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ABSTRACTS

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Mar del Plata, Argentina, October 13–17, 2007

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Juan Jorge Heinrich, Buenos Aires

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Invited Speakers

Prof. Dr. Antonio Carrascosa
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Hospital Materno-Infantil Vall d' Hebron,
Universidad Autónoma de Barcelona
Barcelona, Spain

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Centro de Investigaciones Endocrinológicas – CONICET
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Department of Pediatrics - University of Chieti
Chieti, Italy

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Institut de Recherche Interdisciplinaire en
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Université Libre de Bruxelles.
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The John Hopkins University School of Medicine
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Section Pediatric Endocrinology,
University Children's Hospital
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Assist. Professor. The Mount Sinai School of Medicine
Endocrinology/Diabetes and Bone Disease
New York, N.Y., USA

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Program

Saturday, October 13th

19.00–20.00	Opening Session
20.00–21.00	Opening Lecture **Prof. Dr. Michael RANKE** The Future Paediatric Endocrinologist and His/Her Future
21.00	Venue Cocktail

Sunday, October 14th

08.00–09.00	Conference **Prof. Dr. Alan ROGOL** Gender and Hormonal Regulation of Growth
09.00–10.30	Oral Presentation Session
10.30–11.00	Coffee break
11.00–12.00	Oral Presentation Session
12.00–13.00	Conference **Prof. Dr. Shoshana YAKAR** Metabolic Effects of IGF-I Deficiency: Mouse and Human
13.00–14.00	Lunch with the Professor **Prof. Dr. Alan ROGOL** New Facets of Androgen Replacement Therapy during Childhood and Adolescence
13.00–15.00	Interval
15.00–16.00	Miniposter Session
16.00–17.00	Poster Session (coffee)
17.00–18.30	Oral Presentation Session
18.30–19.30	Conference **Prof. Dr. Suzanne JAN DE BEUR** Clinical and Molecular Pathogenesis of Hereditary Hypophosphatemic Rickets
19.30–21.00	SLEP Business Meeting
21.00	Dinner

Monday, October 15th

07.30–09.00	Oral Presentation Session
09.00–10.00	Short Conferences **Dr. Sabine Costagliola** G Protein-Coupled Receptors and Diseases **Prof. Dr. Suzanne JAN DE BEUR** Pathophysiology (Molecular, Clinical and Genetic) of Pseudohypoparathyroidism Associated with Mutations in Gs Proteins
10.00–10.30	Coffee break
10.30–11.30	Oral Presentation Session
11.30–12.30	César Bergada Conference **Dr. Héctor Chemes** Testicular Abnormal Development: From Cryptorchidism to the Testicular Digenesis Syndrome
12.30–19.00	Free Afternoon
19.00–20.30	SLEP Business Meeting
21.00	Dinner

Tuesday, October 16th

08.00–09.00	Conference **Prof. Dr. Shoshana YAKAR** The Role of the Growth Hormone/Insulin-Like Growth Factor Axis in Tumor Growth and Progression: Lessons from Animal Models
09.00–10.30	Oral Presentation Session
10.30–11.00	Coffee break
11.00–12.00	Oral Presentation Session
12.00–13.00	Conference **Dr. Sabine COSTAGLIOLA** Congenital Hypothyroidism
13.00–14.00	Lunch with the Professor **Prof. Dr. Francesco CHIARELLI** Methods for Evaluation of Insulin Resistance in Children
13.00–15.00	Interval
15.00–16.00	Miniposter Session
16.00–17.00	Poster Session (coffee)
17.00–18.30	ESPE Symposium **Prof. Dr. Antonio CARRASCOSA** Obesity during Infanthood and Adolescence: A Pandemic that Claims Our Attention **Prof. Dr. Francesco CHIARELLI** Early Detection and Prevention of Microvascular Complications in Children with Type 1 Diabetes **Prof. Dr. Michael RANKE** Responses to GH in Children Other than Height
18.30–19.30	Closing Ceremony
21.00	Gala Dinner

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Horm Res 2007;68(suppl 4):1–33

1

Identification and Characterization of Testicular Macrophages (MT) Present During Human Prepuberty and Puberty

J. Accorinti[1], E. Berensztein[1], M. Baquedano[1], N. Saraco[1], C. Pepe[1], R. Ponzio[2], M. Rivarola[1], A. Belgorosky[1]

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Interstitial cells (IC) of human prepubertal testes express aromatase and ERβ particularly during the post natal activation stage (PNA) [Berensztein et al., Ped Res 2006]. In rats during testicular development, an increase in macrophage number closely follows the increase in Leydig cell number. Previous studies have demonstrated the expression of ER in murine macrophages. A role of testicular macrophages in Leydig cell differentiation during prepubertal development of rats has been proposed [Gaytan F et al., J Reprod Fert 1994].

The aim of the study was to identify and characterize the macrophage population in human testes collected at necropsy or after biopsy for grade I varicocele. Three groups were studied: GrPNA, postnatal activation stage (1- to 7-month-old infants), n = 8; GrPPu, early pre puberty (12- to 60-month-old boys), n = 8; and GrPu, pubertal stage (14- and 15-y-old boys), n = 6. ERβ and CD68 immunoexpression (macrophage marker) was analyzed. Among IC, CD68% positive cells in GrPPu (15.8 ± 2.56) and in GrPu (16.8 ± 3.57) was significantly higher than in GrPNA (9.45 ± 1.64), $p < 0.05$ (Bonferroni test). A significant positive correlation between CD68% positive cells and age was found ($r = 0.458$, $p = 0.045$). In GrAPN, co-localization of cytoplasmic CD68 and nuclear ERβ was detected, suggesting that interstitial TM might be estrogen targets.

It could be then proposed that MT, stimulated directly by estrogens, among other factors, could modulate proliferation and differentiation of mesenchymal Leydig cell precursors.

2

Body Composition after Long-Term Follow-Up in Renal and Liver Transplanted Pediatric Patients

G. Alonso, J. Ferraris, D. D'Agostino, A. Galich, T. Pasqualini

Departamento de Pediatría, Hospital Italiano de Buenos Aires, Argentina

Introduction: Renal and Liver transplanted children present alterations in body and bone composition secondary to growth failure, undernutrition, preexistent bone disease, decreased physical activity and adverse effects of immunosuppressive therapies.

Objective: To describe body composition after long-term follow-up in liver (TxH) and renal (TxR) transplanted pediatric patients.

Patients: 13 TxH (4 girls) and 27 TxR (6 girls); age (years) at Tx: 5.5 + 3.4 (TxH), 7.1 + 2.6 (TxR); age at study: 12.6 + 2.4 (TxH), 12.1 + 2.3 (TxR); normal liver function in both groups; serum creatinine (mg/dl): 0.73 + 0.2 (TxH), 1.0 + 0.2 (TxR); 3 TxH and 26 TxR were on glucocorticoid therapy.

Methods: We assessed bone mineral density (Lunar DPXL): lumbar (DMOL) and total body (DMOE); bone mineral content (CMO), lean mass (MM) and fat mass (MG).

Results:

	Z height	Weight for height (%)	DMOL (Z) for chronological age	DMOE (Z) for chronological age	CMO (g/m^2)	MM (g/m^2)	MG (g/m^2)
TxH	−1.58	104	0.1	0.11	1,197	21,799	8,662
TxR	−3.06	128	−1.84	−1.06	934	20,303	7,929
p	<0.001	0.002	<0.001	<0.001	0.007	ns	ns

CMO correlated with MM: TxH r: 0.87, $p < 0.01$; TxR r: 0.92, $p < 0.01$.

Conclusion: (1) In TxR patients alterations in bone mineral density may be related to growth failure; (2) Lean mass was similar in both groups; (3) Correlation between MM and CMO suggests the preservation of bone-muscle unit.

3

New Mutation in the CASR in a Family with Familial Hypocalciuric Hypercalcemia (FHH) and Neonatal Severe Primary Hyperparathyroidism (NSHPT) Cases

A. Arias Cau[1], R. Matsunga Martin[2], L. Bussmann[2], C. Insua[1], M. Pacin[3], G. Bastida[3], P. Corrêa[2], O. Brunetto[1]

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Introduction: FHH and NSHPT represent conditions associated with loss of CASR function due to inactivating mutations. NSHPT represents the most severe expression of familial hypocalciuric hypercalcemia and course as a life-threatening condition.

Methods and Results: We studied a female infant who was referred for dehydration, apathy and lack of sucking. She presented

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severe hypercalcemia (Ca = 18.1 mg/dl, P = 4.1 mg/dl, PTH = 1036 pg/ml). She remained hypercalcemic despite of hydratation, furosemide and bisphosphonate administration and required total parathyroidectomy. Her parents were asymptomatic and laboratorial data did not rule out FHH. The molecular diagnosis for PCR-amplified CASR exons from the patient's DNA was performed. A homozygous mutation (E519X) was identified; her parents had the same mutation in heterozygous state.

Conclusions: This mutation has not been previously described. It determines a truncated protein with lose of the full transmembrane and intracellular domain. Its function is severely affected, determining the FHH phenotype of the parents and the patient's NSHPT condition. Moreover, this study allows a genetic counseling for this family.

4

Nephrogenic Diabetes Insipidus (NDI): Possible Mosaicism for a Mutation in the AVPR2 Gene Resulting in a Partially Expressed Phenotype

M. Arriazu[1], G. Isaac[1], S. Rittig[2], J. Christensen[2], H. Kvistgaard[2], M. Roubicek[1]

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Inroduction: NDI type I is an X-linked recessive disorder due to defects in the arginine vasopressin receptor AVPR2. We present a case with unusual findings.

Case Report: A boy aged 14.5 years was first seen in 1999 for polyuria and polydypsia. No similar cases nor diabetes mellitus are present in the family. The mother remembers changing his very soaked diapers frequently. His diuresis was 5–71 daily, with micturitions of up to 1,350 ml at once. Response to oral medication (indomethacin, amiloride + thiazide) was unsatisfactory. Various water restriction tests were unconclusive; there was no response to two different dosages of nasal DDAVP (Desmopressin). Molecular studies on DNA from blood lymphocytes were undertaken.

Results: Sequencing of the Aquaporin-2 (AQP2) water channel gene was normal, including a known polymorphism. Sequencing the AVPR2 gene disclosed an unexpected heterozygous pattern in exon 2 for a single base substitution (797G>A) predicting a G122D amino acid substitution. Sequences of exons 1 and 3 were normal. Contamination of the sample with extraneous DNA, and Klinefelter syndrome were excluded; the karyotype was 46, XY. DNA from parents, two siblings and the patient's little daughter did not show the mutation.

Conclusion: As a probable explanation for the unusual finding of an apparently heterozygous pattern of an X-linked gene in a male, we propose that our patient presents a mosaic pattern of the AVPR2 gene as a consequence of a postzygotic mutational event, resulting in a phenotype of partial nephrogenic diabetes insipidus.

5

Novel Compound Heterozygous Mutation of the ACTH Receptor Gene (MC2R) in a Familiar Glucocorticoid Deficiency

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Familiar Glucocorticoid Deficiency (FGD) is an autosomal recessive disorder. A 25% of the cases present mutations in ACTH receptor, MC2R, gene.

Aim: To report a new mutation in the MC2R gene.

Patients: A male presenting: hypovolemic shock at 2 years old, height +3.6 SD, hyperpigmentation, Tanner I. Basal cortisol <1.0 ug/dl and post-ACTH: 1.1 ug/dl, PRA: 2.8 ng/ml/h, ACTH: 930 [9–65 pg/ml], 17OH-Progesterone <0.1 ng/dl, Testosterone <10 ng/dl, adrenal autoantibodies (−), normal VLCFA. Bone age: 4 years. Healthy and non-consanguineous parents, 4 siblings, one affected by FGD.

Methods: MC2R gene was amplified by PCR, sequenced and aligned with control and genebank sequences. CHO cells were transfected with pCRE-luc reporter, cotransfected with pcDNA 3.1 vector, expressing wild type (wt) or mutated MC2R cDNA (generated by site-directed mutagenesis), and stimulated with ACTH, 8-bromo-cAMP, or unestimulated.

Results: In both affected members we found: (1) An adenine heterozygous insertion in MC2R gene (named insA1347 or G217fs) producing a premature stop codon. (2) A novel heterozygous insertion, Ala126Ser, affecting the MC2R tertiary structure. Either, father and mother carry one of the heterozygous mutations, Ala126Ser and insA1347 respectively, without symptoms of FGD. pCREluc-mutated-MC2R cells had a lower activity when stimulated with ACTH than pCREluc-wt-MC2R cells.

Conclusion: We present a family with two FGD affected members who carry compound heterozygous mutations in MC2R gene. In vitro studies showed an important effect of the novel mutation Ala126Ser in MC2R gene expression.

6

Growth Hormone Binding Protein (GHBP) Serum Levels are Associated to GH Receptor Exon 3 Polymorphism in Normal Children

M. Ballerini[1], P. Scaglia[2], H. Domené[2], M. Ropelato[1], A. Martínez[2], V. Pipman[3], S. Bengolea[4], L. Karabatas[2], P. Bedecarrás[2], J. Heinrich[1], H. Jasper[2]

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The GH receptor (GHR) mediates GH action. To our knowledge, there is no information about a possible association between a genomic

polymorphism, deletion (D) or retention (F) of GHR exon 3, and GHBP levels in normal subjects. To test this hypothesis we measured GHBP by an in house fluoroimmunoassay (modified from Fisker et al., 1996) and GHR polymorphism determined by multiplex PCR, IGFBP3 by IRMA in 151 normal children (56 boys), aged 5–17 yr. Data were expressed as mean ± SEM. GHBP serum levels were not significantly related to sex, age or pubertal stages. Nevertheless a tendency to higher GHBP levels in pubertal girls than boys was found ($p = 0.059$). Serum GHBP reference interval was (P2.5–P97.5) 0.96–6.42 nM for girls and 0.86–5.79 nM for boys. GHBP significantly correlated with BMI percentiles ($r = 0.45$, $p < 0.001$). Children carriers of the GHR F/F genotype ($n = 90$) presented significantly higher GHBP levels than carriers of one or two copies of GHR D allele ($n = 61$) (2.9 ± 0.2 vs. 2.5 ± 0.2 nM, $p = 0.02$). In a subgroup of 110 children, 68 genotyped F/F presented significantly higher height and IGFBP3 SDS (0.49 ± 0.13 vs. 0.05 ± 0.16, $p = 0.03$ and 0.22 ± 0.13 vs. −0.23 ± 0.13, $p = 0.02$ respectively).

Conclusion: The concomitant finding of greater GHBP and IGFBP-3 levels and a higher height associated to the F/F genotype suggest a regulatory effect of exon 3 GHR polymorphism on GH action.

7

Physiopathology of Human Adrenocortical Tumors (ACT): IGF Receptor 1 and Insulin Receptor might be Involved in Tumor Growth, while Estrogens might Regulate the Steroidogenic Pattern

M. Baquedano[1], G. Guercio[1], M. Costanzo[1], N. Saraco[1], E. Berensztein[1], M. Davila[2], M. Rivarola[1], A. Belgorosky[1]

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Estrogens, produced locally in adrenal medulla, could play a role in zona reticularis functional differentiation through estrogen receptor β (ERβ) (Baquedano et al., JCEM, 2007), whereas the IGF system could be involved in postnatal proliferation and migration of progenitor adrenal cells (Baquedano et al., Ped Res 2005). The IGF system could be involved in ACT progression via type 1 IGF receptor (IGFR1). We analyzed aromatase (ARO), ERβ, insulin receptor (IR) A and B, IGF1, IGF2 and IGFR1 mRNA expression in 8 virilizing ACT of prepubertal children (age range 1.3–4.5 yr) and in 29 normal human adrenal tissues (HAT) (GR1, preadrenarche, GR2, post adrenarche). ACT mRNA levels of ARO (1.48 ± 0.24 AU) and ERβ (1.43 ± 0.38) were similar to GR2 but higher ($p < 0.05$) than in GR1 (1.03 ± 0.43 and 0.79 ± 0.28, respectively). IRA (1.93 ± 0.34) and IRB (1.39 ± 0.28) were higher ($p < 0.05$) than in GR1 and GR2 (1.19 ± 0.44 and 1.34 ± 0.35, 0.52 ± 0.50 and 0.86 ± 0.43, respectively). IGF1 (0.68 ± 0.34) was lower ($p < 0.05$) than in GR1 (1.26 ± 0.42) but similar to GR2, while IGF2 (1.38 ± 0.21) was similar to GR1 and GR2. IGFR1 (2.14 ± 0.43) was higher than in GR1 (1.43 ± 0.4, $p < 0.05$). In ACT, different patterns of utilization of exons 1 were observed, but in contrast to HAT in the majority of ACT the adipose tissue promoter was prevalent. In conclusion we propose that similar to normal HAT, local estrogen production in ACT might play a role in androgen production. In ACT the expression of ARO might be regulated differently. Since cytokines regulate ARO adipose tissue promoter, they might also control ACT steroidogenesis pattern. The signaling activation of IGFR1 and IR might be involved in tumor growth.

8

Sertoli Cell Function in Boys with Central Precocious Puberty (CPP)

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CPP is an interesting model to evaluate the regulation of Sertoli cells by gonadotropins and testosterone (T). To further assess the regulation of Sertoli cell function, 5 boys with CPP (3.4 ± 0.9 yr; mean ± SEM) were included in this observational, retrospective study. Levels of LH, FSH, T, Pro-αC, inhibin B (InhB) and anti-Müllerian hormone (AMH) were determined before, during, and after GnRHa treatment (triptorelin acetate 110–190 mcg/kg). Pre-treatment testis volume (TV) was 6.6 ± 1.1 ml, LH 3.9 ± 1.7 IU/l, FSH 1.5 ± 0.4 IU/l, T 357 ± 99 ng/dl and Pro-αC 160 ± 38 pg/ml. Sertoli cell function was assessed by measuring serum InhB and AMH. Pre-treatment InhB was elevated (396 ± 57 pg/ml) and AMH was low (99 ± 25 pmol/l) for chronological age. During treatment, LH, FSH, T and AMH returned to prepubertal values. The TV decreased to 4.4 ± 0.8 ml and InhB levels to 175 ± 28 pg/ml. After treatment, all variables reached normal pubertal values.

Conclusion: GnRHa treatment curtailed FSH, LH and T secretion and normalized AMH. However, TV and InhB remained moderately elevated, probably reflecting an increased mass of Sertoli cells that was not restored to prepubertal stage. This clinical model supports the hypothesis that prepubertal peripheral InhB reflects the production of two pools, one regulated by LH/FSH and the other by the Sertoli cell mass present in the gonad.

9

Unusual Association of Hypophosphatemic Rickets and 46XX Male Syndrome: A Case Report

D. Braslavsky, H. Cassinelli, G. Del Rey, L. Gruñeiro

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X-linked hypophosphatemia (XLH) is the most prevalent heritable form of rickets. It is characterized by renal phosphate wasting, and a defective bone mineralization. On the other hand, the 46XX male syndrome represents a rare, and poorly characterized form of male hypogonadism. We report the rare association of an adolescent with

XLH and hypergonadotrophic hipogonadism. We present a boy with diagnosis of XLH made at the first year of age, in which his mother and brother have the same pathology. Basal laboratory showed phosphorus 2.5 mg/dl (↓), AP 1115 U/l (↑), PTR 15% (↓), and radiological features of rickets. Normal FGF23. He started treatment with Calcitriol and Neutraphos-K. A total regression of rickets was obtained. The dose was adapted according to his growth. At the age of 12.2 years old, he began his puberty G2, PH2, T4/4 ml, reaching, at the age of 13.8 years old, G4, PH4, T10/10. But, 8 months later, gynecomastia was noted: B3-2, PH4, G4 and testicular volume of 5/5 ml. Laboratory: LH 20 mUI/ml (↑), FSH 71.8 mUI/ml (↑), AMH 4 pmol/l, Testosterone 213 ng/dl (↓), Karyotype: 46XX, ish (Y) (SRY+), ish (X) (DXZ1++). Although XLH rickets is frequent, either in female as male, the 46XX male syndrome is not a usual form of hypogonadism. In spite of both pathologies involve the X sexual chromosomes, they do not have a relation in common. By an abnormal paternal meiotic recombination the SRY was translocated in the X chromosome. The association of XLH and 46XX male syndrome has not been described up to date.

10

Secular Changes of the Body Composition in School Age Children 6 to 15 Years of Age of Metropolitan Region (RM) of Chile

R. Burrows, X. Ceballos Sánchez, M. Burgueño Aguilera, S. Muzzo Benavides

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Introduction: Obesity increased 4 times in children during the last 20 years, in direct relation with the caloric food consumption and the sedentary life style. There is a strong association between physical inactivity and DM2 and isquemic cardiopathies incidence, indicating that sarchopenia could be one of the determinants of the insulin resistance (IR) that characterizes them.

Objective: To evaluate the secular change of fat and lean mass of schoolage children during the last decades.

Method: In two samples of 6 to 15 years of schoolage children of both sexes, one selected between years 1986–88 (G1987: 2,582 children) and the other between the years 2000–2003 (G2002: 2,095 children), the weight, height, triceps skinfold and brachial perimeter were measured, to evaluate BMI and upper arm fat (UAF) and muscle (UMA) (Frisancho). The percentile (p) distribution of this variables was calculated by age and sex (LMS methodology) and was compared with the NHANES referent.

Results: In both sexes, 50th p of BMI and UAF of the G1987, was similar at all ages to 50th p of NHAHES; however, both were highest in the G2002 than NHANES. In both sexes, there is an increase of 50th p of the UAM in the G2002 compared to G1987. Nevertheless, in both generations 50th p of UMA is below NHANES, specially after 10 years old.

Conclusion: The School age children showed a trend to increase BMI and UAF, with a small gain of the UMA. The possible etiology of this type of obesity with 'sarchopenia' and the effect on the risk of degenerative chronic diseases are discussed.

FONDECYT 1985/ FOGARTY 2001 Project.

11

Inflammatory Mediators in Overweight Adolescent: Association with Insulin Sensitivity, Body Composition and Metabolic Syndrome

R. Burrows, L. Leiva, A. Tong, J. Aldunate, E. Díaz

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Introduction: The insulin resistance metabolic syndrome (IR MetS) lays to great cardiovascular disease (CVD) and Type 2 Diabetes risk in adult life and has been associated with an early state of chronic low–grade inflammation (CLGI). The aims of this study was to determine the association between a state of CLGI with insulin sensitivity, body composition and MetS prevalence in overweight adolescents.

Methods: In 158 overweight adolescent (BMI ≥ 85th) (86 men) between 13 to 16 years, we evaluated the BMI, body composition through Fat Body Mass (%FBM) and Free Fat Mass (%FFM) percentage by pletismography, prevalence of MetS for 3 or more of the following: Waist perimeter (WP) ≥ 90th p; HDL – chol ≤ 40 mg/dl; TG ≥ 110 mg/dl, blood arterial pressure ≥ 90th, fasting glucose ≥100 mg/d), HOMA-IR (Glum mmol/l × Ins IU/dl/22.5) and the state of inflammation by C-Reactive protein (CRP). Pearson correlation and Chi2 were used to study associations between variables, Odds Ratio to calculate risk and analysis of variance simple and test of Tukey to compare averages between groups.

Results: The CRP levels were 0.7 mg/l in both sexes. The CRP showed a direct correlation with BMI ($p < 0.05$), WP ($p < 0.02$), %BFM ($p < 0.05$), fasting insulin ($p < 0.001$) and HOMA ($p < 0.001$) and indirect with %FFM ($p < 0.05$). The CRP was associate ($p < 0.01$) with an cardiovascular risk profile. The prevalence and the risk of abdominal obesity (WP ≥ 90th p), IR (HOMA ≥ 3.3) and MetS, were significantly highest (63% and OR: 3.0; 43% and OR: 4.1; and 26% and OR: 4.1 respectively)) in adolescent with CRP ≥ 1.12 mg/l (≥Tercil 3).

Conclusion: This results confirmed that the CRP levels in overweight adolescents is associated with a greater cardiovascular risk and the IR involve inflammatory processes that may play an early role in the development of cardiovascular lesion.

Project DID/SAL 12/02.

12

Pituitary Transcription Factors and β-Catenin Genes in Adamantinomatous Craniopharyngiomas

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Introduction: Craniopharyngiomas (CP) are among the most common childhood brain tumors. Their pathogenesis is poorly understood; however, genetic events may be involved.

Objectives: We performed molecular analysis of three homeodomain containing transcription factors, HESX1, PROP1, POUF1,

involved in anterior lobe of the pituitary gland development, in addition to the β-catenin gene, a co-regulator for transcription activity of PROP1.

Material and Methods: We studied 4 pediatric (1F, 3M; age 6–14 years, median 9.5 years) and 3 adult (1F, 3M; age 20–30 years, median of 23.6 years) patients with adamantinomatous CP. DNA was extracted from tumor tissues; the entire coding region of HESX1, PROP1, POUF1 genes and exon 3 of β-catenin gene were amplified by PCR, followed by automatic direct sequencing. For comparison, we sequenced all genes in 30 to 50 normal subjects.

Results: No mutations were identified in HESX1, PROP1, POUF1 genes. However, we found three polymorphisms (SNP) in PROP1 gene, the IVS + 3G > A at intron A, rs1135320 at exon 1 and rs1800197 at exon 3. The frequency of all these polymorphisms in Brazilian population was similar to that observed in CP patients. We found a mutation in β-catenin gene, the g.304G > A, corresponding to c.94G > A at codon 32 (Asp32Phe) in one patient (14.3%).

Conclusion: Mutations or polymorphisms in pituitary transcription factors or their coregulators are unlike to contribute to the pathogenesis of adamantinomatous CP. In addition, β-catenin gene mutation is not frequent in Brazilian patients with adamantinomatous CP.

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Lower Number of GGC Androgen Receptor Gene Repeats in Prepubertal Girls with Hypertricosis

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Introduction: The transactivation domain of the androgen receptor is required for its genomic activity and it includes two polymorphic aminoacid repeats, whose number is inversely related to androgen receptor function. Our aim was to quantify the number of GGC repeats (which codes for gly), in the androgen receptor gene of prepubertal girls with hypertrichosis compared to normal control girls.

Methods: The hypertrichosis score was determined by the method of Gryngarten (Horm Res 54: 20, 2000). DNA obtained from leukocytes was amplified by PCR with specific primers (antisense marked with 6-FAM) for the region in exon 1 which contains the GGC repeats. The number of repeats in each allele was extrapolated based upon the size of the fluorescent amplicons, which were determined by capilar denaturating electrophoresis.

Conclusions: The girls with hypertrichosis showed a greater prevalence of alleles with less than 17 GGC repeats compared to controls. This finding suggests that some girls with hypertrichosis may have androgen receptors with enhanced sensitivity, which may facilitate the growth of their body hair.

14

Experience with the Neonatal Screening Program for Congenital Adrenal Hyperplasia (CAH)

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Objective: To report the experience of CAH neonatal screening program performed in Buenos Aires, Argentina from 1997 to 2006.

Population and Method: 17OHP was measured with an immunofluorometric assay in filter paper blood samples' collected at neonatal maternity discharge. Filter paper blood levels <40 nmol/l were considered normal. 17OHP levels between 40–90 nmol/l triggered a new assessment to decide on a course of action. Confirmation of CAH was done with levels >90 nmol/l. This led to clinical follow up. For preterm babies (PT) data were adjusted according to percentiles for gestational age and/or birth weight.

Results: From 80,436 screened newborns (46.8% girls), 8,848 (11%) were PT. 15T and 3PT were recalled (0.022%). 9 were confirmed as having CAH (8T and 1PT) (female//male: 0.8). (Incidence 1: 8,937). Mean ages of screening and treatment were 5.7 and 13 days. Only 33% of affected children were clinically suspected of having CAH prior to screening. Four boys and two girls presented salt wasting forms and severe adrenal insufficiency crises were prevented as a result of the screening.

Conclusions: Our findings confirm the benefits of CAH neonatal screening in our country with a high incidence of the classical form. Established criteria of screening and follow up allowed us to detect unrecognized affected males and females and to successfully prevent salt wasting crises.

Results: (of Abstract 13)

	n	Age[a] (years)	Hypertricosis score[a]	Follicles/ ovary[a]	Testosterone[a] (ng/dl)	SHBG[a] (mmol/l)	Alleles <17 (%)
Cases	44	6 ± 2	16 ± 4**	5 ± 2	14 ± 1	71 ± 16*	70*
Controls	29	6 ± 2	3 ± 2	4 ± 1	14 ± 1	82 ± 15	52

[a](mean ± SD)
Comparison of means (Kruskal-Wallis) and proportions (chi^2); **: $p < 0.01$, *$p < 0.05$.

15

Nailfold Capillaroscopy in Turner Syndrome Considering Dyslipidemia and HOMA IR

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Introduction: Turner syndrome patients (TS) have an increased prevalence of impaired glucose homeostasis and dyslipidemia.

Objectives: We compared the morphology of nailfold capillaries using videocapillaroscopy in TS and healthy controls, considering dyslipidemia and HOMA IR.

Method: Videocapilaroscopy was performed in 33 TS and 35 controls to study: loops distribution and length, papilla, edema, form, tortuosity, flow and hemorrhagic extravision. We analyzed serum lipids and glucose, the use of medications. The significance level accepted was $p < 0.05$.

Results: The mean age of patients and controls was 13.4 ± 3.8 and 13.2 ± 3.8 respectively ($p = 0.85$). Thirteen patients had dyslipidemia. The HOMA IR was higher ($p = 0.0005$) in controls than in TS (1.9 ± 0.89 and 1.3 ± 0.69). Abnormal capillaroscopy was found in 22 (32.4%) TS and 2 (9.1%) controls ($p < 0.0001$). Edema occurred in 13 (39.4%) TS and 3 (8.6%) controls ($p = 0.002$). Papillas were ratified in 6 (18.2%) TS and 1 (2.9%) control ($p = 0.04$) and enlarged in 3 (9.1%) TS and 1 (2.9%) control ($p = 0.28$). No control had flow abnormalities while in TS the flow was granulous in 3 (9.1%) ($p = 0.10$), slow in 5 (15.2%) ($p = 0.02$) and fast in 6 (18.2%) ($p = 0.01$). Macrocapillaries occurred in 3 (6%) TS ($p = 0.10$), branched capillaries in 3 (9.1%) TS ($p = 0.10$) and 1 (2%) control. Tortuosity occurred in 5 (15.2%) TS and no control and hemorrhagic extravisions in 1 (2%) TS ($p = 0.48$). Twenty six patients were using sex hormone replacement or GH in 14 (53.8%) had abnormal capillaroscopy, of those 10 (71.4%) were in use of GH.

Conclusion: In the Turner Syndrome there are more alterations in capillaroscopy than in healthy people independently of dyslipidemia or HOMA IR.

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Frequency of Molecular Diagnosis in Brazilian Patients with 46, XY Disorders of Sexual Development (DSD)

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Objectives: To evaluate the frequency of molecular diagnosis in patients with 46, XY DSD.

Patients: A total of 148 patients were studied and classified in 3 groups of 46, XY DSD: ***I*** – due to abnormalities of gonadal development ($n = 44$); ***II*** – due to disorders in the production, action or metabolization of testosterone: 7 LH receptor defect (LHCGR), 16 steroidogenesis defect, 24 androgen insensitivity syndrome (AIS) and 16 5a reductase-2 deficiency (SRD5A2) and ***III*** – undetermined: 41 patients with no established diagnosis.

Methods: The coding regions of the *SRY*, *SF1*, *WT1*, *LHCGR*, *3bHSD2*, *CYP17*, *17bHSD3* genes, androgen receptor and *SRD5A2* were amplified and sequenced. In *Group III*, *SRY* was studied.

Results: In *Group I*, the molecular diagnosis was established in 11.4% of the patients. We identified mutations in the *SRY* (3), *SF1* (1) and *WT1* (1) genes. In G*roup II*, we identified mutations in 100% of the patients with enzymatic defects. The molecular diagnosis was established in 75% of the AIS patients and in 81.2% of those with SRD5A2 deficiency. In *Group III*, a mutation in the *SRY* was identified.

Conclusion: In this sample, 38.5% of the patients had the molecular defect identified. The molecular etiology was established in a small number of patients from *Group I*. All patients with steroidogenesis defects had the molecular etiology established. Regarding the patients with LHCGR defects, AIS and SRD5A2 deficiency, mutations were identified in most cases. The molecular diagnosis must always be directed by an accurate clinical-laboratory diagnosis.

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Duplication of the Dosage-Sensitive Sex Reversal (DSS) Locus in a Brazilian Patient with Partial 46, XY Gonadal Dysgenesis and Gonadoblastoma

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

Objective: To analyze the dosage effect of *DAX1* gene by MLPA technique (Kit Intersex) in a patient with partial 46, XY gonadal dysgenesis and the 46, XY/47, XY, +mar karyotype.

Patient: A non-syndromic 14-yr old patient was referred with primary amenorrhea and absence of breast development. External genitalia showed clitoromegaly (4.6×1.0 cm), urogenital sinus and impalpable gonads. Hormonal measurements revealed elevated LH and FSH levels (32.7 and 72 UI/l, respectively) and testosterone levels of 479 ng/dl. Bilateral gonadectomy was performed and a gonadoblastoma was identified in the right dysgenetic testis as well as Müllerian and Wolffian derivatives structures.

Methods: MLPA technique (Kit Intersex) was performed to screen the dosage of the *DAX1*, *SOX9*, *WNT4* and *SRY* genes. FISH analysis was performed using the RP11-89L23 probe, which binds to the Xp21.2-21.3 region containing *DAX1* and contiguous MAGEB genes (Xp21).

Results: Duplication of *DAX1* gene was demonstrated by MLPA. FISH analysis identified duplication on Xp21 region in the marker chromosome.

Conclusion: We described a non-syndromic girl with a 46, XY partial gonadal dysgenesis and a marker originated from the X chromosome containing a duplication in the Xp21 region. This finding supports the fact that *DAX1* gene overexpression causes gonadal dysgenesis, although the role of the contiguous genes present in Xp21 region should be ruled out.

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Presence of Several Polyadenylation Signal Polymorphisms in IGF1 Gene of Children Born Small for Gestational Age (SGA) with and without Postnatal Catch-Up Growth

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Background: There have been no studies that systematically evaluated the *IGF1* gene in SGA children without catch-up growth.

Objectives: To sequence *IGF1* gene of SGA children that did not present catch-up growth (SGA/CU−) and compare the frequency of found polymorphisms in SGA children with catch-up growth (SGA/CU+).

Patients and Methods: *IGF1* were directly sequenced in 53 SGA/CU−. The frequency of allelic variations identified at *IGF1* was assessed in 72 SGA/CU−, 73 SGA/CU+ and in 180 normal controls.

Results: No mutations were identified in *IGF1* coding region in the SGA/CU− children. In contrast, 6 polymorphisms were identified in the upstream core polyadenylation signal located in *IGF1* 3′ UTR at exon 6. Four of these polymorphisms modify the AATATA polyadenylation signal, yielding an alternative AATATA motif located down or up stream from the original one. The frequency of the different allelic variants identified was similar in SGA/CU−, SGA/CU+ and controls. The polymorphic allele AATATA > AAAATA, previously described by Bonapace et al., as the cause of isolated IGF-1 deficiency, was found in 4% of studied alleles and this polymorphism was found in homozygous state in 4 normal height controls. Children harboring the wild-type allele in homozygous state presented similar clinical features and serum IGF-1 SDS when compared with children carrying any of the described *IGF1* allelic variants.

Conclusion: *IGF1* is a well conserved gene and mutations in *IGF1* are rare in SGA children without catch-up growth. However, a highly polymorphic region located in the upstream core polyadenylation signal at *IGF1* exon 6 has been identified. Our clinical and laboratory data demonstrate that these polymorphisms did not have any influence on phenotype.

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Analysis of LH Determination 2 Hours after Depot Leuprolide Acetate in Monitoring Gonadotropin-Dependent Precocious Puberty Treatment

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Introduction: A single LH determination 2 h after depot leuprolide acetate (LA) represents an useful hormonal parameter for monitoring the adequacy of LA treatment in girls with gonadotropin-dependent precocious puberty (GDPP). Recently, we have established the cut-off value (6.6 U/l) for LH obtained 2 h after LA (3.75 mg every 28 days) in 18 girls with well controlled GDPP.

Objectives: To evaluate a single LH determination 2 h after LA in a larger group of GDPP patients.

Patients and Methods: We selected 41 girls and 6 boys with GDPP in treatment with LA (3.75 mg every 28 days). At the moment of LH assessment, the duration of treatment was 1.6 ± 1.3 yr in girls and 3.2 ± 2.4 yr in boys. All patients were followed for more than 6 months. Clinical data, pubertal stage, growth velocity and bone age were evaluated quarterly. Girls were divided in two groups: adequate and inadequate control. Inadequate control was defined by one or more of the following: growth velocity >6 cm/yr, inappropriate bone age advancement, Tanner stage progression. LH 2 h after LA was assessed by IFMA. Data are presented as mean ± SD and range. Sensibility and specificity of LH cut-off value of 6.6 U/l were calculated.

Results: Adequate clinical control was achieved in 38 of 41 girls and in all boys. 53 samples of LH 2 h after LA were analyzed (11 girls with adequate control had multiple samples). Results are shown at the table. The cut-off for LH 2 h after LA of 6.6 U/l have sensibility and specificity of 100%.

	Basal LH	LH 2 h after LA
Girls: adequate control	0.6 ± 0.1 U/l (<0.6–0.7 U/l)	2.7 ± 1.4 U/l (0.7–6.6 U/l)
Girls: inadequate control	1.2 ± 0.7 U/l (0.6–2 U/l)	9.9 ± 1.8 U/l (7.9–11 U/l)
Boys	<0.6 U/l	1.9 ± 0.6 U/l (1–2.9 U/l)

Conclusions: We have confirmed, in a larger number of patients, which were followed for a long period that the cut-off of 6.6 U/l for LH 2 h after LA present sensibility and specificity of 100%.

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Intensification of Type 1 Diabetes Mellitus (DM 1) Treatment: Efficacy of Detemir as Basal Insulin

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Rapid-acting insulin analogues as preprandial bolus showed more predictable effect and less hypoglycemic events, however the extent of benefit of the new long-acting analogues as basal insulin is still not fully established.

Objective: To evaluate the efficacy of the Detemir 2x/day + rapid-acting analogue regimen in a DM 1 pediatric population.

Methods: 49 patients in NPH human insulin 3x/day + rapid-acting analogue were randomized in a case-control study for 24 weeks. NPH human insulin (syringe) was switched to Detemir insulin (flexpen) in a 1:1 dose rate, splitting 2/3 before breakfast and 1/3 at bedtime. The mean capillary glucose levels and the frequency of hypoglycemia in the previous 90 days of NPH regimen were recorded in Camit-Pro software of Accuchek meters and were compared to those of the last 90 days of Detemir regimen. The mean HbA1c levels in NPH regimen were compared to those of the initial, 60, 120 and 180 days after the beginning of Detemir. Insulin doses (U/kg/d) and BMI (kg/m^2) in both regimens were also compared.

Results: The mean HbA1c level at the end of Detemir regimen was lower than that in the beginning (9.3 vs. 10.7%; $p < 0.05$) and Detemir dose at the end was higher than NPH dose in a 1.26:1 rate ($p < 0.001$). Patients' satisfaction with the pen injector was greater than with syringes.

Conclusion: Detemir 2x/day regimen as basal insulin was more efficient than NPH 3x/day only in a dose rate of 1.26:1, respectively.

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Prevalence of Metabolic Syndrome (MS) in Students of Basic Education of Santiago, Chile. Preliminary Results

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Introduction: MS constitutes a predictive index of metabolic and cardiovascular risk. We have reported a prevalence of 43% of MS in children before being treated for obesity. The aim is to determine the prevalence of MS in healthy children and correlate it with their nutritional state.

Methods: Taking a random sample of 1,946 students of Santiago, Chile in whom weight, height, waist circumference and blood pressure were measured, we selected a random subsample of 271 children of 8 to 15 years (130 obese, 96 overweight and 45 normal). Fasting insulin, glucose and lipid profile were obtained. MS was established, when ≥ 3 of the following criteria: glucose ≥ 100 mg/dl, triglycerides (TG) ≥ 110 mg/dl, HDL < 40 mg/dl, waist circumference >p90 for age and sex and systolic and/or diastolic blood pressure (BP) > p95 for sex, age and height.

Results: The prevalence of MS was 13.7%. In normal, overweight and obese were 0%, 5.2% and 24.6% respectively ($p < 0.0001$). Averages values and SD are shown in table according to presence or absence of MS.

	BMI zscore	Glucose mg%	Chol. HDL mg%	Triglycerides mg%	Insulin mUui/ml
SM (−)	1.3 ± 0.6	91.4 ± 7	49.2 ± 10.6	96.4 ± 36.1	25.1 ± 12.3
SM (+)	2.0 ± 0.3*	93.6 ± 6.8*	37.6 ± 7.2*	165.4 ± 71.7*	50.3 ± 25.3*

*$p < 0.05$.

Conclusions: (1) A significant number of obese and overweight were found to have MS. This suggests that these children should be identified early during the school-life. (2) SM is related to BMI, and insulin levels being significantly greater in obese which are at great risk of cardiovascular and metabolic events.

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Mutations in the SRY Gene Identified in Three Patients with 46, XY Disorders of Sexual Development (DSD) due to Abnormalities of Gonadal Development

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Objective: To describe 3 *SRY* mutations in Brazilian patients with 46, XY DSD.

Patients and Methods: Genomic DNA of 3 patients with 46, XY DSD due to abnormalities in the gonadal development was obtained and *SRY* coding region was amplified and sequenced.

Results: Three *SRY* mutations were identified: the P125A mutation in the patient with Complete Gonadal Dysgenesis; the R30I in the patient with Parcial Gonadal Dysgenesis and the T37A in the patient with no established etiological diagnosis.

Discussion and Conclusion: Three *SRY* mutations were identified. The P125A mutation is located in the *HMG box*. Another mutation, the P125L, was described in 3 members of a family in which the father presented mosaicism for the mutated *SRY* gene. The functional study of the protein P125L showed a decrease in the DNA binding capacity. The R30I mutation, located in the 5′ region outside the *HMG box* was also identified in the patient's father. This mutation had been described previously in several affected and non-affected (father and siblings) members of another Brazilian family. The functional studies showed a reduction in the DNA-binding capacity of this mutated protein when phosphorylated. The new mutation, T37A, located in the 5′ region outside the *HMG box*, was identified in a previously gonadectomized patient with no established etiological diagnosis. Despite the participation of several genes in the gonadal development process, *SRY* gene mutations are still the most often

identified molecular etiology in patients with 46, XY DSD due to abnormalities in gonadal development.

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Frequency of Ovulatory Cycles in Young Adolescents with Type 1 Diabetes Mellitus

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Introduction: Adolescents with type 1 diabetes mellitus (T1DM) may exhibit a delay in pubertal development. The attainment of regular ovulatory function, which occurs after menarche, has not been evaluated in girls with T1DM.

Aim: To determine the proportion of ovulatory menstrual cycles in adolescents with T1DM during the years following menarche (M).

Method: Adolescents with T1DM (n = 16) and healthy girls (C; n = 33) who had developed M during the previous 28 months were recruited. Both groups were matched for gynaecological age (time after M) and BMI. Menstrual cycles were evaluated with a prospective calendar and assessment of ovulation by salivary progesterone (days 13, 18, 23 and 28 of each cycle) during 5.3 ± 0.5 and 5.8 ± 0.5 cycles in each DM1 and C girls, respectively. A progesterone level detecting ovulation (≥0.05 ng/ml) was established from the values obtained from simultaneous saliva and blood samples in 10 adult women with regular menstrual cycles.

Results: A total of 85 and 191 menstrual cycles were evaluated in T1DM and C groups, respectively. Girls with T1DM exhibited a lower rate of ovulatory cycles than C beyond two years post-menarche. However, within the first two years of menarche the frequency of ovulatory cycles was similar in both groups. The menstrual cycle length was similar in both groups.

	T1DM	C
Gynaecologycal age (months)	18.7 ± 3.1	18.0 ± 1.9
BMI-SDS	0.5 ± 0.2	0.9 ± 0.2
Ovulatory cycles (%)		
Cycles ≤2 yr postmenarche	19.4	24.1
Cycles >2 yr postmenarche	36.7	53.7*
HbA1c (%)	8.5 ± 0.2	

*p < 0.05.

Conclusions: The presence of ovulatory cycles after two years post-menarche may be delayed in girls with T1DM.

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Ovulation in Normal Postmenarcheal Adolescents with Polycystic Ovary Morphology (PCOM)

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Aims: To evaluate the frequency of PCOM and to determine its association with ovarian function and body composition in normal post-puberal adolescents.

Subjects and Methods: Study of 29 healthy adolescents (14.0 ± 0.9 years), with a gynecological age of 25.8 ± 4.0 months. PCOM was diagnosed according to the Rotterdam's criteria. A leuprolide test (500 ug sc), with measurement of steroids and gonadotropins (0.3 and 24 h), was performed. Twenty two adolescents were followed during 6 consecutive months with salivary and urinary samples for measurement of progesterone (PG) and LH, respectively, in days 13, 18, 23 and 28th of each cycle. Body composition was assessed with DEXA.

Results: PCOM was observed in 35% of the girls, corresponding to 20.7; 24.1 and 10.3% of the cases with an increased number of follicles, an elevated ovarian volume and both criteria, respectively. Gonadotropins, steroids levels and body composition were similar in both groups.

	PCOM (n = 10)	Without PCOM (n = 19)	p
Cycle duration (days)	31.8 ± 3.6	32.5 ± 4.9	ns
Ovulatory cycles by PG and LH (%)	34	36.2	ns

A new ultrasound was performed one year later in 14 cases, and seven girls showed a change in the sonographic category (3 of them developing PCOM and 4 showing disappearance of PCOM). In the 7 remaining girls, no changes in sonographic features were observed.

Conclusions: Presence of PCOM in normal postmenarcheal adolescents is a frequent and variable finding and does not appear to affect ovarian function or body composition. This study suggests that PCOM should be considered a physiological condition during early adolescence.

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Onset of Puberty in Girls Between Six and Eight Years of Age: Relation Between Final Height and Treatment with GnRH Analogue

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The objective was evaluate final height and treatment with GnRH analogue in girls aged 6–8 yr at the onset of puberty. According to

Herman-Giddens, these girls achieve a normal adult height. To evaluate height in girls with early puberty and its relation with treatment and target height. Forty-two girls with onset of puberty between 6–8 yr, divided in 2 groups: 18 treated with GnRHa (group A) and 24 untreated (group B). The decision to treat was based on several parameters, especially bone age above stature age and predicted height. We determined height at baseline, final height and target height with respectives z-scores (NCHS 2000). Chronological age, bone age and stature age at first evaluation did not differ between groups, although bone age was above stature age in group A [mean ± SD; yr] (10.3 ± 1.6 vs. 8.7 ± 1.3; $p = 0.0035$). Height SD at baseline (0.52 ± 0.98 vs. 1.05 ± 0.92; $p = 0.0782$) and target height [cm] (154.8 ± 5.7 vs. 156.8 ± 3.9; $p = 0.2038$) did not differ between groups. Group A was treated for a median 2 yr and menarche was reached at a median 11.5 and 10.8 yr, respectively, in group A and B ($p = 0.0497$). There was a decrease in height SD between baseline and final height in group A (0.52 ± 0.98 vs. −1.08 ± 1.01; $p < 0.0001$) and group B (1.05 ± 0.92 vs. 0.52 ± 0.94; $p = 0.0550$). However, girls presented with final height within target height. Overall, despite GnRHa use as single therapy, most girls showed a decrease in height, although had a final height within target height. In spite of variation in puberty timing, girls aged 8 yr or less must be individually assessed.

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In Vivo E In Vitro Analysis of the Novel Mutation L848P in the Mineralocorticoid Receptor Gene (MR) Causing Type 1 Pseudohypoaldosteronism (PHA1)

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Introduction: In the dominant form of PHA1 salt waste occurs in the neonatal period due to renal unresponsiveness to mineralocorticoid which is associated with mutations in the MR gene.

Objective: To study the molecular mechanisms in a Brazilian family with PHA1.

Patients and Methods: A 40 days girl presented failure to thrive, vomiting, dehydration, normal genitalia, hyponatremia (124 mmol/l) and hyperkalemia (8.3 mmol/l). Plasma aldosterone was elevated (422 ng/dl); 17-OHP, ACTH, and cortisol were normal. Patient showed marked catch-up growth and electrolytes normalization with oral NaCl supplementation (2 g/d). Her healthy mother presented vomiting and dehydration early in life. MR gene was analyzed by directed sequencing. Functional analysis of MR gene mutation was assessed by site directed mutagenesis, transient transfections and transactivation activity of a reporter gene.

Results: We found a new heterozygous point mutation in exon 7 (c.2543T>C, p. L848P) in the index case and her mother. Renal RCSV3 cells were transfected with expression vectors for either wild type or the mutant MR-L848P receptor and reporter plasmids glucocorticoid response element (GRE2)-luciferase or mouse mammary tumor virus (MMTV)-luciferase. In cells expressing the mutant receptor, aldosterone-induced luciferase activity was markedly reduced (10–15%) compared to that observed in cells expressing wild-type receptors (100%).

Conclusion: The p. L848P mutation affects a conserved residue in the ligand binding domain and markedly impairs MR activity, explaining the PHA1 phenotype found in the index case.

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Found in Children with Idiopathic Short Stature and NS-Associated Signs

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Introduction: Noonan syndrome (NS) is a clinically heterogeneous disorder, with short stature being one of its cardinal signs. PTPN11 gene mutations are involved in NS and have been associated with partial GH insensitivity, at post-receptor level. During mutation screening of NS patients' relatives, some individuals showed to carry the same PTPN11 mutation found in the index case, but exhibited mild NS phenotype or even did not fulfill the NS diagnosis criteria. Therefore, PTPN11 became a good candidate gene to be involved in the pathogenesis of short stature in idiopathic short stature (ISS) children.

Objective: To evaluate the presence of mutations in the PTPN11 gene in NS patients and ISS children that presented NS-related signs.

Patients and Methods: DNA from peripheral leukocytes was extracted from 47 NS patients and 50 ISS children with at least two NS-related signs. Exons 2 to 15 of PTPN11 gene were directly sequenced.

Results: 19 of 46 NS patients (41%) presented mutations in PTPN11 gene. No mutations or polymorphisms were found in the coding region of the PTPN11 gene in any of the ISS children.

Conclusion: Considering that no mutations were found in the present cohort that presented NS-related signs, it is unlikely that mutations would be found in unselected ISS children. We conclude that mutations in the PTPN11 gene are commonly involved in the pathogenesis of NS, but are not a common cause of idiopathic short stature.

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Validation of Changes in Chemiluminescent Methodology (Immulite 1000) for TSH Measurement, and Results in Healthy Newborns

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As from June 2006, changes were made in kits for TSH measurements and manufacturers warned about possible differences in the results obtained. To validate the above-mentioned changes, we evaluated 80 serum samples (patients, controls and pools) with TSH values (mIU/l) ranging from 0.04 to 34, processed by immulite, pre-changes (batch 340) and post-changes (batch 351). A high correlation was found between the results obtained with both batches ($r = 0.995$, confidence interval 95% (CI) = 0.992–0.997; $p < 0.0001$. Spearman Regression analysis). Consistency in TSH measured with both batches was evaluated using the Bland and Altman method, obtaining the ratio: batch 351/340, as a measurement of the difference. Raw data were used because distribution was normal. The ratio was: 1.31 ± 0.03 (Mean ± SEM), CI 95%: 1.26−1.37. Given the inconsistency between both batches, we decided to present the results obtained in 53 full-term healthy newborns, performing the following measurements 48 h after birth: TSH (mIU/l) (Immulite 1000 post-change), T3 (nmol/l), T4 (nmol/l) and T4L (pmol/l). Results obtained (Median and range) were: TSH: 4.79 (1.60–9.64); T3: 2.4 (1.6–4.6); T4: 219 (161–295) and T4L: 29.6 (23.2–43.8). In conclusion, the TSH values obtained show a mean overestimate of 31%, which suggests that a careful clinical evaluation of results should be performed. Therefore, it is essential to obtain reference values in this age group for a proper interpretation of doubtful measurements in neonatal screening

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Identification of SHOX Gene Deletions in Patients with Disproportional Short Stature or Léri-Weill Dyschondrosteosis: Comparison Between the Fish Technique and Microsatellite Study

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Introduction: Around 77% of the individuals with Léri-Weill Dyschondrosteosis (LWD) and 2% of the children with idiopathic short stature (ISS) present *SHOX* gene haploinsufficiency. About 50% of the *SHOX* gene defects are caused by deletions of this gene. The fluorescence *in situ* hybridization (FISH) technique and the microsatellite study (MS) allow the identification of *SHOX* deletion.

Objective: To compare the FISH technique and MS for the detection of *SHOX* gene deletion in patients with LWD and ISS.

Methods: Genomic DNA and peripheral lymphocytes of 7 patients with LWD, 14 disproportional ISS children and 45 family members were obtained. For FISH, the LLNOYCO3"M"34F5 probe was used. For MS, *SHOX* gene markers: CA repeat (5′ untranslated), DYS290 (intra-gene) and the DXYS10096 (200 kb downstream) were used.

Results: Six patients with LWD (4 familial and 2 isolated cases) presented *SHOX* gene deletion in heterozygous form. The results of the two methods were concordant in 19 cases and discordant in 2; 1 case with LWD presented *SHOX* gene deletion only at FISH, while the CA and DXYS10096 markers demonstrated the presence of 2 distinct alleles. In another patient and his mother, both with LWD, the CA marker identified a deletion of the gene, which was not detected by FISH.

Conclusion: We suggest that the search for *SHOX* gene deletion should be carried first through MS, as it is a faster and lower-cost method compared to FISH. In cases where the deletion is not detected by MS or when the microsatellites are not informative, the study must be complemented by FISH.

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Utility of Insulin Resistance Indices vs. Clinical Parameters for the Metabolic Syndrome Diagnosis

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Introduction: The methods described to measure insulin resistance (IR), show difference in sensibility and specificity when detecting high risk patients. The IR indices (IRI) (HOMA, QUIKI, fasting insulin, postabsortive insulin) were compared with clinical parameters for the metabolic syndrome (MS) diagnosis.

Methods: 99 patients (53 women and 46 men), aged 3–17 years, with overweight and obesity, were determined for fasting and postabsortive glycemia and insulin, lipid profile, and components for MS. IRI was calculated and MS was defined if 3 or more criteria: abdominal obesity (>p75), hypertriglyceridemia (>p5), HDL (p90), fasting glucose >100 mgdl. U Mann-Whitney was made for statistical differences, and ROC curve for sensitivity and specificity.

Results: 38 patients had MS criteria and 61 did not. There were significant statistical differences between the mean for each IRI when compared with the clinical diagnosis for MS. 30% of the patients in which IR was discarded have clinical diagnosis for MS. HOMA had the major surface under the curve with a mean value of 5.84 and sensitivity of 26%.

Conclusions: The sensitivity for each mean value for de IRI is low, even when they are above the mean points described in the literature. The MS clinical criteria allow identifying most number of high risk patients. That's why we question if it is worth to measure IRI in patients with MS criteria.

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Final Height (FH) in Recombinant Growth Hormone (rhGH) Treated Patients with Chronic Kidney Disease after Renal Transplantation (RTx)

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Introduction: rhGH treatment has been effective in improving growth in RTx patients, but little is known about FH in this group.

Aims: (1) to evaluate FH in RTx patients treated with rhGH; (2) to evaluate the effect of rhGH treatment on creatinine clearance (crcl); (3) to establish predictive variables for FH. FH was evaluated in 23 RTx patients (M: 17; F: 6) treated with rhGH (GHG) (dose: 30 IU/m^2/w) for a period not less than 36 m and in a control group (CG) of 14 RTx patients (M: 8; F: 6) with similar chronological age, bone age, crcl, corticoid accumulative doses (CD), height SDS (H), target HSDS (TH) and TH-initialH (iH) at the beginning of the study.

Results: FH was significantly greater in GHG vs. CG (-1.9 ± 1.1 vs. -3.5 ± 1.2, $p = 0.0002$). TH-FH was significantly lower in GHG (-1.8 ± 1.1 vs. -3.5 ± 1.2, $p = 0.02$). iH vs. FH showed a significantly height gain (-3.2 ± 1.1 vs. -1.9 ± 1.1, $p = 0.000$) in GHG and a significantly lost of height in CG (-2.96 ± 0.66 vs. -3.5 ± 1.2, $p = 0.03$). TH-FH vs. TH-iH in GHG was significantly lower (-1.8 ± 1.1 vs. -3.1 ± 1.2, $p = 0.000$) and in CG significantly greater (-2.9 ± 1.5 vs. $-2.4 \pm 1.$ $p = 0.03$). Initial crcl was significantly greater than final crcl in both groups (GHG 76 ± 9 vs. 66 ± 14, $p = 0.008$ y CG 72.5 ± 19 vs. 56 ± 9 $p = 0.02$). In a best subject regression test the best model to predict FH included iH and initial CD, being iH the only significant predicted variable ($p < 0.01$).

Conclusion: rhGH was effective in improving FH in RTx patients without affecting renal function. This is the first case control study of RTx patients that reached FH.

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Absence of Mutations in DHH Gene in Brazilian Patients with 46, XY Disorders of Gonadal Development

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Introduction: It is now clear that several other genes, in addition to *SRY*, are necessary for normal testis development. *Desert Hedgehog* (*DHH*) is a gene member of the hedgehog family of signaling proteins that has been implicated in the etiology of 46, XY disorders of gonadal development (DGD 46, XY), associated or not with minifascicular neuropathy.

Objective: Analyze the *DHH* gene in Brazilian patients with 46, XY DGD.

Patients: We evaluated 45 patients with 46, XY DGD: 37 sporadic cases and 4 families with 2 affected individuals in each family.

Methods: Genomic DNA was extracted from the patients' peripheral blood leukocytes; the entire coding region and the splicing sites flanking regions of *DHH* gene were analyzed by PCR amplification, followed by automatic sequencing.

Results: One novel silent polymorphism, H181H (in heterozygous state), was identified in two patients with gonadal dysgenesis, as well as in 2 of 103 normal subjects (T allele frequency = 0.97%). Another polymorphism, also in heterozygous state located at intron 2, IVS 2 + 29 G > A, was identified in one patient with complete gonadal dysgenesis whereas it was not found in 103 normal subjects; however, it does not seem to interfere with the splicing site located at the 3′ end of exon 2.

Conclusion: We describe 2 new rare polymorphisms in a gene related to testis development. The absence of mutations in this gene suggests that it seems to be rarely involved in the etiology of 46, XY disorders of gonadal development in humans.

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TCF21 Gene Analysis in Brazilian Patients with 46, XY and 46, XX Disorders of Gonadal Development

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Introduction: Several genes seem to be involved in the gonadal development; some related do the early development of the bipotent gonad and others only to the ovarian or testicular development. *TCF21*, known as *Pod1* in mice, has a role in the early steps of gonadal development. *Pod1* transcriptionally represses steroidogenic factor 1 (*Sf1*), an orphan nuclear receptor, which regulates the expression of multiple genes that mediate sexual differentiation. To date, there have been no studies of *TCF21* in humans with disorders of gonadal development (DGD).

Methods: We evaluated 46 patients with 46, XY DDG and 16 patients with 46, XX DDG: 42 sporadic cases and 10 families with 2 affected individuals in each family. Genomic DNA was extracted from the patients' peripheral blood leukocytes; the entire coding region and the splicing sites flanking regions of the *TCF21* gene were analyzed by PCR amplification, followed by automatic sequencing.

Results: One novel polymorphism, G22V (in heterozygous state), was identified in five patients: 2, 46, XY DGD sporadic cases with embryonic regression testicular syndrome, one 46, XX familial case (2 affected members) and another 46, XX DGD sporadic case, all three with gonadal dysgenesis. This polymorphism was also found in 3 of 103 normal subjects (T allele frequency = 1.45%).

Conclusion: We describe a new polymorphism in a gene related to gonadal development. The absence of mutations in this gene suggests that it seems to be rarely involved in the etiology of disorders of gonadal development in humans.

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Evaluation of Gonadal Function in Cryptorchid Patients before Treatment

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Treatment of cryptorchidism is often performed without knowing the functional status of the gonads, which impairs the evaluation of treatment efficacy. Our aim was to assess the prevalence of gonadal dysfunction before treatment in cryptorchid boys and the usefulness of gonadotropin determination. In 212 prepubertal cryptorchid patients, gonadal function was classified according to AMH levels measured by ELISA, and FSH and LH were measured by IFMA (table).

Hypo/agonadism was detected in 81% of bilaterally cryptorchid boys with non palpable gonads, 76% of bilaterally cryptorchid patients with palpable gonads and 15% of unilaterally cryptorchid boys. In bilaterally cryptorchid boys, LH and/or FSH elevation predicted hypo/agonadism in 100% of the cases, but did not allow distinguishing between hypogonadism and agonadism. Lack of FSH and/or LH elevation ruled out agonadism in 98% of all cases, but did not allow distinguishing between hypogonadism and functionally normal testes. We conclude that the high prevalence of testicular dysfunction justifies hormonal assessment before treatment in cryptorchidism.

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Ovarian Hyperstimulation Secondary to a FSH Secreting Adenoma in a 13 Years Old Girl

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Gonadotropin oversecretion by pituitary adenomas is extremely rare in childhood and adolescence and infrequently results in a recognizable clinical syndrome. A 13 years old postmenarcheal girl was referred for 6 months amenorrhea with and abdominal mass. She had Tanner IV breast development without galactorrhea and abdominal distension with a mass occupying the whole low abdomen. Ultrasonography showed enlarged ovaries (right 150 × 86 mm; left 148 × 90 mm) with multiple giant cysts. Serum βhCG and αFP were negative. At baseline LH, FSH (IU/l), PRL (ng/ml) and E2 (pg/ml) levels were <0.05, 8.4, 65 and 8,643 respectively. LH was unresponsive to GnRH test while FSH and subunit peaked to 30.5 UI/l and 26,374 ng/l, respectively. TRH test showed normal TSH response, no response of LH, a FSH response of 20 IU and a serum subunit α of 28,203 ng/l. MRI revealed a 20 mm pituitary tumor. The patient returned two months later with visual impairment and galactorrhea. Trans-sphenoidal resection of a macroadenoma was performed. Immunostaining was positive for FSH. Visual function, hormonal levels, ovarian volume and the pituitary image normalized within 3 months after surgery. Menstrual cycles resumed within 3 months.

Conclusion: A pituitary FSH secreting adenoma should be considered in the differential diagnosis of an ovarian multicystic tumor in a young patient when hyper-estrogenism is found in the context of blunted LH responses to GnRH with FSH hyper-response. An equivocal diagnosis in these cases could lead to iatrogenic ovarian resection.

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Hormonal and Metabolic Profile in an Aromatase-Deficient Girl. Response to Estrogen Replacement Therapy

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We are describing the hormonal and metabolic profile of an affected girl who is a compound heterozygote for two point mutations of the CYP19 gene (c655G>A point mutation at the consensus 5′ slice site of exon/intron 5 and DA GLU 412X) before and on estrogen (E) therapy. Metabolic and hormonal parameters were determined periodically. Before E therapy, at 9 years (y), insulin-resistance (IR) (HOMA 5.6 ± 2.97) and glucose intolerance (2-hour glucose value 177 mg/dl) was detected, along with high serum testosterone (T: 0.66 ± 0.46 ng/ml), androstenedione (A: 1.41±1.13 ng/ml) and FSH (13.4 ± 3.5 mIU/ml) levels. Conjugate E therapy was started at 9.6 y,

Table (of Abstract 34)

Cryptorchidism	Functional status	%	AMH pmol/l	LH UI/l	FSH UI/l
Bilat., non palpable	Normal	19	621 ± 188	0.16 ± 0.11	0.78 ± 0.68
n = 36	Hypogonadism	64	109 ± 111	0.32 ± 0.79	3.9 ± 9.2
	Agonadism	17	<3	9.8 ± 15.2	51.3 ± 31
Bilat., palpable	Normal	24	598 ± 326	0.08 ± 0.05	0.89 ± 0.46
n = 42	Hypogonadism	71	188 ± 115	0.12 ± 0.17	1.36 ± 1.74
	Agonadism	5	<3	0.28 ± 0.28	6.6 ± 7.2
Unilateral	Normal	85	705 ± 281	0.14 ± 0.14	0.87 ± 0.62
n = 134	Hypogonadism	15	262 ± 128	0.11 ± 0.08	0.80 ± 0.46

but she continued with severe IR and glucose intolerance. E therapy was also unable to suppress gonadotropin, T and A levels which remained elevated for chronological age and associated with the presence of ovarian follicular cysts. Metformin treatment was started at 10.6 y but she developed type 2 diabetes mellitus. Finally, at 11.4 y, Pioglitazone and GnRH analogue therapy was added improving the hormonal and metabolic profile. Contrarily to affected adult men, in this girl, E was unable to reverse the hormonal profile and carbohydrate intolerance developing type 2 diabetes mellitus in the follow-up. This finding suggests that E deficiency is not the only factor involved in the abnormalities in this patient. In addition, we propose that, in females, the increment of androgens and/or lack of E during fetal life might be involved in the mechanism of fetal programming on insulin sensitivity and on hypothalamic-pituitary-gonadal axis.

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Higher Expression of IGF-I and IGF-I Receptor (IGF-IR) in Placentas from Small for Gestational Age Newborns: Relation with Birth Length

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Introduction: IGF-I plays an important role in the post natal growth of humans. In humans a direct relationship between IGF-I cord blood levels and birth weight has been demonstrated.

Objective: To determine the placental IGF-I and IGF-IR expression in full term pregnancies from appropriate for gestational age (AGA) and small for gestational age (SGA) newborns.

Methods: We selected 30 cases of idiopathic SGA full term pregnancies (birth weight <10th percentile) and 35 AGA full term pregnancies. The IGF-I and IGF-IR placental expression was determined by RT-PCR in the maternal (MS) and fetal (FS) side of the placenta, and normalized using 18S rRNA expression.

Results: (mean arbitrary units (AU) ± SEM).

	IGF-I/18S rRNA		IGF-IR / 18S rRNA	
Placental side	SGA	AGA	SGA	AGA
Maternal	1.22 ± 0.09*	0.85 ± 0.09	0.89 ± 0.07*	0.65 ± 0.05
Fetal	1.21 ± 0.11*	0.81 ± 0.09	0.91 ± 0.08*	0.63 ± 0.07

* $p < 0.05$, Mann Witnney.

The IGF-I and IGF-IR expression in the FS and MS of the placenta was significantly higher in SGA compared to AGA. An inverse correlation between IGF-I and IGF-IR expression with birth length was observed in FS ($r = -0.362$, $p = 0.004$; $r = -0.332$, $p = 0.009$).

Conclusion: These results suggest that the higher of IGF-I and IGF-IR expression observed in the SGA compared with AGA placentas, may be influencing fetal growth.

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Expression of 11β-OH-Steroid Dehydrogenase (11β-HSDS) Type 1 and 2 Enzymes in Human Term Placenta from Small (SGA), Appropriate (AGA) and Large (LGA) for Gestational Age New Borns

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Introduction: In some species fetuses with growth restriction have higher glucocorticoid systemic concentrations. The bioavailability of cortisol at the placental level is regulated by the 11β-HSDs which converts cortisone to cortisol (11β-HSD1), and cortisol to cortisone (11β-HSD2).

Objective: To study the expression of 11β-HSDs in full term placentas from SGA, AGA and LGA newborns and the cortisol cord blood concentration.

Methods: We selected 15 AGA, 15 SGA (birth weight <10th percentile) and 17 LGA (birth weight >90th percentile).11β-HSDs placental expression was determined by RT-PCR in the maternal (MS) and fetal (FS) side of the placenta and normalized using 18S rRNA expression. Cortisol was determined by RIA.

Results: (mean arbitrary units (AU) ± SEM).

	Placental side	SGA	AGA	LGA
11β-HSD1/18S rRNA				
	Maternal	0.91 ± 0.07	0.92 ± 0.07	0.87 ± 0.08
	Fetal	0.80 ± 0.06*	0.93 ± 0.09	0.95 ± 0.07
11β-HSD2/18 S rRNA				
	Maternal	0.88 ± 0.06	0.92 ± 0.07	0.95 ± 0.06
	Fetal	0.88 ± 0.08	0.92 ± 0.07	0.95 ± 0.07
Cortisol (μg/dl)	16.0 ± 1.9	11.0 ± 0.7	10.3 ± 1.0	

*$p < 0.05$ M vs. F (Wilcoxon). **$p < 0.05$ SGA vs. AGA and LGA (Mann Whitney).

Conclusion: The lower expression of 11β-HSD1 observed in the fetal side of the SGA placentas suggests a possible compensatory mechanism to diminish the higher cortisol fetal concentrations observed in fetuses with intrauterine growth restriction.

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Leptin Nocturnal Concentrations Increase after the Administration of the IGF-I/IGFBP-3 Complex in Small for Gestational Age Prepubertal Children

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Introduction: It is not known whether IGF-I regulates circulating Leptin concentrations.

Objective: We determined the effects of IGF-I over Leptin and Insulin concentrations in small for gestational age children (SGA).

Methods: We selected 20 prepubertal SGA with a mean age of 7.3 ± 0.5 years. We studied the Insulin and Leptin nocturnal profiles before and after the sc administration of 1 mg/kg of the IGF-I/-IGFBP-3 complex (Iplex). Blood samples for measurement of Insulin and Leptin (basal and post Iplex) were obtained every 60 min between 11PM and 7AM. We determined serum IGF-I and IGFBP-3, and calculated the mean Insulin and Leptin area under the curve (Ins AUC and Lep AUC respectively).

Results: (mean ± SEM).

	IGF-I (ng/ml)	IGFBP-3 (mg/l)	Ins AUC (μUI/ml × h)	Lep AUC (ng/ml × h)
Baseline	214 ± 18	2.7 ± 0.2	−26.2 ± 14.1	2.7 ± 2.1
Post Iplex	462 ± 27*	3.4 ± 0.2*	−17.5 ± 19.4	11.2 ± 2.6*

*p = 0.011; Wilcoxon test.

As expected, serum IGF-I and IGFBP-3 increased significantly after Iplex administration. In addition, mean nocturnal Lep AUC showed a significant increase after administration of the complex, but mean Insulin AUC did not change.

Conclusion: These findings indicate that the IGF-I/IGFBP-3 complex appears to have a stimulatory effect over circulating Leptin concentrations in SGA children, suggesting that IGF-I may have a positive feed back effect over Leptin secretion by adipose tissue.

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"In Vitro" Ternary Complex Formation (TCF) in Short Stature Patients (SS)

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Introduction: IGFs circulate predominantly (80–90%) bound to a 150 kDa ternary complex also formed by IGFBP-3/IGFBP-5 and ALS. These 3 components are all GH dependent.

Objective: To compare normal TCF with that of SS, with (GHD) or without growth hormone deficiency (idiopatic [ISS]).

Patients and Methods: 113 normal controls (see SLEP 2006), 22 GHD and 65 ISS. Last ones divided according to their IGF-I level: normal IGF-I (ISSN, n = 49) or low IGF-I (ISSL, n = 16). IGF-I was determined by RIA; IGFBP-3 by IRMA; TCF by cross-linking followed by size exclusion chromatography. Statistics: one way ANOVA followed by Tukey's Test, χ^2 Test.

Results: Expressed in SDS, mean ± SEM; ISSN vs. ISSL vs. GHD, ANOVA $p < 0.001$ for IGF-I (−0.89 ± 0.1 vs. −2.24 ± 0.04 vs. −2.19 ± 0.14), IGFBP-3 (0.21 ± 0.18 vs. −2.26 ± 0.18 vs. −2.96 ± 0.34), TCF (−0.51 ± 0.19 vs. −1.40 ± 0.21 vs. −1.73 ± 0.18). In every instance GHD vs. ISSL p = NS; ISSN vs. ISSL $p < 0.05$; ISSN vs. GHD $p < 0.001$. The frequency of low IGFBP-3 values (2/49 vs. 11/16 vs. 17/22) and low TCF (4/49 vs. 2/16 vs. 10/22) was significantly different among the 3 groups, χ^2 $p < 0.001$.

Summary: TCF was significantly reduced in GHD and ISSL.

Conclusions: We found patients with reduced TCF in the 3 groups studied, more frequently in those with the lowest IGF-I and IGFBP-3 levels (GHD > ISSL > ISSN). Our findings suggest the presence of altered IGFBPs in some patients with ISS.

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Variability in X-Chromosome Inactivation Pattern Between DNA Extracted from Peripheral Leukocytes and Androgen-Responsive Hair Bulbs from Normal Women

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Introduction: Androgen receptor genes (AR) are located at X-chromosomes and their exon 1 have polymorphic CAG repeats, varying from 11–30 in the general population. AR transactivation activity is inversely correlated to the CAG repeat number (CAGn) and is associated with idiopathic hirsutism and precocious pubarche. One of the X-chromosomes is inactivated in women to compensate gene dosage in relation to male sex, and the inactivation pattern may vary among different tissues. However, most studies evaluating the X-inactivation pattern in androgen-dependent diseases use peripheral leukocyte DNA.

Objective: To compare the X-inactivation pattern among DNA of three different normal women's tissues.

Material: DNA extracted from peripheral leukocytes, pubic and scalp hair from 28 women heterozygous for CAGn.

Methods: DNA samples were *Hpa II* digested, the CAG region PCR amplified, the products submitted to capillary electrophoresis and analyzed by GeneScan. A skewed X-inactivation pattern was defined when one of the alleles was ≥75% preferentially amplified and random when it was ≤74%. Differences in inactivation patterns among DNA samples of each woman were evaluated by Fisher test.

Results: The skewed inactivation pattern was found in 11%, 25% and 17% of leukocyte, pubic and scalp hair DNA samples, respectively. Leukocyte DNA inactivation patterns differed between pubic and scalp hair in 54% and 50% of women ($p < 0.05$), respectively. The inactivation pattern differed between scalp and pubic hair DNA in 75% of women and among the three DNA tissues in 7% of them.

Conclusion: The inactivation pattern could vary among the tissues, even with those of similar embryologic origin, indicating that leukocyte DNA inactivation patterns cannot be extrapolated to those that occur in other tissues. We demonstrated a need for collecting tissue-specific DNA in X-inactivation analysis.

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Thyroid Axis Impairment in Short Children Born Small for Gestational Age (SGA)

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Fifteen percent of children born SGA fail to catch up and remain short. In them evaluation of the hypothalamic pituitary thyroid axis is seldom assessed. We retrieved and analyzed data of this axis during the initial evaluation of 58 SGA short patients. Five (9%) had primary hypothyroidism with circulating levels of antithyroid antibodies. In the remaining 53 patients (age 2–12 years, mean height SDS -2.7 ± 0.57) data of TSH, freeT4, antithyroid antibodies and a 90′ TRH stimulation test were analyzed. TRH test was considered abnormal if TSH response was absent, increased, retarded or with poor decline compared with data of 30 normal short children. (CG). Patients were grouped according to their response to TRH in G1: normal response ($n = 27$) and G2: abnormal response (n: 26). When compared, no statistical significant differences were found in chronological age, gestational age and birth weight SDS between groups. G2 showed a higher SDSBMI at consultation ($p < 0.05$). FT4 levels were not different between groups. Basal TSH levels were elevated in G2 when compared to G1 and CG (7 ± 2.3; 3.5 ± 1.6; 3.1 ± 1.2 mU/l) ($p < 0.01$). In those patients replaced with LT4, FT4 did not change, TSH levels normalized and, after a year of treatment, no changes were seen in height or BMI SDS. These data suggest the presence of previously unrecognized thyroid abnormalities in short SGA children. As intrauterine growth retardation may permanently influence the programming of the endocrine system during development further follow-up will be required to confirm these findings and their long term impact.

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Congenital Adrenal Hyperplasia (CAH) due to a Small Indel in the CYP21A2 Gene

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Introduction: CAH due to 21-hydroxylase deficiency is a frequent autosomic recessive disorder with a wide phenotypic variability, from salt wasting form in the neonatal period to hyperandrogenic symptoms in the adult life. Different mutations in the *CYP21A2* gene have been described in these various clinical forms. The most common source of mutations in CAH is microconversion with highly homologous pseudogene, In the Brazilian population 5 new mutations have been described, and in four of them a gene founder effect was observed.

Objective: To define the genotype of a female patient with salt wasting form in whom a previous study found only the I2 splice mutation in the paternal allele.

Patient: She presented prenatal external genitalia virilization, Prader III and at 12 days old, developed salt wasting crisis. Basal hormone levels were 17OHP: 252 ng/ml, testos: 464 ng/dl, Na 128 mEq/l and K 6.6 mEq/l.

Methods: Genomic DNA from the patient and their parents was extracted, the *CYP21A2* gene was PCR amplified with specific primers, sequenced through dideoxynucleotide terminator methodology and analyzed in the ABI Prism 3100 Genetic Analyzer.

Results: A new indel mutation was found in exon 8,2145_2151delCCCAGCAinsTGGGT, in compound heterozygosis with I2 splice mutation. This mutation was inherited from the maternal allele and alters the reading frame creating a premature stop codon.

Conclusions: We described a new mutation in a salt wasting form, consists\ing of a small deletion and insertion, and its screening in other cohorts will reveal if it presents a gene founder effect.

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PSA as a Marker of Prostatic Tissue in 46, XX Children and Adolescents with Congenital Adrenal Hyperplasia (CAH)

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Introduction: The presence of prostatic tissue in female pseudohermaphrodites due to Congenital Adrenal Hyperplasia has already been reported. One patient developed prostatic adenocarcinoma. The Skene's paraurethral glands in girls have histological homology with prostate. Early and prolonged dihydrotestosterone stimulation in these patients may lead to the development of prostatic tissue, which could be detected by Prostate-Specific Antigen (PSA).

Objective: To search for prostatic tissue in children and adolescents girls with classical form of CAH through PSA measurement and radiological imaging.

Methodology: 31, 46, XX CAH children and adolescents patients (6 to 20 years old, mean: 11.8 y) were studied. 30 presented 21-Hydroxylase deficiency and one 11-Hydroxylase deficiency. PSA was measured through ultra-sensitive chemoluminescence method. Prostatic tissue was evaluated through pelvic MRI in all patients.

Results: Prostatic tissue was found in five patients (16.1%) with elevated PSA (>0.10 ng/ml; Sensitivity: 100%, Specificity: 96.7%).

Only one patient showed elevated PSA (0.17 ng/ml) and no prostatic tissue was detected.

Conclusion: PSA should be measured as a routine screening test for prostatic tissue in all 46, XX classical form CAH patients. Thus the finding and follow-up of prostatic tissue can avoid possible complications such as prostatic adenocarcinoma.

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Successful Transfer to Sulfonylureas in a Toddler with Permanent Neonatal Diabetes Associated to KIR6.2 Mutation

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A 17 months girl was admitted at 4 months of life due diabetic ketoacidosis. Blood glucose levels were 485 mg/dl and HbA1c of 14%. The patient received treatment with three daily doses at day of NPH insulin and four injections of lispro insulin for 0.95 U/Kg/d. A molecular study of *KCNJ11* gene was performed. This gene codificates for KIR6.2, the inward rectifying potassium channel subunit, part of the β cell K_{ATP} channel. This analysis showed an heterozygous mutation with substitution of arginine for hystidine at codon 201 (R201H). This is an activating mutation with diminishes the K^+ channel closure, therefore decreasing insulin release from β cell. The parents did not carry this mutation. The diagnosis of neonatal diabetes caused a mutation KIR6.2 was made and glibenclamide was initiated. This drug stimulates insulin secretion by inducing closure of K^+ channel. Glibenclamide was started at doses of 0.3 mg/Kg/day (*NEJM 2006.355: 467–477*, and was gradually increased by 50% daily, with simultaneous decrease of insulin dose by 50%. Seven days after this sulfonylurea was introduced, and upon reaching a dose of 0.6 mg/kg/day, insulin was stopped. Continuous glucose sensor monitoring was performed during glibenclamide treatment, which showed normal blood glucose levels in the range of 70–110 mg/dl. Oral glucose tolerance test was made performed before and after the initiation of glibenclamide.

Table: Oral glucose tolerance test

Age	7 month; with insulin before glibenclamide treatment Blood glucose (mg/d) l	7 month 10 d: without insulin receiving glibenlcamide 0.6 mg/kg Blood glucose (mg/dl).
Basal	354	66
15 min	572	183
30 min	599	173
60 min	583	89

At present, the patient has been treated for 10 months with sulfonilureas with an excellent control, with HbA1c levels of 5.4 to 6.2%. She is eating a normal diet. The glibenclamide doses was progressively decreased to 0.1 mg/Kg/d. The last HbA1c was 5.4%. Herein we describe a clinical case of neonatal diabetes due to mutation of *KCNJ11* gene, with an excellent metabolic control with glibenclamide. This case clarifies the utility of pharmacogenomics in helping to choose an optimal treatment.

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Novel Mutation FGF23 and Familiar Tumoral Calcinosis in a Girl: Response to Induction of Tubular Renal Acidosis with Acetazolamide and Non Calcium Phosphate Binder Sevelamer

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A girl of three years old, product of first pregnancy of non consanguineous parents and without familiar or personal history defects of calcium or phosphate metabolism or either renal stones. At 2 years of age developed a left elbow mass with progressive growth. Six months after laboratory showed phosphate 9.5 mg/dl and calcium 10.5 mg/dl (product 99.8), creatinine 0.4 mg/dl, PTHi 20.7 pg/ml and 1.25 dihydroxivitamin D 36 pg/ml (15–90) with renal tubular phosphate reabsortion of 100%. The X ray study showed an heterogeneous and amorphous lesion with calcium density not dependend of bone with 'pop corn' appearance which infiltrate around structures; the density of distal humerus was decreased. Microscopic examination concluded tumoral calcinosis due to findings of fibroseous bands around amorphous calcificated material with macrophage and giant cells. Collagen disease, malignant tumors with 1.25 dihydroxivitamin D production and tuberculosis was ruled out. A CT scan of central nervous system showed calciphylaxis. Diagnosis of familiar tumoral calcinosis was made with the clinical picture of metastasic calcification with hyperphosphemia and normal renal function, normal PTH in the context of high tubular phosphate reabsortion and inappropriate normal 1.25 dihydroxivitamin D. Hospital admission was indicated due to serious growth of the mass with skin rupture. During admission induction of tubular proximal acidosis with acetazolamide (39 mg/Kg/d) and supervision of alimentary regimen to 400 mg day of phosphate and pharmacological absorption restriction was made with sevelamer (20 mg/Kg/d). The patient showed a decrease of the circumference of her left arm mass and of tubular phosphate resortion up to 92% and phosphate serum levels decreased to 6.6 mg/dl and calcium phosphate product to 62.7. The parents evaluation showed normal calcium and phosphate. Her sister has a calcium of 10.6 mg/dl and phosphate of 8.1 mg/dl (product 86). The sister of index case has hyperphosphataemia; DNA of family members showed Novel homozygous FGF23 missense mutation (c.367G>T, p. GlY123Trp) in both sisters, the parents are carriers of the mutation.

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Circulating Levels of High Sensitivity C-Reactive Protein (CRP) and Markers of Vascular Endothelial Cell Activation in Growth Hormone Deficient (GHD) Adolescents

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Objective: To assess the relationship between levels of CRP and markers of vascular endothelial cell activation and their association with brachial artery flow mediated vasodilation in GHD adolescents.

Design/Methods: Twenty eight GHD children on GH treatment (CA of 15.7 ± 2.6 yr) and sixteen untreated GHD adolescents (CA 16.6 ± 3.3 yr) were studied. Levels of CRP were measured and the association between CRP and the fasting soluble markers of vascular endothelial cell activation: ICAM-1, VCAM-1, E-selectin and P-selectin levels was evaluated. Sixteen healthy adolescents (CA 15.1 ± 2.2 yr) served as controls.

Results: CRP and P-selectin levels were higher in untreated GHD adolescents ($p < 0.02$), while VCAM-1 was increased in both untreated and treated GHD adolescents when compared to controls ($p < 0.007$). E-selectin and ICAM-1 levels were similar in all groups. CRP was associated with BMI, P-selectin, E-selectin, ICAM-1 and VCAM-1 concentrations in untreated GHD adolescents. A weak inverse association was observed in a subgroup of patients between brachial artery EDD and P-selectin (r: −0.56; $p < 0.07$).

Conclusions: Low-grade inflammation as manifested by increased circulating levels of CRP seems to be associated to the early activation of vascular endothelial cells in GHD adolescents. This data suggests that GH may play an important role in the modulation of both the systemic inflammatory and the local vascular endothelial response.

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Prevalence of Obesity According to Different Socioeconomic Levels in Three Schools of Bogotá, Colombia

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Background: We are facing a global epidemic of obesity. In Colombia there are very few studies about the prevalence of obesity, and they have been done in small groups.

Objective: To describe the prevalence of obesity and at risk of obesity in a group of school aged children at the three different socioeconomic levels of the city of Bogotá.

Design: Descriptive study that evaluates 1,251 females and 1311 males. According to CDC 2000, obesity was classified as BMI greater than the 95th percentile and at risk of obesity BMI between the 85th and 95th percentile.

Boys	Perc 85–94	%	Perc >95	%
High SEL	49/383	12.8	38/383	9.9
Mid SEL	38/331	11.5	11/331	3.3
Low SEL	21/516	4.1	13/516	2.5
Total	108/1230	8.8	41/1311	3.1
Girls	**Perc 85–94**	**%**	**Perc >95**	**%**
High SEL	66/450	14.7	18/450	4.0
Mid SEL	31/309	10.0	10/309	3.2
Low SEL	66/552	11.9	13/552	2.3
Total	163/1311	12.2	41/1311	3.2

Conclusions: (1) The prevalence of at risk of obesity and obesity is higher as the SEL increases, with a statistically significant difference in boys ($p < 0.001$) but not in girls ($p < 0.5$). (2) The global prevalence of obesity was of 3.1% for the boys and 3.2% for girls, levels that are lower than those published for other countries. (3) Our results show that in Colombia we do not have yet an epidemic of obesity. (4) It is not possible to evaluate the tendency since we do not have previous data in our country.

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Mutations in the Vitamin D Receptor in Four Brazilian Children with Hereditary 1,25-Dihydroxyvitamin D-Resistant Rickets

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Mutations in the vitamin D receptor (VDR) are associated to the hereditary 1,25-dihydroxivitamin D-resistant rickets. The objective of this work was to search for mutations in the VDR and analyze their functional consequences in four Brazilian children that presented with rickets and alopecia. The coding region of the VDR was amplified by PCR e direct sequenced. We identified three mutations (one novel). All the mutations were confirmed by restriction enzyme digestion. Two patients had a mutation in exon 7 changing the amino acid Glutamine to Glutamic acid at position 259 (Q259E). One patient had a novel mutation in exon 8 changing Glycine to Valine at position 319 (G319V). One had a mutation in exon 3 changing Arginine to a stop codon at position 73 (R73X). Fibroblast primary culture from the patients' skin biopsy was performed for the functional analysis of the receptor. Nuclear protein extracts showed reduction of expression of all mutant receptors. Treatment of the patient's fibroblast primary culture with increasing doses of 1,25 $(OH)_2$ vitamin D showed that the activity of the mutants VDRs were impaired with reductions of the 24-hydroxylaseexpression. In conclusion we report three mutations in the VDR. Two mutations in the ligand binding domain and one mutation resulted in a truncated receptor in the DNA binding domain. All mutations result in reduction of receptor expression and function.

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Multivariate Analyses of the Influence of HOMA-IR, Tanner Stage, BMI and Waist Circumference on Leptin Levels in Obese Children and Adolescents

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Introduction: Leptin is well known as an important factor in glycemic homeostasis. Recent data show an inter-relation between leptin and insulin resistance.

Methods: 106 subjects (4–19 years old), median age of 11.2 ± 3.0 years of age (43.5% boys) were studied. They were considered obese (n = 69) when BMI percentile for age and sex was above 95 and overweight (n = 37) when it was between percentiles 85 and 95, according to 2000 NCHS curves. IR was evaluated through HOMA-IR formula. Logarithms were used for statistical analyses for leptin and HOMA-IR. After submitting the variables to univariate analysis, multivariate model was used for those with p value <0.20. A value of $p < 0.05$ was considered to be significant.

Results: In the univariate analysis, HOMA-IR ($p = 0.003$) and waist circumference ($p < 0.001$) were positively correlated to leptin levels. Tanner stage progression ($p = 0.101$) and BMI z-score ($p = 0.361$) did not. However, the former was included in the multivariate analysis ($p < 0.20$). In the multivariate model, pubertal development wasn't relevant for leptin level increase ($p = 0.994$). Waist circumference and HOMA-IR model were chosen, since both showed independently positive correlations ($r^2 = 0.15$) with leptin ($p = 0.003$ and $p = 0.076$, respectively).

Conclusion: In this study, waist circumference was the strongest predictor for leptin levels. IR, calculated by HOMA-IR formula, also influences leptin. Due to its clinical importance, IR was maintained in this model, despite its borderline p value. Larger studies, including further explicatory variables for leptin are desirable.

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Evaluation of Sensitivity and Specificity of Basal and Adrenocorticotropic (ACTH) Hormone-Stimulated Serum 17-Hydroxyprogesterone (17 OHP) in Relation to Molecular Genetic Analysis of the CYP21A2 Gene, in Non-Classical 21-Hydroxylase Deficiency (21OHD)

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Congenital adrenal hyperplasia due to 21OHD is a common autosomal-recessive disorder.

Aim: To determine the sensitivity and specificity of ACTH stimulation test to detect non-clasical 21OHD in relation to molecular genotype. We performed molecular genetic analysis of 205 patients with probable diagnosis of non-classical 21OHD due to hyperandrogenic symptoms, and compared the presence of mutated alleles with basal and 1-h-post-ACTH serum 17OHP. Molecular analysis was performed to screen the 11 most frequent mutations. Receiver operating characteristics (ROC) curve analysis was performed to determine the potential of serum 17OHP value to predict non-classical 21OHD. Patients were classified in three groups, as a function of molecular genotype: Gr0 n = 61 (30%) no mutated allele; Gr1 n = 57 (28%) one mutated allele and Gr2 n = 87 (42%) two mutated alleles. Basal serum 17OHP in Gr2 was significantly higher than in Gr0 and Gr1 ($p < 0.05$) but there was no difference between Gr1 and Gr0. Stimulated serum 17OHP was significantly higher in Gr2 vs. Gr0 and Gr1 and in Gr1 vs. Gr0 ($p < 0.05$). Basal and ACTH-stimulated 17OHP may predict the molecular genotype in non-classical 21OHD using a cut-off of 5.5 ng/ml for basal 17OHP and of 15.6 ng/ml for post-ACTH 17OHP

levels (maximum predictive value 86%, 89% for sensitivity and 87%, 95% for specificity, respectively). We conclude that a 15.6 ng/ml cut-off is a good predictive value for non-clasical 21OHD, but since a percentage of non detection might occur, molecular analysis is mandatory in those cases with a strong clinical suspicion.

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Testicular Adrenal Rest Tumors and Leydig and Sertoli Cell Function in Boys with Classical Congenital Adrenal Hyperplasia

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Context: Infertility observed in adult males with congenital adrenal hyperplasia (CAH) has been associated with testicular adrenal rest tumors (TART) that may originate during childhood.

Objective: To describe the prevalence of TART and Sertoli and Leydig cell function in a group of boys aged 2–10 years with CAH, and to compare prevalence with that of a control group.

Design: From August 2005 to January 2007, nineteen patients with classical CAH (CAH group) were referred from seven endocrinology centers.

Methods: We studied 19 subjects in the CAH group and, as a control group, 13 boys from the community that did not have testicular diseases. A complete physical exam was performed. High resolution ultrasound was used to determinate TART prevalence. Inhibin B (Inh-B) and anti-Müllerian hormone (AMH) were used as Sertoli cell markers. The ratio between basal and 72 h post-βhCG (5000 U/m^2) treatment levels of testosterone $[(T_{72}-T_0)/T_0]$ was used to evaluate the Leydig cell response.

Results (median): CAH and control groups were comparable in chronological age (5.9 vs. 5.6; $p = 0.67$) and bone age/chronological age ratio (1.09 vs. 1.03; $p = 0.09$). TART prevalence was 4/19 (21%) in the CAH group. Lower values for Inh-B (49.2. vs. 65.2 pg/ml; $p = 0.018$), AMH (70.1 vs. 94.2 ng/ml; $p = 0.002$) and $(T_{72}-T_0)/T_0$ (5.6 vs. 13.6; $p < 0.01$) were observed in the CAH group.

Conclusion: TART in prepubertal males with classic CAH could be found during childhood. We also report differences in markers of gonadal function in a subgroup of patients, especially in those with inadequate control.

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Complications, A1C and Anthropometric Variables During 4 Years of Follow Up in Children with T1DM and Insulin Pumps

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Introduction: Pumping insulin (PI) in children with T1DM has increased metabolic control and Quality of life. It also has been described Obesity and inadequate glicemic control. We describe a follow up report of children pumping insulin in Cali, Colombia.

Methods: Cohort observational study. We determined A1C, Body Mass Index (BMI), BMI percentile (BMIp), z-score Weight/Age (zW/A); z-score Height/age (zH/A); Ketoacidosis, Hypoglycemia and Site infections in 4 different times: (t1) before PI, (t2) beginning PI, (t3) less than 3 years PI and (t4) more than 3 years PI. For the descriptive analysis we calculated Medians (Me) and the Friedman test was used with a statisitc significance value of $p < 0.05$ to analized the correlation within continued variables.

Results: Total of 7 patients, 4 female. In t1 Me for age was 55.6 months, for A1C 9.8, for BMI 15.8, for BMIp 42.3, for zW/A 0.27, for zH/A 0.52. On t2 Me for age 136.8 months, A1C 8.0, BMI 17.12, BMIp 47.26, zW/A −0.41, zH/A −0.8. On t3 Me for age 160.7 months, A1C 8.4, BMI 19.2, BMIp 68.9, zW/A 0.04, zH/A −0.36. On t4 Me for age 160.8, A1C 8.5, BMI 19.9, BMIp 82.9, zW/A 0.56, zH/A −0.11. Difference was only find in BMIp ($p = 0.018$) with the highest Median in t3 and t4. No statistics significance was found with ketoacidosis, Hypoglycemia and Site infection.

Conclusion: A1C decreased during pumping insulin to acceptable levels for children. There was an increase in BMI relatived to years of pumping insulin and it was to percentiles below overweight.

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Serum Adiponectin and Leptin Levels in Children Born Small for Gestational Age (SGA) in Relation to Insulin Sensitivity Parameters and Growth Factors

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Introduction: SGA children have increased risk of developing obesity, insulin resistance, type 2 diabetes and cardiovascular disease later in the life. The adiponectin and leptin are fat cell-derived hormones associated with insulin sensitivity, however in SGA their correlations with these parameters are contrasting.

Aim: To investigate the relation between adiponectin and leptin with insulin sensitivity in SGA children with and without catch up growth compared with normal controls. We analyzed 48 healthy prepubertal children (AGA), CA 7.2 yr SDS height −0.16 and 23 SGA with catch up growth (SGAcg), CA 7.0 yr SDS height −0.88 and 26 SGA without catch up growth CA 6.1 yr SDS height −2.27. Serum levels of adiponectin, proinsulin, leptin, IGF-I, IGFBP-1, GHBP, insulin, HOMA-IR and HOMA-B were measured. Analysis of variance and multilevel regression.

Results: Adiponectin in SGA children showed similar values in both sexes, there were higher levels in females AGA $p = 0.03$. No difference in leptin levels in boys were found between the groups. The girls SGAcg showed significantly higher values of leptin $p = 0.02$. Similar results were found with HOMA B $p = 0.02$. No significant difference in Proinsulin, insulin and HOMA-IR levels among the groups. Leptin concentrations were positively correlated with insulin and HOMA IR in AGA group $p < 0.05$.

Conclusions: Our study shown that prepubertal SGA children independently of the catch-up growth present adiponectin levels compared with controls. In contrast to the results observed with leptin, adiponectin was no related to insulin sensitivity parameters.

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C-Reactive Protein and Metabolic Syndrome in Youth: A Strong Relationship?

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Objective: To evaluate the association between MS and its components and hs-CRP in a sample of Brazilian children and adolescents.

Study Design: 407 students (229 girls, 273 with excessive weight, age 11.3 ± 3.2 yr) were evaluated. Measurement included: BMI, WC, blood pressure, lipids, insulin and hs-CRP. Excessive weight was defined using BMI z-score; MS by the modified NCEP-ATPIII.

Results: Subjects were classified into two groups: with MS ($n = 72$) and without ($n = 335$). Hs-CRP means and medians were higher in MS group (1.41 vs. 1.06, $p < 0.001$; 2.9 vs. 1.3, $p < 0.001$). Association between hs-CRP_quartiles and IR, MS, WC, BMI (zs), hypertension, hypertriglyceridemia ($p < 0.001$ for all) and low HDL-C ($p = 0.023$) were found. Adjustment of hs-CRP for BMI (zs) eliminated the previously associations, except for the number of MS components (nMSc, $p < 0.001$). Adjusting for HOMA-IR did not eliminate the relation between hs-CRP and the nMSc. Furthermore, increases in BMI (zs) and nMSc were associated with increased hs-CRP. Excessive weight (OR, 7.9; CI, 4.7–13.4; $p = 0.001$), hypertension (OR, 2.3; CI, 1.3–4.2; $p = 0.003$) and hypertriglyceridemia (OR, 2.3; CI, 1.5–3.7; $p < 0.001$) were independently associated with high hs-CRP.

Conclusion: In youth, hs-CRP is strongly related with MS and its components and also determined by the body composition. This association indicates a precocious pro-inflammatory state and suggests an increasing risk of atherosclerosis in this population.

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Alanine Aminotransferase Plus High Sensitivity C-Reactive Protein: Risk Markers of Cardiovascular and Metabolic Disease in Children and Adolescents

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Objective: To evaluate the association between hs-CRP_plus_ALT and clinical/metabolic parameters in Brazilian overweight/obese children/adolescents.

Study Design: 407 students (229 girls, 273 overweight/obese, 11.3 ± 3.2 yr) were evaluated. The measurements included: BMI, WC, blood pressure, lipids, glycemia, insulin, liver enzymes and hs-CRP. Overweight/obese was defined using BMI_z-score; IR by HOMA-IR; MS according to modified NCEP-ATPIII.

Results: Subjects were classified into weight_quartiles and as weight increased, SBP, DBP, TG, HDL-c, insulin, HOMA-IR, hs-CRP, ALT, ALT_plus_hs-CRP, AST and number of MS components (nMSc) also increased ($r \leq 0.001$ for all). Subjects with hs-CRP and ALT above the median had higher BMI (zs) , WC, SBP, DBP, TG, AST, insulin, HOMA-IR and nMSc than those with both markers below the median ($r \leq 0.002$ for all). Adjusted for age, gender and ethnicity, BMI_z-score (OR, 1.5; CI, 1.38–1.86; $r < 0.001$), WC (OR, 1.3; CI, 1.19–1.43; $r < 0.001$), TG (OR, 1.8; CI, 1.29–2.62; $r < 0.001$), insulin (OR, 1.4; CI, 1.23–1.71; $r < 0.001$), HOMA-IR (OR, 1.2; CI, 1.09–1.29; $r < 0.001$) and nMSc (OR, 2.0; CI, 1.16–3.47; $r = 0.012$) were independently associated with high ALT_plus_hs-CRP. For every 5 cm increase in WC and every one point increase in BMI_z-score, there were a 1.3 and 1.5-fold greater chance of having increased ALT_plus_hs-CRP respectively.

Conclusions: Simultaneous measurements of ALT and hs-CRP should be considered as a screening test for metabolic and CVD risk factors in overweight/obese children/adolescents.

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Thyroid Abnormalities in Newborn from Mothers with Graves Disease

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Maternal hyperthyroidism implies the risk of a wide spectrum of thyroid abnormalities in the newborn. We studied retrospectively the clinical presentation, treatment and evolution of 28 children born from hyperthyroid mothers that came to the Endocrinology Division Patients were grouped according to their biochemical profile at

presentation in: Group A (neonatal hyperthyroidism) (Non-detectable TSH, high FT4) (n = 9) born from 8 mothers hyperthyroid at pregnancy and 1 mother thyroidectomized for Graves disease and recieving thyroid hormone (HT). All patients were clinically hyperthyroid. Children born from untreated mothers consulted during the 1st week of life, while those born from treated mothers came to consultation between 8 and 17 days. 8 children needed treatment with antithyroid drugs (ad) and all of them underwent complete remission. Group B (primary hypothyroidism) (High TSH, normal or low FT4, n = 14) born from mothers treated with ad during pregnancy, detected by neonatal screening. All of them were asymptomatic. 11 had transient hypothyroidism and 3 needed treatment with TH. Two of them were athyreotic and 1 was lost in the follow up. Group C (central hypothyroidism) (Normal or low TSH, low T4/FT4, n = 5) Born from mothers with poor compliance to (ad). All of them were detected during the neonatal follow up (9 to 28 days). Treated with TH, thyroid function normalised before 8 months of life. Our experience reinforces the need of endocrinological assessment in every child born from hyperthyroid mother in face of the risk of possible thyroid disorders.

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Insulin Transport to Mitochondria

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Objectives: Mitochondria are insulin-dependent organelles because insulin regulates the amount required of oxidative substrates and control the synthesis and activity of some key enzymes. We previously found the insulin-degrading enzyme (IDE) in the mitochondrial matrix (MM). Our objective was to determine if IDE facilitates the insulin transport to MM.

Methods: IDE was extracted from rat skeletal muscle homogenates after successive chromatographic steps. Active mitochondria from hepatic source were isolated with Parson's method. Mitochondria were recovered and studied at equilibrium at different times with 100% oxygen and insulin. Substrates or inhibitors of IDE were added in agreement with the experimental design. The reaction was stopped at 2 C with 1 mM N-ethylmaleimide. The MM was isolated after digestion with digitonin. Insulin transport and degradation were studied by electrophoresis in SDS-PAGE, gel chromatography, inmunoblot and autoradiography.

Results: Insulin incorporation in MM by IDE is doses/dependent. Dinitrophenol and vanadate decreases this incorporation in MM and succinate + ADP slightly decrease it. The insulin degradation by IDE is unapparent and the degraded insulin (pg/sec/mg of protein; control: 0.87; IDE: 0.88) is produced by mitochondrial proteases. The chromatography and autoradiography of MM showed insulin incorporation through the mitochondrial transport system, acting IDE as transporter. The results strongly suggest that insulin is actively transported to MM.

Conclusions: IDE induce the insulin transport to MM.

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A New Familiar Case of Congenital Goiter with Hypothyroidism Caused by Homozygous Mutation in the Thyroglobulin Gene

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The prevalence of Congenital Hypothyroidism (CH) in Cordoba city is 1/2185 newborns. Eutopic thyroid glands were seen in 41% of these patients. In order to ascertain a dishormonogenesis in 5 unrelated patients with a marked impairment of thyroglobulin (TG) levels, goitrous thyroid glands with increased radioisotopic uptake, serum thyrotropin augmented and low thyroid hormone levels, genomic DNA was analyzed with the aim of search for a TG synthesis defect. DNA sequencing from one of the studied patients revealed an homozygous mutation in exon 7 (c.886C → T) determining a premature stop codon at amino acid 277 (p. R277X) that results in a grossly truncated protein of only 276 amino acids with marked reduction in the ability to generate thyroid hormone. Two other brothers of this patient, previously diagnosed with CH, exhibited the same mutation, one of them with a severe mental retardation as a consequence of a late diagnosis. The same mutation was previously identified in brazilian and argentinean families. To conclude, we report here a new case of congenital goitrous hypothyroidism, in an Argentinean family, that is homozygous for the p. R277X mutation. The present study suggests that this TG mutation gene is a mutational hot spot.

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Evaluation of the Type 1 Insulin-Like Growth Factor-1 Receptor (IGF-1R) Expression by Immunohistochemistry in Malignant vs. Benign Pheochromocytomas/ Paragangliomas

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Introduction: Pheochromocytomas and paragangliomas (feo/pgl) are rare neuroendocrine tumors derived from chromaffin cells. Although mostly benigns, up to 26% of feo/pgl will undergo malignant transformation. The diagnosis of malignancy requires the presence of distant metastasis. Insulin-like growth factors have been shown to promote chromaffin cells survival and proliferation. In adrenocortical carcinomas, overexpression of IGF1-R has been observed. In contrast, expression of the IGF-1R appears to be similar to normal in adenocortical hyperplasia and adenomas.

Aim: To characterize the distribution and expression of the IGF-1R in feo/pgl of different origin and degree of malignancy, in order to establish a potential role for the IGFs in the pathogenesis of the disease.

Methods: Benign (feo 5/pgl 1) and malignant (feo 2/pgl 3) tumors were processed for immunohistochemistry using a specific anti-IGF-1R antibody. Both, intensity and distribution of the labelling were evaluated (IDP: intense diffuse positive; CP: combined intense and weak positive areas; WDP: weak diffuse positive).

Results:

	IDP	CP	WDP
Feo/pgl benign (n = 6)	1 (16.6%)	3 (50%)	2 (33.3%)
Feo/pgl malignant (n = 5)	3 (60%)	2 (40%)	0 (0%)

Conclusions: These results would indicate that both expression and distribution of the IGF-1R are different in malignat vs. benign pheochromocitomas/paragangliomas, suggesting a potential role for the IGFs in the pathogenesis of the disease. Further studies are necessary to confirm these preliminary results.

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Methylenetetrahydrofolate Reductase and Vitamin D Receptor Genotypes in Turner Syndrome Patients

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Introduction: The genetic factors play a major role in determining bone mass, wich is an important predictors of osteoporotic fracture risk. Although the genetic basis of osteoporosis is not understood completely, Vitamin D receptor (VDR), estrogen receptor and methylenetetrahydrofolate reductase (MTHFR) polymorphisms has been implicated as genetic markers for bone mineral density (BMD). In a previous study, we reported a significative association between BsmI-VDR bb genotype and lower BMD in Turner Syndrome (TS) patients.

Aims: To determine VDR and MTHFR genotypes in TS patients and to analyze the relationship between these genotypes and karyotype and different parameters associated with calcium and phosphorus metabolism.

Subjects and Methods: 65 TS patients (CA 15.9 ± 7.8) and 68 healthy control (CA 17.3 ± 8.3) were analyzed. Fok I and Apa I (VDR) and Hinf I (MTHFR) were the restrictase enzyme used. Serum calcium, phosphorus, PTH, osteocalcin y β-crosslaps and lumbar and femoral BMD were determined. Data were analyzed with one way ANOVA and Bonferroni test.

Results: Apa I genotypes were distributed differently in TS patients (Aa 63.3%, AA 15%, aa 21.7%) vs. control (37.5%, 41.1% and 21.4% respectively; p = 0.004). No association between genotypes and the variables analysed was found.

Conclusion: This polymorphism does not play a major roll in the development of a lower BMD in these patients.

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Analysis of CYP21A1P and CYP21A2 Gene Dosage Alterations in 21-Hydroxylase Deficiency by Multiplex Ligation Probe Amplification Technique

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Introduction: 21-hydroxylase deficiency is one of the most common congenital adrenal hyperplasia (CAH), frequently caused by gene conversions between the active and its homologous pseudogene. Polymerase chain reaction based methods are used to detect the majority of point mutations. However, 25% of the alleles have large gene rearrangements, such as *CYP21A2* deletions and gene conversions that can be challenging to detect by traditional methods.

Objective: We evaluated the specificity of the MLPA technique to detect the different *CYP21* rearrangements in relation to the standardized *Southern blot* analysis.

Patients: Genomic DNA were selected from eight CAH patients bearing the most common *CYP21* rearrangements detected previously by *Southern blot* analysis and delimited through allele-specific PCR. These rearrangements included *CYP21A1P* duplication and deletion, *CYP21A2* deletion and large gene conversion.

Methods: DNA was tested with the new multiplex ligation probe amplification (MLPA) technique according to the manufactures instructions. This method included five *CYP21A2*, three *CYP21A1P* and two *C4* probes.

Results: MLPA and *Southern blot* results were concordant in all samples. It was possible to discriminate the presence of the rearrangements in homozygous and heterozygous states, as well as to achieve the breakpoint of the rearrangement, implicated in the genotype/phenotype correlation.

Conclusion: MLPA compared to Southern blot is less time-consuming, the analysis is less costly, less labor-intensive and essentially the results are specific. Overall, MLPA provide important tools for the genetic evaluation of 21-hydroxylase deficiency patients in clinical practice, in situations where a small amount of DNA is available, such as prenatal diagnosis of 21-hydroxylase deficiency.

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Consequence of Lowering De Cutoff in Neonatal Screening for Congenital Hypothyroidism

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Congenital hypothyroidism (CH) neonatal screening program is carried out by FEI since 1985 measuring TSH with DELFIA method since 1994. Till 2003 the TSH cutoff level was 15 mU/l blood and from then till now was lowered to 10 mU/l. For the internal recall

strategy a cutoff level of TSH of 25 mU/l was set to require serum or DBS for confirmation. We communicate the results on detection with the new cutoff in terms of recall and positive predictive value (PPV) for the confirmed disease. From 1994 to 2003 447.802 newborn were screened detecting 238 congenital hypothyroid (CH) (1: 1881) (recall rate (RR) 0.067%). Afterwards out of 215.409 newborn screened 92CH were found (1: 2341) with a RR of 0.12%. The PPV of this period would have been 68% with a 15 mU/l cutoff and lowered to 34.92% when the level was lessen. The PPV for TSH levels >25 mU/l was 96%, and 12.5%. For levels between 10 and 24.9 mU/l 67/92 detected children (72.8%) had TSH levels >25 mU/l, and 25/92 (27.2%) between 10 y 24.9 mU/l. Lowering the TSH cutoff level allowed the detection of 13 additional congenital hypothyroid. (14.1% of the total detected) (4 transient and 9 definitive: 2 goiters, 5 eutopics, 1 ectopic and 1 without diagnosis).

Conclusion: Diminishing the cutoff level allowed the additional identification of 13 congenital hypothyroid children. Although this procedure duplicated the RR and diminished the PPV of the detection, these parameters are still acceptable when benefits are considered.

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Mutations in PTPN11 Gene are a Common Cause of Noonan Syndrome (NS) but are not a Selective IGF-1 Receptor Kinase Inhibitor (NVP-AEW541) Suppresses Proliferation of the Human Adrenocortical Cancer Cell Line NCI H295 due to Apoptosis Induction

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Introduction: Insulin-like growth factor 1 (IGF-1) receptor kinase inhibitors have been considered a new approach for treatment of several malignant tumors. Recently, we demonstrated *IGF-2* and *IGF-1* receptor overexpression in pediatric adrenocortical cancer (ACC). In this study, we investigated the effects of a selective IGF-1 receptor kinase inhibitor on human ACC cells.

Methods: The human ACC cell line NCI H295 was treated with NVP-AEW541 (0.3 to 30 μM), a selective IGF-1 receptor kinase inhibitor. Cell proliferation was measured by a colorimetric assay in NVP-AEW541-treated cells with or without IGF2 stimulation, from 24 to 96 h. Apoptosis (caspase-3/7 activity) was determined by a luminescent assay after 3 h of NVP-AEW541 treatment.

Results: NVP-AEW541 treatment led to a significant decrease in cell proliferation at 3 μM (mean ± SD, 0.24 ± 0.02 nm), 10 μM (0.21 ± 0.03 nm) and 30 μM (0.1 ± 0.02 nm) vs. control (0.3 ± 0.02 nm) at 24 h ($p < 0.05$). This treatment blocked proliferation even in cells under IGF-2 stimulation (0.3 μM,0.2 ± 0.03; 1.0 μM, 0.16 ± 0.01; 3.0 μM, 0.11 ± 0.01; 10 μM, 0.06 ± 0.004; 30 μM, 0.01 ± 0.001; vs. control, 0.45 ± 0.02 nm; $p < 0.05$) at 72 h. NVP-AEW541 also promoted a significant increase in caspase-3/7 activity (1.0 μM, 3,714 ± 248; 3.0 μM, 5,257 ± 311; 10 μM, 7,069 ± 801; 30 μM, 9,060 ± 733; vs. control, 2,019 ± 329 relative light units; $p < 0.05$).

Conclusion: NVP-AEW541 blocked proliferation of the human ACC cell line NCI H295 due to apoptosis induction. Therefore, IGF-1 receptor represents a promising therapeutic target for ACC.

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Basal and GnRH-Stimulated Luteinizing Hormone (LH)/Follicule Stimulating Hormone (FSH) Ratio by Immunochemiluminometric and Immunofluorometric Assays in Diagnosis of Puberty in Normal Children

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Background: The aim of the study was to compare the diagnostic value of the basal and GnRH-stimulated LH/FSH ratio evaluated by IFMA and ICMA for puberty in order to save time and cost.

Methods: Basal LH and FSH were performed on 315 prepubertal and pubertal children (170 males and 145 females) divided into five groups according to Tanner stage. Of these, 106 subjects (59 males and 47 females) were submitted to GnRH test and GnRH-stimulated LH/FSH ratio was measured on the 30 minutes (peak response). Clinical data of stage of puberty and the results of LH /FSH was compared. Cutoff values for the best sensitivity and specificity were also determined.

Results: Regarding to basal LH/FSH ratio, the sensitivity and/or specificity was lower than 65% for both methods. The sensitivity and specificity of GnRH-stimulated LH/FSH ratio measured by ICMA was 100% for cutoff values of 1.50 and 0.30 in males and females, respectively, while the sensitivity and specificity measured by IFMA was higher than 80% for cutoff values of 1.25 and 0.25 in males and females, respectively.

Conclusions: While the basal LH/FSH ratio measured by ICMA or IFMA is not sensible and specific, the GnRH-stimulated LH/FSH ratio evaluated by both IFMA and ICMA methods seems to be adequate to diagnose puberty in normal children.

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Screening of Thyroid Hormone Receptor Beta Gene Mutations in Unrelated Argentinian Families with Resistance to Thyroid Hormone

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Resistance to thyroid hormone (RTH) syndrome is a genetic disorder with a dominant mode of transmission characterized by a variable clinical state and increased thyroid hormone levels with an inappropriately normal or elevated level of TSH. RTH is linked to the thyroid hormone receptor beta (TRbeta) gene. 11 unrelated argentinian families with clinical evidences of RTH were studied. In order to identify mutations causing this pathology, genomic DNA was isolated from blood cells and the exons 9 and 10 of the TRbeta gene (corresponding to the ligand-binding domain), including the flanking intronic regions were amplified by PCR. DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method and using the specific TRbeta forward and reverse primers. Direct sequence analysis revealed 3 novel missense mutations in exon 9. The first, a c.991A>G transition that results in a p. N331D substitution. The second, a c.1022T>C transition results in a p. L341P. The third, c.1036C>T transition causing a p. L346F change. 4 novel mutations were identified in exon 10: c.1293A>G; p. I431M, c.1339C>A; p. P447T, c.1358C>T; p. P453L and c.1297–1304delGCCTGCCA; p. A433fsX461. In addition, 2 previously reported missense mutations have been identified, one of them in exon 9: c.1012C>T; p. R338W and the other in exon 10: c.1357C>A; p. P453T (detected in three patients). The molecular diagnosis of RTH is important to determinate the appropriate treatment in those symptomatic patients to ameliorate features of hyper or hypothyroidism.

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Increased Ovarian Volume in Girls with Isolated Precocious Pubarche

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Introduction: We postulate that in some girls precocious pubarche may be induced by gonadal androgens. A sign of gonadal activity might be a greater ovarian volume in girls with isolated precocious pubarche compared with normal prepubertal girls.

Methods: We studied 33 prepubertal girls, with isolated precocious pubarche and a control group of 19 normal healthy prepubertal girls. No patients with breast development were included in the study. We performed a physical examination, bone age, pelvic ultrasound, and obtained a baseline blood sample for hormonal determinations.

Results: Are shown in the tables.

Girls	BMI (percentile)	Age (years)	Height (SDS)	Bone age advancement (years)	Mean ovarian vol (cc3)	Uterus length (mm)
Pubarche	81 ± 3.7	6.7 ± 0.1	0.5 ± 0.2	1.3 ± 0.2**	1.5 ± 0.1*	34 ± 1**
Control	79 ± 6.2	6.6 ± 0.3	0.3 ± 0.3	0.1 ± 0.2	0.9 ± 0.1	22 ± 1

Girls	17OHP (ng/ml)	E2 pg/ml	LH (mU/Ml)	FSH (mU/Ml)	A2 (ng/ml)	T (ng/dl)
Pubarche	0.8 ± 0.1	12 ± 0.7	0.3 ± 0.1*	1.4 ± 0.1*	0.6 ± 0.14*	0.3 ± 0.07
Control	0.7 ± 0.1	11 ± 0.6	0.5 ± 0.02	2.2 ± 0.2	0.3 ± 0.04	0.2 ± 0.01

*$p < 0.001$ ** $p < 0.05$.

We observed that the bone age, uterus length and ovarian volume were increased in girls with isolated precocious pubarche. In addition, serum LH and FSH were lower and serum A2 was higher in girls with precocious pubarche.

Conclusion: Our findings suggest that in some girls, precocious pubarche may be induced by ovarian rather than adrenal androgens.

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GH Sensitivity is Related to Nutritional Status in Children with Normal BMI

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Introduction: We postulate that Growth hormone sensitivity is regulated by nutritional status in children with normal BMI, and that children with BMI in the upper normal range might have an increased GH sensitivity compared to children with BMI in the lower normal range.

Table (of Abstract 68)

Nutritional Status	BMI (percentile)	Age (years)	Height (SDS)	GH peak (ng/ml)	0 h IGF-1 (ng/ml)	24 h IGF-1 (ng/ml)	Delta IGF-1 (%)
High BMI	88 ± 1.3**	8.3 ± 0.7	2.1 ± 0.1	11 ± 3.0	189 ± 15	259 ± 23*	36 ± 4.9*
Low BMI	19 ± 3.2	8.7 ± 0.7	2.4 ± 0.2	16 ± 5.3	156 ± 13	185 ± 18	18 ± 6.3

Methods: We studied 30 prepubertal children with idiopathic short stature, comparing children with normal high BMI (BMI p70–97, n = 19) and normal low BMI (BMI p3–30, n = 11). GH secretion was studied with a clonidine GH stimulation test. GH sensitivity was determined with an IGF-1 generation test; IGF-1 concentrations were measured at baseline and 24 hrs after the administration of a single dose of GH (0.1 U/kg).

Results: Are shown in the table. *$p < 0.001$ ** $p < 0.05$.

Children with high normal BMI exhibited a greater GH sensitivity compared to children with low normal BMI, as determined by their higher IGF-1 response to GH.

Conclusion: Our results show that nutritional status modulates GH sensitivity in children with normal BMI. We suggest that the dose of GH should be adjusted by BMI, using a higher dose per kilogram of body weight in children with lower BMI. In fact, current practice may be counterproductive, since children with high normal BMI are usually treated with a higher total GH dose compared to children with low normal BMI.

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STAT5b and JAK2 Phosphorylation Kinetics in Fibroblasts from Children with Aparent GH Insensitivity

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Introduction: Children with apparent idiopathic short stature associated with elevated GH concentrations and low IGF-I levels may exhibit partial GH insensitivity.

Goal: To study basal and post GH JAK2 and STAT5b phosphorylation in fibroblasts of children with apparent GH insensitivity and controls.

Method: We obtained fibroblasts from 10 prepubertal children (age 9.5 ± 2.6 years) with short stature (<−2.2 SDS), decreased growth velocity (below the 10th percentile), a GH response greater than 10 ng/ml to two stimulation tests and decreased serum IGF-I and/or IGFBP-3 levels (<−1.0 SDS). In addition, we studied fibroblasts obtained from 11 prepubertal control children of normal stature (age 6.4 ± 1.7 years). We evaluated STAT5b phosphorylation in cytoplasmatic and nuclear fractions, and JAK2 phosphorylation, after stimulation with GH (200 ng/mL) at 15.30 and 60 minutes.

Results:

	Height (SDS)	STAT5b-P			JAK2-P		
		15′	30′	60′	15′	30′	60′
C	0.1 ± 0.8	3.1 ± 1.2	3.3 ± 1.4	2.7 ± 1.1	1.1 ± 0.5	1.0 ± 0.5	1.2 ± 0.8
P	−3.2 ± 1.0	4.0 ± 2.4	3.5 ± 2.0	2.9 ± 1.8	1.4 ± 0.5	1.3 ± 0.8	1.4 ± 0.8

$p < 0.0001$.

The individual analysis shows an increase of STAT5b phosphorylation (>48%) and JAK2 phosphorylation (>100%) in 2 patients in comparison with controls. In addition, we observed an increase in cytoplasmatic STAT5b phosphorylation in 2 patients, and nuclear STAT5b phosphorylation in 3 patients.

Conclusion: Individually some short patients with apparent GH insensitivity display a pattern of STAT5b and JAK2 phosphorylation and/or nuclear translocation which is different from controls.

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SDHB and SDHD Mutations in Hereditary Paraganglioma/Pheochromocytoma (pgl-pheo) Syndrome

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It has recently been established that in about 25% of cases, pheo and functional pgl are inherited and caused by mutations in one of four genes responsible for: MEN 2, VHL, NF1 and pgl-pheo syndrome. The last is related to mutations in succinato dehidrogenase. Germ-line mutations in SDHD have been identified in multigenerational families and are the predominant causes of head and neck pgl. SDHB mutations have been identified in head, neck and abdominal pgl with malignant behavior. The aim of this study was to establish the mutations in the SDHD and SDHB gene in a group of patients with pgl-pheo syndrome. Seven patients (12–35 yr) affected with pgl and/or malignant pheo were studied. The study was extended to 37 family members. To characterize SDHD mutations we screened the four exons of this gene using PCR followed by direct sequencing and HpyCH4III enzyme digestion. To characterize SDHB mutations PCR-based SSCP strategy followed by direct sequencing was performed. Two patients with neck pgl presented SDHD deletions; in one of them who also had pheo, a dinucleotide deletion c.341–2ATdel was

present. The deletion was detected in 14/27 family members belonging to four generations. Five showed clinical evidence of the disease, while the remaining nine were unaffected carriers. A clear pattern of maternal imprinting was present in these kindred, in agreement with other data on SDHD inheritance. A nucleotide deletion (c.57-Gdel) was found in the other patient with apparently sporadic disease. 3/5 patients with SDHB mutations had abdominal or thoracic pgl (two of them malignant); one had adrenal pheo (malignant) and the remaining a malignant head and neck pgl. Three SDHB mutations were identified: R217G, S198R, g300–304 CCTCAdel.5/10 family members were unaffected carriers. Our date show that in patients with clinical features of pgl-pheo syndrome is mandatory SDHB or SDHD genes testing to establish the etiological diagnosis, clinical management and genetic counseling.

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P450 Aromatase (CYP19) mRNA Expression in Placental Tissue from Children Born Small for Gestational Age

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Estrogens regulate the developmental increase in the ratio of 11βHSD type 2/11βHSD type 1 in placental tissue from primates. Hence, estrogens might modulate transplacental glucocorticoid metabolism. An increased risk for development of metabolic syndrome in adult life has been described in children born small for gestational age (SGA) and has also been associated with prenatal exposure to high glucocorticoid levels. Furthermore, patients with P450 Aromatase deficiency develop lipid and carbohydrate metabolic alterations. We postulate that decreased placental CYP19 expression in SGA could be a mechanism involved in the prenatal programming of the metabolic syndrome observed later on in life.

Total RNA was isolated from 8 placental tissues (Pl) (gestational age: 38 weeks) from: 3 controls (AGA) (3590–3800 g) and 5 SGA (2130–2820 g) newborns and then analyzed CYP19 mRNA expression by Real-time RT-PCR. Lower CYP19 expression was observed in Pl-SGA (23.05 ± 3.71 arbitrary units relative to β-actin) than in Pl-AGA (62.96 ± 23.13 arbitrary units relative to β-actin).

These preliminary results suggest that the reduction in birth weight might be associated to the decrease in placental CYP19 expression. This decrease in placental aromatase activity might be involved in the development of metabolic syndrome, described in adults born small for gestational age. The decrease in 11βHSD type 2/11βHSD type 1 ratio and the increase in placental glucocorticoids levels might be the mechanism involved. Further studies would be necessary to confirm this hypothesis.

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GH Insensitivity in a Patient Compound Heterozygous for a Novel and a Recurrent GHR Gene Mutation. Long Term Effect of rhIGF-I Treatment

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GH insensitivity (GHI) is characterized by severe post-natal growth impairment, IGF-I deficiency, and normal or elevated GH levels. We describe a male patient, the second child from non-consanguineous parents, birth weight 3,420 g, birth length 47.5 cm, presenting many dysmorphic features: flat nasal bridge, bilateral convergent strabismus, frontal bossing, and acromicria. At 1 year of age, the diagnosis of GHI was performed based on high basal and stimulated GH levels, undetectable levels of GHBP and IGF-I, with no response of IGF-I to 4 day rhGH treatment (0.1 mg/kg. dose). DNA sequencing of *GHR* gene revealed two different mutations: a 17 bp deletion in exon 5 [c.328_344del (p. N92FfsX16)] and a point mutation in exon 7 [c.723C > T (p. G223G)]. The parents were heterozygous carriers for one mutated allele (father: p. N92FfsX16; mother p. G223G), while his brother had both wild-type alleles. At the age of 2.6 years (height 69 cm; −5.8 SDS) treatment with rhIGF-I (120 μg/d) was started, attaining a height of 155 cm (−2.4 SDS) at the age of 17.1 years. Triptoreline treatment was added at 12.3 years to prevent pubertal progression. The point mutation in exon 7, which creates a new preferred splicing site, has previously been described in GHI patients from a genetic isolate in the Bahamas, in one patient from Spain, and in two in Argentina. In conclusion, the p. G223G recurrent mutation should be routinely investigated by restriction enzyme digestion in patients with GHI. Early and prolonged rhIGF-I treatment are important to attain a height close to the normal range.

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GH Insensitivity and Immunodeficiency Associated with a Novel STAT5b Gene Mutation

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Mouse KO models and human STAT5b mutations have demonstrated its requirement for normal IGF-I production and postnatal growth, and also for normal immune function. We describe a female patient, adopted at 4 days of age, with extreme growth retardation associated with severe dermatitis, recurrent respiratory infections, failure to thrive and mildly retarded neurological development. Immunological evaluation showed T lymphopenia, (low CD3, CD4,

NK and very low CD8 cell counts), hypergammaglobulinemia, poor proliferative response after antigen stimulation and normal B cell counts. Endocrinological evaluation confirmed GH insensitivity: normal GH response to provocative test, undetectable IGF-I levels, no response of IGF-I or IGFBP-3 to a generation test (11 ng/ml, −4.0 SDS and 1.3 μg/ml, −4.1 SDS respectively), normal GHBP and high PRL levels. She began puberty at 12.5 years of age, reaching menarche at 14.5 years, attaining a near adult height of 124.2 cm (−5.7 SDS). Sequencing of the STAT5b gene exons 8–16, encoding SH2 and DNA binding domains, revealed a homozygous T → C transition in the 2nd base of codon 646, resulting in a missense mutation (p. F646S) which changes a highly conserved hydrophobic residue among STAT proteins. This mutation lies into the SH2 domain, critical for STAT5b recruitment to the activated GHR complex, dimerization, and translocation to the nucleus. These findings support the crucial role of STAT5b in normal IGF-I production and postnatal growth, emphasizing the heterogeneity of the immunodeficiency, with a more consistent severe impact on linear growth.

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Highly Intensive and Prolonged Physical Training: Impact on Glucocorticoid Sensitivity and Nuclear Transcription Factors Gene Expression (GR, NFκB, IκB, IKK)

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Aiming to recognize the impact of exercise on glucocorticoid sensitivity and gene expression, we evaluated 18 healthy sedentary adolescents (17–20 y) recruits of Brazilian Air Force Academy. The intravenous dexamethasone suppression test (20 mcg/m^2) was performed before and after 6ws of intensive physical training. Adequate physical training was confirmed by the improvement in body composition (bioelectrical impedance). The expression of GR and other transcription factors was quantified by qRT-PCR and presented as expression units in relation to the normalizing gene-BCR (Melo, et al., BMC Mol Biol.2004;5(1): 19).

Results are presented as mean (SD) in table:

	Pre-T	Post-T
F basal	20.0 (5.0)	14.4 (3.2)
F dex	13.0 (3.8)	10.8 (2.4)
%↓F	31.7 (22.9)	22.1 (23.8)
GR	1.7 (0.6)	1.4 (0.3)
NFκB	13.3 (11.9)	6.0 (5.2)
IκB	87.5 (35.2)	66.2 (36.2)
IKK	0.9 (0.5)	0.5 (0.2)

Pre-T: pretraining; Post-T: posttraining; F: cortisol (mcg/dl); F dex: cortisol after dexamethasone; %↓F: percent cortisol reduction; GR: glucocorticoid receptor; NFκB: nuclear factor kappaB; IκB: inhibitor of NFκB; IKK: IκB Kinase.

Conclusion: Highly intensive and prolonged physical training is able to reduce cortisol levels, and the expression rate of genes involved in the pathway of glucocorticoid action and glucocorticoid sensitivity. These adaptive responses to physical training can represent one of the mechanisms by which exercise exerts a beneficial effect in metabolic syndrome and prevention of cardiovascular events.

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Polymorphisms of the IGF-I Gene in Children Born Small for Gestational Age (SGA)

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Introduction: We observed that SGA children without postnatal catch-up growth (SGAcg−) had serum IGF-I levels significantly lower than those in AGA children. It has been suggested that minor genetic variation in the IGF-I gene will be able to influence serum IGF-I levels resulting in affected fetal and postnatal growth. Several IGF-I polymorphisms have been identified though their functional significance is not clear.

Aim To analyze the frequency of IGF-I polymorphism and their association with variables of postnatal growth in SGA children.

Methods: 48 healthy prepubertal AGA children, mean CA 7.2 yr and 49 SGA children divided in 23 SGA with catch up growth, mean CA 7.0 yr SDS height −0.88 and 26 SGAcg, mean CA 6.1 yr SDS height −2.27 were studied. Serum levels of IGF-I, IGF-II, IGFBP3, IGFBP1, ALS, osteocalcin, beta cross laps and lumbar and femoral BMD were determined. A cytosine-adenine repeat in the promotor region of the IGF-I gene were analyzed. Chi Squared Test was used.

Results: The most common polymorphisms were 192–192 (51%), 194–192 (16%) and 196–192 (9%), and they were significantly different between the three groups ($p = 0.01$). No important differences were found between the polymorphisms and the variables analyzed.

Conclusions: Our results suggest that this IGF-I microsatellite do not count for the differences in the serum IGF system observed in this population. The real meaning of the different frequencies and their influence on the postnatal growth outcome and the body size will be analysed in further studies.

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Familial Hyperaldosteronism Type I: Variation of Phenotype Expression in the Same Family

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Introduction: The familial hyperaldosteronism type I (FH-1) inheritance of chimeric gene in which the 5-prime regulatory sequences of the CYP11B1 gene were fused to the coding region of the CYP11B2 gene, leading to ACTH-regulated overproduction of aldosterone (causing hypertension) which is suppressible with glucocorticoid treatment.

Aim: To describe the phenotype expression of FH-1 in a Chilean Family.

Results: The presence of a chimeric CYP11B1/CYP11B2 gene was demonstrated by long-PCR in the proband, in the sister and in the father. The proband was a 13 yo man with severe hypertension. Plasma aldosterone (PA) was elevated (48.4 ng/dl), Plasma renin activity (PRA) was suppressed (<0.2 ng/ml/h) and the aldosterone/renin ratio (PA/PRA) was 161.3 (NV < 25), adrenal CT-Scan and plasma K level were both normal. The Father was found to be moderate hypertensive at the age of 25 years, with PA = 69.4 ng/dl, PRA = 1.5 ng/ml/hr and PA/PRA = 46.2. The sister was a 15 yo healthy and normotensive girl with PA = 11.3 ng/dl, PRA < 0.2 ng/ml/hr and PA/PRA = 37.6.

Discussion: FH-1 is characterized by a wide phenotypic variability in blood pressure, renin and aldosterone levels as a result of a variable expression of mutated gen within the affected family members. Considering high aldosterone levels have been related to endothelial damage this patients would be treated even if they have normal blood pressure levels.

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Inhibin A Generation after Gonadotropin Stimulation: A New Method to Detect Ovavian Tissue in Patients with Intersex Conditions such as True Hermaphroditism

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Background: True hermaphrodism (TH), characterized by the presence of ovarian and testicular tissue in the same patient. The evaluation of testicular tissue has been well established but there is no available test to demonstrate the presence of ovarian tissue.

Objectives: To evaluate the effectiveness of the LH/FSH gonadal stimulation in demonstrating ovarian function.

Patients and Methods: Ten patients with congenital adrenal hyperplasia (CAH), 10 with unilateral cryptorchidism, 13 intersex patients with no defined etiology, and seven TH patients have been included in the study. All the patients had a gonadotropic stimulation test with of LH and of FSH (menotropin), for three consecutive days. LH, FSH, estradiol, testosterone, and Inibin A were measured before, 24 h after the first dose, and 24 h after the third dose.

Results: LH did not show significant increase in the four groups. FSH increased in the four groups in a similar way. Estradiol increased in CAH patients and in TH pacients, while testosterone increased in patients with unilateral cryptorchidism as well as in the intersex patients without defined etiology. Inhibin A levels increased in CAH patients and in the TH patients.

Conclusions: The LH/FSH stimulation test demonstrated to be a useful method to diagnose the presence of ovarian tissue in CAH patients as well as in TH patients, becoming an important tool to diagnose TH even before the surgical procedure and histologic studies of the gonads.

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Transient Neonatal Hypothyroidism in a Patient with a De Novo Mutation in the Thyroid Hormone Receptor Beta Gene

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Resistance to Thyroid Hormone (RTH) is an inherited disorder of reduced responsiveness of the target tissues to thyroid hormone (TH) usually suspected when elevated TH are associated with non suppressed TSH. Basal TSH levels are normal or slightly increased and because screening programs are based on the determination of TSH, only few data of RTH are available during the neonatal period. We present a child with transient neonatal hypothyroidism most likely related to iodine overload subsequently diagnosed as having RTH. A 12-day-old boy delivered by cesarean section at term was referred due to borderline TSH at screening (9.2 mIU/l). He was breast fed and the mother was disinfected daily with povidone iodine. TSH was elevated (101 mIU/l), FT4 was normal (1.31 ng/dl) and Tg was markedly elevated (1.200 ng/ml) at 12 days of age. Thyroid US and 99Tc scan revealed an enlargement of the gland. Treatment with T4 was initiated but discontinued after 3 months, owing to significantly elevated FT4. After 1 month without treatment, FT4 was elevated (4.2 ng/dl) with non suppressed TSH (6.6 mIU/l). TRH/TSH test was higher respondent (33.7 mIU/l). RTH was confirmed by detection of a heterozygous mutation (P453T) in the T3 receptor-β gene. The mutation was absent in the parents. We conclude that in this infant the increased iodine load during the neonatal period impaired the ability of the thyroid gland to supply adequate amounts of TH allowing the initiation of a prompt T4 treatment of the hypothyroid state and the early detection of RTH due to a *de novo* mutation in the T3β receptor gene.

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Hyperglycemia During Therapy of Oncologic Disease in Children: An Adverse Effect of Chemotherapy or a Marker of Mortality?

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Hyperglycemia may occur in the induction phase of the treatment for acute lymphocytic leukemia with L-asparaginase. To describe clinical, laboratory findings and outcome in pediatric patients, who presented hyperglycemia during induction therapy. We describe 12 out of 311 patients (3.8%) with undiagnosed diabetes, presented with 15 hyperglycemic episodes, except for one patient who showed hyperglycemia prior to therapy. All patients were considered at high-risk for recurrence. All hyperglycemic episodes occurred during steroids treatment, except in one patient, and 73.3% of episodes were at any L-asparaginase dose. One out of 15 hyperglycemic episodes occurred in an overweight patient and 3/12 (25%) patients had family history of diabetes. No patient was diagnosed as having pancreatitis. All patients needed insulin therapy during the steroid therapy, except for one patient who developed diabetes ketoacidosis and needed insulin up to 3 months. Four out of 12 (33.3%) had a disease relapse and required a new cycle of induction. Four out of 12 died. Two patients presented glucose alterations just after relapses. Overall, we conclude that we would expect a higher incidence of hyperglycemia and pancreatitis in our patients. Leukemia *per si* may alter glucose metabolism and L-asparaginase, a well-known diabetogenic agent, no longer explains our findings. It seems that hyperglycemia might be a predictor of clinical outcome. Thus, glucose measurements should play an important role during leukemia treatment.

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Endocrine Involvement in Children Patient with Langerhans Cell Histiocytosis

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Pituitary function was analyzed in 294 pediatric patients with Langerhans cell histiocytosis (LCH). Posterior Pituitary function was evaluated by water deprivation test and Anterior Pituitary function by serum basal T4, T3, FT4, TSH, ACTH, cortisol, LHRH test and two pharmacological tests for growth hormone (GH). Diabetes insipidus (DI) was diagnosed in 49 of 294 patients (16.6%), 33/49 had multi-systemic disease (MD) and 17/49 had single system disease (SD). Hypothalamo-pituitary magnetic resonance imaging abnormalities were found in 19/27 patients. DI was present before diagnosis in 8%, at diagnosis in 43%, and during follow up in 49% (median: 2.12, range 0.5–9 yr) of the patients. Sixteen patients (32.6%) had GH deficiency (GHD), all with associated DI. In GHDDI group, height SDS at diagnosis of LCH was significantly lower ($p < 0.05$) and age significantly higher ($p < 0.01$) than in non GHD DI group. In the GHD group, low serum T4 and cortisol levels were found in 6 and 5 patients, respectively. Gonadotropin deficiency was found in 4/7 pubertal-age patients and hyperprolactinemia was present in 2. Six patients were treated with rhGH. After one year of treatment, height SDS improved 0.9 ± 0.64, without any adverse effects. During follow up, one SD patient developed thyroid cancer. Precocious puberty was detected in one patient. We conclude that in LCH, GH deficiency is the most frequent hypothalamo-anterior pituitary dysfunction, particularly in those with DI and MD. Health providers should be aware that endocrine dysfunction can appear, either at diagnosis or at any time during long-term follow up.

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Septo-Optic Dysplasia (SOD): Clinical, Endocrinological and Neuroradiological Evaluation. Follow-Up in 46 Pediatric Patients

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SOD is defined by the combination of optic nerve hypoplasia (ONH), midline malformation of the forebrain, aplasia/hypoplasia of the septum pellucidum (ASP) and corpus callosum; and/or hypothalamo-pituitary insufficiency (HPI). We have followed 46 patients with DSO. All patients had ONH confirmed by ophthalmological examination and neuroradiological studies. Pituitary hormone deficiencies were documented using standard endocrine tests. Patients were subdivided in Gr1 ($n = 27$) with HPI, and Gr2 ($n = 19$), no HPI. Groups were similar in age and time of follow-up. GH (GHD) and TSH (TSHD) deficiencies were found in 81.5% of the patients, 48.1% had ACTH deficiency (ACTHD) and 14.8% had Diabetes Insipidus (DI). All patients with ACTHD and/or DI had both GHD and TSHD. Patients developed additional pituitary hormone deficiencies over the time (36% TSHD, 50% GHD). Height SDS at diagnosis in Gr1 was significantly lower than in Gr2, $p = 0.01$. Most common midline CNS abnormalities was ASP (Gr1 63%, Gr2 48% $p = NS$). However cortical dysplasia and heterotopias in Gr2 (57%) were significantly higher than in Gr1 (19%), $p = 0.01$. Hypoglycemia, jaundice and/or seizures were present in all patients in Gr1 ($p < 0.05$). Brain MRI in Gr1 showed pituitary anomalies in 45% of the patients. In Gr1, 13 patients were treated with rhGH, improving 1.5 Height-SDS without adverse effects. In conclusion the variable association within HPI and the neuroradilogical studies suggest that SOD is a multifactorial or polygenic disorder. Physicians should be aware of hormonal deficiencies that could present at birth or appear during follow-up.

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New IVS2-2A>G Mutation in the Anti-Mullerian Hormone Gene in 46, XY Patients with Persistent Mullerian Duct Syndrome (PMDS)

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Introduction: PMDS is characterized by the failure of Mullerian duct regression in 46, XY individuals. It presents an autosomal recessive inheritance, involving the genes that codify the anti-Mullerian hormone (*AMH*) or its receptor. The *AMH* gene contains 5 exons, is 2.75 kb in length and it is located in 19p13. The *AMH* gene mutations are responsible for about 47% of the PMDS cases.

Objective: To search for the presence of mutations in the *AMH* gene in patients with PMDS.

Patients: We evaluated 5 unrelated patients aged 24 to 39 years, with cryptorchidism and uni- or bilateral inguinal hernia and Mullerian derivatives identified at the surgical correction. Testicular tumors were identified in two patients through anatomopathological evaluation.

Methods: The 5 exons had been amplified by PCR and sequenced.

Results: Two patients presented normal *AMH* sequence. In one patient, the R95X and R123W mutations were detected in heterozygous form. The other two presented the allelic variations 146T>G, 252G>A, 303G>A and the new intronic mutation, IVS2-2A>G, all in homozygous form. Through the site *www. fruitfly. org*, it was verified that the new IVS2-2A>G mutation inactivated the acceptor splicing site (ASS) and demonstrated the existence of an alternative ASS in the end of exon 3, what would result in a truncated and inactive protein. One of the patients carrying this mutation presented a testicular tumor.

Conclusion: We identified a new mutation in the ASS in intron 2, probably due to a founder effect, in 2 unrelated patients with PDMS.

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