigate these associations (yielding standardised regression coefficients).

Results: Complete data were available in 287 mother-child pairs. Whole body percentage fat mass(FM) and lean mass(LM) in children were differentially associated with maternal PUFA status: Maternal n-6 concentration was positively related to offspring percentage FM but negatively to percentage LM at 4 years (ß=0.13, p=0.025 & ß=-0.13,p=0.023 respectively), with similar results at 6 years of age. There were less robust relationships between maternal total n-3 PUFA and offspring body composition. However, n-3 PUFA as a percentage of the total fatty acid pool was negatively related to percentage FM and positively to percentage LM at 4 years (ß=-0.17,p=0.003 & ß=0.17,p=0.003); associations at 6 years were in the same direction, but were not statistically significant.
Conclusions: Our results are consistent with adult studies demonstrating an association between PUFA status and obesity and suggest that maternal PUFA levels during pregnancy might influence offspring body composition. These findings may help inform nutritional strategies in pregnancy aimed at optimising offspring health.

P1-d3-365 Programming/Epigenetics 1

Early origins of the metabolic syndrome: role of small size at birth, early postnatal weight gain and adult IGF-I

Gerthe Kerkhof; Ralph Leunissen; Anita Hokken-Koelega
ErasmusMC Sophia Children's Hospital, Pediatrics, Subdivision of Endocrinology, Rotterdam, Netherlands

Background: The relationship between low birth weight and increased risk for Metabolic Syndrome (MetS) in later life has been frequently described. Factors involved in that association might be accelerated early weight gain and decreased IGF-I levels in adulthood, as these factors have been associated with both small size at birth and components of the MetS.
Objective: To unravel mechanisms involved in the association between small size at birth and components of MetS in early adulthood.
Methods: In 280 young adults of the PROGRAM study, aged 18-24 yr, we investigated associations of birth weight, gain in weight for length during early life, and adult IGF-I SDS, with number of MetS components (ordinal regression analyses), prevalence of MetS components and MetS (logistic regression analyses), and other metabolic parameters (linear regression analyses). Revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of MetS. The other metabolic parameters were C-reactive protein (CRP), insulin sensitivity, trunk fat mass, total cholesterol, and LDL cholesterol.
Results: More gain in weight than length SDS in the first three months of life was significantly associated with an increased number of MetS components (Odds ratio: 1.34), prevalence of MetS (Adjusted odds ratio: 2.51, Table 1) prevalence of low HDLc (Odds ratio: 1.49), higher CRP levels (p-value=0.009) and lower insulin sensitivity (p-value=0.007) at the age of 21 years. Low birth weight SDS was associated with lower insulin sensitivity (p-value=0.036), but low birth weight SDS and adult IGF-I SDS were not significantly associated with any of the MetS components, or MetS prevalence at 21 years.

Metabolic Syndrome		
	Odds Ratio	95% Confidence Interval
Birth weight SDS*	0.492	0.224-1.080
Delta weight SDS 0-3 mo#	2.509	1.200-5.247
Adult IGF-I SDS~	0.857	0.409-1.795

Adjusted for sex, age, SES, gestational age, *birth length SDS, #Delta length SDS 0-3 mo, ~IGF-BP3 SDS

Conclusions: Our study demonstrates that higher gain in weight for length in the first three months of life is associated with a higher prevalence of MetS at 21 years, whereas low birth weight and low adult IGF-I are not.

P1-d3-366 Programming/Epigenetics 1

Elevated insulin concentrations at birth and at prepubescent age are associated with an altered BMI course during childhood – results of the ulm birth cohort study (UBCS)

Stephanie Brandt[1]; Anja Moß[1]; Petra Gottmann[1]; Wolfgang Koenig[2]; Melanie Weck[3]; Herrmann Brenner[3]; Martin Wabitsch[1]
[1]University of Ulm, Division of Pediatric Endocrinology, Diabetes and Obesity Unit, Ulm, Germany; [2]University of Ulm Medical School, Department of Cardiology, Ulm, Germany; [3]Cancer Research Center, Division of Epidemiology and Aging Research, Heidelberg, Germany

Background: The natural course of BMI-values of a child shows an increase after birth and reaches a maximum at the age of one. Thereafter BMI-values decrease, reach a minimum at pre-school age and increase until young adulthood . The reversal point is called adiposity rebound.
Objective and hypotheses: The identification of factors which are associated with an altered BMI course is in the focus of current interest.
Methods: The Ulm Birth Cohort study is a prospective study with a longitudinal design.At baseline examination n=1066 mothers and their newborns were recruited for the study. The weight and height development of the children was documented from birth until the latest follow-up at age of 8 yrs. At baseline an umbilical cord blood sample was taken. At the 8-year follow-up a fasting blood sample of the children was taken.The concentrations of insulin in the umbilical cord blood sample and in the fasting blood sample of the children at the age of 8 yrs were measured (ELISA).Insulin concentrations were categorised in quartiles.Three groups of insulin concentrations were defined: elevated insulin concentration at birth and at the age of 8 (both within upper quartile); decreased insulin concentrations at birth and at the age of 8 (both within lower quartile), normal insulin concentrations (all the others).
Results: The BMI courses of the children of the three groups differ significantly (figure 1).Children with elevated insulin concentrations show a BMI course above the BMI course of the other two groups. The most striking difference for the mean BMI values between the three groups of children has been identified at six and eight years of age.There is a strong increase in average BMI values of the children with elevated insulin concentrations between four and six years.
Conclusions: The present data show that higher insulin concentrations in children from birth to 8 yrs of age are associated with an altered BMI course during childhood.Higher insulin levels might be programmed during the fetal period and may stimulate post natal weight gain.

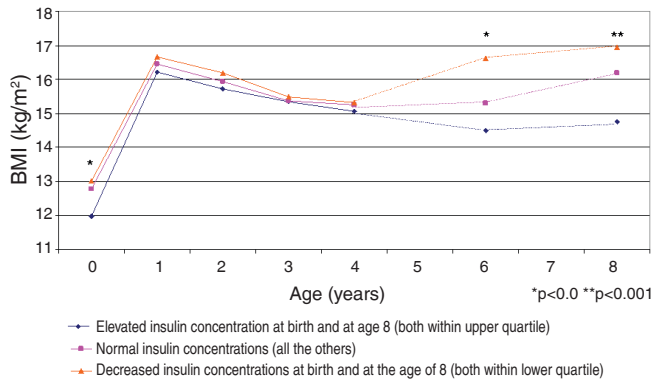


Figure 1: BMI trajectory of the children of the Ulm Birth Cohort Study from birth as a function of insulin concentration at birth and at the age of eight years

P1-d3-367 Puberty and Neuroendocrinology 1

24 months treatment experience of two leuprolide acetate 3 month depot formulations for children with central precocious puberty

Peter Lee[1]; Karen Klein[2]; Nelly Mauras[3]; Lois Larsen[4]; Wangang Xie[4]; Tali Lev-Vaisler[4]; Peter Bacher[4]
[1]Penn State College of Medicine, The Milton S Hershey Medical Center, Hershey, PA, United States; [2]University of California, Division of Endocrinology and Diabetes, San Diego, CA, United States; [3]Nemours Children's Clinic, Division of Endocrinology, Diabetes, and Metabolism, Jacksonville, FL, United States; [4]Abbott Laboratories, Global Pharmaceutical R&D, Abbott Park, IL, United States

Background: We have recently demonstrated short term efficacy and safety of leuprolide acetate (LA) 3 month (M) depot 11.25 or 30 mg in 84 children with central precocious puberty (CPP) treated for 6M (Lee PA, et al. JCEM, May 2012, 97(5).
Objective and hypotheses: To assess long term hypothalamic-pituitary-gonadal axis suppression and the safety of LA 3M depot 11.25 or 30 mg in children with CPP treated for 24M.
Methods: 72 children (baseline mean age 8.5±1.6 yrs, 65 females) with CPP treated with 11.25 (N=34) or 30 mg (N=38) 3M depot LA with continued LH suppression at 6M were followed for up to 24M of additional treatment. Peak stimulated LH <4 mIU/mL was considered suppressed. Adverse events (AEs) data were collected.
Results: Peak stimulated LH was suppressed in 90.3% and 96.9% of subjects at 12M and in 87.5% and 100% of subjects at 24M in the 11.25 and 30 mg groups, respectively. In the 11.25 mg group, 5 subjects escaped during the 24M treatment; 3 of 5 subjects were suppressed at their subsequent visit; 2 of 5 were considered failures at the subsequent visits but had no evidence of progression in Tanner Staging. In the 30 mg group, 2 subjects escaped LH suppression, but were suppressed at the subsequent visit.
Continued positive effects in suppressing pubertal progression and sex steroid levels and slowing bone age maturation were observed. The majority of subjects who discontinued treatment (n=43) were considered ready to progress through physiologic puberty. AEs were comparable between groups with injection site pain being the most common AE (26.5% in 11.25 mg and 23.7% in 30 mg).
One serious AE (shunt malfunction) occurred, and was not considered treatment related. None of the AEs led to discontinuation of study drug. The safety profile was similar to that previously reported at 6M.
Conclusions: The 2 doses of LA 3M depot are safe and provide persistent maintenance of LH suppression in children with CPP over 24 months of additional treatment.

P1-d3-368 Puberty and Neuroendocrinology 1

Utility of basal luteinizing hormone levels for detecting central precocious puberty in girls

Jung Hee Ko; You Jin Kim; Hong Kyu Park; Hae Sang Lee; Jin Soon Hwang
Ajou University School of Medicine, Ajou University Hospital, Pediatrics, Suwon, Republic of Korea

Objective: The hallmark of puberty is the progressive increase in gonadotropin-releasing hormone (GnRH) activity, reflected by an increase in the circulating concentration of luteinizing hormone (LH). The GnRH stimulation test has become widely used in the evaluation of precocious puberty. The aim of our study was to investigate whether the GnRH stimulation test could be simplified with fewer hormonal measurements.
Methods: A total of 1015 girls were referred to Ajou University Hospital for evaluation of precocious puberty between 2008 and 2011. All subjects underwent GnRH-stimulation tests as part of their evaluation. Serum LH and follicle stimulating hormone (FSH) were measured by immunoradiometric assay before and after the GnRH injection.
Results: Of the 1015 subjects, 663 (65.3) were included in the pubertal response group and 352 (34.7%) were in the prepubertal response group. Basal LH level was identified as a significant predictor for central precocious puberty (CPP). Based on the ROC curve, the optimal cut—off point of basal LH related to "pubertal respons" was 1.1 IU/L, which was associated with 72.1% sensitivity and 51.6% specificity, with an area under the ROC curve of 0.649 (95% confidence interval 0.614~0.683)
Conclusion: A single basal LH measurement provides adequate information to discriminate girls with CPP from prepubertal girls and is useful for initial laboratory screening test in most of the girls who were evaluated for early pubertal signs.

P1-d3-369 Puberty and Neuroendocrinology 1

Estrogen receptor alpha polymorphisms and idiopathic central precocious puberty in girls

Jung Hee Ko; Hong Kyu Park; You Jin Kim; Hae Sang Lee; Jin Soon Hwang
Ajou University School of Medicine, Ajou University Hospital, Pediatrics, Suwon, Republic of Korea

Objective: Estrogen plays a crucial role in the development and function of the reproductive physiology. Estrogens regulate cellular activity through binding to estrogen receptor α (ERα) and β (ERβ). ERα polymorphisms have been associated with age at menarche, menopause onset, and fertility. The aim of this study is to investigate the relationship of ERα gene polymorphisms with central precocious puberty (CPP) in girls.
Methods: Two hundred and four (201) Korean girls with idiopathic CPP were included in this study along with 100 healthy Korean female adults with pubertal maturation within normal age, who served controls. Auxological and endocrine parameters were measured, and both patients and controls were genotyped for PvuII (397T→C) and XbaI (351A→G) polymorphisms of ERα gene.
Results: There was significantly lower incidence of the CC genotype of PvuII polymorphism among CPP girls than controls (11.9% vs. 22%, P=0.021). Pubertal onset was also later for carriers of CC genotype of PvuII polymorphism compared with carriers of TT genotype (7.28 ± 1.35 years vs. 7.57 ± 1.40 years, P=0.030). In addition, there was no significant difference in XbaI polymorphism between patients and controls.
Conclusion: The present study indicated that the PvuII polymorphism of ERα gene might function as a modulating factor in the onset and progression of puberty. However, no solid conclusion can be made and further studies are necessary to validate the function of these polymorphisms.

P1-d3-370 Puberty and Neuroendocrinology 1

Timing of puberty: reversal of congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations

Eeva-Maria Laitinen[1]; Johanna Tommiska[2]; Kirsi Vaaralahti[2]; Timo Sane[3]; Jorma Toppari[4]; Taneli Raivio[1]
[1]Helsinki University Central Hospital, Children's Hospital, Helsinki, Finland; [2]University of Helsinki, Biomedicine/Physiology, Helsinki, Finland; [3]Helsinki University Central Hospital, Medicine/Endocrinology, Helsinki, Finland; [4]University of Turku, Physiology and Pediatrics, Turku, Finland

Background: Congenital hypogonadotropic hypogonadism (HH) is a rare cause for delayed or absent puberty. These patients may recover from HH spontaneously in adulthood. To date, it is not possible to predict who will undergo HH reversal later in life.
Objective: To investigate whether Finnish patients with reversal of congenital HH have common phenotypic or genotypic features.
Methods: Thirty-two male HH patients with anosmia/hyposmia (Kallmann Syndrome, KS; n=26) or normal sense of smell (nHH; n=6) were enrolled (age range, 18-61 yrs). The patients were clinically examined, and reversal of HH was assessed after treatment withdrawal. *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *CHD7*, *WDR11*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, *TAC3*, *TACR3*, and *LHβ* were screened for mutations.
Results: Six HH patients (2 KS, 4 nHH) were verified to have reversal of HH. In the majority of cases, reversal occurred early in adulthood (median age, 23 yrs; range, 21-39 yrs). All had spontaneous testicular growth while on testosterone replacement therapy (TRT). One nHH subject was restarted on TRT due to a decline in serum T. Two reversal variants had a same *GNRHR* mutation (R262Q), which was accompanied by another *GNRHR* mutation (R139H or del309F). In addition, both of the KS patients had a mutation in *CHD7* (p.Q51X) or *FGFR1* (c.91+2T>A).
Conclusions: Considerable proportion of patients with HH (8% of KS probands) may recover in early adulthood. Spontaneous testicular enlargement during TRT was highly suggestive for reversal of HH. Those with the *GNRHR*

mutation R262Q accompanied by another *GNRHR* mutation may be prone to reversal, although even patients with a truncating mutation in *CHD7* or a splice-site mutation in *FGFR1* can recover. We recommend that all adolescents and young adults with congenital HH should be informed on the possibility of reversal.

P1-d3-371 Puberty and Neuroendocrinology 1

Vitamin D and calcium levels are associated with glucose metabolism and pubertal timing in Danish children

Kaspar Sørensen[1]; Martin Blomberg Jensen[1]; Lise Aksglaede[1]; Annette Mouritsen[1]; Poul Jannik Bjerrum[2]; Anders Juul[1]
[1]Copenhagen University Hospital, Department of Growth and Reproduction, Copenhagen, Denmark; [2]Holbæk Hospital, Department of clinical biochemistry, Holbæk, Denmark

Background: Vitamin D (VD) is a key regulator of calcium homeostasis. Low VD levels have been associated with adverse effects on glucose metabolism, as well as with early pubertal timing. Whether or not low VD influences glucose metabolism and pubertal timing directly or through effects on calcium homeostasis is unresolved.
Objective and hypothesis: The objective was to evaluate associations of VD, parathyroid hormone (PTH) and albumin corrected calcium (Ca) levels with glucose metabolism and pubertal timing in healthy children and girls with central precocious puberty (CPP).
Methods: One-hundred and eighty healthy children (girls = 115) from the COPENHAGEN Puberty study, and 11 girls with CPP were evaluated cross-sectionally by whole-body DEXA-scan, oral glucose tolerance test (insulin sensitivity (ISI), peak insulin (PI) levels) and pubertal staging. Fasting blood samples were analyzed for VD, PTH, calcium as well as IGF-I and reproductive hormone levels.
Results: Ca, but not VD, levels were positively associated with PI ($p = 0.001$), and negatively associated with ISI ($p = 0.013$) in healthy children. In healthy girls, logistic regression analyses revealed that earlier age at onset of breast development was associated with higher Ca ($p = 0.037$) and lower VD levels ($p = 0.039$), respectively. In healthy girls ≥ 12 years, significant higher levels of LH, FSH, IGF-I and PI were found in the highest tertile compared with the lowest tertile for Ca (all $p \leq 0.04$). No significant differences were found in any of the main outcome variables between VD tertiles. Girls with CPP had significantly lower ISI ($p = 0.04$) and higher Ca levels ($p = 0.005$) than puberty-matched healthy girls.
Conclusion: Calcium levels may influence glucose metabolism as well as pubertal timing in healthy children and girls with CPP. The possible role of calciotropic hormones on activation of the pituitary-gonadal axis needs further elucidation.

P1-d3-372 Puberty and Neuroendocrinology 1

Plasma insulin-like factor 3 from birth to adulthood

Jane McNeilly[1]; Neil Watson[2]; David Shapiro[2]; Maurizio Panarelli[2]; Natalie Smee[3]; Avril Mason[3]; S Faisal Ahmed[3]
[1]Royal Hospital for Sick Children, Department of Biochemistry, Glasgow, United Kingdom; [2]Glasgow Royal Infirmary, Department of Biochemistry, Glasgow, United Kingdom; [3]Royal Hospital for Sick Children, Developmental Endocrine Research Group, Glasgow, United Kingdom

Background: Insulin-like factor 3 (INSL3) is one of the major secretory hormones of Leydig cells in the testis. Circulating INSL3 may reflect Leydig cell maturation and activity displaying a transient peri-natal increase, low levels during childhood, increasing levels during puberty with highest levels found in adulthood. Current biochemical investigations for boys with disorders of sex and pubertal development often include invasive hCG and LHRH stimulation tests. Measurement of INSL3 would enable assessment of leydig cell function/differentiation and avoid the need for dynamic stimulation tests.
Aims: 1) To compare plasma and serum INSL3 concentrations and 2) to establish a normal male reference range from birth to adulthood.
Patients: Plasma and serum samples were obtained from 7 healthy adult males (31.3-56.4yrs) working at RHSC, Glasgow. Plasma samples were obtained from 70 boys (1 day-18.7yrs) attending RHSC and 21 adult males (32.6-70.0yrs) attending GRI, Glasgow. Exclusion criteria included known DSD, testosterone deficiency, chronic inflammatory disease and chemotherapy patients.
Method: INSL3 concentrations were measured using a commercially available enzyme immunoassay (Phoenix Pharmaceutical, Belmont, CA). The lower limit of detection was 0.03ng/ml and intra-assay CV was 8.7%.
Results:

Age Group	Number	Age (years) (Median)	Range (10th, 90th centile)	INSL3 (ng/mL) (Median)	Range (10th, 90th centile)
0-3 months	8	0.13	0.00-0.28	0.23	0.20-0.32
Infants (>3 months - <1yr)	9	0.61	0.35-0.72	0.20	0.11-0.24
1-11yrs	43	9.78	2.41-11.68	0.21	0.14-0.31
>12yrs	10	12.91	12.25-17.97	0.52	0.30-0.91
Adults	21	53.30	32.65-69.96	1.59	0.68-2.54

INSL3 concentrations were ~20% lower in plasma than serum but showed good positive correlation ($r=0.97$). INSL3 was significantly higher in pubertal boys compared to children (1-11yrs) and infants >3 months ($p<0.05$). Despite an overlap, young infants (0-3 months) had higher INSL3 levels than older infants and children. From the age of 3 months to 11yrs INSL3 remained consistently low (median 0.21ng/ml). The data showed a tri-phasic pattern during childhood which supports previously published studies. Adult males had 3-fold higher INSL3 concentrations than children ($p<0.05$).
Conclusion: Plasma INSL3 are clearly higher in boys of pubertal age but their clinical utility in identifying functioning Leydig cells in young infants needs further studies.

P1-d3-373 Puberty and Neuroendocrinology 1

Cryptic chromosomal deletions detected by array-CGH in four cases affected by central precocious puberty and neurodevelopmental disorders

Mariangela Cisternino; Alexandra Madè; Francesca Marabotto; Giulia Rossetti; Laura Losa; Arianna Zaroli; Chiara Visconti
Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Department of Pediatrics, Pavia, Italy

Background: Central precocious puberty (CPP) may be associated with CNS abnormalities including neurodevelopmental disorders (ND), epilepsy (E), CNS structural malformations and/or with dysmorphic features. In the literature, some chromosomal aberrations have been reported in patients with this association.
Objective and hypotheses: The aim of this study was to detect cryptic chromosomal anomalies in patients affected by CPP and CNS disorders using the array CGH technique. Methods: We carried on the array-CGH analysis in 4 girls affected by CPP associated with one or more of following CNS anomalies: ND, E and structural abnormalities detected with MRI. The age at the onset of CPP ranged from 4 to 7.3 years.
Results: Case 1. A de novo distal deletion of the chromosome 9 short arm [del(9)(p24.3-p23)] was found, as in the cases affected by 9p- syndrome. To our knowledge, this is the second reported case of precocious puberty associated to 9p distal deletion. Cases 2 and 3. A deletion of the chromosome 8 short arm was found: in the first case the deletion was localized in the region 8p23.2 and in the latter in the region 8p23.3.1. In both these cases the deletion determines the lost of the gene CSMD1, which is known to be particularly expressed in the ovary during the embryogenesis. Moreover, a duplication of CSMD1 has been found in a girl affected by delayed puberty [1]. Case 4. A deletion of chromosome 8p22 was detected, which is cited in the Database of Genomic Variants as a normal variant. However, this deletion may be related with the age at menarche in normal population.
Conclusions: Our observations confirm the usefulness of the array-CGH analysis for detecting cryptic chromosomal aberrations in patients with CPP associated with CNS abnormalities and or dysmorphic features. Further studies are needed to identify genes responsible for this association.

P1-d3-374 Puberty and Neuroendocrinology 1

Longer androgen receptor CAG repeat alleles are associated with body fat and serum SHBG in boys

Annette Mouritsen; Casper P. Hagen; Kaspar Soerensen; Lise Aksglaede; Kristian Almstrup; Ewa Rajpert-De Meyts; Anders Juul
Universityhospital of Copenhagen, Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark

Background: Longer androgen receptor gene CAG trinucleotide repeat alleles, AR-(CAG)n, are associated with lower sensitivity of the androgen receptor (AR). Shorter AR-(CAG)n have been associated with premature adrenarche in children and with subfertility in adult men. However, it is not known if the length of CAG trinucleotide repeats AR-(CAG)n is associated with age at pubertal onset and body fat accumulation in puberty in normal children.
Objective and hypotheses: The objective was to evaluate associations of the AR-(CAG)n polymorphism with the development of pubic hair, circulating levels of androgens, and body fat in healthy boys.
Methods: A longitudinal study of 78 healthy boys from the COPENHAGEN Puberty study was conducted with clinical examinations and blood samples every half year from 2006 to 2011. We genotyped AR-(CAG)n and measured reproductive hormones. The boys were divided in quartiles (Q1-Q4) according to the number of CAG repeats; Q1: <20 CAG repeats, Q2+Q3: 21-23 CAG repeats and Q4: > 24 CAG repeats.
Results: Median AR-(CAG)n was 22 (range 17-30). The number of repeats was positively correlated with sum of four skin folds at a comparable age (10 years; p=0.026) and at a similar pubertal stage (3 months prior to pubic hair development, p=0.037). The number of CAG repeats was negatively correlated with age at pubarche (p=0.054). The mean age of pubarche was lower in boys with longer CAG repeats (Q4) compared to boys with short CAG repeats (Q1) (p=0.051). The number of CAG repeats was inversely correlated with serum SHBG levels at comparable age (p=0.015) but no significant correlations with circulating levels of androgens (DHEAS, Adione or Testosterone) or free androgen index (FAI) were observed.
Conclusions: The AR-(CAG)n polymorphism was positively correlated with body fat and negatively with SHBG levels and age at pubarche in healthy boys.

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High urinary phthalate concentration associated with delayed pubarche in girls

Hanne Frederiksen[1]; Kaspar Soerensen[1]; Annette Mouritsen[1]; Lise Aksglaede[1]; Casper P. Hagen[1]; Jørgen H. Petersen[2]; Niels E. Skakkebaek[1]; Anna-Maria Andersson[1]; Anders Juul[1]
[1]Universityhospital of Copenhagen, Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark; [2]Universityhospital of Copenhagen, Rigshospitalet, Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark

Background: Phthalates are a group of chemicals present in numerous consumer products. They have anti-androgenic properties in experimental studies and are suspected to be involved in human male reproductive health problems. A few studies have shown associations between phthalate exposure and changes in pubertal timing among girls, although controversies exist.
Objective and hypotheses: The objective was to investigate the association between phthalate concentrations and pubertal timing in girls.
Methods: We determined the concentration of 12 phthalate metabolites in first morning urine samples from 725 healthy Danish girls (aged 5.6-19.1 years) in relation to age, pubertal development (breast and pubic hair stage) and reproductive hormone levels (luteinizing hormone, estradiol and testestorone). Furthermore, urinary phthalates were determined in 25 girls with precocious puberty (PP).
Results: In general the youngest girls with less advanced pubertal development had the highest first morning urinary concentration of the monobutyl phthalate isoforms (∑MBP(i+n)), monobenzyl phthalate (MBzP), metabolites of di-(2-ethylhexyl) phthalate (TAGfontoffTAGsymfontåTAGfontoffTAGstdfontDEHPm) and of di-iso-nonyl phthalate (TAGfontoffTAGsymfontåTAGfontoffTAGstdfontDINPm). After stratification of the urinary phthalate excretion into quartiles, we found that the age at pubarche was increasing with increasing phthalate metabolite quartiles (except for MEP). This trend was statistically significant when all phthalate metabolites (except MEP) were summarized and expressed as quartiles. No association between phthalates and breast development was observed. In addition, there were no differences in urinary phthalate metabolite levels between girls with PP and controls.
Conclusions: We demonstrated that pubarche, but not thelarche, was associated with phthalate excretion in urine samples from 725 healthy school girls. Our findings that high phthalate excretion was assocated with delayed pubarche suggest anti-androgenic actions of phthalates in prepubertal girls.

P1-d3-376 Puberty and Neuroendocrinology 1

Evaluation of urinary bisphenol A and phthalate levels in girls with premature thelarche

Erdem Durmaz[1]; Iffet Bircan[1]; Ali Asci[2]; Hanne Frederiksen[3]; Pinar Erkekoglu[2]; Anders Juul[3]; Sema Akcurin[1]; Belma Kocer-Giray[2]
[1]Akdeniz University, Department of Pediatric Endocrinology, Antalya, Turkey; [2]Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey; [3]University Hospital of Copenhagen, Department of Growth and Reproduction, Copenhagen, Denmark

Background: Endocrine disrupting chemicals (EDCs) are defined as exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, developmental and/or behavior. There is a growing concern over the increasing incidence of pubertal timing and reproductive abnormalities and their possible association with exposure to estrogenic EDCs such as phthalates and bisphenol A (BPA) which are abundantly used plasticizers.
Objective and hypotheses: The aim of this study was to determine the urinary levels of BPA and the main metabolites of di-(2-(ethylhexyl) phthalate (DEHP) namely mono-(2-ethyl- hexyl) (MEHP); mono-(2-ethyl-5-oxohexyl) (MEOHP); mono-(2-ethyl-5-carboxypentyl) (MECPP); and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), in girls with premature thelarche.
Methods: Study group consisted of, 28 newly diagnosed 4-8 aged non-obese premature thelarche cases who were admitted to Akdeniz University, Pediatric Endocrinology Department. The control group composed of 24 age-matched girls without premature thelarche and other endocrine disorders. Urinary BPA and DEHP metabolites concentrations were measured by HPLC and LC-MS/MS, respectively.
Results: The median urinary concentrations of BPA (3.2 µg/g creatinine) and MEHP (18,99 µg/g creatinine) in the study group were found to be higher significantly compared to healthy control group (1.62 µg/g creatinine, p<0.05 and 10.15 µg/g creatinine, p=0.002, respectively). No significant alterations were observed in the levels of MEHHP, MEOHP, MECPP in the girls with premature thelarche.
Conclusions: Our results suggest that exposure of BPA and phthalates may cause abnormal breast development in pre-pubertal girls. Besides, possible combined effects of EDCs might be a triggering factor possibly due to their estrogenic effects and these EDCs may be considered as the etiological factors in the development of premature thelarche.

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Kiss-1 and ERα mRNA expression and puberty onset in the female SD rats with disrupted positive feedback in HPG axis

Baosheng Yu[1]; Xiaonan Li[2]; Ronghua Chen[2]; Ye Shan[1]; Xiaoxiao Zhang[1]; Anru Wang[1]; Lanying Gao[1]
[1]The 2nd Affiliated Hospital of Nanjing Medical University, Department of Pediatric Endocrinology, Nanjing, China; [2]Nanjing Medical University, Institute of Pediatric Medicine, Nanjing, China

Background: The causes of normal and precocious puberty are still unclear. We hypothesized that the positive feedback mediated by ERα-kiss-1 pathway in HPG aixs is required for normal puberty and precocious puberty induced by estrogen agonists. To test this hypothesis, we determine 1. the effect of neonatal exposure to the ERα specific agonist PPT and the ERβ specific agonist DPN on puberty onset in female rats. 2. the effect of disrupted positive feedback in HPG axis on vaginal opening(OV) and mRNA expression of kiss-1 and ERα in hypothalamus in the female rats.
Methods: Group1 were randomly grouped into 3 subgroups and treated with PPT, DPN and placebo, respectively, between p.n. days 16 and 20. Group2, treated with testosterone enanthate sc injected on day 2 and then treated with methods same to group1. OV were observed from p.n. day 21 till the day of

OV in group1 or p.n. day 49 in group2. The mRNA expression of kiss-1 and ERα in hypothalamus were also measured in group2 using real time PCR.
Results: 1. OV in female rats treated with PPT and DPN was earlier than that of the control group (Tab1. The p.n. day of OV in female SD rats with normal positive feedback in HPG axes * p<0.01,vs control).

group	p.n. day of OV
PPT	22.8 ±1.1 *(n= 10)
DPN	27.5 ±2.3 *(n=11)
Control	32.1 ± 0.9 (n=10)

In group2, in which the positive feedback in HPG axes were disrupted, no OV were observed till p.n. day 49, and neither PPT nor DPN could induce earlier OV in them. 2. mRNA expression of kiss-1 and ERα in hypothalamus of female rats with normal or disrupted positive feedback in HPG axes (Tab2. The mRNA expression of kiss-1 and ERα in hypothalamus of female SD rats with Normal or Disrupted positive feedback in HPG axes * p<0.01,vs control).

	Normal positive feedback	Normal positive feedback	Disrupted positive feedback	Disrupted positive feedback
group	Kiss-1 mRNA	ERα mRNA	Kiss-1 mRNA	ERα mRNA
PPT	2.08 ± 0.37* (n=5)	3.85±1.67*(n=5)	1.00 ± 0.48* (n=5)	0.83 ± 0.21* (n=5)
DPN	1.43 ± 0.33 (n=5)	2.98±1.43*(n=5)	0.91 ± 0.35* (n=5)	0.70 ± 0.33* (n=5)
Control	0.60 ± 0.32 (n=5)	0.63±0.47 (n=5)	4.12 ± 1.39 (n=8)	4.77 ± 1.08 (n=7)

Conclusion: Testosterone administration to female rats during the critical period may postpone sexual maturation as revealed by delayed OV. PPT, DPN may inhibit kiss-1 and ERα mRNA expression in hypothalamus in the female rats with disrupted positive feedback in HPG aixs. PPT or DPN can induce earlier puberty onset in female rats through positive feedback mediated by ERα-kiss-1 pathway in HPG aixs.

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Analysis of major causative hypogonadotropic hypogonadism genes in patients with constitutional delay of growth and puberty

Leticia FG Silveira[1]; Cintia Tusset[1]; Lucas Moura[1]; Daiane Beneduzzi[1]; Milena Teles[1]; Alexander AL Jorge[2]; Ana Claudia Latronico[1]; Ericka B Trarbach[2]
[1]Hospital das Clinicas da FMUSP, Laboratório de Hormônios e Genética Molecular/LIM42, Sao Paulo, Brazil; [2]Hospital das Clinicas da FMUSP, Endocrinologia Molecular e Celular/LIM25 e Laboratório de Hormônios e Genética Molecular/LIM42, Sao Paulo, Brazil

Background: Mutations in genes underlying isolated hypogonadotropic hypogonadism (IHH) have been reported in families with a wide phenotypic spectrum, varying from pubertal delay to severe IHH.
Objective and hypotheses: We hypothesized that constitutional delay of growth and puberty (CDGP) might be a mild phenotype of IHH and investigated the occurrence of defects in major causative IHH genes in patients with CDGP.
Methods: Fifty six Brazilian patients (45 males) with CDGP were selected. Genomic DNA was extracted and the whole coding sequences of *GnRHR, TACR3, TAC3, KiSS1R, FGFR1* and *FGF8* was analyzed. The control group consisted of 150 adults with normal pubertal development.
Results: We identified a novel variant c.1345G>T (p.A449S) in *TACR3* in heterozygosis in a female patient with CDGP. Although the p.A449S variant was not observed in the control individuals, this amino-acid exchange involves a non-conserved residue and was predicted to be benign by *in silico* analysis using SIFT and PolyPhen. The recurrent c.137A>G (p.Q106R) *GnRHR* mutation was identified in heterozygosis in a male CDGP patient. This mutation has been previously associated with a partial GnRH receptor loss-of-function in patients with autosomal recessive IHH, but its role in CDGP is probably worthless. We also observed two new synonymous variations, one in *FGFR1* c.1332A>G (p.P444P) and one in *KiSS1R* c.183G>A (p.S61S), both with no apparent effects on gene function. Known polymorphisms in *TACR3* (rs2276973, rs17033889, rs35085919) and *KiSS1R* (rs73507527, rs350132, rs3746147 and rs10407968) occurred in a similar frequency in CDGP and control groups. No genetic abnormalities were found in TAC3 and FGF8 in these CDGP patients.
Conclusions: These results show that GnRHR, TACR3, TAC3, KiSS1R, FGFR1 and FGF8 variations are not a common event in CDGP, suggesting that these genes do not contribute for the pathogenesis of this condition.

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Creating a European consortium to study GnRH deficiency (C.O.S.T. Action BM1105)

Nelly Pitteloud; Andrew Dwyer
Centre Hospitalier Universitaire Vaudois, Endocrinology, Diabetes, & Metabolism, Lausanne, Switzerland

Background: GnRH deficiency is characterized by absent puberty, infertiliy, and psychosocial morbidity. It is a treatable cause of infertility yet patients are often challenged to find appropriate medical expertise given the rarity of their condition (1/10'000). This disorder has a strong genetic component with 16 disease genes disovered via several approaches including cytogenetics (chromosomal aberrations), homozygosity mapping in consanguinous families, and candidate genes.
To date, the pace of discovery has been piecemeal and the majority (2/3) GnRH deficient patients have no known genetic cause.
Objective: To develop a European registry for patients with GnRH deficiency and create a network bringing together clinicians, translational investigators, basic scientists, bioinformaticians, geneticists, and genetic counselors to foster discovery in the field of human reproduction.
Methods: The Clinical Group will develop a web-based database for patient phenotypes and clinical guidelines; the Genetics & Bioinformatics Group will assist in using cutting edge genetic technologies and provide expertise in interpreting data; the Basic Research Group will help prioritize candidate genes identified via whole exome sequencing and explore the biology of novel genes using animal and cellular models; the Education and Training Group will coordinate training program for young investigators.
Results: Twenty European countries have joined the C.O.S.T. Action (funded 2012-2015) and it is still open for interested parties to join at http://www.cost.esf.org/domains_actions/bmbs/Actions/BM1105
Conclusions: We believe this European Consortium will help accelerate scientific discovery in the field of GnRH deficiency yielding important scientific insights including novel biomarkers and targeted therapies for infertility that will benefit patients and families.

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FGFR1 mutations in split hand/feet malformation with or without gonadotropic deficiency

Carine Villanueva[1]; Dov Tiosano[2]; Marion Gérard[3]; Juliane Leger[4]; Clarisse Baumann[3]; Valérie Drouin-Garraud[5]; Hervé Lefebvre[6]; Michel Polak[7]; Dominique Simon[4]; Mireille Castanet[8]; Thérèse Santerre[9]; Géraldine Viot[10]; Alain Verloes[3]; Jean Claude Carel[4]; Sylvie Manouvrier[11]; Nicolas de Roux[12]
[1]Robert Debré Hospital, Pediatric Endocrinology, INSERM U676, Paris, France; [2]Hôpital Meyer Children's de Haifa, Pediatric Endocrinology, Haifa, Israel; [3]Robert Debré Hospital, Genetic, Paris, France; [4]Robert Debré Hospital, Pediatric Endocrinology, Paris, France; [5]CHU de Rouen, Genetic, Rouen, France; [6]CHU de Rouen, Endocrinology, Diabetes and Metabolism, Rouen, France; [7]Hôpital Necker Enfants malades, Pediatric Endocrinology, Paris, France; [8]CHU de Rouen, Pediatric Endocrinology, Rouen, France; [9]CHU d'Amiens, Genetic, Amiens, France; [10]Hôpital Cochin, Genetic, Paris, France; [11]Centre Hospitalier régional de Lille, Genetic, Lille, France; [12]Robert Debré Hospital, INSERM U676, Paris, France

Background: The limb anomaly "split hand/feet malformation" (SHFM) is a malformation of the extremities that can be isolated or associated with other developmental abnormalities including cleft lip and palate, ectodermal dysplasia in EEC syndrome and in few cases with isolated gonadotropic deficiency with or without anosmia. Several loci are described in the isolated form of SHFM. Known causes of syndromic SHFM include TP63 mutations. There are still many cases of syndromic orphans SHFM. Recently, mutations in FGFR1 have been described in the dominant form of Kallmann syndrome (KS), which is defined by the combination of a gonadotropin deficiency and arhinencephaly. A special feature of FGFR1 mutations in KS is the high frequency of syndactyly and cleft lip and palate without being reported to date an association with SHFM.
Objective and hypotheses: During this work, we tested the hypothesis that SHFM associated with at least cleft palate, arhinencephaly or isolated gonadotropin deficiency may be due to FGFR1 mutations.
Methods: For this, we sequenced FGFR1 exons from DNA extracted from

blood lymphocytes of 13 patients having this phenotype without TP63 mutation.
Results: 7 heterozygous missense mutations of FGFR1 were found in 7 patients. These mutations are located in the tyrosine kinase domain but also in the extracellular domain of FGFR1. One mutation occurred de novo. The family analysis for six other mutations showed a highly variable expressivity and incomplete penetrance of a phenotype ranging from normal to Kallmann syndrome with severe gonadotropin deficiency. MRI focused on the olfactory bulbs has not revealed agenesis of the olfactory bulbs in 3 cases.
Conclusions: FGFR1 must thus be tested in patients presenting at birth an EEC syndrome or SHFM associated with a cleft palate and/or an arhinencephaly and an isolated gonadotropic deficiency. This work shows the importance of FGFR1 in the limbs development as it was already known for FGFR2 and FGFR3.

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Response to GnRH agonist challenge is the favorable tool to diagnose hypogonadotropic hypogonadism in boys with delayed puberty

Regina Braun; Roland Schweizer; Gerhard Binder
University Children's Hospital, Pediatric Endocrinology, Tuebingen, Germany

Background: Diagnosis of idiopathic hypogonadotropic hypogonadism (IHH) in males with delayed puberty can be difficult. An exact distinction is important for counseling, treatment, and future fertility.
Objective and hypotheses: We prospectively assessed the accuracy of the GnRH agonist (triptorelinacetat) challenge in comparison to inhibin B (INHB) for diagnosing IHH. GnRH agonist challenge examines the integrity of the pituitary gland and the testicles. INHB is a marker for Sertoli cell activity.
Methods: 37 prepubertal males, testicular volume ≤4 ml, age ranges between 13.6 and 16.5 years. 28 males spontaneously reached a testicular volume ≥8 ml (constitutional delayed puberty), 9 males did not during an 18 months follow-up (IHH). A GnRH agonist test was carried out in all patients at initial presentation. INHB was measured by ELISA (Beckman Coulter, Inc., USA). LH basal (LH1) and LH after 4 hours (LH2) were measured by CLIA (Siemens Health Care Systems, Germany).
Results: ROC plot analysis of the area under the curve (AUC) for diagnosing IHH was greatest for LH2 (100%) followed by INHB (98%; 95%-CI:94%-100%) and LH1 (95%; 95%-CI:88%-100%). A LH2 concentration <6.3 IU/l had a sensitivity and specificity of 100%. For an INHB concentration <114.0 pg/ml the sensitivity was 100% and the specificity was 89.3%. For LH1 ≤0.2 IU/l sensitivity was 88.9%, specificity was 89.3%. The combination of an INHB concentration ≤120 pg/ml and LH1 ≤0.2 IU/l resulted in a sensitivity of 88.9% and a specificity of 96.4%.
Conclusions: LH response 4 hours after triptorelinacetat (LH2) has an excellent sensitivity and specificity to diagnose IHH. INHB has a strong sensitivity but limited specificity. We recommend the GnRH agonist challenge to diagnose IHH in males with delayed puberty.

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Evaluation of gonadal function in 51 adolescents with history of orchiopexy

Özlem Kazanci[1]; Nurçin Saka[2]; Ahmet Uçar[2]; Alaaddin Çelik[3]; Orhan Ziylan[4]; E Yilmaz[5]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatrics, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrine Unit, Istanbul, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatric Surgery, Istanbul, Turkey; [4]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatric Urology, Istanbul, Turkey; [5]Istanbul University, Istanbul Faculty of Medicine, Department of Radiology, Istanbul, Turkey

Background: Despite timely and successful interventions, undescended testicle is still reported as a major cause of male infertility.
Objective and hypotheses: We evaluated the gonadal function of 51 adolescents between December 2010 and July 2011, who had history of orchiopexy due to undescended testes (35 unilateral and 16 bilateral).
Methods: The etiology of undescended testes, location of the testes prior to orchiopexy, history of hCG use, age at orchiopexy, serum LH , FSH , total testosterone (T), AMH , inhibin B levels and scrotal ultrasonography were analyzed.
Results: The testes had an inguinal location in 49 (73%) of 67 undescended testes. hCG treatment had been applied on 16 (%31) boys. Age at orchidopexy was 4.3± 2.6 yr (range 0.6-10 yr). Age at orchiopexy and at current evaluation were similar between boys with unilateral and bilateral undescended testes. Serum LH, FSH, T , AMH and inhibin B levels were not significantly different between boys with unilateral and bilateral undescended testes and they were independent of age at orchiopexy . The testicle volumes had a positive correlation with serum LH and T levels, inverse correlation with serum AMH levels. AMH levels positively correlated with inhibin B levels, negatively correlated with FSH levels. AMH levels did not correlate with T levels. Serum inhibin B levels negatively correlated with FSH levels, and had no correlation with any of the other parameters tested. When all the boys in the study were evaluated, high FSH, reduced AMH and inhibin B were seen in 17.6%, 9.8 % and 51% of the boys, respectively. Scrotal USG revealed 5 boys with testicular microlithiasis and 6 boys with varicocele.
Conclusions: Our study is in line with the previous reports that Sertoli cell function is significantly affected in adolesecent boys with undescended testes, and serum inhibin B levels provide the best determinant of Sertoli cell function.

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Plasma kisspeptin levels in girls with premature thelarche

Aysehan Akinci[1]; Dilek Cetin[1]; Nevin Ilhan[2]
[1]Inonu University, Pediatric Endocrinology, Malatya, Turkey; [2]Firat University, Biochemistry Department, Elazið, Turkey

Background: Premature thelarche (PT) is defined as isolated breast development without secondary sex characteristics in girls below the age of eight.The mechanisms are not fully understood.
Objective and hypotheses: We aim to determine whether the level of kisspeptin, which play a role in the release of gonadotropins, is associated with PT.
Methods: The patient group included the children with PT aged 3-7 years (n=20) and the control group included healthy children of the same age group (n=20). In the patient and control groups, basal follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactine (PRL), and sex hormone binding globulin (SHBG) levels were measured using ICMA. Gonadotropine-releasing hormone (GnRH) test was applied to the patient group, and the peak levels of FSH and LH were determined. Kisspeptin levels were measured using ELISA.
Results: While the plasma basal FSH, LH and E2 levels of the patient and control groups were not significantly different, the PRL level was higher in the patient group ($p<0.05$). In the patient group, kisspeptin levels were significantly higher compared to the levels of the control group (2.96±1.21ng/dl vs. 1.19±0.41ng/dl; $p<0.05$), and kisspeptin levels showed a significant correlation with PRL level ($p<0.05$).
Conclusions: These results support that PT may be related to a premature increase of kisspeptin leading to premature activation of the HPG axis or via kisspeptin-induced increase of PRL.

P1-d3-384 Sex Differentiation 1

Structural analysis of androgen receptor mutant proteins

Reginaldo Petroli[1]; Flavia Leme de Calais[1]; Fernanda C. Soardi[1]; Andrea Trevas Maciel-Guerra[2]; Gil Guerra-Junior[3]; Karla Melo[4]; Ivo Arnhold[4]; Maricilda Palandi de Mello[5]

[1]Universidade Estadual de Campinas - UNICAMP, Centro de Biologia Molecular e Engenharia Genetica - CBMEG, Campinas, Brazil; [2]Universidade Estadual de Campinas - UNICAMP, Departamento de Genética Médica, Faculdade de Ciências Médicas, Campinas, Brazil; [3]Universidade Estadual de Campinas - UNICAMP, Departamento de Pediatria, Faculdade de Ciências Médicas, Campinas, Brazil; [4]Universidade de São Paulo - USP, Unidade de Endocrinologia do Desenvolvimento, LIM42, Endocrinologia, Hospital das Clínicas, São Paulo, Brazil; [5]Universidade Estadual de Campinas, Centro de Biologia Molecular e Engenharia Genetica - CBMEG, Campinas, Brazil

Background: The androgen insensitivity syndrome (AIS) is the most frequent cause of sex development disorders in 46,XY patients. Molecular analyses of androgen receptor (AR) gene including structural and functional studies of AR protein may confirm the clinical diagnosis.
Objective and hypotheses: The purpose of this research was to analyze structural modifications upon AR protein caused by four different mutations identified in Brazilian patients. All mutations were located in the AR ligand-binding domain. The mutations were: p.P694S, p.L768V, p.I898F and p.P904R.
Methods: A modeled protein for each mutant AR was built using the resolved 3D structure of human AR in complex with dihydrotestosterone (PDB-ID 2AM9) as template. Molecular modeling was performed using MODELLER webserver program. The modeled images were examined and edited by PyMOL®program and Millennium STING (CNPTIA Embrapa, Brazil).
Results: The mutation p.P694S was identified in a patient with mild AIS (MAIS). Structural analysis did not show any discrepancy in the internal contacts for wild type and mutant residues indicating no detectable structural changes. The mutation p.I898F was found in patients with partial AIS (PAIS). The structural analysis revealed that residue 898 has lost ten hydrophobic interactions and maintained three hydrogen bonds in the mutant protein. The mutations p.L768V and p.P904R were associated with complete AIS (CAIS). The number of internal contacts for mutant residue V768 increased from 9 to 15 and the number of hydrophobic interactions increased from 12 to 56. Whereas the residue 904 presented equal number of internal contacts in both normal and mutant proteins, the interaction characteristics changed: three hydrophobic interactions were lost and three hydrogen bonds were created for the mutant residue R904.
Conclusions: This study demonstrated that the structural analysis of AR mutant proteins could be used to correlate genotypes to phenotypes associated to PAIS, CAIS or MAIS. However, each mutation described here will be further investigated for functional abnormalities.

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Testis transcriptome analysis in cryptorchid boys: a new insight on the pathogenesis of azoospermia

Faruk Hadziselimovic[1]; Nils Omar Hadziselimovic[2]; Philippe Demougin[3]; Eduard Oakeley[4]

[1]Kindertagesklinik Liestal, Pediatrics, Liestal, Switzerland; [2]Biocentrum Basel, Molecular Genetics, Basel, Switzerland; [3]Biocentrum Basel, Molecular Biology, Basel, Switzerland; [4]Novartis institutes for biomedical research, Molecular Biology, Basel, Switzerland

Background: Despite timely and successful surgery, 32% of patients with bilateral and 10% with unilateral cryptorchidism will develop azoospermia. Cryptorchid boys at risk of azoospermia (HAZR) display a typical testicular histology; impaired transformation of gonocytes into Ad spermatogonia, reduced number of Sertoli cells and severe Leydig cell atrophy indicating a general testicular developmental delay.Aim: This study aimed to analyze data on whole genome expression signatures of undescended testes at risk of developing azoospermia.
Patients and methods: Whole genome expression analysis was performed using Affymetrix method at twenty-three testicular biopsies from 22 boys were analyzed (19 testes from 18 boys with cryptorchidism).
Results: Expression profiling identified 483 genes not or under-expressed in the azoospermia risk group compared with the low risk for azoospermia group. Annotated loci were associated with; spermatogenesis, EGR4, DDX4; Leydig cell; IGRF1 and Sertoli cell; RHOXF1, RHOXF2, NRG1, ETV5 (Sertoli cell). EGR4, which is involved in regulating the secretion of luteinizing hormone, was virtually not expressed. IGFR1, RHOX1, RHOX2,DDX4 are LH while NRG1 FSH dependent gene. Finally, decreases expression of developmental genes FGF9 and FGFR2 was also observed in the HAZR group.
Conclusion: In the HAZR group we observed impaired expression of gonadotropin dependent genes encoding transcription factors that have crucial roles in embryogenesis and development. Multiple differences in gene expression between the HAZR and LAZR groups, confirm the importance of an intact hypothalamo-pituitary testicular axis for fertility

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Functional characterization of a novel mutation (IVS9+5G>A) in the human aromatase gene associated with aromatase deficiency

Nora Saraco[1]; Romina Sainz[1]; Suzana Nesi-Franca[2]; Roxana Marino[1]; Rosana Marques-Pereira[2]; Julia LaPastina[2]; Natalia Perez-Garrido[1]; Romulo Sandrini[2]; Marco Rivarola[1]; Luiz De Lacerda[2]; Alicia Belgorosky[1]

[1]Hospital de Pediatria Garrahan, Endocrine Service, Buenos Aires, Argentina; [2]Federal University of Parana, Pediatric Endocrine Service, Curitiba, Brazil

Background: Aromatase (Arom) is the key enzyme for estrogen biosynthesis and, in humans, is encoded by the CYP19A1 gene. We report a novel CYP19A1 intronic homozygote mutation (IVS9+5G>A) in an affected 46,XX DSD girl born of non-consanguineous parents presenting an aromatase deficient phenotype characterized by ambiguous genitalia, delayed bone age, absence of breast development, multicystic enlarged ovaries and high serum LH and FSH levels.
Objective and hypotheses: The aim of this study was to functionally characterize the IVS9+5G>A mutation. We hypothesize the disappearance of the splicing donor site in intron 9 in the presence of the mutation.
Methods: Arom mRNA was analyzed in the patient's lymphocytes (PBL) by RT-PCR. Splicing assays were performed in Y1 cells transfected with wild type (WT) or mutant (Mut) minigene constructions, which contain full length exon 9 and each flanking intronic sequences. Protein analysis was carried out by western blot after the transfection of an expression vector containing Arom cDNA including part of intron 9 (IN9cDNA); or a complete wild type Arom cDNA expression vector (WTcDNA) as normal control. Enzyme activity was evaluated by measurement of estradiol production after transfection of IN9cDNA or WTcDNA, using testosterone as substrate.
Results: Patient PBL Arom mRNA analysis showed the inclusion of intron 9, confirmed by sequencing. WT minigene expression resulted in a normally spliced mRNA whereas Mut generated an abnormal mRNA. Protein analysis showed the expression of a shorter and inactive protein compared with WT.
Conclusions: We report a novel IVS9+5G>A mutation in the CYP19A1 gene that generates an mRNA that includes intron 9. The presence of an in-frame stop codon 18 bp downstream the splice junction in intron 9 would result in the synthesis of a truncated and inactive aromatase protein lacking the heme-binding region. These findings explain the aromatase deficiency clinical phenotype found in the affected girl.

P1-d3-387 Sex Differentiation 1

Ten novel mutations in the NR5A1 gene cause disordered sex development in 46, XY and ovarian insufficiency in 46, XX individuals

Núria Camats[1]; Amit V Pandey[1]; Mónica Fernández-Cancio[2]; Pilar Andaluz[2]; Marco Janner[1]; Núria Torán[3]; Abdullah Bereket[4]; Teoman Akcay[5]; Primus E Mullis[1]; Antonio Carrascosa[2]; Laura Audí[2]; Christa E Flück[1]

[1]University Children's Hospital Bern, Department of Pediatrics and Department of Clinical Research, Bern, Switzerland; [2]Hospital Universitari Vall d'Hebrón, Pediatric Endocrinology Research Unit, VHIR, CIBERER, Barcelona, Spain; [3]Hospital Universitari Vall d'Hebrón, Pathology Department, Barcelona, Spain; [4]Marmara University, Department of Pediatrics, Division of Pediatric Endocrinology, Istanbul, Turkey; [5]Sisli Etfal Education and Research Hospital, Department of Pediatrics, Division of Pediatric Endocrinology, Istanbul, Turkey

Background: Steroidogenic factor-1 (*NR5A1*/SF-1) is a nuclear receptor which regulates adrenal and reproductive development and function. *NR5A1* mutations have been detected in 46,XY individuals with disorders of sex development (DSD) but normal adrenal function and in 46,XX women with primary ovarian insufficiency (POI).
Objective: To study a group of 102 patients for *NR5A1* mutations and its impact.
Methods: Studied patients originated from Spain (65 46,XY DSD), Switzerland (2 POI) and Turkey (35 46,XY DSD). Clinical, biochemical, histological, genetic and functional analyses were performed. All exons and flanking sequences of the *NR5A1* gene were analyzed. Functional analyses were performed in steroidogenic adrenal and non-steroidogenic cells. Cells were transiently transfected with wild-type and mutant SF-1 and SF-1 regulated promoter reporter constructs. Phenotype-genotype and structure-function correlations were assessed.
Results: Ten novel heterozygote *NR5A1* mutations were detected. Clinical phenotype varied widely with specific mutations and within patients harboring identical mutations. Testes histology showed vacuolization of Leydig cells due to fat accumulation. Promoter transactivation assays revealed that SF-1 mutations located in the DNA binding domain reduced transactivation activity while mutations in other domains showed mixed results. Results were also variable according to cell systems and specific promoters analysed. No dominant negative effect was detected. We were also not able to find structure-function correlation when using bioinformatic tools.
Conclusions: *NR5A1* mutations are frequently found in 46,XY DSD individuals (9%) and manifest with a broad phenotype. Testes histology is characteristic for fat accumulation and degeneration over time similar to findings observed in patients with lipoid congenital adrenal hyperplasia due to *StAR* mutations. Genotype-structure-function-phenotype correlation remains elusive.

P1-d3-388 Sex Differentiation 1

Mutations in the NR5A1 gene in patients with 46,XY disorders of sex development (DSD): high frequency of familial multi-generational occurrence

Mariana Costanzo[1]; Diana Monica Warman[1]; Gabriela Guercio[1]; Marta Ciaccio[1]; Valeria De Dona[1]; Roxana Marino[1]; Pablo Ramirez[1]; Natalia Perez Garrido[1]; Maria Sonia Baquedano[1]; Esperanza Berensztein[1]; Nora Isabel Saraco[1]; Eduardo Chaler[1]; Mercedes Maceiras[1]; Juan Manuel Lazzati[1]; Marcela Bailez[2]; Liliana Ongaro[3]; Marco A Rivarola[1]; Alicia Belgorosky[1]

[1]Hospital de Pediatria Juan P Garrahan, Endocrine Service, Buenos Aires, Argentina; [2]Hospital de Pediatria Juan P Garrahan, Surgery Service, Buenos Aires, Argentina; [3]Hospital de Pediatria Juan P Garrahan, Mental Health Department, Buenos Aires, Argentina

Background: Nuclear receptor SF1/NR5A1 regulates the transcription of genes involved in reproduction, steroidogenesis and male sexual differentiation. Mutations in humans cause gonadal dysgenesis with/without adrenal failure in both 46,XY and 46,XX individuals.
Method: In a cohort of patients with familial 46,XY DSD, we identified 6 heterozygous NR5A1 mutations in 19 subjects from 5 unrelated families. We also identified a de novo heterozygous mutation in one patient with 46,XY DSD and no affected relatives.
Results: Low ovarian reserve with preserved fertility was detected in affected females. Extreme within-family variability was found in 46,XY affected patients, with phenotypes ranging from severe fetal undervirilization, prompting female sex of rearing to spontaneous pubertal development and even preserved fertility. Mutational analysis revealed in the first family a W279X heterozygous mutation and an intronic deletion (g3314-3317delTCTC (IVS 4+8), and in the second a Y183X heterozygous mutation.These mutations had been previously reported. A novel R313H heterozygous variation was found in the third family, and a novel S303R was found in the fourth. A novel heterozygous R69H mutation was found in the only patient studied (46,XY DSD) from the last family, and a G77E de novo mutation in the sporadic case. All new mutations were predicted to affect protein function by prediction models (SIFT, Polyphen and MutationTaster).
Conclusions: We emphasize the extreme phenotypic variability that can be observed even in siblings with the same mutation. As previously reported, we found spontaneous puberty in 46,XY individuals raised as males, and for the first time we report preserved fertility in one of these affected individuals. Individuals with heterozygous NR5A1 mutations and mild phenotypes, such as isolated hypospadias in 46, XY patients, compensated ovarian dysfunction and early menopause in 46,XX subjects, might easily go undetected. A careful family screening of 46,XY as well as 46,XX individuals is recommended whenever an index case is detected.

P1-d3-389 Sex Differentiation 1

Analysis of the gene coding for steroidogenic factor 1 (SF-1, NR5A1) in a cohort of fifty Egyptian patients with 46,XY DSD

Sally Tantawy[1]; Inas Mazen[2]; Hala Soliman[3]; Ghada Anwar[4]; Abeer Atef[4]; Mona El-Gammal[2]; Ahmed El-Kotoury[2]; Ahmad Torky[2]; Agnes Rudolf[1]; Heiko Krude[1]; Heike Biebermann[1]; Birgit Koehler[1]

[1]Institute for Experimental Pediatric Endocrinology, Department of Pediatric Endocrinology, University Children's Hospital, Charite´, Humboldt University, Berlin, Germany, Berlin, Germany; [2]National Research Centre, Department of Clinical Genetics, Division of Human Genetics and Genome Research, Cairo, Egypt; [3]National Research Centre, Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, Cairo, Egypt; [4]Cairo University, Department of Pediatrics, Cairo, Egypt

Background: SF-1 is a key transcriptional regulator of genes involved in the hypothalamic-pituitary-gonadal axis. Recently, SF-1 mutations were found to be a frequent cause of 46,XY disorders of sex development (DSD) in humans.
Objective and hypotheses: To investigate the frequency of SF-1 mutations in an Egyptian XY DSD cohort.
Methods: Molecular genetic analysis of NR5A1, the gene coding for SF-1, in fifty Egyptian XY DSD patients (without adrenal insufficiency) with a phenotypic spectrum including: complete female external genitalia with/without uterus (n=8), ambiguous genitalia without uterus (n=5), vanishing/atrophic testes (n=10), penile to scrotal hypospadias (n=23) and isolated hypoplastic phallus (n=4).
Results: NR5A1 analysis revealed 3 novel heterozygous mutations of the coding region. The p.Arg62Cys mutation lies in the DNA-binding zinc finger region and is predicted to result in conformational change of the protein. The p.Glu121AlafsX25 mutation results in a severely truncated protein. The p.Ala154Thr mutation lies in the hinge region. Furthermore, three novel heterozygous intronic mutations (IVS4-13 C>T, c.1137+105b C>T, c.1137+129b C>A), which are not present in the SNP databases, were detected in patients with severe hypospadias. Sixteen patients (32%) harboured the p.Gly146Ala polymorphism.

Heterozygous exonic mutations	External genitalia	Testis	Uterus
p.Arg62Cys + p.Gly146Ala	Penile hypospadias, hypoplastic phallus	Bilateral small undescended	No
p.Glu121AlafsX25	Hypospadias, hypoplastic phallus	Right undescended atrophic, left normal scrotal	No
p.Ala154Thr	Penile hypospadias, hypoplastic phallus	Bilateral normal scrotal	No

Conclusions: This is the first study of NR5A1 analysis in an oriental cohort of XY DSD patients. We identified exonic NR5A1 mutations in 3 out of 23 Egyptian XY DSD patients with hypospadias, which corresponds to the fre-

quency of 7.5-15% in European patients (Köhler et al, 2009, Allali 2011). Thus, NR5A1 mutations are a frequent cause of XY DSD with hypospadias also in Egyptians. NR5A1 analysis should be considered in XY DSD with hypospadias. Early cryoconservation of sperms should be initiated as there is a risk of early gonadal failure.

P1-d3-390 Sex Differentiation 1

Gonadal dysgenesis and atypic expression of OCT-3/4 in testis of prepubertal patients with complete or partial androgen insensitivity syndrome

Esperanza Berensztein[1]; Mariana Costanzo[2]; Monica Warman[2]; Marta Ciaccio[2]; Elisa Vaiani[2]; Gabriela Guercio[2]; Silvia Gil[2]; Isabel Di Palma[2]; Nora Saraco[1]; Roxana Marino[3]; Pablo Ramirez[3]; Natalia Perez Garrido[3]; Roberto Ponzio[4]; Marcela Bailez[5]; Marco A. Rivarola[2]; Alicia Belgorosky[2]

[1]Hospital de Pediatría Garrahan, Laboratorio de Cultivo Celular y Biología Molecular, Servicio de Endocrinología, Buenos Aires, Argentina; [2]Hospital de Pediatría Garrahan, Servicio de Endocrinología, Buenos Aires, Argentina; [3]Hospital de Pediatría Garrahan, Laboratorio de Biología Molecular Diagnostica, Servicio de Endocrinología, Buenos Aires, Argentina; [4]Facultad de Medicina, Universidad de Buenos Aires, Instituto de Investigaciones en Reproducción, Buenos Aires, Argentina; [5]Hospital de Pediatría Garrahan, Servicio de Cirugia, Buenos Aires, Argentina

Background: In the prepubertal (PP) human testis (TES), the androgen receptor (AR) is expressed in peritubular cells (PC) and interstitial cells (IC), including Leydig cells (LC) but not in Sertoli cells (SC). It has been reported that adult patients with complete androgen insensitivity (CAIS) have dysgenetic TES without differentiated germ cells (GC). The transcription factor OCT-3/4, specific of embryonic stem cells, is found in carcinoma in situ (CIS) and in gonadoblastoma cells.
Objective and hypotheses: To study OCT-3/4 expression in TES of PP patients with total (CAIS) or partial (PAIS) androgen insensitivity as marker of malignancy risk.
Population and methods: We have studied 9 PP patients (CAIS: n=7, PAIS: n=2) gonadectomized between 0.25 and 6.3 years old. In 5/9 subjects, diagnosis was supported by AR gene analysis (M749V, R631X, E603X, L621P mutations and the del1550-1569ex1 deletion), while in 4 subjects by clinical hormonal data (including absence of SHBG response to testosterone stimulus, Belgorosky A JCEM 1987). Histology, OCT-3/4, AR and ERα immunoexpression (n=9) and the testosterone secretion in primary cell culture [n=3] was studied. PP TES without endocrine pathology was used as control (C, n=10).
Results: Signs of testicular dysgenesis were found in 7/9 testes; CIS and/or OCT-3/4 positive expression in 5/9 samples; hyperplasia of LC in 4/9 testes. Positive AR LC immunoexpression was found in 3/9 samples but with atypical cytoplasm localization, different to C tesis (nuclear). Expression of ERα in LC was positive, different from C (ERα negative). Testosterone secretion in vitro confirmed the presence of steroidogenic cells, although no response to hCG was found in patient cell cultures, different from C.
Conclusions: OCT-3/4, a marker of risk of gonadoblastoma, was frequently found in testis of PP patients with CAIS and PAIS. These results suggest that a testicular biopsy is recommended before postponing gonadectomy to adulthood.

P1-d3-391 Sex Differentiation 1

Gonadal histology in patients with complete androgen insensitivity syndrome (CAIS): influence of age, gonadal location and residual androgen receptor activity

Jana Pleskacova[1]; Hans Stoop[2]; Hennie Bruggenwirth[3]; J. Wolter Oosterhuis[2]; Martine Cools[4]; Katja P. Wolffenbuttel[5]; Stenvert L.S. Drop[6]; Marta Snajderova[7]; Jan Lebl[7]; Leendert Looijenga[2]

[1]Charles University, 2nd Faculty of Medicine, Department of Pediatrics, Prague, Czech Republic; [2]Erasmus MC, Daniel den Hoed Cancer Center, Josephine Nefkens Institute, Department of Pathology, Rotterdam, Netherlands; [3]Erasmus MC, Department of Clinical Genetics, Rotterdam, New Caledonia; [4]University Hospital Ghent, Department of Pediatrics, Division of Pediatric Endocrinology, Ghent, Belgium; [5]Erasmus MC – Sophia Children's Hospital, Department of Pediatric Urology, Rotterdam, Netherlands; [6]Erasmus MC – Sophia Children's Hospital, Department of Pediatric Endocrinology, Rotterdam, Netherlands; [7]Charles University, 2nd Faculty of Medicine, University Hospital Motol, Department of Pediatrics, Prague, Czech Republic

Background: Typical changes in gonadal histology of patients with CAIS have been described. These might develop primarily with age, and be influenced also by other factors such as gonadal location and residual androgen receptor (AR) activity.
Objective and hypotheses: To assess the influence of age, gonadal location, and residual AR activity on gonadal histology in patients with CAIS.
Methods: In total 39 gonads from 20 patients were available for assessment. Age at gonadectomy ranged from 3 months to 18.5 years. Twelve patients lacked any AR activity; Eight patients had some residual AR activity. Histological abnormalities were assessed on HE stained slides. Immunohistochemical detection of OCT3/4 and KITLG (malignant germ cell tumor markers) was done on 38 gonads.
Results: The total number of tubules containing germ cells declined with age. AR activity was an independent predictor for germ cell survival ($p<0.001$). OCT 3/4 positive germ cells were present in 13/38 (34.2%) gonads; KITL was detected in matched areas in 6 of those (46.2%). However, neither OCT3/4 nor KITLG positivity was found to be dependent on age, residual AR activity or gonadal location. Tubular atrophy, Leydig cell hyperplasia, Sertoli cell (SC) adenomas and interstitial fibrosis were dependent on age ($p<0.001$), i.e., predominantly found in pubertal or postpubertal gonads. Tubular atrophy and lymphatic enlargement were significantly more prevalent in patients without AR activity ($p<0.01$ and $p<0.05$, respectively). Hyalin deposits, SC nodules and eosinophilic changes of SC cytoplasm did not show any dependence on studied factors.
Conclusions: Survival of germ cells in general is dependent on age and AR activity. However, survival of pre-malignant germ cells is not. Most of the abnormal features develop with rising age. AR residual activity seems to have an impact only on some of these features. Gonadal location is probably not an independent predictor for observed changes.

P1-d3-392 Sex Differentiation 1

A CYB5A gene mutation explains the last unsolved case of our cohort of 30 patients with a diagnosis of 17α-hydroxylase/17,20-lyase deficiency

Delphine Mallet[1]; Michel David[2]; Maguelone G. Forest[1]; Marc Nicolino[2]; Elisabeth Thibaud[3]; Claire Nihoul-Fekete[4]; Bettina Bständig[5]; Yves Morel[1]

[1]Centre de Biologie et de Pathologie Est, Endocrinologie Moléculaire et Maladies Rares, Bron, France; [2]Hôpital Femme Mère Enfant, Endocrinologie Pédiatrique, Bron, France; [3]Hôpital Necker Enfants Malades, Endocrinologie et Gynécologie Pédiatriques, Paris, France; [4]Hôpital Necker Enfants Malades, Chirurgie Pédiatrique, Paris, France; [5]Hôpital l'Archet, Endocrinologie, Nice, France

Background: Combined 17α-hydroxylase/17,20-lyase deficiency is a rare, well defined disease, due to mutations of *CYP17A1*. Isolated 17,20-lyase deficiency is rarer, difficult to diagnose clinically and recent studies have shown that at least 3 genes in addition to *CYP17A1* could be responsible for this disorder.
Objective and hypotheses: Using hormonal and sequencing data of our pa-

tients, we tried to precise the clinical, biological and genetic profiles associated to the different forms of this disorder.
Methods: We studied 30 patients from 26 families. The hormonal profile was first established in details, and then the candidate genes, *CYP17A1*, *POR*, *AKR1C2* and *CYB5A* were sequentially studied.
Results: In all patients with a diagnosis of combined 17α-hydroxylase/17,20-lyase deficiency, we identified mutations in *CYP17A1*(23/23). In the 7 remaining patients, hormonal data were evocative of isolated 17,20-lyase deficiency. In 3 patients the p.R358Q mutation known to cause this isolated disorder was identified. One patient was homozygous, the others being compound heterozygous, with the second mutation causing a complete combined deficiency. *POR* was then sequenced in the remaining patients, allowing us to identify mutations in 3 of them. We failed to found any mutation (or large deletion) in *CYP17A1*, *POR* and *AKR1C2* in the last patient, but identified a homozygous mutation, c.94delC, in *CYB5A*, finally solving this case.
Conclusions: Our study adds 7 cases to the few described cases of isolated 17,20-lyase deficiency and confirms that this disorder could be due to at least 3 different genes. Our detailed hormonal data show that ACTH test can distinguish *POR* deficiency from *CYP17A1* or *CYB5A* deficiency: high progesterone (> 80 nM), pattern of non classical 21-OHD, and no stimulation of cortisol. At this time, with the exception of the dosage of methemoglobin, clear clinical or hormonal criteria differentiating cases due to *CYP17A1* or *CYB5A* mutations remain to be defined.

P1-d3-393 Sex Differentiation 1

Hormone profiles in adolescents and adults with complete androgen insensitivity syndrome (CAIS)

Ulla Doehnert[1]; Silvano Bertelloni[2]; Annette Richter-Unruh[3]; Ralf Werner[1]; Olaf Hiort[1]
[1]University of Luebeck, Department of Pediatric Endocrinology and Diabetes, Luebeck, Germany; [2]University of Pisa, Department of Reproductive Medicine and Pediatrics, Pisa, Italy; [3]Endokrinologikum Ruhr, Pediatric Endocrinology and Diabetes, Wattenscheid, Germany

Background: Due to an increased risk of malignant degeneration of dysgenetic gonads early gonadectomy has often been recommended in people with disorders of sex development. Therefore data about normal values of gonadotropins and sex steroids in postpubertal women with CAIS are rare.
Patients and methods: Hormone profiles (testosterone, estradiol, sex-hormone-binding globulin SHBG, luteinizing hormone LH, follicle-stimulating hormone FSH) were studied in 41 women with proven mutations in the androgen receptor gene and a CAIS phenotype with a breast development corresponding Tanner 5 and absent or sparse pubic hair in three European centers.
Results: The women were aged 14 to 40 years (median = 17). Basal testosterone levels (22 nmol/l +/- 10 nmol/l, mean +/- standard deviation) were within the normal male reference range. Estradiol levels (121 pmol/l +/- 50 pmol/l) were measured in the upper male reference range. At the same time LH levels (20.2 IU/l +/- 9.7 IU/l) were elevated with normal FSH levels (4.5 +/- 3.6 IU/l). SHBG levels (57 nmol/l +/- 31 nmol/l) were in the adult female reference range.
Conclusions: The 41 women show a specific hromone profile with testosterone, estradiol and FSH levels in the male reference ranges and elevated LH levels. Since the prevalence of germ cell tumors in CAIS has been estimated <1% until puberty in recent publications, we tend to recommend to leave the gonads in situ in order to preserve endogenous hormone production. Likewise concepts of hormone replacement therapy after gonadectomy should take these profiles into account and are currently reevaluated in further controlled studies.

P1-d3-394 Sex Differentiation 1

Variable phenotype of 46,XY DSD in three brothers with a novel mutation in NR5A1 gene

Juliana Gabriel Ribeiro de Andrade[1]; Helena Campos Fabbri[2]; Gil Guerra-Junior[1]; Maricilda Palandi de Mello[2]; Andrea Trevas Maciel-Guerra[1]
[1]State University of Campinas (UNICAMP), Faculty of Medical Sciences, Campinas, São Paulo, Brazil; [2]State University of Campinas (UNICAMP), Center of Molecular Biology and Genetic Engineering (CBMEG), Campinas, Brazil

Background: The NR5A1 gene (9q33.3) encodes a nuclear receptor that regulates many aspects of adrenal and reproductive development and function. Mutations in this gene can be associated with a wide range of reproductive phenotypes, including 46,XY Disorders of Sex Development (DSD), premature ovarian failure and spermatogenic failure. Heterozygous changes in NR5A1 can be inherited from a fertile mother in a sex-limited dominant fashion, thus mimicking an X-linked disorder, like partial androgen insensitivity.
Objective and hypotheses: To report three sibs with sex ambiguity due to a novel NR5A1 mutation.
Methods: The three 46,XY sibs, all raised as boys, were born to healthy non-consanguineous parents and were first evaluated as newborns. The index case, now aged 12, had a single perineal opening and gonads were palpable in the labioscrotal folds. Spontaneous puberty began at the age of 11 years with high FSH and normal LH and testosterone levels.
His 5-year-old brother had penoscrotal hypospadias and the gonads were palpable in the labioscrotal folds. In the first months of life he had high levels of LH and normal FSH and testosterone levels. An hCG stimulation test was negative. The younger brother, now 4 years old, had a microphallus and posterior labioscrotal fusion, and gonads were in the inguinal region. In the first months of life he had normal levels of FSH, LH and testosterone, and a positive response to hCG testing.
Results: No mutations were found in androgen receptor and SRD5A2 gene. Molecular analysis of NR5A1 in the three sibs revealed a p.C65Y mutation in exon 3, which was not previously described.
Conclusions: The recent focus on the molecular analysis of NR5A1 has solved many cases of 46,XY DSD labeled as idiopathic. This family adds evidence that mutations in this gene lead to variable genital and hormonal features.

P1-d3-395 Thyroid 1

Neonatal hyperthyrotropinemia is associated with low birth weight: a twin study

Amnon Zung[1]; Arie Yehiel[2]; Shlomo Almashanu[3]
[1]Kaplan Medical Center, Pediatric Endocrinology Unit, Rehovot, Israel; [2]Gamidor Diagnostics Ltd, Gamidor Diagnostics Ltd, Pethach Tikva, Israel; [3]Ministry of Health, National Center for Newborn Screening, Tel Hashomer, Israel

Background: Contradictory reports ascribe neonatal hyperhyrotropinemia to prematurity or small weight for gestational age.
Objective and hypotheses: We aimed to evaluate the association between neonatal hyperthyrotropinemia and birth-weight, the recovery rate of the disorder and its possible association with perinatal stress.
Methods: Based on neonatal screening database, a retrospective twin-study was designed where within-pair differences in thyroid function were evaluated while controlling for differences in gestational age and thyroid affecting environmental confounders. 2595 twin pairs that were screened both for TSH and T4 over three years were included. We evaluated TSH and T4 levels along with birth-weight, birth order, gender and 17-hydroxyprogesterone that was considered as a surrogate marker for stress.
Results: In 156 twins pairs (6.0%), neonatal hyperthyrotropinemia was diagnosed in one of the twins (mean TSH 19.3 ± 4.6; range 15.1-36.3 mIU/L). hyperthyrotropinemia was more prevalent in the smaller twins (64%; $p<0.001$), especially in the discordant pairs (76%; $p=0.001$).
Most twins demonstrated a recovery within the first few weeks of life. 17-hydroxyprogetreone levels were similarly distributed between twins with and without hyperthyrotropinemia.
In a cohort of 1534 twin pairs with normal thyroid function, mean TSH levels were significantly higher in the smaller- than in the larger-twin in the whole group (4.1±3.2 vs. 3.8±2.9 mIU/L; $p<0.001$) and especially among discordant twins (4.7±3.4 vs. 3.8±3.0 mIU/L; $p<0.001$).

Conclusions: Elevated TSH levels are associated with low birth-weight, both in infants with hyperthyrotropinemia and in neonates with normal thyroid function. A rapid recovery rate is expected in most cases. Hyperthyrotropinemia is apparently not stress related.

P1-d3-396 Thyroid 1

Detection of genetic abnormalities in children with congenital hypothyroidism using MLPA analysis

Malgorzata Kumorowicz-Czoch[1]; Anna Madetko-Talowska[2]; Dorota Tylek-Lemanska[3]; Jacek J Pietrzyk[2]; Jerzy Starzyk[4]
[1]Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Department of Pediatric and Adolescent Endocrinology, Cracow, Poland; [2]Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Division of Medical Genetics, Chair of Pediatrics, Cracow, Poland; [3]University Children's Hospital, Division of Mass Screening and Metabolic Diseases, Cracow, Poland; [4]Polish-American Children's Hospital, Collegium Medicum, Jagiellonian University Medical College, Department of Pediatric and Adolescent Endocrinology, Cracow, Poland

Background: Thyroid dysgenesis (TD) is the most common cause of congenital hypothyroidism (CH). Among genetic factors that may contribute to TD etiopathogenesis, the highest importance is ascribed to mutations of transcription factors and TSHR-encoding genes.
Objective and hypotheses: Assessment of incidence of changes involving genes encoding PAX8, FOXE1, NKX2-1, TSHR and TPO detected by multiplex ligation-dependent probe amplification (MLPA) in CH and TD children.
Methods: Investigations included 46 children selected in mass screening tests between 1995 and 2009, in whom CH resulted from TD: ectopy (n=17), agenesis (n=22), hypoplasia (n=7). DNA was isolated from peripheral blood samples using Master Pure DNA Purification Kit (Epicentre Biotechnologies). 50-100 ng of genomic DNA was employed in MPLA analysis using a SALSA MLPA kit P319-A1 THYROID (MRC-Holland). Genetic changes were analyzed within selected fragments of the PAX8, FOXE1, NKX2-1, TSHR and TPO genes. Testing followed a standardized procedure provided by the manufacturer. MLPA analysis included: DNA denaturation and MLPA probe hybridization, ligation, PCR reaction, separation of reaction products by capillary electrophoresis and data analysis.
Results: Four types of heterozygous deletions in probe hybridization regions were identified for the following genes: PAX8 in exon 7, TSHR in exon 2, FOXE1 in exon 1 and TPO in exon 16. Genetic abnormalities of selected gene fragments were identified in 6/46 subjects.
Conclusions: MLPA screening showed genetic abnormalities in 13% of CH and TD children, manifested as deletion at probe hybridization site. Precise determination of the character of such abnormalities and genotype-phenotype correlation requires extending the study of selected regions to include other molecular methods (Sanger sequencing). Table 1. Genetic abnormalities depending on TD type

	Agenesis/ Female	Agenesis/ Female	Agenesis/ Male	Ectopy/ Female	Ectopy/ Female	Ectopy/ Male
PAX8	+			+		
TSHR		+			+	
FOXE1			+			
TPO					+	+

P1-d3-397 Thyroid 1

Iodine deficiency in pregnant women living in the capital city of Turkey, who appear to be iodine-sufficient

Alev Oguz Kutlu[1]; Cengiz Kara[2]
[1]Dr. Sami Ulus Women's and Children's Hospital, Pediatric Endocrinology, Ankara, Turkey; [2]Ondokuz Mayis University, Pediatric Endocrinology, Samsun, Turkey

Background: Previous studies about current iodine status in Turkey have yielded contradictory results. Although urinary iodine concentration (UIC) in school-age children suggests sufficient iodine status, studies on neonatal TSH indicate that iodine deficiency (ID) is still a continuing problem.
Objective and hypotheses: We aimed to assess the iodine nutritional status of pregnant women living in the capital city of Turkey that appears to be iodine sufficient in earlier studies. We hypothesized that if ID is an ongoing problem, pregnant women's iodine intake is insufficient.
Methods: This was a hospital-based, non-interventional, prospective cross-sectional study. A total of 162 pregnant women at second trimester were examined regarding iodized salt use, UIC, goiter rate and thyroid functions. Goiter status was determined by palpation. UIC was measured using colorimetric method based on Sandell-Kolthoff reaction. Serum levels of thyroid hormones and TSH were measured by chemiluminescence immunoassays.
Results: While the proportion of iodized salt use was 80.2%, most women (72.8%) had an UIC below 150 µg/l. The median UIC was 80.5 (8.9-340.3) µg/l, indicating insufficient iodine intake. Total goiter rate was 15.4%. Preferential T3 secretion and relative hypothyroxinemia reflected by elevated molar ratio of FT3/FT4 were present in 89.5% of the women. About 12% had subclinical hypothyroidism or isolated hypothyroxinemia. These thyroid abnormalities supported that the pregnant women were greatly affected by ID.
Conclusions: Our study reveals that ID is a serious problem among pregnant women living in the capital city appearing iodine sufficient. These data confirm that iodine nutritional status in school-age children does not reflect the iodine supply of pregnant women. Nationwide surveillance studies should urgently performed for assessment and monitoring the iodine status of pregnant women directly. The Ministry of Health should provide iodine supplementation to pregnant women to protect them and their offspring against adverse consequences of ID.

P1-d3-398 Thyroid 1

Central regulation of TSH and mutations of the TSH receptor in children with subclinical hypothyroidism

Andrea Paola Rojas Gil[1]; Eirini Kostopoulou[2]; Irini Christopoulou[3]; Athina Chatzikyprianou[2]; Bessie E. Spiliotis[2]
[1]University of Peloponnese, Faculty of Human Movement and Quality of Life Sciences, Department of Nursing, Sparti, Greece; [2]University of Patras, Research Laboratory of the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics School of Medicine, Patras, Greece; [3]University of Patras, Research Laboratory of the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, School of Medicine, Patras, Greece

Background: The diagnosis of subclinical hypothyroidism (SH) during childhood is still controversial. Mutations in the TSH receptor (TSHR) gene (in exons 1,2,4,6 and 10) have been reported in children with SH. Loss-of-function mutations of the TSHR gene have been described in families with TSH resistance. Functional studies of the P68S mutation in exon 2 show loss of function of the TSHR gene.
Objective and hypotheses: To investigate the prevalence of exon 2 TSHR gene mutations in children with SH in relation to the central regulation of TSH secretion.
Methods: SH was diagnosed in 60 children with TSH > 3.0 mIU/ml (P) and low normal concentrations of T-4, fT-4 and T-3. A 60 minute TRH test was performed on all patients using 7 mcg/kg TRH (max: 200 mcg) iv. Genomic DNA was isolated by standard methods from peripheral blood of the 60 P and 25 controls (C). Presence of the P68S mutation of the TSHR gene was confirmed by electrophoretic analysis of PCR products after restriction endonuclease digestion with XmnI using 2U of enzyme and 10 ìl of PCR product.
Results: The P68S mutation of the TSHR gene was found in 10 P (16 % of the total P) with SH but not in any of the controls. Of the P with the P68S mutation, 5P had abnormal TRH-test results, 2 had normal TRH-test results, and 3 were relatives of patients with abnormal TRH tests without the P68S mutation. The range of pre-treatment TSH and f-T4 concentrations of the 10 P with the P68S mutation were: 5P with abnormal TRH tests: 3.9 – 5.0 mIU/ml and 0.9 – 1.48 ng/dl, respectively and in the 2P with normal TRH tests: 3.1 - 4.34 mIU/ml and 1.1 – 1.5 ng/dl, respectively. Of the 10P, 4P presented with goiter, 2P with premature adrenarche and 1 P with polycystic ovarian disease which all resolved after thyroxine therapy.
Conclusions: The P68S mutation of the TSHR gene and TSH resistance is not rare in children with SH although other TSHR gene mutations may also be present. Thyroxine therapy in these children seems to be beneficial.

P1-d3-399 Thyroid 1

Fertility in adult females with congenital hypothyroidism (CH) diagnosed by neonatal screening

Alessandra Cassio[1]; Angela Rizzello[2]; Maria Carolina Salerno[3]; Tommaso Aversa[4]; Maria Cristina Vigone[5]; Sara Monti[2]; Lucia De Martino[3]; Filippo De Luca[4]; Milva Orquidea Bal[2]

[1]Azienda Ospedaliera S. Orsola Malpighi Hospital , University of Bologna, Pediatric Clinic, Endocrine Unit. Bologna, Italy, Bologna, Italy; [2]S. Orsola Malpighi Hospital , University of Bologna, Pediatric Clinic, Endocrine Unit. Bologna, Italy, Bologna, Italy; [3]University Federico II, Pediatrics, Naples, Italy; [4]University of Messina, Pediatrics, Messina, Italy; [5]IRCCS San Raffaele Scientific Institute, Pediatrics, Endocrine Unit, Milan, Italy

Background: CH interferes with human reproductive physiology, reduces the likelihood of pregnancy and adversely affects pregnancy and perinatal outcome. However, in subjects with early treated CH, a normal pubertal development has been reported. There are no systematic studies about fertility and pregnancy outcome in affected adults.
Objective and hypotheses: Fertility and pregnancy evalutation in CH adult females.
Methods: Gynecologic history was evaluated in a semistructurated questionnaire carried out by expert clinicians. The results were compared with data derived from national register of births. 155 girls with permanent CH between 1978 and 1994, in 4 italian regions, were recalled. 69 (44.6%) subjects (mean age 22.1 ± 4.4 yrs; 25 (36%) athyreosis, 32 ectopic gland (47%), 12 (17%) in situ gland) agreed to participate our study.
Results: 9 pts reported 10 pregnancies (15%),3 of which terminated by abortion (1 miscarriage: 6° week of GA in 28.4 yrs pt with ectopic thyroid gland; 2 elective abortion: 4° week of GA in 20.2 yrs pt with hypoplasia, 6° week of GA in 21.3 yrs pt with athyreosis). The abortion rate was 1.45% for miscarriage (0.54% in control population), 2.9% for elective abortion (0.86% in control population).In the fullterm pregnancies the mean increase of L-T4 dose needed in each trimester was 15% (10%-20%), 15%(10%-20%) and 20%(10%-30%) respectively. TSH values >2.5 mU/L were found in 7/7 cases in the 1st trimester, in 4/7 in the 2nd, in 4/7 in the 3th. All newborn showed normal results at neonatal screening and at thyroid ultrasound. The table shows the data of live births subdivided according to maternal age.

	Subjects n,%	CH mothers' gland morphologhy	Live births n, %,% in controls
<20 yrs	27/69(39%)	-	0/27 0% 0.9%
20-25 yrs	26/69(38%)	2 ectopic gland, 1 athyreosis	4/26 15.4% 8%
25-35 yrs	16/69(23%)	2 ectopic gland, 1 athyreosis	3/16 18.7% 24.2%

Conclusions: These preliminary data seem to indicate a normal fertility in patients with CH. The abortion rate seems to be higher than in control population. The monitoring of L-T4 dose adequacy in pregnancy is poor in our patients. These data warrant further investigation to evaluate the impact of genetic or therapeutic factors.

P1-d3-400 Thyroid 1

Intellectual outcome at 8 years of age in a large cohort of children with congenital hypothyroidism: effect of initial LT4 dose

Lucia De Martino[1]; Miriam Polizzi[2]; Maria Cristina Vigone[3]; Dario Bruzzese[4]; Lorenzo Bassi[3]; Clara Pozzi[3]; Raffaella Di Mase[1]; Carmela Bravaccio[5]; Giovanna Weber[3]; Mariacarolina Salerno[1]

[1]University of Naples "Federico II", Department of Pediatrics, Naples, Italy; [2]University of Naples, Department of Psychiatry and Child Neuropsychiatry, Naples, Italy; [3]San Raffaele Scientific Institute, Vita - Salute San Raffaele University, Department of Pediatrics, Milan, Italy; [4]University of Naples "Federico II", Section of Medical Statistics and Informatics, Department of Preventive Medical Sciences, Naples, Italy; [5]University of Naples "Federico II", Department of Psychiatry and Child Neuropsychiatry, Naples, Italy

Background: It is still debated which is the appropriate initial L-Thyroxine (LT4) dose to achieve an optimal intellectual outcome in children with congenital hypothyroidism (CH).
Objective and hypotheses: To evaluate intellectual outcome in patients with CH treated with an initial LT4 replacement dose between 10 and 15 µg/kg/die.
Methods: One hundred ten patients (33 males) affected by CH, detected by neonatal screening program at an average age of 15.2±6.2 days, were longitudinally followed up to the age of 8 years. Thirty healthy children comparable for age, sex and socioeconomic status (SES) were enrolled in the study as controls. At 8 years of age, Intellectual Quotient (IQ) was assessed using the WISH-III. SES was evaluated by Graffar score revised with 1 reflecting the highest and 5 the lowest score.
Results: Mean verbal (VIQ 100.1±15), performance (PIQ 100.8±22) and full-scale (FSIQ 101.6±13.2) IQ scores in patients with CH were normal and comparable to the controls IQ scores (VIQ 101.9±12.1, PIQ 102.8±14.2, FSIQ 102.7±12.7 respectively). No relationship was observed between IQ scores and initial LT4 dose, whereas IQ scores were significantly related to SES (VIQ $p<0.01$, PIQ<0.02, FSIQ<0.01). Additional variables influencing the IQ scores were the severity of CH at diagnosis (FT4 or T4 values) (VIQ $p<0.01$, PIQ $p<0.01$, FSIQ $p<0.02$) and the chronological age at diagnosis only for VIQ ($p<0.04$). However, when a General Linear Model was used only the SES resulted significantly related to IQ scores (VIQ $p<0.01$, PIQ $p<0.02$, FSIQ $p<0.01$).
Conclusions: Our results indicate that an initial L-T4 dose between 10 and 15 µg/kg/day allows to achieve a normal IQ at the age of 8 years. The socioeconomic status seems to be the major factor affecting the intellectual outcome of CH patients.

P1-d3-401 Thyroid 1

Short-term memory in children with congenital hypothyroidism

Olga Chikulaeva[1]; Olga Bezlepkina[1]; Olga Semenova[2]; Tatiana Vadina[1]; Valentina Peterkova[1]

[1]Endocrinology Research Center, Institute of Pediatric Endocrinology, Moscow, Russian Federation; [2]Russian Academy of Education, Institute of Developmental Phisiology, Moscow, Russian Federation

Background: IQ of children with congenital hypothyroidism (CH) in case of timely started therapy is normal. However several patients experience difficulties at school with learning new material. We assume that these difficulties might due to peculiarities of developmental cognitive functions and first of all due to short-term memory state.
Objective and hypotheses: The aim of our study was to examine the state of short-term memory in children with CH.
Methods: Assessment of short-term memory was carried out by analyzing the process of ability to remember verbally described and visually presented subjects – words and figures - 5 for children 5-8 yrs and 6 for children 9-11 yrs. Thirty one children with CH (aged 7.5 yrs; 5.0, 11.9), with early start of treatment (started during first three weeks after birth), were examined. Prior to and during the investigation all patients received adequate L-thyroxin therapy (2.8 ± 0.7 mcg/kg/day). Control group of 79 children (aged 7.8 yrs; 5.1, 10.9) with no developmental disorders and behavior deviations were studied.
Results: Children with CH showed decrease in short-term visual memory in 63% of cases and decrease in audio verbal memory in 60% of cases. Combined decreases in audio verbal and visual memory were discovered in 43.3% of cases. IQ of children with CH was 110.3 ±16.4. We found out reliable correlation among memory decrease and decrease in IQ ($r = 0.5$, $p = 0.029$). Comparison analysis let to discover significant differences in over learning of investigating groups. Children with CH in comparison with healthy peers showed low volume of presentation words and figures ($p < 0.05$), that characterized the volume of short-term memory in children with CH.
Conclusions: Children with CH have significant decrease in short-term memory even with timely started replacement therapy.

P1-d3-402 Thyroid 1

"Block-and-replace" method in pediatric Graves' disease

Maria Cristina Vigone[1]; Elena Peroni[2]; Arianna Passoni[1]; Giulia Maria Tronconi[2]; Sarah Rabbiosi[2]; Giuseppe Chiumello[2]; Giovanna Weber[2]
[1]IRCCS San Raffaele Scientific Institute, Department of Pediatrics, Endocrine Unit, Milano, Italy; [2]Vita Salute San Raffaele University, Department of Pediatrics, Endocrine Unit, Milano, Italy

Background: The "block-and-replace" method is still widely discussed in literature and comprises an association between high dose of antithyroid drugs (ATD) and L-thyroxine (L-T4) in order to block hormone synthesis and ensure a state of euthyroidism secondary to L-T4 replacement.
Objective and hypotheses: To compare efficacy of ATD+L-T4 therapy versus monotherapy in pediatric patients with unstable Graves' Disease (GD).
Methods: 25 pediatric patients (23 female) diagnosed with GD at our center (average age at diagnosis: 8.6 years) with unstable response to ATD (elevated TSH and fT3, and low fT4 levels) were treated with ATD+L-T4 therapy. For each patient we evaluated: 1) percentage of state of hyperthyroidism, hypothyroidism and euthyroidism in both monotherapy with ATD and ATD+L-T4 therapy; 2) remission rate after combination therapy and efficacy of ATD+L-T4 therapy to postpone a definitive treatment; 3) onset of side effects during combination therapy.
Results:
1)

	Hyperthyroidism: major relapse (↓↓TSH, ↑↑fT3, ↑↑fT4)	Hyperthyroidism: minor relapse (↓↓TSH, ↑↑fT3, =fT4)	Hypothyroidism	Euthyroidism
ATD	14,05% ± 12,74	11,08% ± 17	27,77% ± 27,58	47,1% ± 30,94
ATD+L-T4	1,84% ± 4,1	3,56% ± 10,77	6,7% ± 9,04	87,84% ± 18,59
P-value	< 0,001	n.s. (0,075)	0,001	<0,001

2) 1 patient (4%) went into remission with the ATD+L-T4 therapy; 15 patients (60%) required a definitive therapy (4 radioiodine, 11 surgery). In the 4 patients undergoing radioiodine, the ATD+L-T4 therapy has delayed the administration of radioisotope for 4,9 years. In the 11 patients who underwent thyroidectomy, ATD+L-T4 therapy has delayed surgery for 2,9 years.
3) No serious side-effects during the ATD+L-T4 therapy were observed.
Conclusions: "Block-and-replace" therapy is indicated in those cases in which autoimmune hyperthyroidism is difficult to manage, when it is necessary to postpone both the use of radioactive iodine or surgery to a more appropriate age in order to contain the risks that these procedures entail.

P1-d3-403 Thyroid 1

Subclinical hypothyroidism in obese children and metabolic implications

Antonella Petri[1]; Giulia Genoni[1]; Flavia Prodam[2]; Silvia Parlamento[1]; Caterina Balossini[1]; Erica Pozzi[1]; Roberta Ricotti[1]; Valentina Agarla[1]; Simonetta Bellone[1]; Gianni Bona[1]
[1]A. Avogadro University, Division of Pediatrics, Department of Health Sciences, Novara, Italy; [2]A. Avogadro University, Division of Pediatrics, Department of Health Sciences; Endocrinology, Department of Translational Medicine, Novara, Italy

Objective and hypotheses: Aim of this study was to assess the prevalence of subclinical hypothyroidism in a paediatric population of overweight and obese children and its association with metabolic parameters and with metabolic syndrome (MS).
Methods: Clinical and metabolic evaluations in 600 overweight and obese children and adolescents (310 females, 290 males, 260 prepubertal, 240 pubertal, mean age: 10.7 ± 3.1 years) were performed. MS was defined according to paediatric NCEP-ATPIII criteria. TSH levels were evaluated both in quartiles and according to a fixed cut-off level (pathological value > 3.500 µUl/ml). The presence of autoimmune thyroiditis was assessed in those subjects with abnormal TSH values.
Results: 75 subjects (12.5%) had abnormal TSH levels and 10 of them (13.3%) showed antithyroid antibodies. Age decreased ($p<0.0001$) and the number of prepubertal children increased ($p<0.006$) with the rise of TSH levels. non-HDL cholesterol ($p<0.03$), triglycerides ($p<0.0001$), PNFI index ($p<0.0001$), fasting glucose ($p<0.04$), HOMA-IR ($p<0.04$), glucose peak ($p<0.03$) and glucose area ($p<0.01$) during OGTT increased with the rise of TSH levels. No differences in the prevalence of MS were found among the TSH quartiles and in subjects with altered TSH levels. A negative correlation between age and TSH levels was found (â= -0.122; $p<0.003$). Furthermore a positive correlation between TSH and non-HDL cholesterol (â=0.095; $p<0.02$), triglycerides (â=0.232; $p<0.0001$), PNFI (â=0.185; $p<0.0001$) and fasting glucose (â=0.107; $p<0.01$) was shown independently of confounding factors.
Conclusions: Subclinical hypothyroidism and autoimmune thyroiditis are frequent in overweight and obese children. The association between TSH levels and metabolic parameters suggests a role of the hypothalamus-pituitary-thyroid axis in the regulation of lipids and glucose metabolism. Conversely the absence of an association with the metabolic syndrome suggests that this axis may modulate specific metabolic alterations.

P1-d3-404 Thyroid 1

Familial brain-lung-thyroid syndrome due to a new *NKX2-1* mutation p.Q172L causing disabling benign hereditary chorea

Stefanie Graf[1]; Nemya Bösch[2]; Sara Bachmann[1]; Urs Zumsteg[1]; Karl Heinimann[2]; Gabor Szinnai[1]
[1]University Children's Hospital Basel, Paediatric Endocrinology, Basel, Switzerland; [2]University Children's Hospital Basel, Medical Genetics, Basel, Switzerland

Background: Brain-lung-thyroid syndrome is characterized by the triad of congenital hypothyroidism (CH), benign hereditary chorea (BHC) and surfactant deficiency syndrome. It is caused by mutations in the homeobox containing transcription factor NK homeobox 2 / thyroid transcription factor 1 gene (*NKX2-1/ TITF1*).
Objective and hypotheses: Description of the variability of the neurologic phenotype.
Methods: Case report and direct sequencing of *NKX2-1*.
Results: A term newborn was detected by the neonatal screening with increased TSH (17 mU/L). The confirmatory laboratory test revealed subclinical CH (TSH 25 mU/L, normal fT4) in the context of normal thyroid morphology. L-T4 was started immediately. Family history revealed that the patient's father was suffering from CH due to athyreosis. L-T4 had been started at day 6 of life. Despite normal TSH and T4 under substitution, he developed progressive hypotonia from 10 month of life on, evolving to severe choreoathetotic cerebral palsy until the age of 5 years. The father is non-ambulatory and wheel chair dependent. The combination of BHC and CH suggested brain-lung-thyroid syndrome without pulmonary disease. Direct sequencing of *NKX2-1* revealed a new heterozygous missense mutation (c.515A>T, p.Q172L) in father and daughter. The mutation lies within the homeodomain in exon 3. Pathogenicity of the mutation is further supported by *in silico* analysis. Genetic counselling of the family has taken place. Under close neurological follow-up, the daughter showed slight hypotonia only at 12 months. No pulmonal abnormalities occurred until now.
Conclusions: Our case report illustrates how haploinsuffficiency of a new *NKX2-1* mutation resulted in an unusually late onset of hypotonia evolving to particularly severe BHC. Thus, unexplained hypotonia in patients with initially isolated CH despite adequate substitutive therapy should evoke *NKX2-1* mutations before onset of BHC.

P1-d3-405 Thyroid 1

Transient neonatal hypothyroidism resulting from maternal ingestion of a traditional Korean seaweed soup

Tony Hulse
Evelina Childrens Hospital, Department of Paediatric Endocrinology, London SE1 7EH, United Kingdom

Background: Neonatal hypothyroidism secondary to neonatal exposure to iodine containing medication or skin preparation has been well described. Hypothyoidism resulting from maternal ingestion of high iodine containing food through breast milk is less well recognised.
Objective and hypotheses: A male infant presented at 21 days of age with unconjugated hyperbilirubinaemia starting at the third day of life. Both his parents originated from South Korea.
Methods: Investigation showed an unconjugated bilirubinaemia of 346

umol/l, TSH 87.3 IU/l [0.27-4.2], free T4 7.3 pmol/l[12-22] thyroglobulin 250 mg/l. The neonatal TSH screen had been normal and this was confirmed on a repeat sample. An I123 thyroid scan showed a normally positioned, slightly enlarged thyroid.
Results: He was treated with thyroxine until the age of 2 years when treatment was successfully discontinued. It then emerged that from birth his mother had consumed 3 to 4 bowls a day of a traditional seaweed soup called ‚Miyeok Guk' made from brown seaweed (Undaria Pinnatifidia) and thought to improve lactation. This can result in an iodine intake of over 2000ug/day.
Conclusions: There seems little doubt that this moderately severe, later onset neonatal hypothyroidism resulted from maternal ingestion of large amounts of iodine derived from the seaweed soup and transmitted via breast milk. This is an expression of the acute thyroid suppression from the Wolff-Chaikoff effect. This possibility needs to be considered when disorders of thyroid function are seen in babies of Korean mothers.

P1-d3-406 Turner Syndrome 1

Effects of karyotype on birth size, pretreatment growth, and response to GH treatment (GHTx) in Turner syndrome (TS): data from 1383 patients followed in a global observational study

Charmian A Quigley[1]; Judith L Ross[2]; Cheri L Deal[3]; Alan G Zimmermann[4]; Christopher J Child[5]; Werner F Blum[6]
[1]Lilly Research Laboratories, Endocrinology, Indianapolis, United States; [2]Thomas Jefferson University, Pediatric Endocrinology, Philadelphia, United States; [3]Hopital Ste Justine, Pediatric Endocrinology, Montreal, Canada; [4]Lilly Research Laboratories, Statistics, Indianapolis, United States; [5]Lilly Research Laboratories, Endocrinology, Windlesham, United Kingdom; [6]Lilly Research Laboratories, Endocrinology, Bad Homburg, Germany

Background: TS phenotype varies greatly, at least in part due to karyotype, which ranges from complete loss of the 2nd sex chromosome (45X) to mosaic karyotypes (45X + 2nd cell line [46XX;46XY;47XXX;other], or structural rearrangements of 2nd X). While 45X is generally regarded as the most "severe" form of TS, data on karyotype-related differences in growth & response to GH are limited.
Objective: To evaluate karyotype effects on birth size, preTx height(H) & response to GH (1st-yr & near-adult height [NAH]) in TS.
Methods: We assessed relationships between karyotype & growth in 1383 pts with TS. Differences among karyotype groups overall, & pairwise differences between 45X & each other group, were assessed by ANOVA. ANCOVA models were developed to assess factors (e.g. karyotype, baseline age, HSDS, target HSDS [THSDS]) associated with 1st-yr height gain & gain from baseline to NAH (HV<2cm/yr or bone age ≥14yr).

Group; Number	45X; 623	45X/46XX; 184	45X/46XY; 46	45X/47XXX-; 52	Mosaic other; 28	Isochromosome X; 229	Ring X; 111
Birth weight SDS for gestational age	-0.9±1.3	-0.6±1.3*	-0.9±1.1	-1.0±1.1	-0.8±1.0	-1.1±1.3	-1.2±1.3†
Birth length SDS for gestational age	-1.2±1.6	-0.8±1.6*	-1.5±1.6	-1.2±1.5	-0.3±1.7*	-1.4±1.6	-1.4±1.4
Age at GH start (yr)	8.3±4.0	9.6±3.7*	10.3±3.5*	9.4±3.8†	10.1 ±3.5†	9.6±3.7*	8.4±3.5
PreTx height SDS	-2.6±1.0	-2.5±1.0	-2.7±0.9	-2.4±0.9	-2.5±0.9	-2.9±0.9*	-2.7±0.9
Height SDS-target height SDS	-2.6±1.1	-2.2±1.1	-2.5±1.0	-2.3±1.0	-2.6±1.1	-2.9±1.0	-2.8±1.2
Number at 1yr	283	93	18	27	15	120	43
1st-yr height SDS gain	0.47 ±0.36	0.49±0.34	0.36±0.44	0.44±0.38	0.49 ±0.32	0.47±0.34	0.52±0.49
Number at NAH	166	56	12	16	12	70	24
Age at NAH (yr)	16.5±1.5	16.0±1.8†	16.8±1.6	15.3±1.6*	16.5±1.6	16.7±1.5	15.9±1.6
Duration GH (yr)	6.1±2.9	4.2±2.5*	6.2±3.1	4.2±2.5*	4.6±1.9	5.8±2.4	5.4±2.4
NAHSDS	-1.6±0.9	-1.9±1.1	-1.5±1.1	-1.7±0.9	-1.8±0.8	-1.8±1.0	-1.6±0.9
Height SDS gain at NAH	1.1±0.9	0.7±1.0*	1.2±1.0	0.6±1.1†	1.1±0.9	1.1±0.8	1.1±0.7

Footnotes: data are mean±SD; SDS=standard deviation score; NAH=near-adult height; ~and variants;*P<0.01 vs 45X; †P<0.05 vs 45X; 46XXp-, isodicentric X, Y-containing karyotypes not shown (≤25 pts each)

Results: All karyotype groups were relatively small at birth, but 45X/46XX & other mosaics were not as small as 45X. Isochromosome X was shortest at GH start. There were no significant differences among karyotypes for GH dose (0.31±0.08 mg/kg/w overall). 1yr GHTx data were available for 648 pts & NAH data for 388. Mean 1yr HSDS gain was greatest for pts with Y-containing karyotypes (0.9±0.4 SDS;N=9, not shown) but no other significant differences were seen. At NAH GHTx duration was shorter for 45X/46XX & 45X/47XXX so HSDS gain was lower, but NAHSDS not significantly different. By ANCOVA significant factors for NAH were shorter preTx HSDS, taller THSDS & longer GHTx. Karyotype was not significant.
Conclusions: Prenatal growth was less impaired with mosaic karyotypes vs 45X. However HSDS at GH start was similar across karyotypes, likely due to referral/selection bias for GH Tx. Karyotype did not appear to significantly affect GH response at 1st yr or NAH.

P1-d3-407 Turner Syndrome 1

Patients with Turner syndrome and isolated SHOX deficiency have similar bone geometry at the radius

Ondrej Soucek[1]; Jan Lebl[1]; Jirina Zapletalova[2]; Dana Novotna[3]; Ivana Plasilova[4]; Stanislava Kolouskova[1]; Dana Zemkova[1]; Miloslav Rocek[5]; Katerina Hirschfeldova[6]; Zdenek Sumnik[1]
[1]2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Department of Paediatrics, Prague, Czech Republic; [2]Faculty of Medicine, Palacky University and University Hospital Olomouc, Department of Paediatrics, Olomouc, Czech Republic; [3]Faculty of Medicine, Masaryk University and University Hospital Brno, Department of Paediatrics, Brno, Czech Republic; [4]Faculty of Medicine, Charles University in Prague and University Hospital Hradec Kralove, Department of Paediatrics, Hradec Králové, Czech Republic; [5]2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Department of Radiology, Prague, Czech Republic; [6]1st Faculty of Medicine, Charles University in Prague and General University Hospital, Institute of Biology and Department of Medical Genetics, Prague, Czech Republic

Background: Girls with Turner syndrome (TS) have altered bone mineral density and geometry at the radius, which may pose an increased fracture risk in adulthood. The etiology of their low bone strength remains unknown. Since short stature homeobox (SHOX) gene plays a major role in long bone growth and patients with SHOX deficiency share some skeletal features with TS, SHOX gene has been suggested as a causal one.
Objective and hypothesis: We hypothesized that bone geometry and mineral density is similar between girls with TS and patients with isolated SHOX deficiency.
Patients and methods: Fourteen patients with SHOX deficiency (median age 11.8 yrs, range 6.0-36.8, 10 children and 4 adults) were examined by peripheral quantitative CT (pQCT) at the non-dominant forearm. Results were expressed as Z-scores using published reference data and then compared to the results of 67 girls with TS (median age 13.7 yrs, range 6.0-19.4). The differences from reference data were tested by one-sample T test and differences between TS and SHOX deficiency were tested by two-sample T test.
Results: Trabecular volumetric bone mineral density (vBMD) was decreased in TS (mean Z-score -0.7±1.3, p<0.001) but normal in isolated SHOX deficiency (Z-score 0.3±1.3, n.s., group difference p<0.01). Cortical vBMD was low in both groups (Z-score -1.6±1.5, p<0.001 and -1.9±2.3, p<0.01, respectively) without significant group difference. Both groups had increased total bone cross-sectional area (CSA) at the diaphysis (Z-score 0.7±1.7, p<0.01 and 2.3±1.6, p<0.001, respectively), but normal cortical bone CSA (0.0±1.0, n.s. and -0.7±2.3, n.s., respectively).
Conclusions: Patients with Turner syndrome and isolated SHOX deficiency have similar bone geometry at the radius. Our results support the hypothesis that skeletal abnormalities in patients with TS are caused by SHOX deficiency. Low trabecular vBMD in TS is probably a consequence of hypogonadism due to ovarian failure.

P1-d3-408 Turner Syndrome 1

Cardiovascular assessment using echocardiogram and MRI in Turner syndrome may provide complementary information

Sze Choong Wong[1]; Sarah Ehtisham[2]; Michael Cheung[3]; Margaret Zacharin[1]
[1]The Royal Children's Hospital, Department of Endocrinology, Melbourne, Australia; [2]Royal Manchaster Children's Hospital, Department of Endocrinology, Manchaster, United Kingdom; [3]The Royal Children's Hospital, Department of Cardiology, Melbourne, Australia

Background: Recent studies suggest that assessment with MRI is gold standard for evaluation of aortic dimensions and aortic valve leaflets.
Aims: To describe abnormalities detected on echocardiogram and cardiac MRI in adolescents and adults with TS where routine surveillance with MRI is undertaken at 10 years of age or earlier if clinically indicated.
Methods: Patients were included if both echocardiogram and MRI were performed. Aortic dimensions were adjusted for body surface area (BSA), aortic sized index (ASI). Results reported in median (range).
Results: The study included 50 patients(21XO, 24mosaic, 5Y-positive. 8/50(16%) had a known past history of coaractation of the aorta at the time of MRI. 17/50(34%)had structural abnormalities on echocardiogram. 24/50(48%)had structural abnormalities on MRI. Twenty patients (40%) had abnormalities detected solely on MRI. Nine of those patients (18%) had clinically significant abnormalities: coarctation (n=5), bicuspid aortic valve (n= 2), coarctation and bicuspid aortic valve (n=2). Twelve patients (24% of whole cohort)had abnormalities detected solely on echocardiogram. Four of these (8% of whole cohort) had clinically significant abnormalities: bicuspid aortic valve(n=4). Using MRI measurements, median ASI aortic root(n=9) was 1.77 cm/m2(1.17, 2.21). 2/9(22%)had ASI aortic root>2 cm/m2.Median ASI ascending aorta(n=31)was 1.50 cm/m2(0.97, 2.19). 2/31(6.5%) had ASI ascending aorta>2.0 cm/m2. Median ASI aortic arch(n=31)was 1.20cm/m2(0.93, 2.4).1/31(3%)has ASI aortic arch> 2.0cm/m2. Absolute ascending:descending aorta dimensions(A:D)for 29 patients with measurements available was 1.43(1.06, 2.30).10/29(35%) had A:D>1.5.
Conclusion: 18% of adolescents and women with TS had significant cardiac abnormalities missed if assessed using echocardiogram alone but 8% had abnormalities missed if only assessed using MRI. Comprehensive cardiac evaluation in TS should include echocardiogram and cardiac MRI as they may provide complementary information.The relationship between the various aortic dimensions with clinical outcome is unclear and deserves future studies.

P1-d3-409 Turner Syndrome 1

Turner syndrome detection using multiplex ligation dependent probe amplification (MLPA): preliminary data

Anna Grandone[1]; Federica Messa[1]; Ruggero Coppola[1]; Adalgisa Festa[1]; Raffaella Nacca[1]; Rosaria Gaeta[1]; Manuela Rinaldi[1]; Rosaria Marotta[1]; Francesca Del vecchio blanco[2]; Marco Savarese[2]; Vincenzo Nigro[1]; Giuseppina De Luca[2]; Emanuele Miraglia del Giudice[1]; Lucia Perone[2]; Laura Perrone[1]
[1]Seconda Università degli Studi di Napoli, Pediatrics, Naples, Italy; [2]Seconda Università degli Studi di Napoli, Dipartimento di Patologia Generale, Naples, Italy

Background: Turner syndrome (TS) is one of the most common genetic conditions affecting females, with an incidence of one in 1500–2000 live births. TS occurs when an entire X-chromosome is deleted, a portion of an X-chromosome is deleted, or the X-chromosome is deleted in a subset of cells (TS mosaicism). Clinical features include primary hypogonadism, renal abnormalities, structural cardiac problems, and short stature, but with early diagnosis and initiation of GH therapy, normal or near-normal adult stature can be achieved. It is estimated that one in 50–100 girls with short stature have TS. Experts in the field recommend that short girls be tested for this condition. Unfortunately, many girls with TS are not diagnosed until after 10 yr of age. At the moment, cytogenetic analysis by karyotype is the standard test used to diagnose TS. Karyotype analysis is labor intensive and is impractical for large-scale population. In contrast, multiplex ligation-dependent probe amplification (MLPA) based on relative quantification of different DNA target sequences in a single reaction is a quantitative method, faster and cheaper.
Objective and hypotheses: We aimed to apply MLPA technique to detect X monosomy and compare the results to conventional karyotyping.
Methods: We studied 33 females by MLPA and conventional cytogenetic. The MLPA kit (SALSA P095), used to detect the most frequent aneuploidies, includes 36 genomic targets, eight probes for chromosomes 13, 18, 21 and X, and four probes for Y chromosome (MRC-Holland, Amsterdam, The Netherlands).
Results: 10 females had mosaic Turner syndrome and all were detected by MLPA. 23 patients had normal karyotype and only one had a doubtful MLPA result. Sensitivity of MLPA was 100% and specificity 95,6%.
Conclusions: We suggest that MLPA could represent a rapid, economic, automated, reliable and accurate method to diagnose Turner syndrome in girls with short stature, not requiring culturing cells. Of course, larger samples are needed.

P1-d3-410 Turner Syndrome 1

Determination of BMP15 gene dosage in a woman with TS and spontaneous menarche

Annalisa Nicoletti; Laura Mazzanti; Soara Menabò; Emanuela Scarano; Federica Tamburrino; Giuseppe Cangemi; Piero Pirazzoli; Lilia Baldazzi
S. Orsola Malpighi Hospital, University of Bologna, Pediatric Clinic, Endocrine Unit, Bologna, Italy

Background: Chromosome X genes haploidy is the basis of phenotype of Turner Syndrome with 45,X karyotype, then it is reasonable to assume that patients non 45,X (mosaicism), may have partial X chromosome genes diploidy that can result in a milder phenotype. A "critical region" for ovarian development and function and a "POF critical region" have been mapped in Xq13.3 to Xq27 and in Xp11.2 respectively. Gonadal dysgenesis and oocyte loss occurring in TS are probably due to monosomy in genes located in these regions, therefore a double dosage of these genes may be associated to spontaneous menarche reported in mosaic- and non mosaic cases.
Objective and hypotheses: In order to find a possible correlation with gene dosage and phenotype, we select BMP15 (Xp11.2), a candidate gene for POF, to determine if a normal BMP15 gene dosage can be responsible of spontaneous menarche in TS in combination with karyotype specificity.
Methods: In 20 TS patients with spontaneous menarche, MLPA analysis was performed on genomic DNA, using KIT P018 (MCR Holland), with the addiction of two "home made" specific probes for BMP15 gene.
Results: 12 pts resulted haploid for BMP15 gene, 2 pts resulted diploid (according to karyotype), in 6 pts a BMP15 dosage was > 1 allele for the presence of mosaicism.
Conclusions: MLPA analysis resulted a powerful test for gene copy number determination, in addition to probe specificity, it allows the simultaneous analysis of several probes of a chromosomal section. Most of the patients resulted with the expected number of copies of BMP15 gene with a good correlation with karyotype. Given the high number of patients haploid for BMP15 gene (12/20), it seems that the dosage of this gene does not have a primary role in determining the menarche, or that it is not the variation in copy number the genetic basis of mechanism of BMP15 gene dosage. The presence of mosaicism and perhaps trans acting factors on X chromosome should be deeply investigated.

P1-d3-411 Turner Syndrome 1

Growth outcome of Turner girls with 45X/46XY mosaicism after GH therapy

Franciska Verlinde[1]; Muriel Thomas[1]; Sylvie Tenoutasse[2]; Raoul Rooman[3]; Jean De Schepper[4]
[1]BSGPE, Belgium; [2]HUDERF, Pediatric Endocrinology, Brussels, Belgium; [3]UZA, Pediatric Endocrinology, Antwerp, Belgium; [4]UZ Brussel/UZ Gent, Pediatric Endocrinology, Brussels/Ghent, Belgium

Background: About 4% of the girls with Turner syndrome (TS) in Belgium have a 45X/46XY karyotype. Little is known about the possible influence of the presence of the Y chromosome on the growth response to GH therapy.
Objective and hypotheses: To compare the adult height (AH) of TS girls with 45X/46XX mosaicism and girls with a 45X/46XY constitution.
Methods: The growth data of TS patients with a 45X/46XX and a 45X/46XY karyotype, who had attained AH after a standardized GH therapy (50 µg/kg.day) with at least one year of GH treatment before puberty induction with ethinyloestradiol, were retrieved from the database of the BSGPE. Height dat are expressed for National References (NR). Corrected mid parental height

(CMPH), changes in height (cm and SDS) and remaining height defeicit (RHD)(CMPH-AH) were calculated Data are represented as median (range).
Results:

	45X/46/XY (N = 12)	45X/46XX (N = 6)	P value
Age at start GH (yr)	13.4 (4.2 - 15.8)	12.7 (9.6-19.1)	0.925
Height at start GH (cm)	133.7 (93.6 - 148.0)	129.0 (117.4 -153.0)	0.708
Height at start SDS (NR)	-3.0 (-5.0 - -1.07))	-3.49 (-4.88 - -2.25)	0.399
Duration of GH therapy (yr)	4.9 (1.5 -11.8)	3.4 (1.8 -6.6)	0.261
Age at AH (yr)	17.6 (5.0)	17.0 (6.4)	0.925
AH (CM)*	158.5 (146.8 - 164.7)	147.3 (137.8 - 159.0)	0.025*
AH SDS (NR)	-1.31 (-3.33 - -0.27)	-2.87 (-4.78 - -1.28)	0.039*
Age at start EE2 (yr)	15.4 (12.6 - 17.8)	15.1 (14.0 -20.5)	0.874
Height at start EE2 (cm)	151.7 (137.2 - 154.8)	141.4 (135 - 158)	0.399
Height SDS (NR) at start EE2	-2.35 (-4.39 - -0.57)	-3.24 (-4.67 - -1.44)	0.246
CMPH SDS (NR)	-0.38 (-2.38 - 1.5)	-1.29 (-3.13 - 0.37)	0.190
AH SDS - CMPH SDS (NR)	-1.04 (-3.33 - 0.48)	-1.66 (-3.07 - 0.42)	0.160
Delta height (cm)	21.3 (9.1 - 70.4)	18.3 (6.0 - 27.7)	0.349
Delta height SDS (NR)*	1.74 (0.16 - 3.16)	0.72 (-0.8 - 1.44)	0.031*
RHD (cm)*	5.8 (-2.8 - 14.3)	10.8 (-2.5 - 18.6)	0.044*
Delta height before start EE2 (cm)	14.8 (5.6 - 60.8)	13.7 (5.7 - 24.1)	0.640
Delta height after start EE2 (cm)*	5.9 (1.1 - 11.7)	2.5 (0.3 - 6.4)	0.031*

Conclusions: Compared with 45X/46XX patients, GH treated 45X/46XY Turner girls had a greater height increase, especially after the start of puberty induction, and a smaller RHD, suggesting that the presence of the Y chromosome might improve the response to GH in Turner girls. Confirmation of these results in larger groups of patients is indicated.

P1-d3-412 Turner Syndrome 1

Ovarian tissue cryopreservation in girls with Turner syndrome: feasibility and acceptability

Lise Duranteau[1]; Sylvie Beaudoin[2]; Helene Martelli[3]; Catherine Poirot[4]; Pierre Bougneres[1]
[1]Hopitaux Paris Sud, Bicêtre and University Paris Sud, Pediatric Endocrinology and National Reference Center for DSD, Le Kremlin Bicêtre, France; [2]Hopital Necker Enfants-Malades and University René Descartes, Pediatric Surgery, Paris, France; [3]Hopitaux Paris Sud, Bicêtre and University Paris Sud, Pediatric Surgery, Le Kremlin Bicêtre, France; [4]Groupe Hospitalier Pitié-Salpétrière and University Pierre et Marie Curie, Reproductive Biology Unit, Paris, France

Background: Ovaries of children and adolescents with Turner syndrome (TS) are considered by most pediatric endocrinologists to contain a minimal number of oocytes if any, precluding attempts to collect and store gonadal tissue for future procreation. There are clinical data in the literature suggesting that a yet undetermined proportion of Turnerian ovaries could harbor follicles.
Objective and hypotheses: We have started a clinical study which primary aim is to test if cryopreservation of ovarian tissue could allow fertility in young women with TS. The short term objective is to assess the feasibility of the procedure, the demand and the medical characteristics of TS patients volunteering for the protocol.
Methods: 18 girls with TS aged from 4 to 18 years referred in our center by pediatric endocrinologists for ovarian preservation were included. Clinical examination, blood samples for hormonal measurements and surgical procedure, were performed according the protocol; the tissue is shipped to an expert lab to be prepared in strips and freezed for cryopreservation; acceptability of the procedure was assessed through self-questionnaires
Results: -14/18 of girls included are of puberty age with spontaneous puberty in 9/14 (64%); - karyotypes were: 45X, 3; 10 mosaicisms with absence or structural anomalies in one of the X chromosome; 5 mosaicisms with complete chromosomes. -serum FSH range from 4 to 109 UI/L; AMH concentrations were measurable (>2 pmol/L) in 7/18 girls; - the number of strips made from the ovarian tissue varies from 3 to 23. None of the girls had any complications with surgery. In general, the procedure was considered as slightly aggressive
Conclusions: Girls with TS volunteering for fertility preservation are mostly of post-pubertal age with spontaneous puberty. Surgery was not complicated and the overall procedure was considered as well-accepted by the girls with TS.

P2-d2-413 Adrenals and HPA Axis 2

Early pubertal onset in congenital adrenal hyperplasia due to 21-hydroxylase deficiency

Marie Noufi Barhoum[1]; Yardena Tenenbaum Rakover[2]
[1]Clalit HMO, Pediatric endocrinology, Nahariya, Israel; [2]HA'Emek MEDICAL Center, Pediatric endocrinology, Afula, Israel

Background: Diminished cortisol synthesis in patients with congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency results in increased ACTH secretion that enhances adrenal androgen production.Hyperandrogenism in childhood may cause premature pubarche,accelerated growth and consequently short final height. Whether patients with CAH exhibit early true puberty is a matter of debate.
Objective and hypotheses: The aim of the current study was to assess pubertal characteristics and growth patterns in children with CAH.
Methods: Fifty-five subjects (33F/22M) with CAH (22,salt wasting (SW);15,simple virilizing (SV);18,non classical (NC)) were enrolled. The design was a retrospective longitudinal study. All subjects underwent genetic analysis of the CYP21 gene.
Results: The most common mutation in the entire group was V281L (22%) followed by I2splice (21%), I172N (14%), 8del (11%) and others (32%). The mean age of onset of pubarche was earlier in females 5.6±2.3 years (1.9-10.0) than in males 7.3±2.4 years (1.8-11.3) in all forms of CAH. Onset of pubarche was earlier in SV (5.0±2.6 years) than in SW (6.9±1.6 years) and NC (7.1± 2.4 years) forms. In females, despite early onset of telarche 8.9±2.1 years,the mean age of menarche was within the normal range 12.5±1.9 years,though highly variable 9-16.5 years. High variability in onset of gonadarche was shown in males as well 10.5±1.5 years. Seven patients were treated with LHRH-analogs due to early onset of puberty. Low final height was shown in both females 155.7±9.0 cm and males 164.4±8.0 cm.
Conclusions: Our results indicate that onset of puberty is earlier in children with CAH despite early initiation of therapy. Patients with SV form are at higher risk for earlier pubarche and advanced bone age(BA).The earlier timing of pubertal onset plays a critical role in final height attainment. Earlier diagnosis in children with SV form, stricter control and future medical therapy such as aromatase inhibitors may delay pubertal onset and improve the final height attainment in these patients.

P2-d2-414 Adrenals and HPA Axis 2

Prevalence and clinical presentation of adrenarche in healthy prepubertal children

Aino Mantyselka[1]; Jarmo Jaaskelainen[1]; Virpi Lindi[2]; Timo Lakka[2]
[1]Kuopio University Hospital and University of Eastern Finland, Department of Pediatrics, Kuopio, Finland; [2]University of Eastern Finland, Department of Physiology, Kuopio, Finland

Background: Adrenarche refers to the onset of increased production of adrenal androgens, mainly DHEAS. Clinical signs of adrenarche include adult type body odor, oily hair, comedones, acne, and pubarche. Adrenarche is defined premature (PA) if clinical signs appear before the age of 8 years in girls and 9 years in boys and serum DHEAS concentration is ≥ 1 µmol/l. The prevalence and presentation of adrenarche in prepubertal children is not well known.
Objective and hypotheses: Our objective was to examine the prevalence and clinical presentation of adrenarche in a population based sample of prepubertal healthy Finnish children aged less than 9 years. We hypothesized that the prevalence of adrenarche is higher than generally thought in boys when studied in a population based setting.
Methods: Healthy prepubertal children (209 girls and 228 boys; age ± SD 7.6 ± 0.4 years and 7.6 ± 0.4 years, NS) taking part in the Physical Activity and Nutrition in Children (PANIC) Study were included in this study. Clinical signs of adrenarche were assessed by a physician. Serum DHEAS concentration was determined by enzyme immunoassay.
Results: In all studied children, girls had more often one or more signs of androgen action (24.5 vs. 9.6 %; $p<0.001$) but biochemical adrenarche was less common in girls than in boys (8.1 % vs. 16.7%; $p = 0.045$). Only four girls (1.9%) and none of the boys had pubarche. Total prevalence of PA was 4.7 % (8.6% in girls and 1.8 % in boys)
Conclusions: Though clinical signs of androgen action are more common in prepubertal girls than in boys, biochemical adrenarche is more common in boys. Thus, girls are more sensitive to adrenal androgens. This difference may be explained by their more active androgen receptor or by modulation of other hormones and factors, including gonadal steroids.

P2-d2-415 Adrenals and HPA Axis 2

The frequency of mutations in the CYP21A2 gene in the general population of Cyprus

Vassos Neocleous[1]; Alexia AP Phedonos[1]; Christos Shammas[1]; Meropi Toumba[2]; Charilaos Stylianou[3]; Elena Andreou[4]; Michalis Picolos[5]; Tassos C Kyriakides[6]; Nicos Skordis[2]; Leonidas A Phylactou[1]

[1]The Cyprus Institute of Neurology & Genetics, Molecular Genetics, Function & Therapy, Nicosia, Cyprus; [2]Makarios III Hospital, Paediatric Endocrine Unit, Nicosia, Cyprus; [3]Paphos General Hospital, Department of Paediatrics, Paphos, Cyprus; [4]Makarios III Hospital, Department of Endocrinology, Nicosia, Cyprus; [5]Alithias Endocrinology Center, Endocrinology, Nicosia, Cyprus; [6]Yale University, Department of Epidimiology & Public Health, New Haven, United States

Background: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is the most common autosomal recessive disorder and is mostly attributable to mutations in the CYP21A2 gene. The carrier frequency of CYP21A2 mutations in the general population has been estimated to be 1:25 to 1:10.
Objective and hypotheses: So far the true carrier frequency for CAH due to 21-OHD has not been determined by comprehensive mutation analysis of the CYP21A2 in a specific European population and the present study aims in doing so.
Methods: The present study screened for mutations in the CYP21A2 gene a statistically valid number of 300 clinically asymptomatic subjects (150 males and 150 females) from the general population of Cyprus. The methodology used for the CYP21A2 genotyping involved multiplex ligation-dependent probe amplification (MLPA) and direct sequencing of PCR products of the CYP21A2 gene.
Results: Genotyping of the 600 unrelated alleles from the 300 Cypriot asymptomatic individuals revealed a carrier frequency of 9.83%. The most frequent mutations among the tested subjects of the present study were the mild p.V281L (4.3%), followed by p.Q318stop (2.5%), p.P453S (1.33%), p.V304M (0.83%), p.P482S (0.67%) and p.M283V (0.17%). In conclusion, the detected 9.83% CAH carrier frequency suggests one of the highest prevalence of CYP21A2 carriers reported by a genotyping analysis and the previously described major mutations are found to dominate the mutation spectrum of the Cypriot population. In addition, the rare V304M mutation which to our knowledge was reported only once before in a female patient of Asian origin seems to be quite frequent (0.83%) in the Cypriot asymptomatic population and imply a possible founder effect.
Conclusions: Knowing of the prevalence and the nature of the genetic defects in our population will be of immense help in our understanding and awareness of NC CAH in females presenting with hyperandrogenemia.

P2-d2-416 Adrenals and HPA Axis 2

Idiopathic precocious pubarche and late-onset congenital adrenal hyperplasia: distinctive features

Carla Bizzarri; Francesca Crea; Romana Marini; Danila Benevento; Cappa Marco

Bambino Gesù Children's Hospital, Unit of Endocrinology and Diabetes, Rome, Italy

Background: Precocious pubarche (PP) may reveal late-onset congenital adrenal hyperplasia (LO-CAH) in 5 to 20% of cases, and the ACTH stimulation test is usually recommended in children presenting with PP. This test is expensive and stressful and the results are normal in most cases.
Objective and hypotheses: Aim of our study was to determine clinical and plasma predictors of LO-CAH among children presenting with PP.
Methods: We performed a prospective study including all children evaluated for PP at our Hospital from January 2009 to December 2011 (n° 294, 238 females). Patients with concomitant signs of central precocious puberty (breast development in girls or testicular enlargement in boys) were excluded. All children underwent the ACTH stimulation test. Children showing a post-ACTH 17-hydroxyprogesterone (17-OHP) plasma level >10 ng/ml underwent confirming mutational analysis of the CYP21 gene. The correlations between clinical features and hormonal indicators of LO-CAH were analyzed.
Results: Thirty-three patients (11.2%) had basal 17OHP levels> 2 ng/ml. Fourteen patients (4.76%) showed post-ACTH 17OHP levels> 10 ng/ml (12 children with basal 17OHP> 2 ng/ml and 2 children with basal 17OHP< 2 ng/ml), mutational analysis confirmed LO-CAH in all these children. The clinical and hormonal characteristics of PP and LO-CAH children are showed in table 1 as medians (range).

	PP	CAH	p
number (%)	280 (95.2)	14 (4.8)	
males/females	52/228	4/10	0.315
age at PP onset (years)	6.5 (0.1 - 8.9)	5 (0.1 - 8)	0.004
bone age advance (years)	1.03 (-2.9 - 4.63)	1.64 (0.0 - 5.1)	0.08
height SDS	0.81 (-2.6 - 3.48)	0.74 (-0.75 - 2.59)	0.99
BMI SDS	0.89 (-1.9 - 4.75)	0.03 (-0.94 - 2.26)	0.006
basal 17OHP (ng/ml)	0.8 (0.1 - 6.2)	20.5 (0.9 - 60)	<0.0001
post-ACTH 17OHP (ng/ml)	2.8 (0.3 - 9.0)	47 (10 - 180)	<0.0001
testosterone (ng/dl)	23 (10 - 63.6)	37.1 (19.2 - 101.7)	0.0003
delta-4-androstenedione (ng/ml)	0.59 (0.1 - 2.24)	1.05 (0.38 - 7.2)	0.01

Conclusions: On the average LO-CAH children show significantly elevated basal levels of hormonal precursors and active adrenal androgens, but a wide variability is evident and a few children show normal basal values. Therefore, all children with PP should undergo ACTH stimulation test. No clinical feature at PP onset seems to be a good predictor of LO-CAH. Even though elevated height SDS and bone age advance are relatively common in LO-CAH children, they do not represent distinctive characteristic of LO-CAH. Conversely, a later onset of PP may be predictive of idiopathic PP, in particular when it is associated with higher BMI SDS, overweight or obesity.

P2-d2-417 Adrenals and HPA Axis 2

Early BMI and height growth and exaggerated adrenarche in 7 years old Chilean children

Camila Corvalan[1]; Ricardo Uauy[2]; Veronica Mericq[3]

[1]Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile; [2]London School of Hygiene and Tropical Medicine (UK) and INTA, University of Chile, Santiago, Chile; [3]University of Chile, Institute of Maternal and Child Research, Santiago, Chile

Background: Accelerated weight and height gain in infancy have been associated with adrenarche. However, the exact temporality of these events remains unclear.
Objective: To assess the relationship between early body mass index (BMI) and height growth and DHEAS at 7 years.
Methods: In 975 children (46% girls) of birth weights 2500-4500g we abstracted weight and height 0-4y from health records and measured them annually thereafter. We calculated BMI: weight/heigth2 and defined 5 periods of interest: prenatal, 0-6, 6-24, 24-48 and after 48 months. At 7y we measured DHEAS plasmatic concentrations. We used general lineal models to test associations, adjusting for age and sex.
Results: BMI was over the WHO standards from birth on (0.91 BMI-SDS at 7y) while height was slightly below until 4y and increased thereafter (0.18 height-SDS at 7y). At 7y, mean DHEAS was 35.2±21.8 µg/dl; 19.7% children had ≥50µg/dl. BMI and length at birth were inversely associated with DHEAS at 7y (Table 1). BMI gains, particularly from 2-4y, increased DHEAS levels while only height gain in the 4-7y period had a positive association (Table 1). Children with DHEAS ≥50µg/dl compared to remaining children presented significantly higher BMI from 4y onwards (Dif BMI-SDS at 4y: 0.35 95% CI(0.51-0.19), p<0.05) and higher height beyond 5y (Dif height-SDS at 5y: 0.18(0.32 to 0.03), p<0.05). Analyses did not differ by sex (p>0.05). BMI 95 % CI Height 95% CI are in the Table

Months	Beta	inferior	superior	Beta	inferior	superior
0	-0.11	-0.20	-0.03	-0.16	-0.24	-0.07
0-6	0.03	-0.05	0.11	-0.03	-0.12	0.05
6-24	0.12	0.30	0.21	0.06	-0.03	0.14
24-48	0.19	0.10	0.27	0.15	0.04	0.25
48-84	0.05	-0.04	0.13	0.21	0.12	0.31

Conclusions: In normal birthweight children, smaller size at birth, increased weight gain before 4y, and increased linear growth after 4y were associated with higher DHEAS at 7y; in exaggerated adrenarche increased adiposity precedes and probably leads to subsequent linear growth. Fondecyt #1100206 & 1090252

P2-d2-418 Adrenals and HPA Axis 2

Loss of length in patients with congenital adrenal hyperplasia is associated with elevated hydrocortisone dosage during the first year of life

Abdulsalam Abu-Libdeh[1]; Yaarit Ribak[1]; Yardena Rakover[2]; Naomi Weintrob[3]; Eli Hirshkovitz[4]; David Zangen[1]
[1]Hadassah Hebrew University Medical Center, Division of Pediatric Endocrinology, Jerusalem, Israel; [2]Ha'Emek Medical Center, Pediatric Endocrine Unit, Afula, Israel; [3]Dana Children Hospital, Pediatric Endocrine Unit, Tel Aviv, Israel; [4]Soroka University Medical Center, Pediatric Endocrine Unit, Beer-Sheva, Israel

Background: Decreased final height in congenital adrenal hyperplasia is mainly caused by advanced bone age and compromised pubertal growth. Elevated glucocorticoid levels are associated with decreased chondrocyte proliferation and linear growth.
Objective and hypotheses: We studied the correlation between hydrocortisone (HC) dosage and growth velocity during the fastest growth period the 1st year of life.
Methods: Ethnicity, mutation, clinical phenotype, HC dosage, and growth parameters at 1, 3, 6 and 12 months of age were assembled from 71 patients with salt-wasting CAH at 5 pediatric endocrine centers in Israel. Normal average 1st y growth from the Israeli Ministry of Health registry was used as the control data .
Results: Six-months-old CAH males and females were significantly shorter than controls (2.23cm; p= 0.001, 1.64 cm; p <0.05, respectively). This deficit increased further at 1y of age. A strong negative correlation was found in males between the HC dosage at 3 months and the length at 6 and 12 months and between the HC dosage at 6 months and the length at 12 months of age (r = -0.609, r = -0.517, respectively). A weaker yet statistically significant relation was found between HC dosages at 3-6 months, and length at 6-12 months for the entire group (r = -0.3, p <0.05).
Conclusions: Patients with CAH may lose significant height already at 6-12 months of age. Higher dosages of glucocorticoids are associated with a slower growth velocity during the 1sty of life mainly in boys. Intensive infantile optimization of the HC dosages may improve 1sty growth and final height in CAH patients.

P2-d2-419 Adrenals and HPA Axis 2

Assessment of adrenal function in female-to-male adolescents with gender identity disorder (GID)

Mahalakshmi Gopalakrishnamoorthy[1]; Michal Ajzensztejn[1]; Fionnghuala Mills[1]; Anne Dawnay[2]; Russell Viner[2]; Caroline Brain[2]; Gary Butler[3]
[1]University College London Hospital, Adolescent Endocrinology, London, United Kingdom; [2]University College London Hospital, Biochemistry, London, United Kingdom; [3]University College London Hospital NHS trust, Adolescent Endocrinology, London, United Kingdom

Background: Most adolescents with GID have no overt functional or phenotypic abnormalities to explain their presentation. Currently all female to male (FtM) persons undergo detailed evaluation of adrenal function.
Objective: We aimed to determine whether subtle adrenal abnormalities were present in the above group and thereby develop a suitable investigation schedule.
Methods: Over the past 4 years , 56 biological females with mean age 16.57 years (13.5-18.4) were referred to the National GID service and underwent a standard short synacthen test (250ug) with baseline androstenedione, dehydroepiandrosterone sulphate(DHEAS) and testosterone measurements. One additional patient with previously known non-classic 21-OH deficiency CAH was excluded. Results were compared with 15 age-matched (mean 16.2 years) controls.
Results: Baseline, 30 and 60 minute mean 17-Hydroxyprogesterone(17OHP) concentrations were 1.78 (1-8.4), 4.22 (1-12.4) and 4.31 (1.2-10.7) nmol/l respectively, not different from the control group means of 2.1(1-4.9), 4.75(2.5-7.4) and 5.02(1.8-10.9) nmol/l at 0, 30, 60 mins respectively. Parallel cortisol concentrations were 354 (101-975), 731 (206-1228) and 874 (496-1298) nmol/l at 0, 30, 60 mins respectively compared with 272.53 (79-681), 694 (419-1166) and 840 (506-1426) nmol/l in the controls. The one GID adolescent with a baseline 17OHP of 8.4 nmol/l, greater than the normal laboratory range (<6) did not have an exaggerated rise on stimulation and other adrenal androgens were normal.
Conclusion: In our national cohort of FtM GID adolescents, we have not been able to demonstrate any variations, subtle or otherwise in adrenal steroid secretion to differentiate them from the control group. Baseline adrenal steroid profile may be evaluated but unless the androgens and precursor concentrations are elevated, Short Synacthen testing does not appear to be indicated.

P2-d2-420 Adrenals and HPA Axis 2

Allgrove syndrome: a study of 10 cases

Mongia Hachicha[1]; Hajer Aloulou[1]; Safa Hadj Hmida[1]; Lamia Sfaihi[1]; Sana Kmiha[1]; Imen Chabchoub[1]; Hassen Kammoun[2]; Thouraya Kammoun[1]
[1]Hedi Chaker Hospital, Pediatric, Sfax, Tunisia; [2]Hedi Chaker Hospital, laboratory of human medical genetic, Sfax, Tunisia

Background: Triple A or Allgrove syndrome is a rare autosomal recessive disease with alacrimia, achalasia cardia, and ACTH-resistant adrenal insufficiency.
Objective and hypotheses: Study the epidemiologic, clinical, biological, genetic, therapeutic and evolving asppect of Allgrove syndrome among our patients.
Methods: A retrospective study of cases of the Allgrove syndrome hospitalized in the pediatrics department at the CHU Hédi CHAKER Sfax over a period of 22 years (1990 - 2011).
Results: During the period of study, we collected 10 cases of triple A syndrome from 7 families. There were 3 girls and 5 boys. The average age was 3 years 5 month with extremes ranging from 13 months to 4 years 6 months. All our patients had a proven alacrimia. The adrenal insufficiency was inaugurated by a seizure related to hypoglycemia in 2 cases and a melanodermia in all patients. Achalasia was noted in 5 cases. The cortisol was low in all cases. Synacthene test was conducted at 3 children, it was negative in 2 cases. Plasma levels of ACTH was high in all cases. Mineralocorticoid deficiency was found in 8 cases. The genetic study found the same mutation in AAAS gene (IVS14+1G-->A in 9 cases; it has not been performed in a patient. Our patients have been treated by hydrocortisone and Florinef, 5 patients had balloon dilatations and 1 patient had surgery with Heller's myotomy. After an average decline of 5 years 7 months, growth retardation was found in 5 cases, one patient was lost sight and a patient died of malnutrition.
Conclusions: The Allgrove syndrome is a serious disease, despite the surgical therapeutic, medical treatment of achalasia, treatment of adrenal insufficiency and the alacrimia seems to be the best alternative therapy in child.

P2-d2-421 Adrenals and HPA Axis 2

Endonasal transsphenoidal endoscopic pituitary surgery; Results in four paediatric patients with Cushing's disease

Helen L Storr[1]; William M Drake[2]; Scott A Akker[2]; John P Monson[2]; Martin O Savage[1]; Ghassan Alusi[3]; H Ian Sabin[4]
[1]William Harvey Research Institute, Queen Mary University of London, Barts and the London School of Medicine and Dentistry, Centre for Endocrinology, London, United Kingdom; [2]St Bartholomew's Hospital, Department of Endocrinology, London, United Kingdom; [3]St Bartholomew's Hospital, Department of Otolaryngology, London, United Kingdom; [4]St Bartholomew's Hospital, Department of Neurosurgery, London, United Kingdom

Background: Selective transsphenoidal adenomectomy remains the accepted first line treatment for Cushing's disease (CD), until recently by microscopic (sublabial) transsphenoidal pituitary surgery. Endonasal transsphenoidal endoscopic surgery is emerging as a less invasive treatment for pituitary adenomas with lower post-operative complications and morbidity.
Objective: There are no published series of the treatment of paediatric CD by endoscopic pituitary surgery and we report our centre's preliminary results.
Methods: Four paediatric patients (median age 14.4yr; range 11.7-16.8yr) fulfilled standard diagnostic criteria for CD. Preoperatively no abnormality was identified on pituitary MR scanning in 3 (75%) patients. Bilateral petrosal sinus sampling demonstrated central ACTH secretion (IPS/P ACTH ratio >2.0, pre or post-CRH) in 3 (75%) patients with lateralisation of ACTH

secretion (IPSG post CRH ≥1.4) in 2 patients. The same neurosurgeon and endoscopic nasal surgeon undertook all the operations. ‚Cure' was defined as a 09.00h cortisol level of <50nmol/L post-operatively on two separate occasions associated with regression of the clinical features of CD. Results: Clinical recovery and biochemical ‚cure' was achieved in 3 (75%) patients and a corticotroph adenoma was confirmed histologically in all cured cases. One case developed post-operative CSF leak requiring lumbar drain insertion and patching. At a mean interval of 5.9 years (0.4-9.8 yr) post-operatively, cured patients have shown no recurrence. One patient, who had a large diffuse adenoma requiring more extensive surgery has panhypopituitarism, another patient has GH and gonadotrophin deficiencies.
Conclusions: Our experience shows that endonasal transsphenoidal endoscopic surgery for removing corticotroph adenomas in children, in most cases not visualized on MRI imaging, is minimally invasive and gave excellent post-operative recovery/results. In skilled hands this technique provides an alternative to conventional transsphenoidal microscopic surgery in managing paediatric CD.

P2-d2-422 Adrenals and HPA Axis 2

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: genotype-phenotype correlations

Inês Vaz Matos; Luís Ribeiro; Ana Novo; Maria João Oliveira; Helena Cardoso; Teresa Borges
Centro Hospitalar do Porto, Pediatric Endocrinology Unit, Porto, Portugal

Background: Congenital adrenal hyperplasia (CAD) due to 21-hydroxylase deficiency (21-OHD) is a common autosomal recessive disorder. It is caused by distinct mutations in the CYP21A2 gene and in the majority of cases the disease severity correlates with CYP21A2 allelic variation.
Objective and hypotheses: Our aim was to describe the mutational spectrum of CYP21A2 and evaluate genotype-phenotype correlations in a cohort of Portuguese patients with 21-OHD.
Methods: Molecular analysis of CYP21A2 was performed in 23 patients with clinical and laboratorial 21-OHD. A variety of genotyping techniques were used.
Results: Genotyping was performed in 23 unrelated patients: 4 salt-wasters, 8 simple-virilizers, 11 non-classical patients. CYP21A2 mutations were detected in all patients. Homozygosity was found in 7 patients (30,4%), compound heterozygosity in 12 patients (52,2%) and heterozygosity for one mutation in 4 patients (17,4%). The most frequent mutations were V281L (37,8%), I172N (17,8%), I2 splicing (13,3%), Q318X (11,1%) and CYP21A2 deletions or gene conversions (6,7%). The overall concordance between genotype and phenotype was 73,9%, with complete concordance in the salt-waster phenotype.
Conclusions: The frequency of the underlying genetic defect in our patients has some differences compared with other Portuguese cohorts probably due to a smaller sample size and the use of different genotyping techniques. In most cases there was a good correlation between genotype and phenotype, which highlights the concept that the molecular analysis of CYP21A2 provides useful information in terms of prediction of disease severity, genetic and prenatal counseling. The discrepancies may be explained by novel mutations, incorrect genotyping, compound heterozygosity for two or more mutations and other genetic variations in androgen biosynthesis or sensitivity to androgens.

P2-d2-423 Adrenals and HPA Axis 2

CT characteristics of adrenocortical adenomas in children

Aygul Voronova; Maria Kareva; Oleg Remizov; Valentina Peterkova
Endocrinology Research Centre, Pediatric Endocrinology, Moscow, Russian Federation

Background: 70% of adenomas in adults contain high intracellular fat (lipid-rich adenomas) and have low density on non-enhanced CT (up to 20 HU). It is supposed that the children are observed the same pattern.
Objective and hypotheses: To assess the attenuation on non-enhanced CT in children with adrenocortical adenomas.
Methods: The study included children with a clinical manifestation of an excess of adrenal hormones. We performed CT examination in 7 patients with adrenocortical adenomas from 2 to 15 years old (median 9 years). After surgical treatment histological studies confirmed the diagnosis of adrenocortical adenoma.
Results:

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)	14	2	4	5	13	15	10
Sex	female	female	female	male	male	female	female
Clinical manifestation	hypercorti-solism	virilization	virilization	hyperaldo-steronism	hyperaldo-steronism	hypercorti-solism	hypercorti-solism
Tumor size (mm)	23-25-27	36-37-38	35-38-64	4-5-9	17-18-24	31-32-26	24-32-21
Non-enhanced attenuation (HU)	35	42	46	6	26	32	38

Conclusions: Pediatric adrenocortical adenomas have a higher non-enhanced density than adult. Only one patient with hyperaldosteronism has low non-enhanced attenuation (6 HU). We can suggest that the children with large adenomas and the clinical manifestation of virilization syndrome are at higher risk of developing adrenocortical carcinoma and should be watched more closely than other.

P2-d2-424 Adrenals and HPA Axis 2

Virilizing adrenocortical tumor in a 7,5-year-old boy

Elzbieta Moszczynska[1]; Karolina Kot[1]; Agnieszka Lecka Ambroziak[1]; Wies³awa Grajkowska[2]; Maciej Pronicki[2]; Katarzyna Malczyk[3]; Elzbieta Jurkiewicz[3]; Mieczyslaw Szalecki[1]
[1]The Children's Memorial Health Institute, Department of Endocrinology, Warsaw, Poland; [2]The Children's Memorial Health Institute, Department of Pathology, Warsaw, Poland; [3]The Children's Memorial Health Institute, Department of Radiology, Warsaw, Poland

Background: Ectopic adrenocortical tumors arising in the nervous system are very rare. We report an unusual case of an intradural, extramedullary adrenocortical adenoma of a spinal region in a 7,5-year-old boy.
Objective and hypotheses: 7,5-year-old boy with symptoms of virilisation: acne, pubarche, penile growth, acceleration of growth velocity for 2 years and pain of a right thigh for one year. Clinically he presented with height >97 ct, weight 10 ct according to the height, acne, A1, P3, testes 2 ml, with neurological symptoms- walk disorders, increased reflexes in the legs, right site foot clonus.
Methods: Endocrine and imaging studies were performed.
Results: Examinations revealed: advanced bone age of 2 years, significantly raised serum levels of DHEAS (4748 ng/ml), androstenedione (512 ng/dl) and testosterone (3000 pg/ml), very high levels of androgen metabolites in urine steroid profile, suggesting a virilizing tumor. Levels of cortisol and ACTH were normal. There was no androgen suppression during the 4-day Dexamethasone test (2 days of 2 mg/d, 2 days of 8 mg/d), with normal cortisol suppression after the small dose of Dexamethasone. Urine catecholamines and their metabolites were normal. Abdomen CT and MRI, with adrenal imaging, didn't reveal abnormalities. Testes ultrasound was normal. Adrenal scintigraphy (131J-norcholesterol) didn't show adrenal autonomic function. There was a spinal MR performed due to the pain of the right thigh and the neurological symptoms. It revealed an intradural, extramedullary tumor at L3-L5 level, size 28x21x60 mm. An adrenocortical hormonal active tumor in the spinal cord was suspected. The patient underwent surgery, the tumor was removed totally. Histopathological diagnosis was ectopic adrenocortical adenoma without atypia, Ki proliferation index 3%. Hormonal resolution was observed after the surgery.
Conclusions: In the case of symptoms of virilising tumor in patients with normal adrenal imaging examinations, ectopic adrenocortical tumor should be considered.

P2-d2-425 Adrenals and HPA Axis 2

Five-year follow-up of 24-hour blood pressure profiles in children with classic congenital adrenal hyperplasia (21-OHD, CAH)

Thomas M.K. Völkl; Stefanie M. Hacker; Wolfgang Rascher; Helmuth G. Dörr
Division of Paediatric Endocrinology and Diabetology, Department of Paediatrics and Adolescent Medicine, Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Germany

Objective: Five years ago, we have shown that 24-hour blood pressure profiles are altered in CAH patients with elevated systolic levels correlated with the degree of obesity, whereas normal-weight patients tended to diastolic hypotension (Völkl et al. 2006, JCEM).
Design: We included 39 children with CAH, aged between 6.9 and 17.1 years (median 13.5, n=21 females) into this single-centre, cross-sectional, prospective, observational study. All patients had proven CAH (genetic groups: 0, n=13; A, n=16; B, n=8; D, n=2), received steroid substitution therapy and underwent standardized 24h-blood pressure (BP) monitoring (Mobil-O-Graph-TM, I.E.M., Solberg, Germany). N=26 of them had participated in the previous study. Eleven different variables of 24h-BP were analysed and compared with current German reference data.
Results: BMI SDS slightly improved over the five years, but are still significantly different from zero (from 1.09 SDS, 0.21;2.01, to now 0.61 SDS, -0.28;1.68). Daytime and night-time systolic BP did not change significantly and remained elevated (from 0.35 SDS, -0.10;0.85, to now 0.46 SDS, -0.47;1.2, and from 0.39 SDS, -0.03;0.68, to now 0.67 SDS, -0.13;1.1), whereas the lowered daytime diastolic BP improved (from -1.2 SDS, -1.5;-0.71, to now -0.62 SDS, -1.2;0.10; p=0.007) and stayed normal during the night (from -0.18 SDS, -0.86;0.11, to now 0.29 SDS, -0.40;0.83). Nocturnal dropping of systolic BP did not change (from 13.2%, 9.1;15, to now 12.6%, 6.1;17). The different parameters of systolic and diastolic BP were significantly correlated with BMI SDS and the skinfold thicknesses (r_s=0.323 to 0.660, p<0.05). There was no clear correlation with equivalent hydrocortisone and fludrocortisone dosage, bone age, and various laboratory parameters (e.g. renin, 17OH-progesterone).
Conclusions: Despite an improvement of BMI SDS among our CAH patients, these follow-up data confirm our previous findings of altered 24h-BP profiles with elevated systolic BP levels correlated with the parameters of overweight.

P2-d2-426 Adrenals and HPA Axis 2

Primary adrenal insufficiency caused by a new mutation on DAX1 gene

Olcay Evliyaoglu[1]; Ipek Dokurel[1]; Feride Bucak[1]; Oya Ercan[1]; Serdar Ceylan[2]
[1]Istanbul University Cerrahpaþa Medical School, Pediatric Endocrinology Department, Istanbul, Turkey; [2]Intergen, Genetik Merkezi, Ankara, Turkey

Introduction: Primary adrenal insufficiency can be caused by a deficiency in steroid biosynthesis or abnormal adrenal gland development. One of the genes involved in adrenal development is DAX1 which also functions in gonadal and pituitary development. We aimed to present a new mutation on DAX1 gene in a patient with adrenal insufficiency and normal male sexual development.
Case: 33 days old male infant admitted with dehydration, hyponatremia , hyperkalemia and prerenal kidney failure. His physical examination revealed severe dehydration with normal anthropometric measurements (length: 52cm (3-10%), weight: 3150gr(10%), head circumference:37 cm (25-50%)), normal male genital development and scrotal hyperpigmentation. Prenatal and natal histories were unremarkable. Parents were fourth degree relatives. Laboratory evaluation showed high ACTH level (>1250pg/ml). Baseline and corticotropin stimulated cortisol, 17-OH-progesterone, 1-4 androstenedione and DHEA-S levels were 1,3 µg/dl-1,6 µg/dl, 0.18 ng/ml-1,4 ng/ml, 0.06ng/ml-0,1ng/ml, and 110 µg /dl-115,8 µg /dl. Plasma renin activity (32,32ng/ml/hr) was high whereas aldosterone level (7,1ng/dl) was relatively low. Karyotype of the patient was 46,XY.With the diagnosis of adrenal insufficiency hydrocortisone, fludrocortisone, and salt treatments were initiated, to which the patient responded well. Hormonal and clinical features of the patient suggested adrenal insufficiency was related to abnormal adrenal development rather than a steroid biosynthesis defect. Thus a new frame shift mutation on DAX1 gene (c.543delA hemizygous mutation) which can alter gene function and cause the disease has been identified.
Conclusion: In the patient presenting with adrenal insufficiency not related to steroid hormone biosynthesis deficiency suggested an abnormality in adrenal gland development and genetic evaluation revealed a new frame shift mutation on DAX1 gene. Although for the present time only adrenal gland seems to be affected, pituitary and gonadal functions will be followed in the patient.

P2-d2-427 Adrenals and HPA Axis 2

Congenital adrenal hyperplasia, CAH, a cohort of 606 patients, 1915-2011

Anna Nordenström; Sebastian Gidlöf; Henrik Falhammar; Anna Wedell
Karolinska Institutet, MMS, Stockholm, Sweden

Background: Treatment of Congenital adrenal hyperplasia, CAH, with hydrocortisone was first performed in the 1950's. The diagnostics for the disease have changed drastically with the development of molecular genetics and the introduction of screening. Neonatal screening for CAH, has been performed in Sweden since 1986, more than 2.5 million infants have been screened.
Objective and hypotheses: To investigate the effect of screening on a large clinical cohort of identified CAH patients and to analyze the change in clinical presentation observed over time in relation to disease severity and CYP21A2 genotype.
Methods: 609 patients known from the screening, laboratory follow-up, or CYP21A2 genotyping were included in the study. The sex of the patients, the CYP21A2 genotype or disease severity when known were recorded.
Results: Twenty-one patients born before 1950 were identified. The oldest patient was born 1915, with V281L genotype. In the 40's 18% of the patients had the SW form of CAH. Four males born in the 20's and 40's had 46,XX caryotype. An increase in the number of identified patients was seen after 1970. The sex ratio was 1,6 in the 1970's. Since the introduction of neonatal screening 312 patients were diagnosed. Among the patients detected by the screening the sex ratio was close to 1. Late clinical diagnosis of non classic (NC) CAH in females accounted for the female preponderance (ratio 1.35) observed also during the period 1980-1999. In 404 patients the CYP21A2 genotype was known. 86% of the patients diagnosed after the start of the screening and more than 70 % of the patients had known CYP21A2 genotype. In 15% of the patients the disease severity was not known. 15% had the NC form, 20% SV, and 50% CAH.
Conclusions: 50% of the patients had SW CAH, increasing to 75% at the end of the study period. The majority of patients with the NC form were women with V281L genotype and late diagnosis, which accounted for the higher sex ratio seen over time.

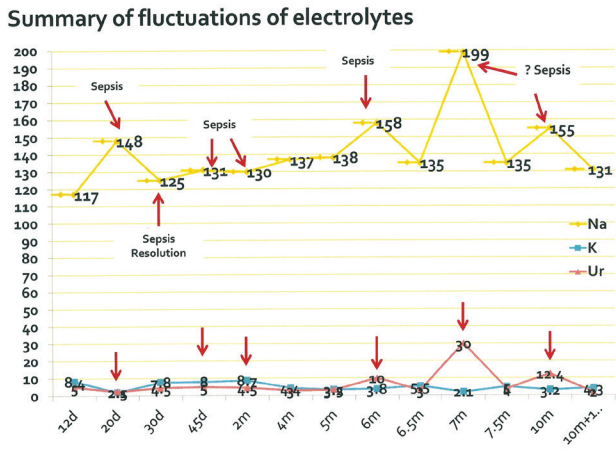
P2-d2-428 Adrenals and HPA Axis 2

Novel mutation of SCNN1B gene in a patient with pseudohypoaldosteronism causing intermittent resolution of salt loss accompanied by life threatening fluctuations in electrolytes

Raja Padidela[1]; Indi Banerjee[2]; Bindu Avatapalle[2]
[1]Manchester Children's Hospital, Dept of Paediatric Endocrinology, Manchester, United Kingdom; [2]Royal Manchester Children's Hospital, Dept of Paediatric Endocrinology, Manchester, United Kingdom

Background: Pseudohypoaldosteronism (PHA) is characterized by renal resistance to the action of aldosterone. Multiple target organ defects (MTOD) is the most severe form of PHA caused by loss-of-function mutation in the α, β or γ subunits of the epithelial sodium channel (ENaC), resulting in defective sodium transport in many organs containing the ENaC. Affected patients develop life-threatening salt loss, hyperkalaemia, and acidosis due to end-organ resistance to aldosterone.
Objective and hypotheses: We present a severe neonatal case of MTOD-PHA with a novel homozygous mutation in SCNN1B gene. We describe a novel finding of intermittent resolution of severe salt loss during episodes of sepsis with accompanying hypernatraemia.
Methods: A female neonate presented with hyponatraemia (117mmol/l), hyperkalaemia (8.5mmol/l) and high urinary sodium (180mmol/l). Renin and Aldosterone were elevated confirming the diagnosis of PHA. Proband's electrolytes were normalised on sodium supplements (45mmol/kg/day) and sodi-

um resonium. Subsequent progress of proband was characterised by multiple episodes of sepsis (Fig-1). In some of these episodes, proband had complete resolution of salt loss with accompanying hypernatraemia. These episodes resolved by complete cessation of sodium supplementation and resolution of sepsis (Fig-1).
Results: Genetic testing revealed pathogenic homozygous donor splice site mutation in intron 12 of SCNN1B gene: c.1542+1G>A. Mutation is anticipated to cause skipping of exon 12 and defective subunit of ENaC.
Conclusions: We describe a novel mutation in SCNN1B gene of ENaC. Phenotype of intermittent resolution with sepsis could be secondary to the novel genotype or increased mineralocorticoid sensitivity. Other hormones controlling water balance may have a role. Functional studies of the genotype and further hormonal studies during episodes of resolution may help in determining the cause.



P2-d2-429 Adrenals and HPA Axis 2

Surgical management of congenital adrenal hyperplasia in girls: a population-based study

Céline Castaings-Carlioz[1]; Thomas Blanc[2]; Bénédicte Coulm[3]; Yves Morel[4]; Véronique Tardy[4]; Pierre Mouriquand[5]; Stephen Lortat-Jacob[6]; Alaa El Ghoneimi[2]; Joël Coste[3]; Jean-Claude Carel[1]
[1]Hôpital Robert Debré, Pediatric Endocrinology and Diabetology, Paris, France; [2]Hôpital Robert Debré, Pediatric Surgery and Urology Departement, Paris, France; [3]Groupe Hospitalier Cochin-Saint Vincent de Paul, Biostatistics and Epidemiology Unit, Paris, France; [4]Groupe Hospitalier Lyon Est, Molecular Endocrinology, Lyon, France; [5]Groupe Hospitalier Lyon Est, Pediatric Surgery and Urology Departement, Lyon, France; [6]Hôpital Necker - Enfants Malades, Pediatric Surgery and Urology Departement, Paris, France

Background: Despite the long-term influence of feminizing surgery in girls with CAH and several consensus conferences, little is known of when and which type of surgery is performed at a population level.
Objective and hypotheses: Describe the surgical management of girls with classical form of congenital adrenal hyperplasia due to 21-hydroxlase deficiency.
Methods: We analyzed the first surgical procedure performed in a national population-based cohort of 166 girls born from 1996 to 2003.
Results: Patients who had surgery were classified according to Prader, as I (n=3), II (n=19), III (n=43), IV (n=49), V (n=6) and not available (n=22). Five of them had received a prenatal dexamethasone treatment. 142/166 (85,5%) had genital surgery at a mean age of 289,5 ± 427 days. Data concerning surgery were missing for 6 girls and the data are presented on 136 patients Preoperative genitography was done in 99/136 girls, and could evaluate the level of uretro-vaginal confluence in 79. Endoscopy was performed in 64 girls, before surgery (n=16) or during genitoplasty (n=48). Among the 34 girls who had both, the result was consistent in 30. Clitoridoplasty and vaginoplasty were performed during the same procedure in 118 patients (87%) whereas 4 patients had a two-stage procedure. Neurovascular sparing dismembered clitoridoplasty was performed in the majority of cases (n=76/121). Fortunoff flap with posterior colpotomy was the most frequent technique for vaginoplasty (56%). The 142 patients were operated at 29 institutions throughout the country: 10 institutions operated a single patient while 5 institutions operated more than 8 patients during the 8-year period.
Conclusions: Our study demonstrates a wide heterogeneity of surgical techniques performed by a large number of institutions throughout the country despite the rarity of the condition. Similar studies should be undertaken in other countries to allow the establishment of evidence-based guidelines for the surgical management of CAH.

P2-d3-430 Adrenals and HPA Axis 3

The use of serum steroids in monitoring therapy of congenital adrenal hyperplasia due to 21-hydroxylase deficiency

Huamei Ma[1]; Zhe Su[1]; Sinian Pan[2]; Hongshan Chen[1]; Yanhong Li[1]; Qiuli Chen[1]; Minlian Du[1]
[1]The first Affiliated Hospital of Sun Yat-Sen University, Pediatric department, Guangzhou, China; [2]The third Affiliated Hospital of Sun Yat-Sen University, Pediatric department, Guangzhou, China

Background: The management of children with congenital adrenal hyperplasia (CAH) due to 21- hydroxylase deficiency (21OHD) remains difficult, and assessing the adequacy of treatment has always been a matter of great concern to the clinician.
Objective: To assess the utility of serum steroids measurement in monitoring the treatment of patients with CAH 21OHD, and determine the valuable steroid parameters for optimal hydrocortisone replacement therapy.
Methods: Nineteen-one Patients with CAH 21OHD aged (3.67±1.54) yrs treated with hydrocortisone and fludrocortisones replacement were followed in an intervals of (0.55±0.23) yrs over a period of (1.47±0.7)yrs. At each visit, bone ages were estimated, peripheral blood were collected to test serum E1 (estrone),E2 (estradial), T (testosterone), P (progesterone), DHEA-S (dehydroepiandrosterone sulfate), 17OHP (17-hydroxyprogesterone), Δ4-A (androstenedione) and FT (free testosterone) concentrations. The patients were classified as being in good control (GC) or in poor control (PC) based on clinical criteria and bone age (G-P) advancement (ΔBA/ΔCA). Comparisons were carried out between the serum steroid concentrations of the two groups. The receiver operating characteristic curve (ROC) was used to determine the cut-off value for diagnosing ‚Poor Control'.
Results: Each of serum E1, E2, Δ4-A and 17OHP levels was higher in PC group than GC group($P<0.05$). ROC analysis showed that any of serum 17OHP or/and Δ4-A level was of significance in diagnosing ‚Poor Control', with the diagnostic efficacy being serum Δ4-A, serum 17OHP and serum Δ4-A in combination with 17OHP in descending order. Serum Δ4-A of 3.9 nmol/L has 77.8% of sensitivity and 75% of specificity in diagnosing ‚Poor Control'. Serum 17OHP of 7.1 ng/mL has 66.7% of sensitivity and 77.8% of specificity in diagnosing ‚Poor Control'.
Conclusions: Serum Δ4-A/17OHP level can be used as the biochemical indicators to monitor the treatment of CAH 21-OHD.

P2-d3-431 Adrenals and HPA Axis 3

Screening for hypothalamic-pituitary-adrenal axis suppression in asthmatic children on corticosteroids is not possible when employing clinical and static biochemical parameters

Ekkehard Zoellner[1]; Carl Lombard[2]; Ushma Galal[2]; Stephen Hough[3]; Elvis Irusen[4]; Eugene Weinberg[5]
[1]University of Stellenbosch, Paediatric Endocrinology, Cape Town, South Africa; [2]Medical Research Council, Biostatistics Unit, Cape Town, South Africa; [3]University of Stellenbosch, Endocrinology, Cape Town, South Africa; [4]University of Stellenbosch, Allergy unit, Cape Town, South Africa; [5]University of Cape Town, Lung Institute, Cape Town, South Africa

Background: It is impractical to test all asthmatic children for hypothalamic-pituitary-adrenal axis suppression (HPAS) with dynamic adrenal function tests.
Objective: To determine which parameter is the most useful screening test for HPAS in asthmatic children.
Methods: 143 asthmatic children, 5-18 years old, treated with corticosteroids were recruited. Height velocity (HV), weight velocity (WV), height standard deviation score (SDS), weight SDS, change in systolic blood pressure from

supine to standing (ΔSBP) were recorded. Early morning urinary free cortisol (UFC), morning serum cortisol (C), adrenocorticotropin hormone (ACTH) and dehydroepiandrosterone sulphate (DHEAS) were collected. UFC was expressed as a ratio of creatinine (Cr) excretion and as a ratio of body surface area. A metyrapone (MTP) test was performed if the 08:00 hr C was >83nmol/l. Spearman correlation coefficients (r) were calculated between the post-MTP ACTH, 11-deoxycortisol (11DOC), 11DOC+C, and each variable. Diagnostic statistics were calculated for the most promising test.

Results:

Screening Variable	ACTH		11DOC		11DOC+C	
	r	p	r	p	r	p
Height SDS	0.12	0.186	-0.13	0.120	-0.05	0.542
Weight SDS	0.10	0.262	-0.01	0.195	-0.10	0.279
HV SDS	0	0.999	0.07	0.420	0.07	0.452
WV SDS	-0.04	0.638	0.07	0.421	0.09	0.302
ΔSBP	0	0.992	0.05	0.538	-0.04	0.616
cortisol	0.05	0.538	0.08	0.374	0.12	0.176
ACTH	0.10	0.248	0.04	0.640	0.10	0.263
DHEAS	0.20	0.025	0.21	0.017	0	0.995
UFC (nmol/m2)	0.08	0.379	0.19	0.033	0.20	0.022
UFC (nmol/mmolCr)	0.08	0.397	0.14	0.111	0.16	0.064

The area under the receiver operating characteristics (ROC) curve for DHEAS in boys at 10-14 yrs (n=37) is the highest with 76 %.
The screening performance at 2.0 µmol/l: sensitivity 100 (95%CI 63.1-100.0) %, specificity 38 (95%CI 17.9-54.3) %, accuracy 51 (95%CI 31.9-65.6) %, positive likelihood ratio (LR) 1.6 (95%CI 1.4-2.4), negative LR 0.0 (95%CI 0.0-4.5).

Conclusions: No useful screening test for utilization at all ages could be identified.

P2-d3-432 Adrenals and HPA Axis 3

24-hour blood pressure monitoring: might be useful in the individualization of the hydrocortisone supplementation?

Malgorzata Wojcik; Dominika Janus; Agata Zygmunt-Gorska; Katarzyna Dolezal-Oltarzewska; Katarzyna Tyrawa; Jerzy B. Starzyk
Jagiellonian University Collegium Medicum, Pediatric and Adolescent Endocrinology, Krakow, Poland

Background: Patients on lifelong HCT supplementation are at risk of long-term cardiovascular complications due to glucocorticoid exposure, while even slight disruptions in the cortisol diurnal rhythm may affect the blood pressure (BP) profile.

Objective and hypotheses: (1) to evaluate daily BP profile in AI patients on HCT supplementation, and its correlation with blood and urine cortisol level, (2) an attempt to use the 24-hours ambulatory BP monitoring for individualization the HCT supplementation.

Methods: The pilot study involved 15 (8 girls) AI patients (14 secondary AI due to combined pituitary hormone deficiency; 2 primary AI: congenital adrenal hypoplasia and after bilateral adrenalectomy due to primary pigmented nodular adrenal disease) at the mean age of 12.1 (4.96-17) years. 24-hour BP monitoring was performed using Ambulatory BP Monitor (Spacelabs90217,USA). It was set to take a reading every 15 minutes (day-6AM-10:59PM), and every 30 minutes (night). Cortisol level was assessed in blood samples (every 6 hours), and in 24-hour sample of urine.

Results: There were significant correlations between mean blood cortisol level and day mean arterial pressure (MAP) ($r=0.76$, $p<0.05$); mean systolic BP (SBP), day mean SBP, maximal SBP ($r=0.73$; 0.8; 0.68 respectively $p<0.05$); and SBP load >95 centile, SBP load >90 centile, and SBP load >75 centile for height ($r=0.71$; 0.7; 0.73 respectively $p<0.05$). There was no correlation between urinary cortisol level and 24-hour BP measurement parameters. In 6 cases the nocturnal dip was reduced and the dip episodes during the day were noticed. According to the BP monitoring data the HCT dose was reduced/increased up to 20%, the hours of administration were changed. Re-evaluation revealed normal BP rhythm.

Conclusions: In patients on HCT supplementation MAP and SBP are correlated with blood cortisol level. The 24-hour BP monitoring may be used for the adjustment of the HCT supplementation in AI patients.

P2-d3-433 Adrenals and HPA Axis 3

A rare association: neonatal ovarian cyst and 21-hydroxylase deficiency

Aurelie Valade[1]; Eric Dobremez[2]; Sylvie Cabrol[3]; Yves Morel[4]; Muriel Houang[3]
[1]Centre hospitalier de la Cote Basque, pediatrie, Bayonne, France; [2]Hopital des enfants Pellegrin, chirurgie pediatrique, Bordeaux, France; [3]Hopital Armand Trousseau, Explorations fonctionnelles endocriniennes, Paris, France; [4]CHU de Lyon-GH Est, Service d'endocrinologie moléculaire et maladies rares, Bron, France

Background: Neonatal ovarian cysts are rare in 21-hydroxylase deficiency (21OHD).

Objective and hypotheses: We describe a patient with classic form of 21OHD and endometrial bleeding at 2 months due to ovarian cyst.

Results: At 26 weeks of gestation, because of clitoris enlargement in a 46 XX fetus, the diagnosis of classic form of 21OHD has been done and confirmed by molecular studies of CYP21A2 gene (homozygous for IVS2-13A/C>G). Because of late diagnosis, the mother was not treated with dexamethasone. At birth, the neonate had a male phenotype (Prader V), and high levels of testosterone (16 ng/ml), 17OH-progesterone (78 ng/ml) and renine (110 mUI/l). Hydrocortisone and salt were given on day 2, then fludrocortisone on day 8. At 1 month, testosterone level was 0.5 ng/ml. At 2 months, because of vaginal bleeding and breast development, pelvic ultrasound was performed and showed enlarged uterus of 4 cm and a giant cyst of 6 cm diameter on left ovary. To prevent ovarian torsion, she underwent cystectomy. Estradiol level in cyst fluid was very high. Bleeding stopped 2 weeks following surgery. At 3 months, she still had breast buds; pelvic ultrasound showed a small cyst of 2 cm on right ovary, normal left ovary and enlarged uterus. Hormonal measurements showed normal levels of testosterone (0.1 ng/ml), LH (2.0 mUI/ml) and FSH (3.1 mUI/ml), and high estradiol level (365 pg/ml). At 4 months, we performed GnRH test: baseline LH 1.0 mUI/ml, peak LH 17.0 mUI/ml, and baseline FSH 10.0 mUI/ml, peak FSH a 48.6 mUI/ml. Estradiol was normal (<50 pg/ml).

Conclusions: High steroid levels in neonatal period act as positive feedback on gonadotrop axis. Besides we speculate that glucocorticoid treatment mediated androgen suppression led to a marked rise in gonadotropin levels that favoured follicular growth and estrogen secretion. Interestingly, this severe virilised female neonate had higher FSH than LH levels when compared to usual higher LH levels in infant males, keeping the gender dichotomy in gonadotropin secretion.

P2-d3-434 Adrenals and HPA Axis 3

A review of patients with congenital adrenal hyperplasia

Maryam Razzaghy-Azar[1]; Mona Nourbakhsh[2]; Mitra Nourbakhsh[3]
[1]Tehran University of Medical Sciences, Endocrine Research Center (Firouzgar), Tehran, Islamic Republic of Iran; [2]Tehran University of Medical Sciences, H. Aliasghar Hospital, Tehran, Islamic Republic of Iran; [3]Tehran University of Medical Sciences, School of Medicine, Biochemistry, Tehran, Islamic Republic of Iran

Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder stemming from one of the enzymatic defects in cortisol biosynthesis from cholesterol. In the majority of instances the disorder comprises deficiency of 21-hydroxylase (21-OHD). This defect causes excessive androgen production from adrenal source, which leads to virilization of varying severity (Prader grade 1- 5) in female fetus.

Objective and hypotheses: To find out frequency of different types of congenital adrenal hyperplasia, rate of consanguinity, family occurrence, birth weight, final height and weight.

Methods: Medical records of patients with CAH between 1968 and 2011 in 3 endocrine centers were reviewed.

Results: Out of 617 patients, 79.6% had 21-hydroxylase deficiency (21-OHD). In 21-OHD group 73.7% had salt wasting, 20.8% simple virilizing (SV) and 5.5% non classic type. Frequency of other types were as follow:11-hydroxylase deficiency (11-OHD), 13.3%; 3—βhydroxysteroid dehydrogenase deficiency (3—βOHD), 4%; lipoid adrenal hyperplasia,1%; 17-hydroxylase deficiency, 1%; hypoaldostronism, 0.6% and Antley-Bixler, 0.3%. Parental consanguinity was present in 62.6% and familial occurrence was reported in 42.6% of the patients. Sixteen girls had grade 5 virilization of Prader staging. The most prevalent Prader stage was 4 in 21-OHD-SW and 11-OHD. In

21-OHD-SV, 9 patients had Prader 4 & 5 virilization. The difference between midparental height and final height SDS was lowest in 21-OHD-SV (-1.2 ± 1.1). Birth weight of all the patients was normal except 5 patients with 21-OHD-SW, 2 with 3—βOHD and 2 with 21-OHD-NC that the mean ± SD was 1.2 — 2.25 kg. All the patients were assigned as their genetic gender except 5 patients due to delayed diagnosis or resentment of the parents.
Conclusions: The prevalence of different types of CAH, grade of virilization, final weight and height and birth weight have detected among referral patients.

P2-d3-435 Adrenals and HPA Axis 3

DAX-1 (NR0B1) mutations in four patients affected by adrenal hypoplasia congenita

Aleksandra Rojek[1]; Monika Obara-Moszynska[2]; Elzbieta Malecka[3]; Malgorzata Slomko-Jozwiak[3]; Marek Niedziela[2]
[1]University of Medical Sciences in Poznan, Department of Pediatric Endocrinology and Rheumatology, Molecular Endocrinology Laboratory, Poznan, Poland; [2]University of Medical Sciences in Poznan, Department of Pediatric Endocrinology and Rheumatology, Poznan, Poland; [3]Karol Jonscher's Clinical Hospital, Department of Pediatric Endocrinology and Rheumatology, Poznan, Poland

Background: X-linked Adrenal Hypoplasia Congenita (AHC) is an inherited disorder of adrenal gland development which is caused by mutations in *DAX-1* (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) (*NR0B1*) gene located on X-chromosome. *DAX-1* (OMIM*300473) encodes for an orphan nuclear hormone receptor that functions as a transcription factor regulating expression of other genes.
Patients and methods: Four boys in a life-threatening state came to our attention and were suspected for AHC. Laboratory studies at diagnosis, in neonatal period, showed in all hyponatremia and hyperkaliemia. Severely low serum cortisol levels and high plasma ACTH levels confirmed primary adrenal insufficiency. The hydrocortisone therapy with saline and glucose infusions were started immediately. In each of these patients, two exons of the *DAX-1* gene were directly sequenced after polymerase chain reaction amplification of the entire coding region.
Results: Molecular analysis of the *DAX-1* gene coding region and the adjacent splicing sites revealed one novel mutation (patient 1) and two already known mutations (patients 2, 3 and 4). In patient 1 we found a novel mutation c.T1118>G (p.Ile373Ser) in exon 1. This mutation was also found in the patient's mother. In patient 2 and 3 a known mutation c.A1319>T (p.Asn440Ile) in exon 2 was identified. Both patients' mothers were not available for the genetic testing. Both mutations are located in ligand-binding domain (LBD) that functions as a transcriptional repression domain. In patient 4 a known mutation c.C109>T (p.Gln37X) in exon 1 was found, that resulted in a premature stop codon generation destroying the N-terminal domain of DAX-1. This mutation was also found in the patient's mother.
Conclusions: We show that molecular analysis of *DAX-1* gene is very important for the confirmation of clinical diagnosis of adrenal insufficiency and highlights the role of further genetic counselling in families with AHC patients.

P2-d3-436 Adrenals and HPA Axis 3

Premature adrenarche: no difference between sexes with respect to metabolic complications and pubertal timing in children born appropriate for gestational age

Ahmet Uçar; Nurçin Saka; Firdevs Baþ; Rüveyde Bundak; Feyza Darendeliler
Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrine Unit, Istanbul, Turkey

Background: Premature adrenarche (PA) refers to the isolated development of pubic and/or axillary hair under the age of 8 years in a girl and 9 years in a boy.In girls, PA has been associated with obesity, decreased insulin sensitivity, coronary arterial disease and polycystic ovary syndrome (PCOS) in adulthood. Data about PA in boys with respect to metabolic complications and puberty are scarce and conflicting.
Objective and hypotheses: To determine whether premature adrenarche (PA) has a different impact on metabolic issues and pubertal timing in boys and girls born appropriate for gestational age (AGA).
Methods: Growth, puberty and metabolic work-up of 47 girls and 23 boys with PA born AGA followed-up from our outpatient endocrinology clinic between 2000-2009 were reviewed.
Results: Initial anthropometric measurements except for body mass index (BMI) standard deviation score (SDS) being higher in boys than girls (p=0.01), bone age (BA) SDS, homeostasis model assessment-insulin resistance (HOMA-IR) and fasting plasma glucose / insulin ratio (FGIR) and plasma lipids were similar between sexes. Hormone levels except for significantly higher dehydroepiandrosterone sulfate (DHEA-S) levels in boys than girls (p=0.0006) were also similar between the sexes. BA SDS and BA / CA were significantly advanced ($p<0.05$) with respect to initial evaluation in 28 girls at onset of gonadarche unlike the case in 9 boys with PA ($p>0.05$).
Conclusions: PA in children born AGA does not herald any significant differences with respect to metabolic complications between sexes, and it appears to be a discrete process from onset of puberty in girls unlike boys, in whom it is likely a variant of normal puberty.

P2-d3-437 Adrenals and HPA Axis 3

Decline of cortisol levels over time before the first dose of intravenous immunoglobulin in patients with Kawasaki disease

Shinichiro Sano[1]; Yuichi Nakagawa[1]; Satoru Iwashima[1]; Takamichi Ishikawa[1]; Takehiko Ohzeki[2]; Tsutomu Ogata[1]
[1]Hamamatsu University School of Medicine, Pediatrics, Hamamatsu, Japan; [2]Kyoritsu Women' s University & Junior College, Nursing, Tokyo, Japan

Objective: Cortisol is a stress hormone and is secreted in response to stress or inflammatory insults as a normal counter regulatory hormone. However, little is known about the changes in cortisol levels in Kawasaki disease (KD) before the first intravenous immunoglobulin (IVIG) treatment. The aim of this study was to investigate changes in cortisol levels before the first IVIG treatment, and to compare cortisol levels between responders and non-responders to initial IVIG.
Methods: Blood samples were obtained in 46 children (23 females) with KD before their first IVIG treatment. Serum cortisol levels, white blood cell (WBC) counts and C-reactive protein (CRP) levels were measured.
Results: Seventy seven blood samples were obtained from 46 patients before their first IVIG treatment. The day of illness was negatively correlated with the cortisol level ($r = -0.47$, $P < 0.001$), but not with the WBC count or CRP level. Cortisol levels before IVIG treatment were higher in non-responders than in responders.
Conclusions: Cortisol levels in patients with KD decreased over time, despite the persistence of elevated WBC and CRP, before initial IVIG treatment. Cortisol levels were higher in non-responders than in responders to initial IVIG.

P2-d3-438 Adrenals and HPA Axis 3

Molecular characterization of 21-hydroxylase deficiency in patients with the congenital adrenal hyperplasia (CAH) in two Balkan countries

Mirjana Kocova[1]; Tanja Milenkovic[2]; Violeta Anastasovska[1]
[1]Univeristy Pediatric Clinic, Endocrinology & Genetics, Skopje, Macedonia, Fyrom; [2]Mother and Child Health Institute, Endocrinology, Belgrade, Serbia

Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused in 95% of cases by 21-hydroxylase deficiency. Molecular characterization of CYP21A2 gene mutations in many countries revealed specific mutations associated with different forms of the disease. No data are available for the Balkan region.
Objective and hypotheses: The aim of this study was molecular characterization of CAH in Macedonian and Serbian patients with all three forms of CAH and comparison with findings in other countries.
Methods: Methods used for the molecular characterization included: differential polymerase chain reaction (PCR), amplification created restriction site (ACRS), restrictional endonuclease digestion, agarose and polyacrilamide gel electrophoresis.
Results: We studied 71 patients with 21-hydroxylase deficiency (53 Macedo-

nian patients and 18 Serbian patients) and 109 family members. Six different CYP21A2 point mutations were detected in 76,4%(81/106) of alleles in Macedonians, and in 75% (27/36) of Serbian patients (Table). In salt- wasting(SW) form 87% of Macedonians and 100% of Serbians had a mutation, 87.5 of simple virilizing(SV) and 47.2% of late onset(LO) form. IVS(656nt) mutation was the most frequent in SW form in both populations. Homozygosity for severe mutations was detected in Macedonian and Serbian patients (34% vs 38.4%). Genotype-phenotype correlation was found in all patients with SW and LO form in both populations, and in 90% of SV form. High percentage of P30L mutation in Serbian patients with SV form was found with a frequent homozygosity(16,7%). Analysis of the siblings showed high frequency of IVS2 and P30L mutations, and low frequency of V281L mutation.
Conclusions: Most of the mutations in CAH patients and siblings of the two Balkan populations are similar to other European populations. Unusual findings are high number of P30L and low number of V281L mutations.

Mutation	Macedonians (% of alleles with a mutation)	Serbians (% of alleles with a mutation)
IVS2	37.7	22.2
P30L	17.9	30.6
Q318X	10.4	11.1
V281L	4.7	/
I172N	3.8	5.6
R356W	1.9	5.6

P2-d3-439 Adrenals and HPA Axis 3

Renin- angiotensin-aldosterone system and insulin sensitivity in adolescent boys with hypertension

Oksana Khyzhnyak[1]; Elena Nosova[2]; Tatjana Sulima[1]
[1]Institute for Endocrine Pathology Problems, Departments of Age-Releated Endocrinology, Kharkiv, Ukraine; [2]Institute for Children and Adolescent Health Problems, Department of Cardiorheumatology, Kharkiv, Ukraine

Background: Puberty is a crucial time for early onset of arterial hypertension in adolescent boys.
Objective and hypotheses: 156 lean, overweight and obese boys (13-18 years) with arterial hypertension (AH) were under investigation.
Methods: Physical examination included height, weight, waist circumference measurements and blood pressure diurnal rhythm (BPDR) monitoring. Blood samples for glucose (Gl), insulin (IRI), plasma renin activity (PRA), angiotensin-II(A-II) aldosteron(ALD) measurements were taken in fasting state. Systemic analysis between RAAS and carbohydrate metabolism was performed by factor (F) analysis. Data are given as factor loadings (FL) after varimax rotation procedure. Results after multifactor ANOVA application are given as M [95%CI].
Results: Increased levels of PRA = 0.92 [0.75-1.11] ng/ml*h; A-II = 21.05 [19.1-22.8] pmol/L; ALD = 37.8 [32.17-45.11] pg/ml were found out in adolescent boys with AH. Overweight patients (HOMA = 2.98 [2.71 - 3.24]) were less sensitive to insulin than lean individuals (HOMA = 3.83 [3.53 - 4.12]). Interrelations between RAAS and carbohydrates metabolism were described by two factors model. F1 is composed of GL (FL = - 0.67), PRA (FL = 0.71) and ALD (FL = 0.65); F2 consists of IRI (FL = 0.81) and A-II (FL = - 0.71). It was found out association between factor's value and type of blood pressure diurnal rhythm and body mass. F1 in overweight non-dipper (ND) and over dipper (OD) was higher ($P< 0.05$) than in dipper (D) on the account of PRA. F2 was higher ($P< 0.01$) in overweight boys irrespective to the type of BPDR on the account of IRI level.
Conclusions: It was found out negative association between glucose level and plasma activity of renin and aldosteron concentration as well as between angiotensin-II and IRI in boys with arterial hypertension. This fact supports previous finding about supressive action of angiotensin-II on insulin synthesis. Patient's type of blood presure circadian rhythm was strongly associated with RAAS activity and isulin sensitivity.

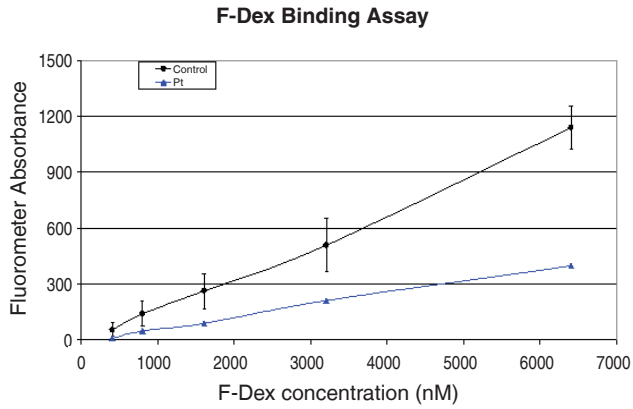
P2-d3-440 Adrenals and HPA Axis 3

Monogenic approach to PCOS

Divya Khurana[1]; Aristotle Panayiotopoulos[1]; Natia Pantsulaia[1]; Maushumi Assad[1]; Duanjun Tan[2]; Felcitas Lacbawan[2]; Josef Michl[3]; Amrit Bhangoo[1]; Svetlana Ten[1]; Steven Ghanny[1]
[1]SUNY Downstate Medical Center, Pediatric Endocrine, Brooklyn, United States; [2]SUNY Downstate Medical Center, Molecular Pathology, Brooklyn, United States; [3]SUNY Downstate Medical Center, Pathology, Brooklyn, United States

Background: PCOS is a heterogeneous group of disorders. Recently some monogenic disorders were recognized to present as PCOS: NC-CAH forms, mutations in WNT 1 and lamin A/C gene, primary insulin receptors defects, lipodystrophies, as well as glucocorticoid(GC) resistance with mutations in the GC receptor. We hypothesized that using a candidate gene approach; new monogenic reasons for PCOS can be found in the GC sign pathway.
Objective: To study the role of GC signaling proteins in the etiology of PCOS. GC signaling was evaluated by GC sensitivity in vitro by F-Dex binding assays and by sequencing 3 genes from GC pathway: Glucocorticoid receptor gene (NR3C1) and 2 co-receptor protein genes FKBP4 and FKBP5
Methods: Recruited 25 controls and 28 PCOS pts. Pts had ACTH stim test, 21-OH gene analysis, OGTT, lipid profile. GC sensitivity was evaluated by F-Dex binding assays. GC index was calculated as log of AUC from the difference in binding to the GC receptor between control and PCOS pts. NR3C1, FKBP4, FKBP5 genes were sequenced
Results: Using the F-Dex binding assay, we found a cohort of PCOS patients that were GC resistant, with dec binding of F-Dex molecules to the GC receptor in comparison to controls(fig 1). GC receptor number was found to be no different from the control patients. From these results, we were able to calculate a glucocorticoid index(GI). We found that the degree of resistance, as calculated by this index, correlated with biochemical data such as degree of elevation of testosterone and other androgens, as well as pt phenotype. Also, we found that this also correlated with the elevated stimulated ratios of delta 5/cortisol, DHEA/ delta 4, as well as DHEA/Testo. Mutational analysis is pending.

Fig 1:



Conclusions: It was found that the degree of GC resistance, as calculated by the GI, correlates well with phenotype and biochemical data for these pts. This indicates that the cause of PCOS of this subgroup was due to a candidate gene in the GC receptor signaling pathway.

P2-d3-441 Adrenals and HPA Axis 3

Influence of ethnicity on the origin and clinical and hormonal characteristics of premature pubarche

Inge Gies[1]; Jesse Vanbesien[1]; Stephanie Verheyden[1]; Ellen Anckaert[2]; Jean De Schepper[1]
[1]UZ Brussel, Pediatrics, Brussels, Belgium; [2]UZ Brussel, Clinical Chemistry, Brussels, Belgium

Background: Smallness for gestation, prematurity and obesity are known risk factors for premature pubarche (PP), whereas PP can be a forerunner of polycystic ovary syndrome in girls.

Objective: We wanted to analyze 1) whether Magreb girls with PP are at higher risk for exaggerated adrenarche and higher serum AMH concentrations, given their known risk for increased adiposity and insulin resistance, and 2) whether the presence of acne was associated with a more severe form of adrenarche.
Patients and methods: 27 (6 boys and 21 girls) children evaluated for PP in 2010 and 2011 at our hospital, in whom a non-classical form of congenital adrenal hyperplasia by ACTH testing and an adrenal tumor by ultrasound were excluded, were studied. Anthropometric data, basal and ACTH stimulated serum androgens, serum SHBG as marker of insulin resistance, serum AMH and bone age readings at the left hand and wrist were collected.
Results: Seven of the 11 Magreb children and 13 of the 16 Belgian girls who were investigated, had a premature adrenarche (PA), defined by a basal DHEAS > 0.6mg/L. Mean gestational age, birth weight SDS, BMI SDS, serum AMH and SHBG concentrations were not different between the Magreb and Belgian girls with PA. Degree of pubic hair development, bone age advancement and the prevalence of acne were also similar. Children with associated acne (n=11) had similar basal serum DHEAS and bone age advancement.
Conclusion: Magreb girls with PP have a comparable frequency of premature adrenarche. No correlation between PA, insulin resistance and potential later risk for polycystic ovary syndrome was found. Serum AMH did not differentiate girls with PA from an idiopathic form of PP. Associated acne does not necessarily indicate an exaggerated form of PP.

P2-d3-442 Adrenals and HPA Axis 3

Phaeochromocytoma- clinical presentation of three cases

Emese Boros; Cécile Brachet; Claudine Heinrichs
Hôpital des Enfants Reine Fabiola- ULB, Pediatrics, Brussels, Belgium

Background: Classically pheochromocytoma presents with headaches, palpitations, and diaphoresis in association with severe hypertension.
Objective and hypotheses: To present the heterogeneity of the clinical presentation of pheochromocytoma in children.
Methods: We present 3 cases of pheochromocytoma diagnosed in our department between 2007- 2011
Results: Case 1: a 5,8 years- old boy presented with headache and severe hypertension. High urinary levels of norepinephrine and normetanephrine were found. The abdominal magnetic resonance (MRI) showed bilateral adrenal masses, confirmed by the 18F- FDG- positron emission tomography. Right adrenalectomy and left tumorectomy was performed. An inactivating mutation in the exon 3 of VHL gene was found. Case 2: a 10,5 years - old girl with a retinal hemangioblastoma. The physical examination was normal. Von Hippel Lindau disease was suspected and an abdominal CT scan was performed as a screening and showed an adrenal mass. Urinary levels of norepinephrine and normetanephrine were elevated. MIBG scan showed a left adrenal mass while MRI showed bilateral adrenal masses. Right adrenalectomy and left tumorectomy were performed, then left adrenalectomy was performed. An inactivating mutation in the exon 2 of VHL gene was found in the index case, his mother and one of his brothers. Case 3: a 15-year-old patient presented with transient and acute left lumbar pain. His clinical examination including blood pressure was normal. An abdominal ultrasonography showed a left adrenal mass. The urinary norepinephrine and normetanephrine were high. The MRI confirmed the left adrenal mass. The MIBG scintigraphy and the Ga 68 DOTATATE PET SCAN showed uptake in the left adrenal mass. Left adrenalectomy was performed.
Conclusions: Pheochromocytoma can be nearly asymptomatic even in the presence of very high levels of catecholamine production.

P2-d3-443 Adrenals and HPA Axis 3

Central adrenal insufficiency (AI) after childhood acute lymphoblastic leukemia (ALL)

Martina Zanotti[1]; Federico Baronio[1]; Claudia Balsamo[1]; Annalisa Martini[1]; Anna Marton[2]; Elena Facchini[2]; Piero Pirazzoli[1]
[1]University of Bologna & S.Orsola-Malpighi H., Pediatric Clinic Endocrine Unit, Bologna, Italy; [2]University of Bologna & S.Orsola-Malpighi H., Pediatric Clinic Oncology Unit, Bologna, Italy

Background: In patients with ALL adrenal insufficiency can arise in the induction phase, due to the high dose corticosteroid therapy, but rarely has been reported during post treatment follow-up.
Objective and hypotheses: We investigated the adrenal function of 7 patients (pts) with a suspected AI arisen during follow-up after treatment for childhood ALL.
Methods: We evaluated pituitary adrenal axis of a cohort of 56 consecutive pts in follow-up (3.6 ± 1.6 years) after childhood ALL (age at diagnosis 5.6 ± 3.4 years; protocol AIEOP ALL 2000, 10/56 treated with cranial radio-prophylaxis). 7/56 pts showed biochemical and clinical (2/7 pts) signs of suspected central AI (table) at median time of 2.4 years after treatment (range 1.2-5.5 years) . All pts did not show signs of AI before and immediately after ALL treatment. In pts with suspected central AI, 1 microgram ACTH test was performed.
Results: In 5/7 cases the ACTH test ruled out AI. In the two symptomatic cases, reduced cortisol basal levels were confirmed with inadequate cortisol response after stimulation (peak <500 nMol/L) in case n. 6 and at the lower limit of the standard (555 nMol/L) in case 2 (table). These 2 pts started hydrocortisone replacement therapy (8 mg/m^2/day) with prompt clinical improvement.
Conclusions: Our study shows that central AI could arise during follow-up of childhood ALL, even treated without cranial radio-prophylaxis. It is however not possible, given the small number of cases, to determine the risk factors and predict the time of onset of central AI in these pts. In our study 1 microgram ACTH test confirmed the suspicion of central AI in symptomatic cases.

Pt	Age at stop ALL treatment (yrs)	Cranial radiotherapy (cGy)	AI onset (year of f-up)	symptoms	Basal ACTH h 8 (pMol/L)	Basal Cortisol h 8 (nMol/L)	ACTH test Cortisol (nMol/L)
1	8.7	1800	5.5	-	2.6	141	223/362
2	4.5	ND	2.4	++	1.8	97	77/555
3	4.8	ND	2.9	-	3.7	102	141/590
4	7.5	ND	5.0	-	2.6	108	483/803
5	5.1	ND	2.1	-	2.2	138	472/660
6	12	ND	1.2	++	5.5	138	105/422
7	5	ND	2.0	-	2.2	113	403/593

P2-d3-444 Adrenals and HPA Axis 3

Molecular mechanisms of action of a novel point mutation in the human glucocorticoid receptor gene causing primary generalized glucocorticoid resistance

Nicolas C. Nicolaides[1]; Amalia Sertedaki[2]; George P. Chrousos[2]; Evangelia Charmandari[2]
[1]Biomedical Research Foundation of the Academy of Athens, Division of Endocrinology and Metabolism, Athens, Greece; [2]University of Athens Medical School, Division of Endocrinology, Metabolism and Diabetes, First Department of Pediatrics, ‚Aghia Sophia' Children's Hospital, Athens, Greece

Background: Primary Generalized Glucocorticoid Resistance (PGGR) is a rare genetic disorder characterized by partial, generalized target-tissue insensitivity to glucocorticoids. The molecular basis of the condition has been ascribed to mutations in the human glucocorticoid receptor (hGR) gene, which impair the molecular mechanisms of hGR action and decrease tissue sensitivity to glucocorticoids. We recently identified a new case of PGGR caused by a novel mutation in the hGR gene.
Objective and hypotheses: To present the findings of the genetic testing of the above patient and the molecular mechanisms through which the natural novel hGR mutant impairs glucocorticoid signal transduction.
Methods and results: DNA was extracted from peripheral lymphocytes and the entire coding region of the hGR gene was amplified and sequenced. A single, heterozygous nucleotide (A to G) substitution was identified at nucleotide position 2177 (exon 8), resulting in histidine (H) to arginine (A) substitution at amino acid position 726 in the ligand-binding domain (LBD) of the receptor. In transient transfection assays, the mutant receptor hGRαH726R demonstrated decreased ability to transactivate the MMTV promoter in response to increasing concentrations of dexamethasone compared with the wild-type receptor.
Western blot analysis showed equal protein expression of hGRα and hGRαH726R, indicating that the above differences do not reflect differences at protein expression levels. Further molecular studies are currently underway to complete the functional characterization of the natural mutant receptor hGRαH726R.

Conclusions: Mutations in the hGR gene impair glucocorticoid signal transduction and alter tissue sensitivity to glucocorticoids. The location of this mutation in the LBD of the receptor may predict impaired affinity for the ligand, while manifestation of the disease at the heterozygote state may indicate a dominant negative effect of the mutant GR upon the wild-type receptor.

P2-d3-445 Adrenals and HPA Axis 3

Transient generalized glucocorticoid hypersensitivity: clinical manifestations and transcriptomics profile

Evangelia Charmandari[1]; Eleni Katsantoni[2]; Panagiota Triantafyllou[3]; Athanasios Christophoridis[3]; George Katzos[3]; Tomoshige Kino[4]; Nicolas C. Nicolaides[5]; Amalia Sertedaki[1]; George P. Chrousos[1]
[1]University of Athens Medical School, Division of Endocrinology, Metabolism and Diabetes, First Department of Pediatrics, ‚Aghia Sophia' Children's Hospital, Athens, Greece; [2]Biomedical Research Foundation of the Academy of Athens, Division of Hematology, Athens, Greece; [3]Aristotle University Medical School, First Pediatric Department, Thessaloniki, Greece; [4]National Institutes of Health, Unit on Molecular Hormone Action, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland, United States; [5]Biomedical Research Foundation of the Academy of Athens, Division of Endocrinology and Metabolism, Athens, Greece

Background: Generalized Glucocorticoid Hypersensitivity is a rare disorder characterized by generalized increased tissue sensitivity to glucocorticoids and compensatory decreases in plasma ACTH and serum cortisol concentrations. The molecular basis and the underlying mechanisms in subjects with the condition have not been fully elucidated.
Objective and hypotheses: To present the clinical manifestations, endocrinologic evaluation and transcriptomics profile in a rare case of transient Generalized Glucocorticoid Hypersensitivity.
Methods and results: A 9 year old girl presented with an 8 month history of progressively deteriorating clinical manifestations of Cushing's syndrome, including central obesity, buffalo hump, purple striae, hirsutism and decreased growth velocity. Endocrinologic evaluation showed undetectable 08:00h ACTH (< 1 pg/mL) and cortisol (0.025 mcg/dL) concentrations that remained similarly decreased throughout the 24h period and did not respond to ovine CRH stimulation (1 mcg/kg). Sequencing of the human glucocorticoid receptor gene following extraction of genomic DNA revealed no mutations or polymorphisms. Transcriptomics analysis (RNA-seq) in the disease and the resolution phase identified 1064 significantly differentially expressed genes. Of those, 200 genes were downregulated, while 864 were upregulated in the disease versus the resolution phase. Bioinformatics analysis on the differentially expressed gene networks is currently underway.
Conclusions: The transcriptomics profile of this patient will elucidate the molecular pathways underlying Generalized Glucocorticoid Hypersensitivity and provide expression signatures for the therapeutic management of glucocorticoid-related disorders.

P2-d3-446 Adrenals and HPA Axis 3

Congenital adrenal hyperplasia in republic of Ireland - should we be screening?

Ciara McDonnell[1]; Aoife Carroll[1]; Mary White[1]; Philip Mayne[2]; Nuala Murphy[1]
[1]Children's University Hospital, Department of Endocrinology & Diabetes, Dublin, Ireland; [2]Children's University Hospital, Biochemistry, Dublin, Ireland

Background: Controversy persists regarding the efficacy of neonatal screening for detecting congenital adrenal hyperplasia [CAH]. The French national newborn screening program [1996 – 2003] reported that screening preceded clinical diagnosis in 42.3% of neonates. A recent two year prospective audit in the UK identified an incidence of 1 in 18,000 newborns with 70% of those with salt wasting crisis likely to be identified by newborn screening.
Objective and hypotheses: This study reviews the presentation of all cases of congenital adrenal hyperplasia within the Republic of Ireland in the first decade of the 21st century to ascertain whether a case exists for the introduction of CAH to the Irish Newborn Screening programme.
Methods: Case records of all children diagnosed between January 1st 2000 and December 31st 2010 were reviewed. Diagnosis was confirmed by raised serum 17OH progesterone level or characteristic urinary steroid profile. Clinical features, biochemical findings, treatment and outcome of all cases were reviewed.
Results: Forty two children [Male=21] were diagnosed over the decade studied [average 3.8 cases annually, range 1-6/year]. Fourteen of 42 [33%] were diagnosed in the first seven days of life which would precede a screening result [8 females with virilised genitalia, 4 males]. A further 15/42 children [14 male, 1 female] presented with salt losing crisis after the first week of life. The average incidence of CAH was calculated as 1 per 26,000 births which is an incidence of 0.44 per 100,000 population less than 14 years old.
Conclusions: Two thirds of children presenting with CAH in the Republic of Ireland between 2000 and 2010 would have been identified earlier through a newborn screening programme. The incidence of CAH is comparable to countries where newborn screening has been implemented successfully.

P2-d1-447 Autoimmune Endocrine Disease 2

Low-dose cyclosporine A in a patient with severe APS1-associated keratoconjunctivitis

Antonella Meloni[1]; Anna De Magistris[1]; Manuela Gherardini[2]
[1]Clinica Pediatrica II, Ospedale Microcitemico, Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Cagliari (Sardinia), Italy; [2]Clinica Oculistica, Azienda Ospedaliero-Universitaria, Dipartimento di Chirurgia e Scienze Odontostomatologiche, University of Cagliari, Cagliari (Sardinia), Italy

Background: Autoimmune Polyendocrine Syndrome type1 (APS1, OMIM 240300) is a rare AR disorder caused by mutations in the AIRE gene, primarly involving the endocrine glands. The patients manifest also mucocutaneous candidiasis (CMC) and ectodermal distrophies.
Chronic keratoconjunctivitis is the most commonly observed ophthalmic finding in APS1 (25%-50%), its pathogenesis is unclear, appropriate intensive management is required. Corneal transplants and keratoplasty have little success, medical treatment often fails in severe keratoconjunctivitis/ progressive forms.
Objective: To describe a case with APS1 with bilateral chronic keratoconjunctivitis, who developed severe photophobia and corneal ulceration, which was successfully treated with cyclosporine A orally.
Patient and methods: The patient, a 35-yr-old male, was diagnosed in infancy with APS1 (CMC, Addison's disease, hypoparathyroidism). Over time, he developed additional manifestations and severe phenotype (Table). Disabling chronic keratopathy appeared since 17 yr of age with dry eye, photophobia, decreased vision, corneal ulceration. Conservative management with lubrificants and artificial tears, associated with topical corticosteroids, antibiotics and oftalmoplegic agents in the acute phase of keratitis did not prevent the progression of the ulcerative process. Addition of low-dose cyclosporine A orally (5 mg/kg/day), in a single dose for 4 months, led to arrest of the ulcerative process, reduce corneal edema and greatly improve photophobia. Minimum short-term side effects were noted (slight increase in creatinine, hypomagnesemia).
Results and conclusions: The benefit of systemic cyclosporine A in the short treatment of keratoconjunctivitis has been reported in isolated cases with APS1. Our experience confirms efficacy of immunosuppressive therapy and supports the use of oral cyclosporine A in selected patients with severe APS1-associated keratoconjunctivitis. Further studies are needed to confirm our preliminary findings.

Patient sex	Origin	Current age	Age at diagnosis	Classic features	Ectodermal distrophy	Other features	AIRE genotype
Male	Sardinia	35.1 yr	4 yr	CMC (1) Addison's disease (4), Hypoparathyroidism (4,5)	Dental enamel defects (18), Nail dystrophy (22), Keratoconjunctivitis (17)	Polineuropathy (17) Hypogonadism (16), Malabsorption (19), Psoriasis (23) Alopecia (26), Vitiligo (22) GH deficiency (30)	R139X/ R139X

P2-d1-448 Autoimmune Endocrine Disease 2

Clinical characteristics of patients with autoimmune polyglandular syndrome type 1 (APS 1)

Anna Malinowska; Agnieszka Rudzka-Kocjan; Mieczyslaw Szalecki
Children's Memorial Health Institute, Department of Endocrinology and Diabetology, Warsaw, Poland

Background: Autoimmune Polyglandular Syndrome type 1 (APS 1) is a rare autosomal-recessive disease caused by mutations in the autoimmune regulator (AIRE) gene. Although typical manifestations of APS 1 include candidiasis, hypoparathyroidism and Addison's disease, other autoimmune conditions can be associated with the syndrome.
Objective: To present clinical course of APS 1 in patients from Caucasian population diagnosed at pediatric age. Patients:8 patients (4 girls, 4 boys), currently aged 10-26 years, diagnosed in one department of endocrinology between year 2000 and 2010. Mean age of boys and girls is 21 and 15 years respectively. The diagnosis of APS 1 was made on the basis of presence of at least two cardinal features of the disease.
Results: In every patient mucocutaneous candidiasis and hypoparathyroidism were found. So far adrenocortical failure was diagnosed in 4 cases. The disease manifested the earliest at the age of 15 months as the mucocutaneous candidiasis. The highest number of disturbances (6 in total) were diagnosed in 1 patient. In 2 patients 5 disturbances, in 2 patients 4 abnormalities, in 2 patients 3 components and in 1 patient 2 disturbances were diagnosed. Among rare abnormalities megaloblastic anemia, keratitis, asplenia, and central diabetes insipidus were found. In 1 boy profound short stature of undetermined etiology was noticed. In the studied patients the presence of the following autoantibodies: against 21-hydroxylase, against LKM (liver-kidney microsomes), against GAD65 and against IA2 were found. In every patient molecular analysis of AIRE gene was performed. The most common Finnish R257X mutation was found in 5 patients.
Conclusions: APS 1 is a rare syndrome in Caucasian pediatric population. In the studied patients many components of the syndrome manifested at early age. Patients with APS 1 require constant and regular specialist care with knowledge that new components of the APS 1 may appear over the years.

P2-d1-449 Autoimmune Endocrine Disease 2

Interruption of insulin treatment after anti-CD20 therapy in a boy with type 1 diabetes, thyroiditis and immune thrombocytopenia

Letizia von Laer Tschudin[1]; Annette von Scheven-Gête[2]; Valérie M Schwitzgebel[3]; Sophie Stoppa-Vaucher[1]; Michael Hauschild[4]; Michael Hofer[2]; Franziska Phan-Hug[1]
[1]Centre Hospitalier Universitaire Vaudois, Paediatrics, Unit of Endocrinology and Diabetology, CHUV, Lausanne, Switzerland; [2]Centre Hospitalier Universitaire Vaudois, Paediatric rheumatology, CHUV Lausanne and HUG Geneva, Lausanne, Switzerland; [3]Hôpital Universitaire de Génève, Paediatrics, Unit of Endocrinology and Diabetology, HUG, Génève, Switzerland; [4]Centre Hospitalier Universitaire Vaudois, Paediatric diabetology and endocrinology, CHUV, Lausanne, Switzerland

Background: Type 1 diabetes (T1D) is a component of autoimmune polyglandular syndrome (APS). No cure for T1D exists but immune modulatory approaches are under investigation. Specific antibody treatment targeting CD20 expressed at the surface of B-lymphocytes would increase C-peptide level and reduce daily insulin dose.
Objective and hypotheses: We report a case with APS type 3 associating thyroiditis, immune thrombocytopenia (ITP) and T1D where insulin therapy was stopped for 28 months after treatment with anti-CD20.
Results: ITP and thyroiditis were diagnosed in a 13-year-old Caucasian boy. L-T4 therapy was started. ITP was resistant to immunoglobulins and dependent on high dose steroids. Under such therapy, the patient developed T1D (Glycemia 22mmol/l, insulin 36.2U/l, HbA1c: 6.1 %, anti-GAD 3530E/l (<9.5)) and insulin treatment was initiated. Because of persistent severe thrombocytopenia even under Cyclosporine treatment, anti-CD20 therapy was introduced for 2 months, allowing stabilization of thrombocytes >50G/l and stopping steroids 7 months later. Three and five months after anti-CD20 therapy, l-T4 and insulin therapy could be stopped, respectively. Twelve months later normal C-peptide level, HbA1c (5.6%) and reduced anti-GAD (672E/l) were measured. A second anti-CD20 trial for relapse of ITP, thyroiditis, elevated HbA1c (7.2 %) and anti-GAD (2612E/l) was initiated one year later. Despite decreasing antibody levels (anti-GAD 157E/l) HbA1c stayed elevated and glucose monitoring showed elevated postprandial glycemia with normal fasting glucose and C-peptide level. Low dose insulin therapy was reintroduced after 28 months.
Conclusions: At our knowledge this is the first report where anti-CD20 therapy led to an interruption of insulin in T1D in the context of APS-3. Anti-CD20 treatment allowed this boy to go through puberty without an often-constraining insulin therapy at this age. Even if the first diabetes manifestation was probably triggered by corticosteroid treatment anti-CD20 therapy seems to be efficacious in the early course of T1D.

P2-d1-450 Autoimmune Endocrine Disease 2

Enteropathy with loss of gastrointestinal endocrine cells in a child with type 1 diabetes and immundysregulation

Thomas Barth[1]; Georgia Lahr[2]; Wilhelm Friedrich[2]; Martin Wabitsch[3]; Carsten Posovszky[2]
[1]Ulm University, Department of Pathology, Ulm, Germany; [2]University Medical Center Ulm, Department of Pediatrics and Adolescent Medicine, Ulm, Germany; [3]University Medical Center Ulm, Department of Pediatrics and Adolescent Medicine, Division of Endocrinology and Diabetes, Ulm, Germany

Background: Gastrointestinal endocrine cells (GIECs) are necessary for the regulation of gastrointestinal function; e.g. the lack of intestinal enteroendocrine cells in enteroendocrine cell dysgenesis causes severe malabsorptive diarrhea and leads to severe, life-threatening watery diarrhea. Recently, we described paucity of GIECs in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) as specific gastrointestinal (GI) feature.
Objective: Other autoimmune polyendocrine disorders such as X-linked syndrome (IPEX) are often accompanied by GI-symptoms and may be also affected.
Results: We report a patient who developed severe diarrhea and juvenile-onset diabetes in his second year of life. Endoscopy revealed a massive inflammation in the bowel. Histology showed a chronic inflammation of the GI-tract, with duodenal villous blunting, crypt hyperplasia and focally increased intraepithelial lymphocytes. FOXP3 expression in lymphocytes in the GI-tract was reduced. However, neither mutations in the FOXP3 gene nor in the promoter were found. Therefore, the patient was classified as IPEX-like syndrome. Interestingly, immunehistochemistry showed a marked reduction of GIECs in the gastric and duodenal mucosa similar to other APECED patients, however an AIRE mutation was excluded. Growth retardation and an autoimmune thyroiditis followed in the next two years. At 6 years, B- and NK-cell lymphopenia developed with reduced immunoglobulin making regular intravenous substitution of immunoglobulin necessary. At 8 years, he suffered from salmonella enteritis, developed recurrent infections (e.g. otitis, bronchitis) and photodermatitis. The following year, he suffered from bronchiolitis obliterans and became neutro- and thrombopenic. In addition, a posterior reversible encephalopathy syndrome and a toxic hepatopathy developed; the patient died at the age of 9 years due to multi-organ failure.
Conclusions: The reduction of GIECs is a specific and important early event in the pathogenesis of autoimmune polyendocrine disorders with GI dysfunction.

P2-d1-451 Autoimmune Endocrine Disease 2

Multiorgan autoimmunity in a Turner syndrome patient with partial monosomy 2q and trisomy 10p

Armando Grossi[1]; Alessia Palma[2]; Graziamaria Ubertini[1]; Ginevra Zanni[3]; Antonio Novelli[4]; Marco Cappa[1]; Alessandra Fierabracci[2]

[1]Bambino Gesu' Children's Hospital, Endocrine and Diabetes Unit, Rome, Italy; [2]Bambino Gesu' Children's Hospital, Research Laboratories, Rome, Italy; [3]Bambino Gesu' Children's Hospital, Molecular Medicine Unit, Rome, Italy; [4]Institute Css Mendel, Cytogenetic Laboratory, Rome, Italy

Background: Turner syndrome (TS) is a condition caused by numerical and structural abnormalities of the X chromosome, characterized by a series of clinical features, the most common of which are short stature and gonadal dysgenesis. An increased frequency of autoimmune diseases as well as an elevated incidence of autoantibodies has been observed in TS.
Objective and hypothesis: What follows is a unique case of TS with chromosomal aneuploidia and rearrangements on chromosome 2 and chromosome 10 [45, X/46XX/del2 (q37.1), dup(10) (p12.32)]. The patient presented partial empty sella and association with a cohort of multiorgan autoimmunity-related manifestations including Hashimoto's thyroiditis, celiac disease, insulin dependent diabetes mellitus (Type 1 diabetes), alopecia, onycodystrophy, possible autoimmune inner ear disease with sensorineural deficit, preclinical hyposurrenalism. We aimed to investigate the genetic background that could be responsible for the complex association of multiorgan autoimmune manifestations in this patient.
Method: Screening of the autoimmune regulator gene (AIRE) and of protein tyrosine phosphatase non receptor type 22 (PTPN22) gene was performed on patient's DNA. SNP-array analysis was conducted to verify in which extent autoimmunity-related genes could have been affected by the peculiar TS karyotype and identify genotype/phenotype correlations.
Results: AIRE gene screening revealed heterozygous c.834 C>G polymorphism and IVS9+6 G>A variation, thus excluding autoimmune polyendocrine syndrome Type 1. Heterozygous R620W polymorphism of the PTPN22 gene was detected in patient's DNA. SNP-array analysis revealed that autoimmunity-related genes could be affected by the partial monosomy 2q and trisomy 10p including IL2 receptor/CD25.
Conclusion: These data suggest that early genetic analysis is recommended in TS patients with complex associations of multiorgan autoimmune manifestations for their diagnostic classification as well as indicator of undiscovered pathogenetic mechanisms.

P2-d1-452 Autoimmune Endocrine Disease 2

Vitamin D status in children with Hashimato's thyroiditis

Mahmut Orhun Camurdan; Esra Doger; Nurullah Celik; Hamdi Cihan Emeksiz; Peyami Cinaz; Aysun Bideci

Gazi University Medical School, Pediatric Endocrinology, Ankara, Turkey

Background: Vitamin D is involved in immune system and, in particular, on T cell-medicated immunity. Vitamin D receptor is profoundly present in the immature immune cells of thymus and the CD8.
Objective: To investigate vitamin D status in children with Hashimato's thyroiditis.
Subjects and methods: The study group consisted of 78 children with newly diagnosed Hashimato's thyroiditis and 74 subjects as the control group. Parameters of calcium metabolism, thyroid function tests and 25(OH) vitamin D levels were measured.
Results: Vitamin D deficiency rate was significantly higher in the Hashimato group compared with the control subjects (73.1% vs 17.6%, p<0.0001). In the Hashimato group, mean 25(OH) vitamin D levels were significantly lower compared with the control group (12.5±7.0 vs 22.3±7.9 ng/mL, p<0.001) and was inversely correlated with the AntiTPO levelsm (r = - 0.30, p = 0.007).
Conclusion: The higher vitamin D deficiency rates besides lower vitamin D levels in the Hashimato group together with the inverse correlation between vitamin D and AntiTPO suggest that vitamin D deficiency may have a role in the autoimmun process in Hashimato's thyroiditis in children.

P2-d1-453 Autoimmune Endocrine Disease 2

Coeliac disease and risk for other autoimmune diseases in patients with Williams-Beuren syndrome

Elisabetta Lapi[1]; Stefano Stagi[2]; Maria Gabriella D'Avanzo[3]; Marika Petrillo[3]; Cecilia Cecchi[4]; Francesco Chiarelli[5]; Salvatore Seminara[4]; Maurizio de Martino[4]

[1]Anna Meyer Children's University Hospital, Medical Genetics Unit, Florence, Italy; [2]Mugello's Hospital, Paediatric Unit, Borgo San Lorenzo, Italy; [3]San Giuseppe Moscati Hospital, Medical Genetics Unit, Avellino, Italy; [4]Anna Meyer Children's University Hospital, Department of Paediatrics, Florence, Italy; [5]University of Chieti, Department of Paediatrics, Chieti, Italy

Introduction: In Williams-Beuren syndrome (WBS) an higher prevalence of coeliac disease (CD) has been reported, but the coexistence with other autoimmune diseases has not been evaluated.
Objective: To study the prevalence of the more frequent autoimmune diseases and organ- and non-organ-specific autoantibodies in WBS.
Patients and methods: We longitudinally analyzed 46 WBS patients to evaluate the prevalence and co-occurence of the main autoantibodies and the HLA typing for CD diagnosis. These data were compared with healthy age- and sex-matched controls, Down (DS) and Turner (TS) syndrome patients.
Results: In one (2.2%) WBS patient CD was diagnosed, differing not significantly from healthy controls (0.55%; P = NS), but differing from DS and TS (respectively, 9.9% and 8.3%; P = < 0.005). However, no patients with WBS showed anti-thyroid antibodies and other organ- and non-organ-specific autoantibodies, not significantly differing from healthy controls, but with a significant difference with DS (respectively, 10.5% and 6.7%; p < 0.05) and TS (respectively, 9.37% and 7.8%, p < 0.00) patients. The frequencies of CD specific HLA-DQ heterodimers were not significantly higher than controls, even if WBS patients showed more frequently (56% vs 39%; P < 0.05) DQA1*0505 allele.
Conclusions: CD may be no more frequent in patients with WBS, such as showed in some past papers. However, unlike other genetic conditions such Turner and Down syndromes,in WBS no evidence of a significantly higher prevalence of other autoimmune diseases or positivity of the main organ and non-organ-specific autoantibodies was found. A different pathogenesis of CD in WBS may be considered

P2-d1-454 Autoimmune Endocrine Disease 2

Unusual presenting forms of primary adrenal insufficiency

Maria Chueca Guindulain[1]; Nora Lecumberri Garcia[2]; Sara Berrade Zubiri[1]; Sergio Aguilera[3]; Mirentxu Oyarzabal Irigoyen[2]

[1]Complejo Hospitalario de Navarra, Pediatric Endocrinology, Pamplona, Spain; [2]Navarra Hospital Complex, Pediatric Endocrinology, Pamplona, Spain; [3]Navarra Hospital Complex, Pediatric Neurology, Pamplona, Spain

Background: Primary Adrenal Insufficiency (PAI) is rare in childhood. More than 40% of all PAI cases are diagnosed late because the symptomatology has not been suspected.
Objective: To describe physical clues that guide to diagnose PAI. Patients: 3 cases of PAI of rare prevalence.
Results: 1) A 14-year old male, athletic with progressive, generalized hyperpigmentation since the age of 6 years. No asthenia or weight loss. Lab: cortisol 2.58 ug/dL; corticotropin 2333 ng/L; aldosterone 46 ng/L; Renin 3.7ng/dL; DHEA-S 22.8 ug/dL. Suspicion of familiar glucocorticoid deficiency/ACTH resistance. Genetic analysis ACTH receptor negative. 2) A 7-year old male referred from Neuropediatrics due to visual and auditory impairment for the last 5 months with diplopia and difficulty for reading; poor motor control; behavioral issues. Dark skin color (racial) with hyperpigmentation. Lab: basal cortisol, 3.7 ug/dL; DHEA-S, 1.38 ug/dL; ACTH, 3060 ng/L; aldosterone, 53 ng/L; renin 16 ng/L; cerebral MRI: parietal-occipital white matter demyelination. Suspected diagnosis: X-linked adrenoleukodystrophy, confirmed with increased VLCFA in blood and p(R66oW)c(1978C-T) mutation. 3) A 12-year old female who presents in the ER due to vomiting and abdominal pain. She reports asthenia, anorexia, weight loss for the last 3 months. Controlled in Psychology with a diagnosis of anxiety-depression disorder. History of Atopic dermatitis. Mother and maternal family with thyroid disease. Examination:muscle weakness,hyperpigmentation. Lab: cortisol 3.73 ug/dl;

ACTH 380 ng/l; aldosterone 10 pg/ml; antibodies against adrenal capsule positive; TSH 6.5; FT4 1.2; anti-thyroglobulin antibodies +; anti-GAD and anti-IA2 + without clinical diabetes. Suspected type II polyglandular A-I syndrome.
Conclusions: We remark on the vagueness symtomatology of the cases often leads to late and mistaken diagnostic approaches. Hyperpygmentation is a common sign in these patients to suspect the disease. Addison's disease involves long-term monitoring given the association of other endocrine diseases.

P2-d1-455 Autoimmune Endocrine Disease 2

Autoimmune polyendocrine syndrome type 1 due to homozygous missense mutation in autoimmune regulator gene AIRE in a consanguineous Saudi family

Mohammed Al Dubayee[1]; Mohammed Al Balwi[2]; N Katsumata[3]; Ibrahim Al Alwan[1]; Hessa Al Kandari[4]
[1]King Saud Bin Abdulaziz University for Health Sciences, Pediatric, Riyadh, Saudi Arabia; [2]King Saud Bin Abdulaziz University for Health Sciences, Moleculargenetic, Riyadh, Saudi Arabia; [3]National Research Institute for Child Health and Development, Department of Molecular Endocrinology, Tokyo, Japan; [4]Farwaniya Hospital, Pediatric, Kuwait, Kuwait

Background: Autoimmune polyendocrine syndrome type I (APS-I) or autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) is a rare autosomal recessive disease caused by mutations of the Autoimmune REgulator (AIRE) gene. Affected individuals typically have chronic mucocutaneous candidiasis, primary hypoparathyroidism, and primary adrenocortical insufficiency (Addison's disease). Other autoimmune diseases such as autoimmune hepatitis, diabetes mellitus, keratitis, and pernicious anaemia occur less frequently. The causative AIRE gene has important role in induction and tolerance to self antigens
Objective and hypotheses: The objective of this study was to describe the phenotypic features of APS-I in a consanguineous Saudi family and correlate it to their mutation in the AIRE gene
Methods: Clinical data were obtained from medical records of a Saudi family with clinical and biochemical features of APS-I, DNA sequencing of the AIRE gene was done for all family members
Results: Seven out of eight children fulfill the diagnostic criteria of APS-I, one sibling was disease free. The phenotypic presentation varies significantly despite the fact that they all are pediatric and carries the same genetic mutation. Both parents were clinically asymptomatic. Analysis of AIRE gene demonstrated that, the affected family member are homozygous for pathogenic missense mutation (c.202A>G (p.T68A)). Subsequent molecular analysis of the parents showed that they are heterozygous for (c.202A>G (p.T68A))
Conclusions: Autoimmune polyendocrine syndrome type I (APS-I) is a rare monogenic disease. Clinical presentation can vary among sibling with same genetic mutation. Genetic counseling is highly recommended, since no specific therapy available

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P2-d1-456 Autoimmune Endocrine Disease 2

A new mutation in autoimmune polyendocrine syndrome type 1

Ayse Derya Bulus[1]; Nesibe Andiran[1]; Handan Akil[2]; Pascale Saugier-Veber[3]
[1]Keçiören Training and Education Hospital, Pediatric Endocrinology, Ankara, Turkey; [2]Numune Training and Education Hospital, Ophtolmology, Ankara, Turkey; [3]Laboratoire de Génétique Moléculaire, Faculté de Médecine et de Pharmacie, Genetics, France

Background: Autoimmune polyendocrine syndrome type 1(APS 1) APECED is an autosomal recessive disorder that causes progressive endocrine tissue destruction, cell-mediated immune deficiency and ectodermal dystrophies. The disabling ocular manifestations include chronic persistent keratoconjunctivitis, dry eye, iridocyclitis, retinal detachment , optic atrophy and rarely cataract. We report a new AIRE gene mutation in a patient with hypoparathyroidism, mucocutaneus candidiasis, ectodermal displasia, pernicious anemia and cataract.
Case: A 9 years and 10 months of age female patient admitted because of alopecia.In physical examination, weight 25.5kg(25–50 p), height130.2cm(25-50 p). She had multiple local alopecia, bilateral loss of eyelashes and eyebrows, oral candidiasis, ectodermal dysplasia of nails. The patient was first child of firstdegree consanguineous Turkish parents.In history, it was learned that one year ago, she admitted to an other hospital because of lethargy and convulsion. Laboratory results:Ca:3,5,P:9,3 mg/dl,PTH: 1,2(11-75)pg/ml. The diagnosis of hypoparatyroidism was made and rocaltrol was given. The history of untractable diaper dermatitis and recurrent oral candidiasis during her infancy were also taken. Laboratory Ca:9,1, P:9,3 mg/dl,ALP:345 IU/L,PTH:1pg/ml.Hb:11,6 g/dl, MCV:102,3 fL.She was diagnosed as megaloblastic anemia. Vit B12:34 pg/mL.She had normal cortisol (25 µg/dL) response to ACTH stimulation test. Due to complaint of blurred vision ophthalmology consultation was perfomed . Her best corrected visual acuity was 20/50 OD and 20/50 OS. Slit lamp examination showed a posterior polar cataract on left eye. Genetic analysis:Sequencing of the entire coding region of the AIRE gene led us to identify an homozygous mutation in exon 2:c.267_275del(p.Tyr90_Arg92del).
Discussion: Although the association between APS 1 and keratoconjuctivitis has been frequently established our patient had unilateral posterior polar cataract which was very rare. The 9bp deletion c.267_275del (p.Tyr90_Arg92del) has never been reported.

P2-d1-457 Autoimmune Endocrine Disease 2

HLA markers of autoimmune endocrinopathies

Elena Kondratyeva[1]; Natalya Tarasenko[2]; Irina Chernyak[3]; Asiet Tlif[1]
[1]Kuban State Medical University, Department of Pediatrics of the Advanced Training and Professional Development Faculty, Krasnodar, Russian Federation; [2]Research Institute of Medical Genetics SB RAMS, Laboratory of Molecular Genetics, Tomsk, Russian Federation; [3]Children's Regional Clinic, Department of Endocrinology, Krasnodar, Russian Federation

Aim: to study associations of polymorphic alleles of HLA-DQB1, HLA-DQA1 genes with diabetes mellitus 1 type (DM1), diabetes mellitus 1 type in combination with autoimmune thyroiditis (AT), celiac disease (CD) in probands of Krasnodar region.
Materials and methods: The alleles of HLA-DQB1, HLA-DQA1 genes in 110 children with DM1, 20 children with DM1 in combination with AT and in 24 patients with celiac disease were determined. Genotyping of specifities of HLA-system were conducted with use of commercial sets of «DNA-Technology» firm, Moscow.
Results: For patients with DM1 of Krasnodar Region following classic predisposing alleles:DQA1*0301,DQB1*0201, DQB1*0302 and DQB1*0304 were typical. DQB1*0304 allele, revealed only in Russian populations and was not meet in European populations, was selected in 4,5% patients with DM1of Krasnodar Region type, which is higher than in patients with DM1 in Moscow and Vologda population. Combination of AT and DM1 was registered in 17%, CD in 0,3%. In Krasnodar population of patients with DM1 in combination with AT, DQB1*0305 allele was registered significantly more frequent than in comparison with group of patients with diabetes without AT ($p<0,0206$). For patients with celiac disease without diabetes it was typical: DQA1*301-38,2%, DQA1*0501-43,8% alleles and of DQB1*0201-41,8%, DQB1*0302-10,4% genes. It was conducted a comparative analysis alleles of DQA1 and DQB1 genes in groups of patients with CD and DM1. Protecting DQA1*0103 allele for DM1 was registered more frequent at CD. Predisposing to DM1 DQB1*0302 allele was noted more frequent in patients with DM1. DQB1 0601 allele also was revealed more frequent in patients with DM1. QB1*0303 allele was registered 5 times more frequent at CD, protective for DM1 DQB1 0602/8 - 4 times more frequent was registered at CD.
Conclusions: Obtained results testify the necessity of further studying of HLA-system genes at given diseases, in their combinations, including other populations of Russian Federation.

P2-d2-458 Bone, Growth Plate and Mineral Metabolism 2

Different mechanisms of intestinal calcium absorption during pregnancy,lactation and childhood: therapeutic implications and long-term responses to treatment in patients with hereditary vitamin D-resistant rickets (HVDRR)

Deepti Chaturvedi[1]; Michèle Garabedian[2]; Jean-Claude Carel[1]; Juliane Leger[1]

[1]Hospital Robert Debre, Pediatric Endocrinology and Diabetes, Paris, France; [2]INSERM U986, Reference Center for rare diseases of calcium, Paris, France

Background: Intestinal calcium absorption is regulated principally by 1,25-dihydroxyvitamin D, but other regulators are also involved in this process.
Objective and hypotheses: We describe three cases of HVDRR that provide support for insight from clinical practice.
Methods: All three children were born with unaffected bones, because calcium flux across the placenta was normal. Two of the cases were referred at about 20 months of age, with classical clinical features of severe rickets, including bowing of the legs. They were treated with calcium intravenously for 12-18 months, following an initial lack of response to oral calcium and vitamin D. The third patient (a younger sibling of case 1), who was exclusively breast fed, was diagnosed at four months of age, due to alopecia. His condition was successfully managed with high doses of oral calcium and vitamin D. All three patients were homozygous for a loss-of-function mutation of exon 3 introducing a premature stop codon at residue 73 (normally an arginine residue) in the ligand-binding domain of VDR.
Results: At the most recent evaluation of these patients, currently maintained on oral calcium and vitamin D treatment, at the ages of 7.2 years, 4 years and 4.7 years, respectively, clinical, radiological and serum bone metabolism markers were found to be normal.
Conclusions: These data clearly indicate that placental lactogen and prolactin stimulate intestinal calcium absorption during pregnancy and lactation, respectively, preventing deleterious bone disease in patients with HVDDR and demonstrating the existence of VDR-independent mechanisms during these periods. The maintenance of breast-feeding for several months and an increase in calcium intake therefore constitute an effective approach to ensuring adequate absorption and preventing rickets. During childhood, after the initial treatment of rickets with high-dose parenteral calcium to bypass intestinal calcium absorption, it was possible to maintain normal bone metabolism and structure through long-term oral calcium supplementation.

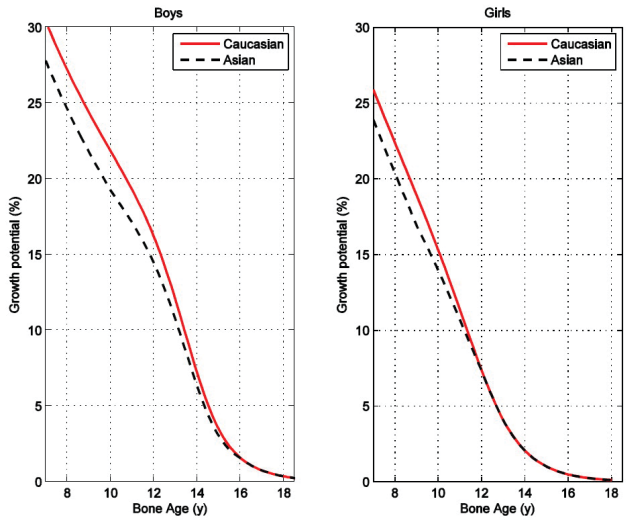
P2-d2-459 Bone, Growth Plate and Mineral Metabolism 2

Adult height prediction for Han children based on automated bone age determination

Shao-Yan Zhang[1]; Toshiaki Tanaka[2]; David Martin[3]; Gang Liu[1]; Chen-Guo Ma[4]; Yi-San Han[5]; Xun-Zhang Shen[6]; Rui-Long Xu[7]; Hans Henrik Thodberg[8]

[1]Hebei, Research Institute of Sports Sciences, Shijiazhuang, China; [2]Child Health Institute, Tanaka Growth Clinic, Tokyo, Japan; [3]Tubingen University Children's Hospital, Pediatric Endocrinology and Diabetology, Tubingen, Germany; [4]Dalian Sports Science Research Institute, Dalian, China; [5]Wenzhou Sports Science Research Institute, Wenzhou, China; [6]Shanghai Sports Sciences Research Institute, Shanghai, China; [7]Guangdong Sports Science Research Institute, Guangzhou, China; [8]Visiana, EO, Holte, Denmark

Background: Adult height prediction (AHP) methods have been studied for more than 60 years, but until recently these have considered only Caucasian children.
Objective and hypotheses: To present an AHP method for Asian (Han) children based on automated determination of bone age.
Methods: The Asian AHP model is constructed on the basis of an existing model for Caucasian children. This study determines the necessary adjustment to describe normal Chinese children. Bone age is determined by the BoneXpert method.
Population: 6,026 healthy children of age 2-19 from a cross-sectional study in five cities in China in 2005 are used to design the model. The modelling is based on the expected secular trend of adult height in young adult Chinese. The model is validated on a longitudinal study performed in Tokyo 1988-2006 on 27 boys and 30 girls with untreated, idiopathic, short stature (ISS).
Results: The Caucasian AHP method overestimates adult height in normal, prepubertal Asian boys and girls by 4.5 cm and 3.2 cm respectively. The Asian model is set up to correct this bias. The figure compares the growth potential for the two models for children with bone age = age. When the model is validated on the ISS data it yields a root mean square error of 3.3 cm for boys in the bone age range 6-15 years and 3.5 cm for girls in the bone age range 6-13 years.
Conclusions: The accuracy of the new AHP model for Asian children has been validated in untreated ISS children. The same accuracy is expected in clinical practice in this patient group because the method is based on automated bone age determination. However, the model has not yet been validated on an independent population of normal-stature Han children with known adult height, and the authors therefore solicit investigators in possession of such image data to join this research project for its completion and final publication.



P2-d2-460 Bone, Growth Plate and Mineral Metabolism 2

The impact of vitamin D in pregnancy on extraskeletal health in children: a systematic review

Henrik Thybo Christesen[1]; Claes Elvander[1]; Ronald F Lamont[2]; Jan Stener Jørgensen[2]

[1]Odense University Hospital, H.C. Andersen Children's Hospital, Odense, Denmark; [2]Odense Univeristy Hospital, Obstetrics and Gynaecology, Odense, Denmark

Background: Maternal vitamin D (VD) status in pregnancy may affect the extra-skeletal health of the offspring.
Objective and hypotheses: Evidence level A and B was systematically reviewed.
Methods: Cohrane, PubMed, Embase were searched using "vitamin D" AND "pregnancy" for randomized controlled trials (RCTs), cohort and case-control studies up to January 1.
Results: VD supplementation resulted in increased birth weight in one randomized controlled trial (RCT), but not in 5 others. Cord 25OHD was (mean) 36% lower than maternal 25OHD. In cohort and case-control studies (total n=24), higher VD intake, or 25OHD, were associated with: Birth weight (increased in large studies only, modified by VD receptor polymorphisms and by race (U-shaped in whites)); HIV mother-to-child transmission (lower); respiratory infections (conflicting data); wheezing (lower in five studies, higher in one); inhalant allergen-specific IgE at 5 years (U-shaped, one study); rhinitis symptoms or eczema (lower in one larger study each); type 1 diabetes at 15 years (lower in one study, no association in another); schizophrenia (U-shaped, one study).
Conclusions: Observational studies suggest an effect of VD on several outcomes. U-shaped associations warrant caution.

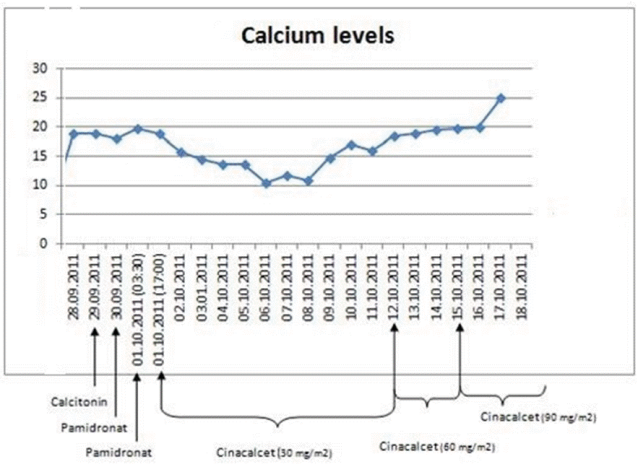
P2-d2-461 Bone, Growth Plate and Mineral Metabolism 2

Challenging clinical course of a case of neonatal severe hyperparathyroidism (NSHPT) associated with homozygous CASR mutations

Zeynep Atay[1]; Abdullah Bereket[1]; Belma Haliloglu[1]; Saygin Abali[1]; Tutku Ozdogan[2]; Tatiane Vilaca[3]; Lucie Canaff[3]; David E. Cole[4]; Geoffrey N. Hendy[3]; Serap Turan[1]

[1]Marmara University, School of Medicine, Pediatric Endocrinology, Istanbul, Turkey; [2]Marmara University, School of Medicine, Neonatology, Istanbul, Turkey; [3]McGill University, Calcium Research Laboratory, Montreal, Canada; [4]Toronto University, Laboratory Medicine & Pathobiology, Toronto, Canada

Background: NSHPT is a rare life threatening disease caused by homozygous inactivating calcium-sensing receptor (CaSR) mutations.
Objective: To describe an unusual case of NSHPT that illustrates the difficulties in management.
Patient and results: A 23-day-old female neonate was admitted for respiratory distress and hypercalcaemia. Physical examination: hypotonia, coarse face, bell-shaped chest and respiratory distress requiring ventilatory support. The parents were third degree cousins. Laboratory evaluation: serum Ca: 19 mg/dl (N: 8.4-10.5), P: 2.6 mg/dl (N: 4.8-8.2), PTH: 1096 pg/ml (N: 9-52) and urinary Ca/Cr: 0.5 mg/mg (N<0.86). At 25 days of age, the sc calcitonin (10 IU/kg-4 doses) and one day later, pamidronate (0.5 mg/kg) for 2 days were given, without immediate effect on serum Ca but increase in PTH to 1600 pg/ml. The po cinacalcet (30 mg/m2/day) had been started on 28th day of life. On the 4th day of cinacalcet treatment, which was 5 days after last pamidronate dose, serum Ca levels decreased to 10 mg/dl but thereafter, started to increase to 20 mg/dl in 7 days, in spite of increasing dose of cinacalcet to 90 mg/m2/day. Total parathyroidectomy without autotransplantation was performed at postnatal 45 days. Severe hungry bone disease after surgery required daily Ca replacement up to 250 mg/kg/day for 15 days. She is now 3-mo-old and mildly hypercalcaemic (12-12.5 mg/dl), without any Ca or calcitriol supplements, a PTH of 12 pg/ml and urinary Ca/Cr:0.00. By CASR mutation analysis, the infant was homozygous and both parents heterozygous for two mutations in the gene: c.[222_226delGATAT;740C>T], p.[M74Ifs*24; S247F]. Asterisk * indicates a stop codon.
Conclusion: A completely inactive CaSR would be predicted consistent with lack of response to cinacalcet. Despite total parathyroidectomy the patient remained mildly hypercalcemic, probably related to the inactive renal CaSR preventing Ca excretion.



P2-d2-462 Bone, Growth Plate and Mineral Metabolism 2

Hypomagnesemia with secondary hypocalcemia mental retardation and short stature: a first Japanese patient with a novel W1397X mutation in TRPM6 gene

Koujyu Katayama; Nataliya Povalko; Shuichi Yatsuga; Junko Nishioka; Yasutoshi Koga

Kurume University School of Medicine, Pediatrics, Kurume, Japan

Background: Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare genetic disorder of magnesium (Mg) metabolism, characterized by neurological symptoms such as tetany, and seizures. An HSH patient is usually diagnosed at seizure in infancy and has normal development with proper diagnosis and medication.
Objective: Our patient showed neurological damage and failure to thrive even early diagnosis. Our aim is to confirm whether our medication is appropriate. Patient: Female patient, the first daughter to consanguineous parents, was occurred the seizure at 72 day-old. At 81 day-old, she had hypomagnesemia (0.20 mEq/l: reference value; 1.5-2.5 mEq/l), and we administered Mg to her intravenously, then, orally for maintenance. (3.97 mEq/kg/day, 1.2 mEq/l). Since she had not been nursed very well for daily life, and poor compliance of the scheduled medication because of her parents divorce event, she showed neurological damage with failure to thrive during infancy.
Methods: We designed 41 primer sets based on the sequence of the human TRPM6 gene. A sequence was used from the patient blood. Corresponding statistical tests were performed in between exon number on TRPM6 and amount of oral Mg administration in previous literatures.
Results: A novel STOP-codon homozygous mutation [c.G4190A] W1397X in exon 26 was detected. The father and the father fs mother (grandmother) were heterozygous for this mutation. Our statistical data showed that patients impaired lower number of exon require the higher amount of Mg. In this case, our amount of oral Mg administration was appropriate for maintenance.
Precise conclusions: A first Japanese HSH patient with a novel nonsense mutation on TRPM6 gene is detected. We cannot explain why severe complications exist. The causes of the complications may be that the patient did not well-controlled drug compliance due to family problems. Preventing severe complications requires early diagnosis, avoiding repeated seizures, and paying attention to family problems.

P2-d2-463 Bone, Growth Plate and Mineral Metabolism 2

Reduced bone quality and altered geometry in children born prematurely, appropriate and small for gestational age

Silvia Longhi[1]; Laura Carloni[1]; Federico Mercolini[1]; Monica Benassai[1]; Roberto Franceschi[2]; Giorgio Radetti[1]

[1]Regional Hospital of Bolzano, Department of Pediatrics, Bolzano, Italy; [2]Regional Hospital of Trento, Department of Pediatrics, Trento, Italy

Background: Prematurity and being born SGA are both associated with many adverse conditions, in particularly with a decrease in bone mass.
Objective: The aim of the study was to investigate bone geometry and quality in a group of children born prematurely, appropriate (AGA) and small for gestational age (SGA).
Methods: 63 patients (30m/33f), 43 born AGA and 20 born SGA, mean age 11.3 years, entered the study. Bone geometry was evaluated from digitalized X-rays taken at the level of the 2nd metacarpal bone. The following parameters were assessed: outer (D) and inner (d) diameter, cortical area (CA), medullary area (MA) and metacarpal index (MI). Bone quality was evaluated by ultrasound, measuring the amplitude dependent speed of sound (AdSos), and bone transmission time (BTT). The results were evaluated according to bone age and expressed as SDS compared to a group of 325 control children born at term of normal weight and height, matched for age and sex.
Results: Children born AGA: D -1.19±1.10 (p<0.0005), d -0.72±0.97 (p<0.0005), MI 0.34±1.13 (p<0.05), MA -0.80±0.83 (p<0.0005), CA -0.90±0.81 (p<0.0005), AdSos -0.09±1.09 (NS), BTT -0.62±0.99 (p<0.0005). Children born SGA: D -1.45±1.13 (p<0.0005), d -0.82±0.83 (p<0.0005), MI 0.23±0.83 (p=NS), MA -0.81±0.72 (p<0.0005), CA -1.19±0.71 (p<0.0005), AdSoS -0.09±1.03 (NS), BTT -0.93±0.96 (p<0.0005). No difference was seen between AGA and SGA, apart from the outer diameter (D) which was significantly smaller only in the group of males SGA.

Conclusion: Children born prematurely have smaller bones with normal thickness and low quality. Moreover, being born SGA seems to be a further negative factor in males only.

	N	D SDS	d SDS	MI SDS	MA SDS	CA SDS	AdSos SDS	BTT SDS
AGA m	20	-0.82 ±0.91*°	-0.52 ±0.72*	-0.01 ±0.77	-0.65 ±0.55*	-0.69 ±0.71*	-0.31 ±0.93	-0.64 ±1.06
AGA f	23	-1.51 ±1.16*	-0.91 ±1.13*	0.65 ±1.31*	-1.01 ±0.92*	-1.08 ±0.86*	0.09 ±1.21	-0.61 ±0.96*
SGA m	10	-1.58 ±1.02*°	-0.84 ±0.85*	0.05 ±0.67	-0.82 ±0.76*	-1.19 ±0.60*	-0.41 ±0.88	-1.29 ±0.81*
SGA f	10	-1.31 ±1.28*	-0.80 ±0.85	0.41 ±0.99	-0.81 ±0.71*	-1.20 ±0.83*	0.22 ±1.12	-0.57 ±1.01
AGA	43	-1.19 ±1.10*	-0.72 ±0.97*	0.34 ±1.13*	-0.80 ±0.83*	-0.90 ±0.81*	-0.09 ±1.09	-0.62 ±0.99*
SGA	20	-1.45 ±1.13*	-0.82 ±0.83*	0.23 ±0.83	-0.81 ±0.72*	-1.19 ±0.71*	-0.09 ±1.03	-0.93 ±0.96*

* $p<0.05$ against zero; ° $p<0.05$ for the difference between AGA and SGA

P2-d2-464 Bone, Growth Plate and Mineral Metabolism 2

Hypomagnesemia with secondary hypocalcemia (HSH): apparent homozygosity for one novel TRPM6 mutation in two Italian siblings

Diego Rinaldini; Martina Zanotti; Alessandra Cassio; Emanuela Zazzetta; Sara Monti; Piero Pirazzoli; Antonio Balsamo
Azienda Policlinico S.Orsola-Malpighi; University of Bologna, Gynecology, Obstetrics & Pediatric Sciences, Bologna, Italy

Background: Familial hypomagnesemia with secondary hypocalcemia is a rare autosomal recessive disease that results in electrolyte abnormalities shortly after birth.
Objective and hypotheses: Characterization of 2 cases with familial hypomagnesemia.
Methods: 2 patients analysed by means of TRPM6 gene.
Results: Patient 1 was a girl born in 2006 after a normal pregnancy from two unrelated parents.At the age of two months she was admitted to another hospital because of generalized seizures.She presented hypomagnesemia and hypocalcemia (0.68-9.28 mg/dl).Treatment started with anticonvulsive and magnesium administration.Electroencephalograms and MRIs had never found specific alterations.Neurological assessment confirmed expressive language delay. When she was admitted to our Center she showed good electrolyte balance.We had not found obvious abnormal findings on general, neurologic or nephrology examinations. Patient 2 was the brother of patient 1.He was born in 2010 after an uneventful pregnancy.At two month's age he had an episode of acute dyspnea. During hospital admission, hypomagnesemia with secondary hypocalcemia (0.51- 7.8 mg/dl) was detected.It was begun replacement therapy.He was admitted to our Center where laboratory, neurologic, nephrology, reevaluations didn't found significant alterations. As suspected, according to the clinical presentation, the mutation c.2922delA was detected in exon 24 of the TRPM6 gene of both patients.Parents analysis is ongoing so we can't be sure of the homozygosity for the mutation.This frameshift mutation has not yet been previously described.However, since this one basepair mutation leads to a shift in the open readinig frame of the mRNA and results in a nonsense mediated generation of a truncated protein, this variant is definitely pathogenic.
Conclusions: Clinical suspicion is essential for an early diagnosis and to avoid abnormalities of neurological and psychomotor development.When HSH is suspected molecular analysis of TRPM6 gene is required to obtain the diagnostic certainty and provide adequate genetic counseling.

P2-d2-465 Bone, Growth Plate and Mineral Metabolism 2

Familial hypocalcaemia – think about calcium sensor receptor (CaSR)

Sanjay Gupta[1]; David Finn[1]; Stephanie How Yaw[1]; Katrina Prescott[2]; Verghese Mathew[1]
[1]Hull Royal Infirmary, Paediatrics, Hull, United Kingdom; [2]Chapel Allerton Hospital, Department of Clinical Genetics, Leeds, United Kingdom

Background: Mutations in the Calcium Sensor Receptor (CaSR) have been identified in patients with autosomal dominant hypocalcaemia. CaSR is located in the parathyroid gland but also expressed in the kidneys, thyroid gland, bones, intestine, brain and stomach. Binding of calcium (Ca++) to CaSR initiates signalling cascade which regulates parathyroid hormone (PTH) to maintain plasma Ca++ within a narrow range. Activating mutations upregulate the CaSR despite low Ca++ concentration such that the secretion of PTH remains low.
Objective and hypotheses: Activating mutations of CaSR gene have been shown to cause hypercalciuric hypocalcaemia. Such mutations of CaSR cause increased sensitivity to Ca++ and hence down regulation of PTH secretion.
Methods: This is a case series where we report a pedigree with clinical picture consistent with familial autosomal dominant hypoparathyroidism. The proband, an 18-month-old girl was found to have incidental hypocalcaemia. We identified 4 other affected individuals in the family, including the father of the proband and his twin brother. All affected individuals had mild hypocalcaemia, low normal serum parathyroid levels and borderline high phosphate levels. Treatment with vitamin D and oral calcium did not improve their serum calcium levels. The father of proband has shown evidence of hypercalciuria and has recently been treated for urolithiasis.
Results: Mutation analysis identified a novel activating mutation of the CaSR gene [c.407C>T(p.Pro136Leu)] in all 3 family members who were tested.
Conclusions: Activating mutation of the CaSR inhibits PTH secretion and renal calcium reabsorption despite hypocalcaemia. Treatment with vitamin D and calcium can increase hypercalciuria leading to nephrocalcinosis and renal impairment. Asymptomatic individuals should not be overtreated in an attempt to normalise serum calcium.

P2-d2-466 Bone, Growth Plate and Mineral Metabolism 2

Specific bone mass acquisition in elite athletes throughout the peripubertal period

Laurent Maimoun[1]; Olivier Coste[2]; Thibault Mura[3]; Florence Galtier[4]; Pascal Philibert[1]; Denis Mariano-Goulart[5]; Françoise Paris[6]
[1]Hopital Lapeyronie, CHU Montpellier and UMI, Hormonologie, Montpellier, France; [2]CHU and UMI and Direction Régionale de la Jeunesse, des Sports et Cohésion Sociale, Hormonologie, Montpellier, France; [3]Centre d'Investigation Clinique et Département d'information Médicale, Hopital St Elois, Montpellier, France; [4]Hopital St elois, CHU Montpellier, CIC 1001, INSERM, Montpellier, France; [5]Hopital Lapeyronie CHU Montpellier and UMI, Médecine Nucléaire, Montpellier, France; [6]Hopital Lapeyronie CHU Montpellier and UMI, Hormonologie and Unité d'Endocrinologie Pédiatrique, Montpellier, France

Background: Various cross-sectional studies have demonstrated that physical activity may improve bone mass acquisition. However, this design is not adapted to describe the specific kinetics of bone mass gain throughout the peripubertal period.
Objective and hypotheses: The aim of this study was to compare the kinetics of bone mass evolution in female adolescent athletes submitted to various mechanical loadings and in untrained controls throughout the peripubertal period.
Methods: A total of 72 peripubertal girls with ages ranging from 10.8 to 18.0 years (mean 14.2 ± 1.7) were recruited for this study: 24 rhythmic gymnasts (RG, impact-activity, mean hours training per week: 23.0 ± 2.7), 24 swimmers (SW, non-impact activity; 14.5 ± 5.9 hr/wk), and 24 age-matched controls (CON; leisure physical activity <3h/wk). Areal bone mineral density (aBMD) was determined at whole body, lumbar spine, femoral region and radius using DEXA, and bone remodeling markers were analyzed. All the investigations were performed at baseline and after one year.
Results: At baseline and after one-year of follow-up, RG compared with CON and SW, presented noticeably greater aBMDs adjusted for age, fat-free-soft tissue and fat mass only at femoral region. When aBMD variation throughout

the peripubertal period was modelled for each group from individual values, the aBMD at femoral region was significantly higher in RG compared to the other two groups from 12.5 to 14 yr and this difference lasted up to 18 yr. At radius and lumbar spine, no difference was demonstrated between groups. Moreover, the mean annual aBMD gain tended to be higher in RG compared to SW and CO only at femoral region and this gain lasted longer with time in RG.
Conclusions: High-impact activity had a clearly favorable effect on aBMD at mechanically loaded bones site throughout the peripubertal period.

P2-d2-467 Bone, Growth Plate and Mineral Metabolism 2

High prevalence of vitamin D deficiency in healthy school children aged 11-18 years

Gülay Karagüzel[1]; Beril Dilber[2]; Gamze Can[3]; Orhan Deger[4]; Aysenur Ökten[1]
[1]Karadeniz Technical University, School of Medicine, Pediatric Endocrinology, Trabzon, Turkey; [2]Karadeniz Technical University, School of Medicine, Pediatrics, Trabzon, Turkey; [3]Karadeniz Technical University, School of Medicine, Public Health, Trabzon, Turkey; [4]Karadeniz Technical University, School of Medicine, Biochemistry, Trabzon, Turkey

Background: Vitamin D is necessary for bone health, but little is known about the vitamin D status of school children in our country.
Objective: To determine seasonal levels of serum 25-hydroxyvitamin D [25(OH)D3] in healthy school children aged 11-16 years in our region (latitude: 40° N).
Populations and methods: The healthy school children aged 11-16 years, selected by stratified sampling, were visited during spring (n: 375) and autumn (n: 371) at schools. After physical examinations were done, blood samples were taken for 25(OH)D3, calcium, phosphorus, alkaline phosphatase (ALP) measurements. Serum 25(OH)D3 levels were categorized as <10µg/ (vitamin D deficiency) and 10-30µg/l (vitamin D insufficieny).
Results: The mean 25(OH)D3 levels were 10,1±4,2 µg/l in girls and 11,4±6,8 µg/l in boys during spring (p<0,001) and 14,6±8,1 µg/l in girls and 18,0±7,4 µg/l in boys during autumn (p<0,001). There was a negative correlation between 25(OH)D3 and PTH levels in both seasons. Threshold for vitamin D level which causes hyperparathyroidism was detected as 14.3 µg/l. The prevalences of vitamin D deficiency and insufficiency were 49% and 51% during spring and 20% and 74% during autumn, respectively. If the threshold is changed to <15 µg/l, 81% of the children is vitamin D deficient in spring and 49% in autumn. 25(OH)D3 level was significantly lower in girls than those boys, moreover, the girls who wear concealing clothing had significantly lower levels of 25(OH)D3. Hyperparathyroidism was detected 24% of the children. Hypocalcemia was not detected.
Conclusions: The prevalences of vitamin D deficiency and insufficiency among the healthy school children are high in our region. We recommend vitamin D suplementation to the school children in this age group. Gender and seasonal differences should be taken into account for the dose adjustments, since 25(OH)D3 levels were significantly lower both in girls and in autumn.

P2-d2-468 Bone, Growth Plate and Mineral Metabolism 2

Bone mass and quality in juvenile idiopathic arthritis: comparison of the role of bone mass determinants using DXA, PQCT and QUS

Stefano Stagi[1]; Loredana Cavalli[2]; Laura Masi[2]; Maria Luisa Brandi[2]; Marco Matucci Cerinic[2]; Maurizio de Martino[3]; Fernanda Falcini[2]
[1]Mugello's Hospital, Paediatric Unit, Borgo San Lorenzo, Italy; [2]University of Florence, Department of Internal Medicine, Florence, Italy; [3]University of Florence, Department of Paediatrics, Florence, Italy

Background: There are few prospective data on bone mass and quality using dual energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and quantitative ultrasound (QUS) in JIA patients.
Objective and hypotheses: To evaluate bone mass and quality in a large cohort of children, adolescents and young JIA adults, and to identify the main predictors of reduced Bone Mineral Density (BMD) and bone quality using these techniques.
Patients: Two hundred and five patients (144 females, 61 males; median age at study entry 15.6 years, 144 oligoarticular, 46 polyarticular, 17 systemic, and 18 enthesitis-related-arthritis onset (ERA)), fulfilling the criteria for JIA were evaluated. Of these, 141 patients (112 females, 29 males; 83 oligoarticular, 27 polyarticular, 15 systemic, and 16 ERA) were followed longitudinally with a second scans. The data obtained were compared with 80 ages- and sex- matched healthy subjects.
Results: JIA patients showed a reduced spine BMAD SDS value in comparison to controls (p <0.005). JIA patients showed significantly lower levels of TrabBMD, muscle CSA, and SSIp, AD-SoS, and QUS z-score, but not CortBMD and CBA, and showed fat CSA significantly increased than controls. These data were confirmed also in longitudinal evaluation, with no differences in comparison to the first evaluation. JIA patients presented no more significant lower levels of SSIp than controls. Analyzing the therapies, a significant negative correlation among spine BMAD values, TrabBMD, AD-SoS, and systemic corticosteroids exposure or number of intra-articular corticosteroids injections, and a positive correlation among TNF-alpha-blocking agents and spine BMAD, TrabBMD, and AD-SoS were observed.
Conclusions: Patients with JIA have a low bone mass and, after a first increase due to the therapy, do not reach the normal condition over time despite the current more effective drugs. our data show that the pronounced bone deficits in JIA are due to reduction in muscle cross-sectional area. Thus, bone alterations in JIA likely represent a mixed defect.

P2-d2-469 Bone, Growth Plate and Mineral Metabolism 2

Neonatal presentation of McCune Albright syndrome: therapeutic considerations for thyrotoxicosis and severe fibrous dysplasia

Athreya Rajath; Akash Deep; Alastair Baker; Charles Buchanan
King's College Hospital, Paediatrics, London, United Kingdom

Background and objective: McCune Albright syndrome (MAS) in neonates is rarely reported, notably with hypercortisolism, hyperthyroidism, liver dysfunction, skin lesions, and fibrous dysplasia. We report a case with thyrotoxicosis and extensive, life-threatening fibrous dysplasia, and summarise clinical course and experience with Pamidronate.
Case report: A boy, born 2.3kg at 36wks, presented with high output cardiac failure on day 2. Ultrasound suggested Infantile Hepatic Haemangioendothelioma (IHE); heart failure was managed with diuretics, digoxin and ventilatory support pending definitive treatment of IHE. At transfer to our Paediatric Liver unit age 6wks he was emaciated, wt 1.8kg. Biochemistry showed high calcium 3.8mmol/L, high urea 8.5mmol/L and markedly raised liver enzymes. IHE may cause thyroid dysfunction, and tests revealed thyrotoxicosis (TSH <0.1mIU/L, fT4 67pmol/L; fT3 9pmol/L). Mother was euthyroid and TRAb negative. A large abdominal café au lait patch was noted. Treatment with propylthiouracil and thyroxine readily restored euthyroid status. Hypercalcaemia resolved with rehydration. Serum 25(OH)VitD was normal and PTH level appropriately suppressed until normocalcaemia restored. Skeletal survey showed osteopaenia, multiple cortical erosions and fractures of ribs and long bones. Urinary phosphate fractional excretion was >90%. Cortisol levels were normal. Once euthyroid, cardiac failure resolved and liver function partially improved. Hepatic lesion was no longer thought significant. MAS seemed the unifying diagnosis; DNA from bone biopsy confirmed GNAS R201 mutation. Serial pamidronate doses were given over 4 months to improve bone disease, in particular the ribs. There was reduction in urinary NTX and phosphate, improved pain control, progressive weight gain and weaning off ventilatory support.
Conclusions: Reported neonatal MAS with severe fibrous dysplasia is rare. Treatment options are limited. Prognosis and value of bisphosphonate are uncertain.

P2-d2-470 Bone, Growth Plate and Mineral Metabolism 2

Genetic defect in *CYP24A1* in a patient with idiopathic infantile hypercalcemia (IHH)

Diana-Alexandra Ertl; Adalbert Raimann; Werner Schlegel; Gabriele Haeusler
University Clinic of Paediatrics and Adolescent Medicine, Paediatric Pulmology, Allergology and Endocrinology, Vienna, Austria

Case report: A female patient was admitted at the age of 6 months because of stage IIB nephrocalcinosis and failure to thrive. Serum calcium was elevated (3,44 mmol/l). Vitamin D intoxication was suspected, as 25-OH-Vit.D levels

were elevated (283 nmol/l, normal range: 75 - 250 nmol/l), but the infant had received usual rickets –prophylaxis, (800 IE Cholecalciferol) and parents denied any accidental Vitamin D- overdose. 1,25-(OH)2 Vit. D was in the upper normal range (61 pg/ml) and PTH was low (2,1 pg/ml, normal range: 16-65 pg/ml). Williams- Beuren syndrome and mutations in the calcium sensing receptor gene (*CASR*) were excluded. In order to further investigate 25-OH Vit. D metabolism, sequencing of *CYP24A1* was performed and showed two mutations: c.443T>C and c.1186C>T. Only c.1186C>T has been described in the pathogenesis of infantile hypercalcemia so far, in both compound heterozygous and homozygous form. The c.443T>C mutation has been, so far, not described in relation to the disorder in question, however mutational analysis programs strongly suggest involvement in pathogenesis. A low-calcium diet was started. 25-OH-Vit. D normalized within weeks, while PTH remained low.

Discussion: Hypercalcemia is a rare symptom in infancy and childhood. Recently, Schlingmann et al. showed that mutations within *CYP24A1* are found in individuals diagnosed with IIH. Our patient was admitted under suspicion of Vitamin D intoxication, however proved to be a new case of compound heterozygosity.

Conclusion: *CYP24A1* mutations should be considered in infants under rickets- prophylaxis that are suffering from hypercalcemia and regarded as a potential cause for increased 1,25- Vitamin D serum levels.

P2-d2-471 Bone, Growth Plate and Mineral Metabolism 2

Serum vitamin D levels in children with recurrent otitis media

Atilla Cayir[1]; Ozalkan Ozkan[2]; Avni Kaya[1]; Salih Davutoglu[1]; Behzat Ozkan[3]

[1]Ataturk University, Pediatric Endocrinology, Erzurum, Turkey; [2]Ataturk University, Otolaryngology, Erzurum, Turkey; [3]Istanbul Medeniyet University, Pediatric Endocrinology, Istanbul, Turkey

Background: Recurrent otitis media is common in early childhood and basic preventive measures are undertaken with chemoprophylaxis, immune-prophylaxis, surgery and improvement of environmental risk factors for treatment without any sequels.

Objective and hypotheses: The objective of this trial is to evaluate serum vitamin D levels in cases with recurrent otitis media and to investigate the effect of vitamin D therapy on the risk of re-occurrence of the disease.

Methods: A total of 42 children between 1-7 years of age, diagnosed with recurrent otitis media were enrolled as the Study Group. A total of 54 children with similar demographic characteristics with an age range of 1-7 years and with no chronic systemic disease were enrolled as the Control Group (Group II). In patients with low initial serum vitamin D levels (vitamin D <15 ng/mL), vitamin D (5000 IU/day) was administered in addition to conventional treatment for otitis media while vitamin D treatment of 400 IU/day was administered in patients with initial serum 25OHD levels >15 ng/mL and all cases were followed-up in due course. The trial was approved by local ethics committee.

Results: Mean serum 25(OH) vitamin D in the study group was 11.2 ng/mL. In the control group, mean serum 25(OH) vitamin D level was 29.6 ng/mL. Serum 25(OH) vitamin D levels were under 20 ng/mL in 30% (n=16) of the control group. Comparison of serum 25(OH) vitamin D levels and PTH in Study and Control groups revealed a statistically significant difference ($p<0.05$). Treatment was initiated in cases diagnosed with vitamin D deficiency and patients were followed-up in due course. In 1-year follow-up of 21 patients with regular visits, no recurrence was observed during this period, apart from three cases where one attack was detected in two patients and 2 attacks were observed in one patient.

Conclusions: We believe that co-administration of supplementary vitamin D together with conventional treatments is appropriate in the management of upper respiratory infections like otitis media.

P2-d3-472 Bone, Growth Plate and Mineral Metabolism 3

Vitamin deficiency in adolescents: 2 different patterns of radiological changes related to IGF-I level, calcium intake and BMI

Ashraf Soliman[1]; Ashraf Adel[1]; Magda Wagdy[1]; Elsaid Bedair[2]; Rania Elalaily[3]

[1]Hamad Medical Center, Pediatrics, Doha, Qatar; [2]Hamad Medical Center, Radiology, Doha, Qatar; [3]Hamad Medical Center, Primary Health Center, Doha, Qatar

Background: Vitamin D deficiency (VDD) is still a major public health problem specially during the winter. The symptoms and signs of VDD in adolescents are less specific and are easily missed.

Objective and hypotheses: Describe the clinical manifestation, radiological changes in relation to calcium intake and IGF-I level in adolescents with severe vitamin D deficiency (25OH vit D < 10 ng/dL).

Methods: 40 adolescents with VDD were studied. Clinical exam included symptoms and signs related to VDD and dietary intake of calcium. Plain x rays of their knees, hips and wrists were studied. Endocrine tests included levels of Vitamin D, PTH and IGF-I.

Results: The manifestations of 40 adolescents with severe VDD included arthralgia in the knees hips and ankle joints and backache (32/40), difficulty in climbing stairs and/or running (9/40), muscle cramps (12/40) , facial twitches (4/40) and carpo-pedal spasms (2/40). High Alkaline phosphatase (ALP)(40/40). Serum ALP and PTH were significantly high and serum phosphate significantly low in adolescents with VDD. Two patterns of radiological changes have been recorded in adolescents with VDD. Pattern (I) (n = 8) appears as metaphyseal multi-locular cystic lesion with sclerotic margins, exocentric subcortical location without significant cortical erosions, periosteal reaction, osteoporosis, or other metaphyseal manifestations. Pattern (II) (n = 18) appears as generalized diminished bone density with prominent primary and 2ry bone trabeculations, widening of the metaphyseal zone with relatively more lucency (zone of poor ossification) with rather loss of all bone trabeculation. No cupping or fraying of the metaphyses was identified. Patients are treated with injecting a mega dose of cholecalciferol (10,000 IU/kg, max 600,000 IU) every 3-6 months according to their serum 25-OH-vitamin D measured every 3 months. Radiological changes improved after six months but complete cure took 2 years in patients with cystic lesions.

Conclusions: Repeated Mega doses of cholecalciferol are effective therapy for adolescents with VDD.

P2-d3-473 Bone, Growth Plate and Mineral Metabolism 3

Body composition and bone mineral density in Egyptian children (Delta Region)

Magdy El-Ziny; Amany Elhawary; Ashraf Elsharkawy; Nanes Salem

Mansoura University Children Hospital, Endocrinology and Diabetes Unit, Mansoura, Egypt

Background: Weight gain is associated with changes in body composition during infancy. Clinical assessment of growth and nutrition status is enhanced by accurate measurement of body composition. Dual energy X-ray absorptiometry (DXA) provides an important means of quantifying total body and regional fat mass, skeletal muscle mass and bone mineral mass and density.

Objective and hypotheses: This study aimed to assess body composition; total fat percent, arm fat percent, trunk/lower limb fat and trunk fat percent in different Egyptian pediatric age groups in Delta region.

Methods: A 310 healthy subjects (135 females and 175 males), ranging in age from one to 13 years included in this study. Subjects were categorized according to age into 4 groups. Total body composition and BMD of lumbar spine L2-L4 were measured by dual-energy x-ray absorptiometry (DXA) with a Lunar DPX-IQ.

Results: The percentage of total body fat, percentage of arm fat and trunk fat / lower limb fat values in male groups tend to be high in infancy compared to early childhood; however they start to increase in late childhood and adolescence. Their is steady increase of total body BMD, arm and leg BMD and BMD of L2-L4 by advancement of age group in female groups. Total body BMD, arm and leg BMD and BMD of L2-L4 showed significant steady increase by age group advancement. Correlation studies show that there is significant positive correlation between age and BMD in both males and females and between age and percentage of total body fat in females. Moreover, percentage of total body fat and percentage of arm fat showed significant correlation with trunk fat / lower limb fat, L2-L4 and total BMD.

Conclusions: There is steady increase in fat mass in male by increasing age. While fat mass in female is high in infancy, decline in childhood, then steady increase in adolescence. There is significant difference between male and female infants and adolescence as regard fat mass and BMD.

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A rare and unexplainable case of hypocalcemic rickets with underlying double pathology; vitamin D deficiency and primary hypoparathyroidism

Azriyanti Anuar Zaini; Fiona Ryan
Oxford Radcliff Children's Hospital, Paediatric Endocrine and Diabetes, Oxford, United Kingdom

Introduction: Hypocalcaemic seizures in infants are most commonly secondary to Vitamin D deficiency, especially in families of Asian origin but can also result from primary hypoparathyroidism. The occurrence of both conditions simultaneously in a patient presenting with rickets is extremely rare.
Case report: We report a boy presenting to our hospital at the age of 5 months. He was born at term via C-section, birth weight 2.3kg. His parents were first cousins with Asian backgrounds. He was exclusively breastfed until 4 months old. Development was normal. He presented with seizures secondary to severe hypocalcaemia with corrected serum calcium 1.24mmol/l (normal range (NR) 2.12-2.62). His kidney function and magnesium was normal. PTH levels were undetectable on 2 occasions (<0.3 pmol/L, NR 1.3-7.6). His total 25OH Vit D <10nmol/L (NR > 80 nmol/L). Alkaline phosphatase was 2553 IU/L (NR 200-1100). X-rays showed severe osteopenia and rickety changes. There was no calcium loss in his urine. Initial treatment with ergocalciferol was ineffective in raising calcium levels but once alphacalcidol was added, marked improvement occurred. After 3 months, there was radiological healing of his rickets.
Discussion: In vitamin D deficiency, serum calcium falls, stimulating PTH release. PTH is required to release calcium from the bone, retain calcium in distal tubules and activate conversion of 25OH Vit D to 1-25 OH Vit D. The action of PTH causes the rickety changes seen on Xray. In our patient, PTH remains undetected (even once Vit D replete) and therefore we cannot explain the phenomenon of rickets changes.
Conclusions: In hypocalcaemic seizures, Alphacalcidol should be prescribed until vitamin D and PTH levels are available. Further molecular study may be warranted to explore the possibility of bone resorption and deposition without PTH in our patient.

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Increasing prevalence of vitamin D deficiency in Korean children and adolescents: results from the Korea national health and nutrition examination surveys (KNHANES) 2008 - 2010

So Hyun Park[1]; Lee Moon Hee[1]; Cho Won Kyung[1]; Cho Kyung Soon[1]; Se Min Lee[2]; Jung Min Ho[1]; Suh Byung Kyu[1]
[1]The Catholic University of Korea, Pediatrics, Seoul, Republic of Korea; [2]Hanyang Univ. Guri Hospital, Pediatrics, Guri-si, Republic of Korea

Background: Vitamin D plays an important role in bone metabolism and its deficiency causes rickets in children. Recently, medical researchers became interested in nonskeletal effects of vitamin D deficiency such as increase in the risk of cardiovascular diseases, diabetes mellitus, infection, and autoimmune diseases.
Objective and hypotheses: This study was performed to investigate the prevalence of vitamin D deficiency and yearly change in its prevalence among Korean children and adolescents, and to identify association of vitamin D deficiency with metabolic risk factors.
Methods: We assessed the data of 2,934 Korean children and adolescents aged 10 to 18 years obtained from the Korea National Health and Nutrition Examination Surveys 2008-2010. We analyzed the measured serum 25-hydroxyvitamin D levels and defined vitamin D deficiency as 25-hydroxyvitamin D <20 ng/mL.
Results: Overall, vitamin D deficiency was found in 70.1% of children and adolescents in the years 2008-2010. The prevalence of vitamin D deficiency was higher in 2010 than in 2008 (74.77% vs. 61.26%, respectively). Vitamin D deficiency was more prevalent among girls (75.1%) than boys (65.8%), and in urban (37.2%) than rural areas (31.1%). We divided the children into three groups by age (group I 10 to 12-years old, group II 13-15, and group III 16-18). The mean 25-hydroxyvitamin D level was lowest in group III. 25-hydroxyvitamin D level was negatively correlated with diastolic blood pressure.
Conclusions: Vitamin D deficiency is very common among Korean children and adolescents, especially among late adolescents and its prevalence increased between 2008 and 2010. Serum 25-hydroxyvitamin D level was inversely correlated with diastolic blood pressure. The nonskeletal consequences of vitamin D deficiency in children and adolescents should be further investigated.

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Kenny Caffey syndrome

Sameh Tawfik[1]; Nermeen Salah[2]; Heba El Sedfy[3]; Rasha Hamza[3]
[1]Maadi Hospital, Pediatrics, Cairo, Egypt; [2]Cairo, Pediatrics, Cairo, Egypt; [3]Ain Shmas University, Pediatrics, Cairo, Egypt

Background: Kenny-Caffey Syndrome is an extremely rare hereditary skeletal disorder, in most cases it is autosomal dominant trait,others are inherited as autosomal recessive trait.
Objectives: Is to report an Egyptian patient with Kenny -Caffey Syndrome.
Methods: An 8.5 year old Egyptian male who had convulsions at the age of 5 days. At the age of 4 years, the parents noticed that the child was markedly stunted.
On examination, the child was short (height=106.5 cm, SDS= -4.0), weight=20 kg with a SDS of weight for height of +2.1, head circumference = 47.5cm with a SDS of skull circumference for height of -3.1 and his sitting height was 60 cm (sitting height SDS = -3.96). He had a widely open anterior fontanel and metopic suture. He had some dysmorphic features (hypertelorism, long nose and high arched palate), with brachydactyly and short 4th and 5th metacarpals and slight bowing of both tibiae.
Investigations showed low serum calcium, high serum phosphorous together with low serum parathyroid hormone. His peak growth hormone was 1.2 ng/ml and 4.5 ng/ml after clonidine and insulin provocation tests respectively.
Plain X-rays of extremities showed slender long bones together with cortical thickening and short metacarpals. CT brain revealed bilateral basal ganglia calcification. Hypoparathyroidism with the typical picture of growth retardation, cortical thickening of the long bones, and delayed closure of the anterior fontanel suggested the diagnosis of Kenny Caffey syndrome.
Results: Mutation in the TBCE gene is the tubulin specific chaperone E, Mutational analysis done and all exons were sequenced,excited to identify a homozygous change that is not known single nucleotide polymorphism(SNP). causing replacement of a conserved amino acid,this fits with our expectation that mutation should neverthless preserve some activity of the protien. The patient is currently on growth hormone therapy along with calcium and vitamin D lsupplementation.
Conclusions: Early detection & diagnosis improves quality of life and prevents comorbidities.

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PRKAR1A mutation in a Japanese patient with pseudohypoparathyroidism type Ia-like phenotype

Keisuke Nagasaki[1]; Maki Fukami[1]; Ogata Tsutomu[2]
[1]National Research Institute for Child Health and Development, Molecular Endocrinology, Tokyo, Japan; [2]Hamamatsu University School of Medicine, Pediatrics, Hamamatsu, Japan

Background: Pseudohypoparathyroidism type Ia (PHP-Ia) is an autosomal dominant disorder characterized by unresponsiveness to several hormones and unique body phenotype referred to as Albright's hereditary osteodystrophy (AHO). Although PHP-Ia is usually caused by abnormalities of *GNAS* mediating the G-protein-coupled receptor (GPCR) signaling, molecular bases remain to be clarified in a small fraction of patients. In this regard, *PRKAR1A* has been identified as a causative gene for resistance to multiple hormones and acrodysostosis reminiscent of endocrine and skeletal abnormalities in PHP-Ia.
Objective and hypotheses: To report a Japanese female patient with PHP-Ia-like phenotype and *PRKAR1A* mutation.
Methods: This patient had PHP-Ia-like phenotype including mildly elevated

serum PTH and TSH values and AHO-like clinical features such as short stature, round face, brachydactyly, and mental retardation.
Results: While neither mutation nor imprinting defect was identified in *GNAS*, a novel de novo heterozygous missense mutation (p.T239A) was identified at the cAMP-binding domain A of *PRKAR1A*.
Western blot analysis using primary antibodies for the phosphorylated cAMP responsive element (CRE)-binding (CREB) protein showed markedly reduced CREB phosphorylation in the forskolin-stimulated lymphoblastoid cell lines of this patient. CRE-luciferase reporter assays indicated significantly impaired response of protein kinase A to cAMP in the HEK293 cells expressing the mutant p.T239A protein.
Conclusions: The results indicate that PHP-Ia-like phenotype is caused by a heterozygous *PRKAR1A* mutation impairing cAMP-mediated GPCR signaling. Since *GNAS* and *PRKAR1A* are both involved in the GPCR signal transduction cascade and have some different characters, this would explain the phenotypic similarity and difference between *GNAS* abnormalities and *PRKAR1A* mutations.

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Sexual dimorphism in bone geometry of adult patients with classical congenital adrenal hyperplasia: data using peripheral quantitative computed tomography

Susanne Bechtold[1]; Andreas Beyerlein[2]; Walter Bonfig[1]; Robert Dalla Pozza[1]; Stephanie Putzker[1]; Ragna Otto[1]; Hans Peter Schwarz[1]; Heinrich Schmidt[1]
[1]University Children´s Hospital, Pediatric Endocrinology and Diabetology, Munich, Germany; [2]Institute of social pediatric and adolescent medicine, Division of epidemiology, Munich, Germany

Background: Long-term glucocorticoid treatment may influence bone and muscle development in patients with congenital adrenal hyperplasia (CAH). Objective: The aim of this study was to evaluate bone mineral density (BMD), bone geometry and muscle mass.
Methods: Seventy-three adult patients with classical CAH due to 21-hydroxylase deficiency were included. BMD, bone geometry and muscle mass were measured at the non-dominant forearm using peripheral quantitative computed tomography (pQCT). Glucocorticoid equivalent doses throughout life were calculated and at time of pQCT androgen levels were measured. Anthropometric data of patients were compared with a reference population by standard deviation (SD) scores (mean±SD).
Results: In males, mean SD score for trabecular BMD (-0.33±0.71) was reduced, whereas mean cortical BMD (1.05±1.11) was elevated.
In females beside normal values of BMD SD scores (trabecular, cortical), mean total (0.86±1.12) and medullary cross-sectional area (CSA) (1.12±1.17) were significantly increased ($p<0.001$).
In all patients SD scores for cortical thickness (-0.65±0.91) and muscle CSA (-0.83±0.91) were reduced. Treatment duration was associated with lower trabecular BMD ($r=-0.56$, $p=0.001$). As a consequence of suppressed androgens and simple virilising CAH a lower muscle CSA SD score was observed (OR: 0.58 and 0.46, respectively, $p<0.05$).
Conclusion: There was a sexual dimorphism with enlarged total and medullary CSA in females, whereas in males trabecular BMD was reduced and cortical BMD elevated. Cortical thickness and muscle CSA were reduced in CAH patients with possible long-term impact on bone development. Monitoring of bone and muscle development might be warranted.

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Biochemical markers of bone turnover in Turkish children and adolescents

Gülay Karagüzel[1]; Beril Dilber[2]; Orhan Deger[3]; Aysenur Ökten[1]
[1]Karadeniz Technical University, School of Medicine, Pediatric Endocrinology, Trabzon, Turkey; [2]Karadeniz Technical University, School of Medicine, Pediatrics, Trabzon, Turkey; [3]Karadeniz Technical University, School of Medicine, Biochemistry, Trabzon, Turkey

Background: The measurements of biochemical markers of bone turnover is important in the evaluation of bone health and diseases. Since rate of bone growth shows variations in different age groups, genders, and ethnics, age-specific reference ranges for biochemical markers should be established.
Objectives: To determine biochemical markers of bone turnover in healthy Turkish children and adolescents aged 11 to 18 years and evaluate in relation to their ages and pubertal development.
Population and methods: Serum osteocalcin and alkaline phospatase (AP) as bone formation markers and C-terminal telopeptide of type I collagen crosslinks (CTx) as a bone resorption marker were measured in 746 healthy children and adolescents (349 girls and 397 boys).
Results: In girls, all the markers of bone turnover changed significiantly with pubertal stage, were maximal at midpuberty, and decreased to the lowest level by Tanner stage 5. We observed similar changes in boys, but the levels of all these markers were significantly higher in boys than those girls, especially between 12 and 17 years old. Serum osteocalcin, AP, and CTx reached a peak at ages 11 and 12 years in girls, however they reached a peak at ages 13 and 14 years in boys. There was a negative correlation among the age and all of these markers.
Conclusions: We established age- and gender-specific reference ranges of these bone turnover markers in Turkish children and adolescents first time. We observed a marked effects of ages and pubertal development on bone turnover markers in both girls and boys. Our data will be useful in the clinical investigation on bone turnover in bone health and diseases.

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A novel mutation in CYP24A1 in familial "idiopathic" infantile hypercalcemia

Mireille Castanet[1]; Eric Mallet[2]; Jean-Pierre Basuyau[3]; Marie-Laure Kottler[4]
[1]CHU, Pediatrics department, Rouen, France; [2]CHU, Reference Center for Rare Disorders of Calcium and Phosphorus Metabolism, Rouen, France; [3]Henri Becquerel Centre, Department of Nuclear Medicine, Rouen, France; [4]CHU, Department of Genetics, Caen, France

Background: Idiopathic Infantile Hypercalcemia (IIH), Lightwood like but without facial dysmorphism or heart murmur is a disorder so far not well understood. High level of 1,25-OH2-D was previously frequently observed and recently, mutations in CYP24A1, which encodes 25-hydroxyvitamin D24-hydroxylase, the enzyme of 1,25-dihysroxyvitamine D3 degradation have been identified in several IIH cases.
Objective: The objective of our study was thus to investigate CYP24A1 in a cohort of children affected by IIH with high level of 1,25-OH2-D.
Result: Direct sequencing of CYP24A1 revealed a new homozygous mutation in two siblings, inherited from both heterozygous parents. Clinical and biological analysis showed that the older brother which received a milk enriched with 1500 IU /d of vitamine D showed at 6 months of age a decrease appetite, weight stagnation, and intermittent vomiting revealing severe hypercalcaemia (3.68 mmol /l), with very low PTH (1 ng/l), mild elevated 25-OH-vitD (140 nmol /l), and normal 1,25- OH2-vit D levels and hypercalciuria (Ca/creat at 4.3) explaining the nephrocalcinosis. Stop of vitamine D supplementation, hyperhydration and furosemide and locasol therapy allowed a normalization of the serum calcium level within 3 weeks. Subsequently, the child received milk enriched with 600U/d and vitamine D levels were adequate, except after the summers when 1,25-OH-vitD levels raised up to 360 pmol/ l in spite of sun protection. Based on these observations, the younger brother did not received vitamin D but only enriched milk (600 IU/d) and biological monitoring showed normal serum (maximum 2.62 mmol/l), and urinary calcium levels, but low PTH (between 4-24 ng/ l) and moderate elevated 1,25-OH-vitD levels (maximum 140 nmol/ l). In addition, renal ultrasound revealed no nephrocalcinosis.
Conclusion: Our study confirms that CYP24A1 plays a causal role in some IIH cases and that preventive measures including vitamine D suspension could protect affected relatives particularly in the infant period.