Symposia

S1-13 Rickets is Not What it Used to be

The Rachitic Bone

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Rickets and osteomalacia are disorders of bone mineralization in which osteoblastic activity and the production of bone matrix continue, but the rate of mineralization is impaired leading to an accumulation of unmineralized matrix. These changes lead to rickets in the growing child with open epiphyses and osteomalacia in the adult. The morphological changes of rachitic bones occur primarily at the epiphyses of growing long bones, where there is proliferation and maturation of he cartilage cells. Failure of the cartilage matrix to mineralize is followed by defective bone formation in which osteoid accumulates without mineralizing. A broad zone of proliferative cartilage and osteoid develops which lacks the rigidity of the normal bone cartilage junction, and becomes compressed and deformed laterally by pressure. Radiological findings are visible relatively late, and include an increased width of the uncalcified growth plate and metaphyses with irregularity and distortion. Marked angulation, thickening, and deformity are common. Hypocalcemic tetany and seizures can be the presenting features of rickets. The clinical features of calciferol deficiency are weakness, bone pain, bone deformity, and fracture. The most rapidly growing bones show the most striking abnormalities. In the first year of life the most rapidly growing bones are the skull, ribs, and wrists. Calciferol deficiency at this time leads to widened cranial sutures, frontal bossing, posterior flattening of the skull, bulging of costochondral junctions ("rachitic rosary"), and enlargement of the wrists. Nutritional deficiency of vitamin D remains a leading cause of rickets worldwide, despite the diminished prevalence of vitamin D deficiency that followed the fortification of milk with vitamin D in the 1930's. More recently calcium deficiency has emerged as an important cause of nutritional rickets. In other patients rickets is caused by genetic defects that impair vitamin D action or phosphate metabolism.

S1-14 Rickets is Not What it Used to be

Hypophosphatemic Rickets: A PerPHEXing Trip From Bench to Bedside

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X-linked hypophosphatemia (XLH), the prototypic vitamin D resistant disease, is due to inactivating PHEX mutations. However, the mechanism(s) by which mutated PHEX influences P transport and bone mineralization, generating the XLH abnormalities, remains unknown. Thus, bridging discovery of mutated PHEX to optimizing clinical management of the disease is perPHEXing. The limited progress in understanding XLH is related to: 1) failure of *Phex* targeted overexpression to normalize *hyp*-mouse osteoblast mineralization *in vitro* and rescue the *Hyp* phenotype *in vivo*; and 2) inability to identify a PHEX/Phex substrate, which functions as phosphatonin/minhibin. Nevertheless, discovery of phosphaturic factors (phosphatonins), FGF-23, MEPE, and FRP-4, supports the hypothesis that the pathophysiological basis for XLH is: 1) an inactivating PHEX mutation produces inadequate amounts of PHEX endopeptidase; 2) resultant inactivation or enhanced production of a phosphatonin(s) elevates circulating levels of a bioactive protein(s); and 3) a consequent repressed expression of NPT2 causes renal P wasting and hypophosphatemia. Resolution of the pathophysiological basis for the disease depends upon data, which have become available and address the possibility that: 1) a developmentally or temporally sensitive lack of Phex expression in osteoblasts is central to impaired bone mineralization and P homeostasis; 2) some characteristics of the *HYP* phenotype may occur as a secondary consequence of a metabolic abnormality caused by the *PHEX*-PHEX-Phosphatonin(s) physiological derangement and are not mediated directly by the *PHEX*-PHEX-Phosphatonin(s) cascade; and 3) phosphate and renal phosphate transport play an important role in bone mineralization and regulation of vitamin D metabolism. Discussion of these observations will highlight a pathway to link the discoveries at the bench to improvement in diagnosis and management of XLH.

S1-15 Rickets is Not What it Used to be

Calcium Deficiency Rickets

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Nutritional rickets has been reported from nearly 50 countries in the last 20 years. Data regarding the prevalence of nutritional rickets are sparse, but studies in countries like Nigeria, Bangladesh, and India report prevalence ranges of 1-9%. Many of these countries in the tropics have abundant sunlight, and measurements of 25-hydroxyvitamin D values in children with rickets in some countries indicate adequate vitamin D status. Characteristically, these children with rickets have daily calcium intakes below 300 mg, and they respond significantly better to treatment with calcium alone than they do to vitamin D (61% vs 19% of Nigerian children healed in 6 mo, respectively). However, compared with control children, children with rickets do not appear to have lower calcium intakes, poorer nutritional status, reduced calcium absorption, or important differences in breast milk calcium intake. The reason why some children develop rickets in response to calcium deprivation, while others do not, is unclear. Dietary inhibitors of calcium absorption (e.g. phytates, oxalates) or genetic factors may be contribute to the pathogenesis of calcium deficiency rickets. Adequate calcium intake in infancy and childhood should be an important nutritional emphasis for children in developing countries at risk for rickets.

S2-16 Endocrinology of the Acutely Ill Child

Inherited and Acquired Parameters in Stress and Anxiety Responses

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Stress and anxiety responses involve complex, incompletely understood multiple small effects of genomic, environmental and experience-derived factors and are currently measured by psychological criteria. We have recently found significant interrelationships between expression variations and nucleotide polymorphisms of the chromosome 7q21-22 acetylcholinesterase-paraoxonase (ACHE/PON1) locus with the trait and state anxiety measures of healthy subjects from the HERITAGE family study. Cortisol levels, in contrast, appeared non-informative. Stress-induced overproduction of the AChE protein controls the termination of the stress-enhanced acetylcholine signaling. Furthermore, tetracycline-dependent antisense suppression of the stress-induced AChE overproduction suppressed fear responses in conditional transgenic mice. Also, the PON protein displays peroxidase-like activity, and can protect AChE from oxidative stress damages. Serum AChE and PON activities, both affected by demographic parameters, showed inverse, reciprocal associations with anxiety measures of healthy individuals. Moreover, the transient score of state anxiety and the susceptibility score of trait anxiety both appeared to be linked to enzyme activities, supporting the notion of corresponding

gene expression relationships. Parallel polymorphisms in the ACHE and PON1 genes displayed apparent associations with both trait and state anxiety scores. Our findings indicate that a significant source of anxiety feelings involves inherited and acquired parameters of acetylcholine regulation that can be readily quantified, which can help explain part of the human variance for state and trait anxiety.

S2-17 Endocrinology of the Acutely Ill Child

The Hypothalamic-Pituitary-Adrenal Axis and Critical Illness

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Like the stress response, the inflammatory reaction of an individual is crucial for survival of the self and species. Also like the stress response, inflammation is tailored to the stimulus and time-limited. A fully fledged systemic inflammatory reaction consists of activation of immune and immune accessory cells and resultant stimulation of four major programs: (1) the acute phase reaction, (2) the sickness syndrome, (3) the pain program, mediated by the afferent sensory and autonomic systems, and (4) the stress program, mediated by the stress system, i.e. the hypothalamic-pituitary-adrenal (HPA) axis and the locus ceruleus- norepinephrine / sympathetic system. The main effector substances of the systemic inflammatory response are inflammatory cytokines, such as TNFalpha, IL-1 and IL-6, chemokines,, and other mediators of inflammation; the acute phase reactants, such as C-reactive protein (CRP), cell adhesion molecules, fibrinogen and plasminogen activator inhibitor 1; the effectors of the sensory afferent system, such as substance P ; and, of the stress system, namely hypothalamic CRH and vasopressin, cortisol, norepinephrine and epinephrine, and peripheral neuronal CRH. The sickness syndrome consists of anorexia/nausea, fatigue and/or depressed affect, somnolence, hyperalgesia, sleep disturbances, elevated temperature and an increased metabolic rate, all manifestations suppressed by glucocorticoids. Yet, peripheral neuronal CRH activated by stress or the inflammatory reaction, and substance P, activated by the inflammatory reaction potentiate inflammation. We recently found that during a systemic inflammatory reaction, as in ARDS or sepsis, there is inadequate activation of cortisol secretion and significant cytokine-induced glucocorticoid resistance, both suggesting beneficial actions of added glucocorticoids.

S2-18 Endocrinology of the Acutely Ill Child

Early Endocrine Predictors of Outcome

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Septic shock is the most severe clinical manifestation of meningococcal disease, with mortality still ranging between 20-40%. We evaluated endocrine and metabolic parameters in 61 children (4-185 months) with meningococcal sepsis, on admission at the pediatric intensive care unit. Nonsurvivors (n=8) had compared to survivors significantly higher pediatric risk of mortality scores (PRISM score) and cytokine levels (IL-6 and TNF-alfa). They had extremely elevated GH levels (mean GH 131 mU/l) during a 6-h profile that were significantly different compared to the very low GH levels of survivors (mean GH 7 mu/l). In all acutely ill children, serum IGF-I, free IGF-I and IGFBP-3 levels were below normal, but these levels were even more reduced (significantly) in nonsurvivors compared to survivors. Nonsurvivors had very high IGFBP-1 and lactate levels and low insulin, glucose and nonesterified fatty acids levels. They also had an inadequate cortisol stress response in combination with very high ACTH levels, whereas survivors showed a normal stress response. Nonsurvivors had compared to survivors significantly higher T3 and lower reverse T3 (rT3) levels, indicating that they had less signs of the so-called 'euthyroid sick syndrome' which was clearly found in the survivors. Serum T4 levels were not different. The PRISM score correlated positively with levels of IGFBP-1, IGFBP-3 protease activity, ACTH, IL-6, TNF-alfa and negatively with levels of total IGF-I, free IGF-I and cortisol. In conclusion, our study suggests that nonsurvivors show insufficient or inadequate adaptations of their endocrine axes during the acute life threatening disease. This might be due to an overwhelming endotoxin load, but a genetic predisposition for inadequate adaptations and/or age-related maturation of the various systems might also play a role. It is yet unknown if the abnormalities play a crucial role in the outcome of the disease or just represent an end-stage before death.

S3-721 Recent Advances

WNT4 is Essential for Sexual Development in Women

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Differentiation of a testis or an ovary from the bipotential gonad is a complex developmental process involving various genes and hormones. Additional elements of the reproductive tract develop from an indeterminate stage via the differentiation of Wolffian (male reproductive tract anlage) and Müllerian (female reproductive tract anlage) ducts. Whereas factors involved in male sex differentiation are well studied, the pathways that regulate female sexual differentiation remain incompletely defined. To date, no genes have been demonstrated to play an equivalent role to that of SRY or SOX9 genes in testes development. Wnt4, one of a few factors with a demonstrated function in the ovarian-determination pathway has been noted to be involved in sex differentiation in mice. We now describe a young woman who showed absence of structures derived from Müllerian ducts (monolateral renal agenesis and clinical signs of androgen excess. Her phenotype resembles the Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH) and is also strikingly similar to that of the Wnt4-knockout female mice. This constellation of findings prompted us to search for mutations in the WNT4 gene in this patient. . The WNT4 gene of our patient carries a heterozygote single-base exchange corresponding to a Glu226Gly mutation in the WNT4 protein. The mutated WNT4 is unable to suppress the expression of the androgen-synthetic enzymes CYP17 and HSD3B2 in human ovarian cells. The mutant WNT4 is not lipid-modified, cannot be secreted and is therefore unable to activate its own signaling pathway. The mutated WNT4 has dominant negative properties, providing a clear genotype-phenotype correlation. This firstly-described loss-of-function mutation in WNT4 gene appears to cause developmental abnormalities in humans and identifies WNT4 as a major player in the development and maintenance of the female phenotype in women, by regulating Müllerian duct formation and controlling steroidogenesis in the ovary.

S3-722 Recent Advances

A Genetic Isolate Form of Late-onset Congenital Lipoid Adrenal Hyperplasia

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Congenital lipoid adrenal hyperplasia (CLAH), the most severe form of CAH, comprises combined mineralocorticoid, glucocorticoid and sex steroid deficiency, causing sex reversal in 46,XY infants and life-threatening salt-losing crises early in infancy of both sexes. CLAH results from mutations in the steroidogenic acute regulatory protein (StAR), commonly by the Q258X mutation found in most Japanese and Korean patients. Only one CLAH patient has been described presenting after 6 months of age, having a mutation (M225T) retaining partial function. We describe 8 patients in 6 Saudi families with CLAH. All were phenotypically female and hyperpigmented, presenting with hyponatremia, hyperkalemia, grossly elevated ACTH, and very low concentrations of serum cortisol, 17OH-progesterone and 17OH-pregnenolone; 5 were 46,XY and 3 were 46,XX. However, the ages of clinical presentation ranged from 1 month to 3 years, with four presenting at > 6 months. Genetic analysis of the StAR gene by PCR amplification of genomic DNA and sequencing showed that 7 patients in 5 families were homozygous for the novel missense mutation R182H, and the eighth patient was homozygous for the novel mutation M144R. Each mutant was re-created in a human StAR cDNA expression vector. Activity was assayed as pregnenolone produced by COS-1 cells co-transfected with a vector expressing a fusion protein of the cholesterol side-chain cleavage enzyme system and the following StAR constructs: empty vector (negative control), M144R, R182H, and wild-type StAR (positive control). Surprisingly, the M144R and R182H mutants were wholly inactive, despite the late onset of clinical symptoms. These cases identify a new genetic isolate of CLAH in Saudi Arabia and illustrate that this potentially lethal disease may exhibit subtle presentations, long after infancy.

S3-723 Recent Advances

Pseudo-SIADH: Identification of Novel Activating Mutations in the V2 Vasopressin Receptor Causing a New Genetic Disease

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The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a common cause of hyponatremia. Increased concentrations of vasopressin cause retention of free water, increased sodium excretion, and hyponatremia. We report two unrelated children, ages 3 and 6 months, who presented with hyponatremia (serum Na ~120 mmol/L). Their clinical appearance, chronic symptoms and laboratory findings were consistent with SIADH, yet they exhibited very low or unmeasurable ADH levels on repeated occasions. We hypothesized that these children had a gain of function defect in the ADH signaling pathway. ADH binds to the 7 transmembrane, G protein-coupled V2 vasopressin receptor (V2) on the basolateral side of renal collecting duct cells to induce cAMP production and mobilization of aquaporin-2 channels to the apical cell membrane. The aquaporin channel allows free water to be reabsorbed, reducing serum sodium. Activating mutations in G protein-coupled receptors have been reported in other diseases, hence we considered that an analogous mutation in the V2 receptor might cause pseudo-SIADH in the absence of ADH in these patients. DNA sequencing of each patient's V2 receptor gene identified mutations (R137C or R137L) in each; R137H mutations have been previously shown to cause nephrogenic diabetes insipidus. We recreated each mutation by site-directed mutagenesis in a V2 receptor expression vector and co-transfected COS-7 cells with wild-type and mutant V2 receptor vectors and a cAMP responsive luciferase reporter plasmid. The R137L and R137C mutants induced 8 and 4 fold more luciferase activity than the wild-type V2 receptor, the empty vector or the inactivating R137H mutant. These novel gain of function mutations in the V2 receptor are the likely etiology of the patients' SIADH-like clinical picture causing constitutive activation of the receptor and hyponatremia. These findings represent a previously unrecognized genetic disease, which we designate as pseudo-SIADH.

S3-724 Recent Advances

Disturbed Co-Development of Thyroid Gland and Cervical Arteries as a New Model for Thyroid Dysgenesis

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In the majority of patients with congenital hypothyroidism due to thyroid dysgenesis the pathogenesis is still unknown. Mutations in the coding region of the transcription factors NKX2.1, FOXE1 and PAX8 were found in few particular cases of thyroid dysgenesis associated with additional malformations. To further unravel the molecular cause of thyroid dysgenesis, additional genes involved in early thyroid developmenet need to be identified. In order to get more insight into the mechanism of proper organ localisation we investigated the effect of one key molecule of early organogenesis in vertebrate embryogenesis e.g. sonic-hedgehoc (shh). By immunohistochemestry, in situ hybridisation and three dimensional reconstruction we studied the thyroid phenotype of shh deficient mice (dsh/dsh mice). It turned out that all studied dsh/dsh mice are affected by thyroid dysgenesis in terms of asymmetric unilateral thyroid glands. This phenotype resembles the most frequent thyroid dysgenetic defect in humans (thyroid hemiagenesis, occurring in 1 in 1000 (mostly euthyorid) individuals). Detailed description of the cervical region of the dsh/dsh mice revealed that the asymmetric thyroid gland was always accompanied by asymmetric non-lateralised carotid arteries. This co-development feature could be further demonstrated in a rescue experiment in which double-mutant shh and gli3 deficient mice were investigated. In this model, which has shown recently to result in the rescue of limb and CNS phenotypes, we observed proper repositioning of the thyroid and the cervical arteries in a symmetric midline position. Together these results demonstrate for the first time that size, shape and position of the thyroid gland depend on co-developing major arteries. The molecular pathways connecting the co-developing structures represent new promising candidate genes for the understanding of the pathogenesis of thyroid dysgenesis.

S3-725 Recent Advances

Tolerance to Proinsulin is due to Expression by the Thymic Epithelium: Implications for Diabetes Immunotherapy

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Autoimmune diabetes is due to a failure of self tolerance resulting in autoreactivity against several beta cell antigens. Proinsulin plays a primary role as demonstrated by early T and B cell responses in humans and mice. Polymorphisms in the 5'VNTR region of the human insulin gene are associated with variable expression of the protein and diabetes susceptibility. However, the mechanism linking this polymorphism and diabetes risk is unknown. The site of proinsulin expression (i.e. bone marrow-derived antigen presenting cell vs thymic epithelial cell vs beta cell) relevant to self tolerance is debated. In the mouse, insulin is encoded by two independent genes with proinsulin-2 (pro2) highly expressed in the thymus. In previous studies, we have demonstrated that 129 mice (non autoimmune prone) with a knock-out of the pro2 gene (pro2KO) are intolerant to pro2, while wild type (wt) mice are fully tolerant. In the present study, we investigated the site of expression of pro2 relevant to tolerance induction using thymus and bone marrow chimeras. CD4 T cells from wt animals transplanted with a pro2KO bone marrow were tolerant to pro2 (i.e. did not produce gamma-interferon in response to immunization and in vitro stimulation). In contrast CD4 T cells from pro2KO animals transplanted with a wt bone marrow responded to pro2. This rules out a role for expression of pro2 in bone marrow-derived cells in induction of self tolerance. CD4 T cells from wt mice, thymectomized and grafted with a pro2KO thymus and a wt bone marrow were intolerant to proinsulin-2. This indicates that loss of pro2 expression in thymic epithelial cells is sufficient to abolish tolerance to pro2. Our results demonstrate the functional role of thymic proinsulin expression in the regulation of beta cell autoreactivity. Future research in diabetes immunotherapy should aim at restoring central (thymic) T cell tolerance rather than pursue the so far unsuccessful attempts to manipulate peripheral tolerance.

S3-726 Recent Advances

Dissecting the Role of Glucocorticoids on Pancreas Development

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We have previously shown that intra-uterine food restriction during the last third of pregnancy induces a rise in maternal and fetal corticosterone levels, which in turn was responsible for the decreased beta-cell mass observed in the fetus. To determine whether glucocorticoids were also involved in normal pancreas development, glucocorticoid treatment of rat pancreatic buds in vitro was combined with the analysis of transgenic mice lacking the glucocorticoid receptor (GR) in specific pancreatic cell populations. In vitro treatment of embryonic pancreata with dexamethasone, a glucocorticoid agonist, induced a decrease of insulin-expressing cell numbers and a doubling of acinar cell area, indicating that glucocorticoids favored acinar differentiation; in line with this, expression of Pdx-1, Pax-6 and Nkx6.1 was downregulated, while the mRNA levels of the exocrine transcription factors Ptf1-p48 and Hes-1 were increased. The expression of the pro-endocrine marker ngn3 was unchanged under the same experimental conditions.The selective inactivation of the GR gene in insulin-expressing beta cells in mice (using a RIP-cre transgene) had no measurable consequences on beta- or alpha-cell mass whereas the absence of GR in the expression domain of Pdx-1 (Pdx-cre transgene) led to a 2-fold increased beta-cell mass, with increased islet numbers and size, but normal alpha-cell mass in adults. These results demonstrate that glucocorticoids play an important role on pancreatic beta-cell lineage, acting after ngn3 but before hormone gene expression onset, and possibly also modulating the balance between endocrine and exocrine cell differentiation.

S4-19 Long-acting Growth Hormone

Mechanisms in the Pulsatility of Growth Hormone Signaling

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Gene expression in mammalian liver is sexually dimorphic, and is regulated by sex-dependent patterns of pituitary GH secretion. DNA microarray studies and liver nuclear proteome analyses have established that GH is the major hormonal regulator of sex-dependent hepatic gene expression. Analysis of mice deficient in STAT5b, a GH pulse-activated intracellular signaling molecule and transcription factor, revealed that this factor is required for normal pubertal growth and the profile of male liver gene expression. STAT5b deficiency leads to a loss of male-specific gene expression and is associated with increased expression of female-specific genes in male liver. Liver-enriched transcription factors, such as HNF4a, are also required for, and may act in concert with STAT5b to regulate sex-dependent liver gene expression. STAT5b is repeatedly activated in male liver by each successive plasma GH pulse, via JAK2-catalyzed tyrosine phosphorylation, followed by STAT dimerization and nuclear translocation. Down-regulation of the GH receptor-JAK2-STAT5b pathway in hepatocytes exposed to GH continuously underlies the much lower level of active liver STAT5b that is characteristic of adult females. Termination of GH receptor signaling to STAT5b is in part mediated by GH-inducible SOCS/CIS proteins, which bind to and inhibit the GH receptor-JAK2 complex by various mechanisms, rendering the hepatocyte temporarily unresponsive to GH. SOCS/CIS proteins synthesized in liver cells stimulated with GH continuously may contribute to the down-regulation of STAT5b signaling seen in adult female rat liver, by a mechanism that involves enhanced proteasome degradation of the GH receptor-JAK2 signaling complex. The recent discovery of human mutations in STAT5b linked to impaired growth highlights the significance of these studies for normal human physiology and development. [Supported by NIH grant DK33765].

S4-20 Long-acting Growth Hormone

Generation of GHBP and Modulation of Cellular GH Sensitivity

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Growth hormone (GH) action begins with binding of GH to the extracellular domain (ECD) of the cell surface GH receptor (GHR) in target tissues. The GH binding protein (GHBP) is a circulating form of the GHR that derives in humans and some other species from metalloproteolysis of the full-length GHR and shedding of the receptor ECD. This proteolysis, which is inducible in cell culture by phorbol ester or platelet-derived growth factor (PDGF) stimulation, is catalyzed by tumor necrosis factor-α converting enzyme (TACE) and for the rabbit GHR occurs in the ECD stem region eight residues outside of the membrane. In addition to generating the GHBP, GHR proteolysis yields the receptor remnant, which contains remaining ECD residues and the transmembrane and cytoplasmic domains. In this presentation, I will discuss the potential impact of inducible GHR metalloproteolysis on cellular and tissue sensitivity to GH. In addition, our recent evidence for further processing of the GHR remnant by a different protease activity (so-called γ secretase activity) and its effects on remnant disposition will be reviewed.

S4-21 Long-acting Growth Hormone

Treatment with Long-acting GH

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Growth hormone (GH) replacement therapy is routinely administered by daily s.c. injections at bedtime in order to mimic the major secretory episodes of GH during sleep. In both children and adults with GH-deficiency, however, the requirement of daily injections may compromise compliance with GH-therapy. To circumvent these problems, sustained release formulations of GH have been developed, of which Nutropin depot® is approved in the US for the treatment of children with GHD. This preparation is injected s.c. once or twice monthly. This galenic formulation has GH encapsulated in biodegradable polylactic glycolic acid (PLG) microspheres. During the first two days after injection circulating GH-levels mostly are found above 10 ng/ml, followed by a steady decline which in a dose-dependent manner results in tonic GH-levels above 1 ng/ml for 1 – 2 weeks. As a consequence IGF-levels raise to a peak at approximately 3 days after injection and remain elevated above baseline for two weeks in GHD children. Both pharmacokinetic (PK) and pharmacodynamic (PD) profiles at doses of 0.75 and 1.5 mg/kg are reproducible without signs of accumulation during twice-monthly injections over 6 months. In adult GHD patients this preparation exhibits similar PK and PD profiles, however, similar to daily s.c. application of GH, doses are lower in adults than in children and women taking oral estrogen preparations require twice the dose of men. At doses of 0.3 mg or 0.6 mg/kg twice-monthly in adult GHD patients this preparation has been shown to effectively decrease fat mass and increase lean body mass, as well as elevating IGF-I initially to the upper edge of the normal range with a subsequent decline within the normal range for 2 weeks. Overall, by comparing the mass amounts of GH applied over time, efficacy per dose in comparison to daily s.c. injections of GH appears reduced for this preparation. LB03002 (LG Life Sciences / Biopartners), a different sustained release formulation incorporating GH into sodium hyaluronate microparticles was injected weekly for 5 weeks in a phase-2 study in adult GHD patients at 25 % above the previously titrated s.c. dose. PK- and PD-data show the maximum GH-concentration after approximately 12 hours with a peak concentration 3-fold that observed during daily s.c. injection. IGF-I-levels peaked after 2 days and declined almost to baseline within one week. Both by GH-level AUC calculations and IGF-generation capacity this sustained release preparation on a mass basis appears equipotent to daily s.c. injected GH. Since GH signals through different pathways which may respond differently to pulsatile versus tonic receptor occupancy and GH mediates a multitude of physiological effects also beyond clinical endpoints routinely monitored, vigilant monitoring of long-term treatment effects with sustained released GH formulations is advisable. In particular, long-term consequences for carbohydrate metabolism need to be prospectively investigated and the metabolic consequences of GH-replacement with slow-release preparations merit further investigations. If these concerns prove groundless, the improved convenience and resulting better compliance may result in slow release GH-preparations becoming a popular alternative to the commonly used daily s.c. injections.

S5-22 Paediatric Gynaecology

Ovarian Development

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A critical event in mammalian differentiation is the development of the indifferent gonads into either testes or ovaries. Studies in knockout mice and analyses of human subjects with disorders of sex differentiation have identified a number of genes that play key roles in testes development and male sex differentiation, but we know much less about the mechanisms that specify ovarian development. A number of genes, such as steroidogenic factor 1 (SF-1), Wilms tumor- related 1 (WT1), and Lhx9 are essential for development of the indifferent gonads in both sexes, but identifying genes that function specifically in ovarian development has been problematic. This talk will summarize efforts to use expression profiling to identify genes that play key roles in ovarian development and to use the Cre-loxP strategy to effect targeted gene inactivation in specific cell types in the developing gonads.

S5-23 Paediatric Gynaecology

Amenorrhea: Diagnosis and Management

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Both congenital and acquired anomalies in the structure of the uterus and vagina could produce amenorrhea: neverheless, in the vast majority of patients, amenorrhea is related to an ovarian malfunction. Diagnostic work-up includes history, physical examination, laboratory data and imaging. Amenorrhea resulting from ovarian malfunction is associated with 4 distinct endocrine conditions. Hyperprolactinemic amenorrhea is often associated with a pituitary adenoma. Surgical adenomectomy is a very infrequent option. Prolactin-lowering drugs, cyclical progestogen and hormone replacement therapy are the different choices of treatment for cycle disturbance. Hypogonadotrophic amenorrhea is frequently associated with stress and nutritional deficiency. If this is the case the patient should simply be counselled. A sequential use of estrogen and progestogen can be

suggested to prevent estrogen deficiency or for physychological reasons. If contraception is needed, oral contraception may be the choice for both cycle and fertility control. Hypergonadotrophic amenorrhea is the result of the premature ovarian failure. There is no curative therapy for these patients, however, a long term hypoestrogenic condition should be treated with estrogen to cure symptoms and to prevent osteoporosis. Normogonadotrophic amenorrhea is caused by some disturbance in the ovarian function. Since these women have some ovarian activity, they are not hypoestrogenic and will bleed in response to progestogen withdrawal. Most of these patients are likely to have polycystic ovarian disease (PCO). Menstrual bleeding can be induced in these women by cyclical progestogen administration or the sequential use of estrogen plus progestogen. Oral contraception is indicated not only in patients who desire to be protected against pregnancy but also in women with acne and hirsutism.

S5-24 Paediatric Gynaecology

Contraception During Adolescence

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Sexual health for adolescents is based on three components: Recognizing sexual rights, sexuality education and counselling, and confidential high quality services. Sexuality education needs to balance between prevention of pregnancies, prevention of STIs, and allowing for sexuality as a positive resource rather than a threat Contraception needs to include prevention of both STIs and pregnancies. The first option is condoms backed-up by emergency contraception. A recent WHO study showed, that emergency contraception can be taken as a single dose of 1.5 mg levonorgestrel. Later there is a switch to oral contraceptives or other hormonal contraception in a longer relationship. Breast, pelvic and genital examination, and routine laboratory tests are not necessary before starting hormonal contraception. Condom use should not be stopped before it is reasonable certain that the partner is STI-negative. Other alternatives can be considered in special cases. Improved contraceptive methods do not automatically lead to reduced numbers of abortions. The prevention of unintended pregnancies requires a desire to use protection, a good contraceptive method, ability to obtain the contraceptive method, and ability to use it. High quality sexual health services for adolescents calls for special clinics. These should have a youth-friendly atmosphere, where young people can feel comfortable. Unquestionable confidentiality is important. The providers must not moralize and judge the adolescents, but treat adolescents with respect indicating that young people are important. Services should be available at an affordable price, which preferably means free of charge. The threshold to come to the clinic should be low. When adolescent sexuality is not condemned but sexuality education and sexual health services are provided, it is possible to profoundly improve adolescent sexual health with comparatively small costs. Each year new groups of young people mature, requiring new efforts.

S6-25 Sexual Dimorphism in Child Growth

Actions of Sex Steroids on the Growth Plate

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Longitudinal growth is determined by a variety of hormones and growth factors. During puberty, estrogen contributes to the pubertal growth spurt through a stimulation of the somatotropic axis. A male patient with inactivated estrogen receptor (ER)-alfa gene and patients with aromatase mutations have confirmed the importance of estrogen in the regulation of growth plate fusion in females and males. This effect may be mediated via a direct effect on growth plate chondrocytes. Indeed, we now that both known estrogen receptors, ERalfa and ERbeta are expressed in the growth plate, in both boys and girls, throughout pubertal development. Any functional role of ERalfa has yet not been defined in the human growth plate. However, recent data obtained in female mice with inactivated ERalfa and/or ERbeta, suggest that stimulation of ERalfa has the capacity to inhibit skeletal growth and also to mediate growth plate fusion. An increased understanding about the effects of estrogen and interactions between estrogens and other endocrine factors within the epiphyseal growth plate is important for development of new treatment strategies in different disorders affecting longitudinal bone growth. Selective estrogen receptor modulators (SERMs) might be useful tools for modulation of pubertal growth. This possibility is supported by a recent report in rabbits showing that the SERM, raloxifene, acts as an estrogen receptor agonist on the growth plate without affecting the uterus. More studies are needed to further define the functional role of different ERs in the regulation of epiphyseal growth and growth plate fusion. This could open the door to more specific treatment modalities affecting longitudinal growth and growth plate fusion.

S6-26 Sexual Dimorphism in Child Growth

Sexual Dimorphism in Intrauterine and Infantile Growth

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It is well known that there are gender differences in birth size in many species. The human is no exception with males displaying a greater length, weight and head circumference than females. The increase in male body weight arises predominantly from an increase in muscle mass whereas in females fat mass is greater – a situation analogous to puberty. These differences in body size can be traced back to early stages of intrauterine growth as at 20 weeks of gestation significant gender differences exist in terms of head circumference. This process of encephalisation is unique to humans but places a considerable strain on energy delivery. The key to energy delivery is placental function and changes in placental size markedly influence intrauterine growth and appear to be key predictors of post natal growth up to 6 months of age. An important component of the fetal growth process is the effects of insulin-like growth factors (IGF) 1 and 2 and both of these peptides display sexual dimorphism with higher concentrations observed in male infants. The circulating concentration of these peptides are influenced by a number of adverse events in pregnancy such as smoking which suggests, along with clinical and animal studies, that the IGF system is the final common pathway for the promotion of intrauterine and early post natal growth. Although sexual dimorphism exists in the growth hormone and IGF-1 axis at birth, this reflects differential feed back effects of IGF-1 rather than a programming effect on the growth hormone axis at this stage. Understanding the early growth process may play an important role in unravelling the gender differences in mortality rates for cardiovascular disease, where the likelihood of disease curves in males antedate those in females by some 4 to 5 years and appear to be set from an early stage in human development.

S6-27 Sexual Dimorphism in Child Growth

Sexual Dimorphism in Body Composition

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Sexual dimorphism in the development of body composition during puberty has important implications for development of later adult disease risk. Overall, females gain 60% body weight between 11-18 years and males 78% between 12-19 years. This weight increase is partly attributed to gain in fat free mass of 30% in females and 50% in males. However, during later puberty, percentage fat mass tends to decrease in boys, while fat mass continues to rise by 1.14 kg/year in females. Fat mass is closely related to circulating leptin concentrations, and during puberty these levels tend to rise in girls, and fall in boys, reflecting their different changes in body composition. In girls, but not boys, leptin levels at the onset of puberty are predictive of subsequent gains in percentage fat mass. Sexual dimorphism in Adiponectin, IGF-I and insulin levels is also observed during puberty, but the extent to which these reflect or determine change in body composition is unclear. Sex steroids are important determinants of 'android' and 'gynaecoid' fat distribution; visceral adiposity being closely related to insulin resistance and cardiovascular risk factors. In males, central fat accumulation is associated with elevated blood pressure and subsequent cardiovascular risk. In females, central fat accumulation and insulin resistance are related to elevated androgen levels, particularly in girls with low birthweight followed by postnatal catch-up growth and precocious pubarche: a sequence which leads to ovarian hyperandrogenism. In these subjects, body composition can be normalised by combination therapy with metformin and flutamide. Body composition in puberty 'tracks' into adult life and common genetic variation is likely to be important. As yet, apart from recent links between aromatase and androgen receptor gene variants and androgen levels and body composition in girls, there are few data, however larger studies are currently in progress.

S7-28 Capillary Blood Glucose Determination

The Theoretical Basis of Glucose Monitoring

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The aim of glucose monitoring is to give the possibility to the diabetic patient to master the control of his (her) glycaemia. In type 1 diabetes, the main aim is to control the effect of the administered insulin. In type 2 diabetes, to estimate the quality of the achieved metabolic control. It is at the beginning of the 80s in the last century that glucose monitoring took its development, essentially due to the appearance of the first fingerprick devices. Within a few years, glucose monitoring widely replaced urinary monitoring, this one remaining nevertheless indispensable mainly in type 1 diabetes to detect ketosis episodes. During the 20 years which followed, glucose meters became smaller, the time of measurement dropped from two minutes to a few seconds, and the volume of the necessary drop of blood is now in the order of a few microliters only. More recently, a new concept appeared: continuous glucose monitoring; using a glucose sensor. Several configurations are possible, and some systems are already on the market. Thus, the CGMS developed by Minimed-Medtronic uses a glucose sensor having the shape of a needle implanted in the subcutaneous tissue; the GlucoDay system developed by Menarini uses a microdialysis fibre also implanted in the subcutaneous tissue. The GlucoWatch system developed by Cygnus is less invasive and measures the glucose extracted from the skin by iontophoresis. The ultimate goal is the control by the glucose sensor of the flow rate of a pump delivering insulin, leading to the development of an artificial beta cell. It is important to consider carefully the physiology of glucose concentration in blood an tissues to understand the results of glucose monitoring.. We shall take two examples : 1) one suggests currently to measure glycaemia in alternate sites, at the forearm for example. It is necessary to pay attention to the fact that there are differences between blood glucose measured at the forearm and at the fingertip, where blood is more arterial. In blood sampled at the forearm, changes in glycaemia are slower, and one risks to underestimate episodes of hypoglycaemia. 2) Systems for continuous glucose monitoring often measure glucose not in blood but in interstitial fluid. There are also important differences between changes in glucose concentration in blood and in interstitial space, because insulin pulls glucose from this space to the surrounding cells. This explains for example that concentration in glucose in this liquid is often lower than glycaemia. The existence of lags between changes in blood glucose and in interstitial glucose concentration must be taken into account if one envisages this type of system as a hypoglycaemic alarm. It will also be necessary to take into account this issue for the development of closed-loop insulin delivery systems: slow kinetics would lead to abnormal oscillations between hyper and hypoglycaemia.

S7-29 Capillary Blood Glucose Determination

Continuous Subcutaneous Glucose Monitoring in Children

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Pediatric experience with continuous glucose sensors is increasing. As maintaining blood glucose levels within the target range can be an elusive goal in children with diabetes mellitus these monitors are used predominantly to identify factors that may contribute to glycemic instability. Several studies using such systems demonstrated facilitated and improved pediatric diabetes treatment, and patients received new insight and increased motivation. Also they have been shown to be of diagnostic value in assessing glucose fluctuations in MODY and Type 2 diabetes cases. A second reason for using these devices in children is to identify asymptomatic hypoglycaemia particularly at night. In this regard it is important whether under all circumstances changes in blood glucose are paralleled by glucose changes in the interstitial fluid. Recent data studying the two most widely applied continuous sensors, the GlucoWatch G2 Biographer and the continuous glucose monitoring system (CGMS), in pediatric patients indicated that they do not reliably detect hypoglycaemia. Thus, these devices perform better at higher glucose levels, suggesting they may be more useful in reducing HbA1c levels than in detecting hypoglycemia. Finally, the sensors may also allow to characterize the day-to-day and within-day blood glucose variability. This is of particular importance as differences in blood glucose fluctuations may contribute to the development of diabetic late complications independent of HbA1c. Postprandial hyperglycemia can exist despite excellent HbA1c and target preprandial glucose levels. The HbA1c is known to mask high and low fluctuations. Calculating the glucose area under the continuous glucose profiles has been shown to serve as indicator of glycemic variability. Thus, in addition to HbA1c and self-blood glucose monitoring, continuous subcutaneous glucose monitoring may turn out to be the third pillar in the assessment of metabolic control in pediatric diabetes patients.

S7-30 Capillary Blood Glucose Determination

Glucose Monitoring Systems in the Future

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Nature´s biological master model for a lifelong truly continous glucose monitoring(GM) system in vivo, e.g. within the pancreatic islet or the hypothalamus, and their inclusion into a feed back system for insulin secretion/production and a rather complex regulation of glucohomoeostasis represents the challenging standard that has not been met by any marketed GM device so far. Despite the fact that for the foreseeable future of the next 5 – 10 years such a perfect GM system will probably not be available (except for a few 100 pancreas transplants/year), considerable efforts are invested globally into a large variety of minimally or non-invasive GM techniques for spot as well as continous GM at different skin sites and depths, but also in the eye, the saliva or even the breath, to replace the current use of invasive strip based systems for capillary blood. The industrial technological research examines for non-invasive GM e.g. different spectral regions (ultra violet, visible, near infra red, mid infra red, infra red, microwave), various spectroscopies (magnetic resonance, fluorescence, raman, photothermal, photoacoustic, radio wave), electrical impedance or swept frequency acoustic interferometry. At a more invasive level mainly s.c. implantation models with a “lifetime” up to several months are examined either of completely arteficial GM devices or of “biosensors” derived from isolated islets of different species with the obvious need for some form of encapsulation to avoid local and/or systemic destructive reactions in vivo. Which of these GM system developments will finally satisfy the daily needs particularly of our pediatric patients remains speculative at present. Intravenous placement of an enzyme-based needle glucose sensor has been successfully studied in few adult patients with type 1 diabetes for several months, including 24 hours pilot closed loops with an insulin pump, but this approach is of questionable value for general use due to its very invasive nature and the related potential serious side effects. The combination of a more generally applicable GM system with an insulin pump remains the ultimate longterm goal of an arteficial pancreas.

S8-31 Endocrine Regulation of Adiposity

Regulation of Adipocyte Differentiation and Function

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Adipocytes serve a main function as a site of storage of excess energy in the form of triacylglycerides. However, equally important is the secretion of factors (so-called adipokines) from adipocytes, many of which regulate whole body metabolism and homeostasis. Adipocyte differentiation and function are controlled by a large number of endocrine and paracrine factors. Some of the paracrine factors originate from the preadipocytes/adipocytes themselves, whereas others originate from non-adipocytes cell types like macrophages and endothelial cells. It has recently become increasingly clear that obesity can be seen as an inflammation of the adipose tissue in which macrophages accumulate in the tissue. The cytokines released by the macrophages have profound effects on adipocyte function. This talk will review the current knowledge on how endocrine and paracrine factors affect adipocyte differentiation and function. A large body of evidence suggests that specific fatty acids like isomers of conjugated linoleic acid (CLA) may have significant effect on adipose tissue function. Our recent data on how isomers of CLA affect both differentiation and function of adipocytes will be presented. Using cultures of primary human in vitro differentiated adipocytes, we have shown that trans-10, cis-12 CLA, but not cis-9, trans-11, inhibits both differentiation of preadipocytes as well insulin sensitivity and expression of adipocyte-specific markers in mature adipocytes.

S8-32 Endocrine Regulation of Adiposity

Genetics of Severe Childhood Obesity

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The identification and characterization of patients with morbid obesity due to mutations in single genes has shed light on the molecular mechanisms underlying the hypothalamic regulation of appetite, body weight and endocrine axes. Two severely obese cousins in a consanguineous family were found to have undetectable levels of serum leptin and were homozygous for a frameshift mutation in the ob gene. These children were severely hyperphagic, constantly demanding food and developed severe disabling obesity, impaired T cell mediated immunity and hypogonadotropic hypogonadism. Treatment with recombinant human leptin for up to six years led to sustained, beneficial effects on appetite, fat mass, hyperinsulinaemia and hyperlipidaemia. The major impact of leptin was on food intake with a marked reduction in caloric consumption during a test meal. Leptin administration permits the full progression of appropriately timed puberty but does not appear to cause precocious activation of puberty in younger children. Mutations in the leptin receptor result in a similar phenotype. We have recruited over 1000 patients with severe, early onset obesity as part of the Genetics of Obesity Study (GOOS). Complete loss of pro-opiomelanocortin derived peptides results in isolated ACTH deficiency, red hair, pale skin and obesity. We have recently identified a second patient who is a compound heterozygote for mutations in pro-hormone convertase-1 which results in a complex endocrinopathy and enteropathy due to a failure of prohormone processing. Loss of function mutations in the melanocortin 4 receptor (MC4R) cause a dominantly inherited obesity syndrome that accounts for up to 6% of patients with severe, early-onset obesity. MC4R deficiency is characterised by hyperphagia, severe hyperinsulinaemia and increased linear growth. These studies provide evidence for the pivotal role of the leptin-melanocortin system in energy balance and neuroendocrine function in humans.

S8-33 Endocrine Regulation of Adiposity

International Consensus Development on Childhood Obesity

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In light of the current worldwide epidemic in childhood obesity, a multidisciplinary group of professionals with a special interest in obesity met to develop a wide ranging consensus statement on all aspects of the problem. We hoped that by drawing upon current evidence that we could highlight issues relating to the care of obese children contribute towards prevention strategies and examine areas requiring further research.

The consensus process: Sixty five professionals (paediatric endocrinologists, community paediatricians, adult endocrinologists, dietitians, psychologists and exercise physiologists) from eight countries convened for 3 days at the Dead Sea in Israel. Prior to the meeting seven groups were formed to address the areas of prevalence, treatment, prevention, psychology, diagnosis, risks and causes. Participants each undertook to address key questions, appraise the literature and draft a document which was circulated to their group. At the meeting the documents were discussed in groups and then by the entire meeting, recommendations were drawn up and agreement was reached.

The consensus conclusions: Debate was extensive, with emphasis on the environmental (as opposed to endocrine) causes of obesity, the need for lifestyle change and how this can be achieved. Controversial issues included

· The use of BMI as a measure and the importance of the IOTF criteria
· The value of waist circumference
· The problem of obesity in infancy
· Investigation for comorbidities
· The need for prenatal prevention strategies
· The definition of the metabolic syndrome in childhood

By the end of the meeting consensus was achieved and a document is in preparation for publication for the medical community. We believe that the development of the consensus statement will provide a valuable contribution towards the development of improved clinical care, help delineate approaches to prevention and signpost areas requiring further research.

S9-34 Gene Imprinting in Paediatric Endocrinology

Basic Mechanisms of Imprinting

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Although most genes are expressed from both alleles, some genes are genomically imprinted such that one allele, the maternal or paternal, is active while the other is silenced. This process is carried out at the molecular level by marking the maternal and paternal alleles in the gametes and maintaining this differential epigenetic pattern throughout development. While DNA methylation plays an important role in this regulatory mechanism, these gene regions are also characterized by asynchronous replication timing whereby one allele replicates early in S phase, while the other replicates late. This differential marking probably affect expression by modifying chromatin structure. Genomic imprinting is actually part of the more general phenomenon of random allelic exclusion, whereby some cells express the maternal allele while others express the paternal allele. This type of monoallelic expression is characteristic of the immune and olfactory receptor loci where it plays a key role in the generation of gene diversity.

S9-35 Gene Imprinting in Paediatric Endocrinology

Role of Genomic Imprinting in Fetal Growth Restriction

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Genomic imprinting is an epigenetic phenomenon resulting in monoallelic expression of genes in a parent of origin dependent manner. It is found almost exclusively in eutherian mammals, and is known to be an important regulatory pathway in growth, development and behaviour in the fetus. About 70 imprinted genes have been found so far in the mouse, and for the majority the imprinting status is conserved in humans Phenotypic effects observed from the disruption of imprinting in mouse and man are often growth-related and occur in utero. The paternal and maternal genomes have been shown to be non-complementary in terms of murine embryonic development, and it is these experiments that first illustrated the involvement of imprinting in normal fetal growth. Parthenogenotes, containing two maternal genomes, give rise to embryos that do not develop well and die after implantation due to failure of the extra-embryonic components. Conversely, androgenotes have development of extra-embryonic tissues but failure of the embryo. Paternally expressed genes appear to be critical for placental development. Recently it has been reported that some genes in the mouse are only imprinted in the placenta, and therefore may affect growth by influencing the fetal demand for, and the placental supply of, nutrients from the mother. Generally, imprinted genes that are paternally expressed enhance growth and maternally expressed genes suppress it. This paternal versus maternal genome tug-of-war is the basis of the genetic conflict hypothesis. It is suggested that paternally derived genes influence nutrient acquisition by selecting more nutrients for the current fetus, while maternally derived genes balance the provision of nutrients to the current fetus protecting her potential for future successful pregnancies. These observations will be discussed with respect to human fetal growth restriction and the genetic aetiology of the growth restriction phenotype in Silver-Russell syndrome.

S9-36 Gene Imprinting in Paediatric Endocrinology

Pseudohypoparathyroidism: a Spectrum of Imprinted Disorders Caused by Different Coding and Non-Coding Mutations in GNAS

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Pseudohypoparathyroidism (PHP), i.e. hypocalcemia and hyperphosphatemia due to renal PTH resistance, was first described by Fuller Albright and his colleagues. These patients furthermore presented with clinical features now referred to as Albright's hereditary osteodystrophy. Inactivating stimulatory G protein (Gs-alpha) mutations cause this form of PHP, PHP-Ia. Gs-alpha is encoded by exons 1-13 of GNAS, a complex gene which undergoes parent-specific methylation and gives rise to at least five different alternatively spliced mRNAs that are, with the

exception of Gs-alpha, transcribed from the non-methylated parental allele. Similar or identical GNAS mutations are also found in patients with pseudohypoparathyroidism (PPHP) and progressive osseous heteroplasia (POH). In contrast, most patients affected by PHP-Ib have no mutations in one of the 13 exons of GNAS that encode Gs-alpha. However, a familial form of PHP-Ib with an autosomal dominant mode of inheritance and paternal imprinting (AD-PHP-Ib) was mapped to a 2.5-Mb locus on 20q13.3 comprising a portion of GNAS. Moreover, most familial PHP-Ib cases show a loss of methylation at the exon A/B differentially methylated region (DMR) of GNAS. Recently, a 3-kb deletion located approximately 220-kb upstream of exon A/B was identified in more than 20 AD-PHP-Ib kindreds, but not in healthy controls. The deletion was shown to be inherited maternally and to be associated with loss of methylation at exon A/B alone. The identified deletion likely disrupts a cis-acting element necessary for establishment and/or maintenance of the methylation imprint at GNAS exon A/B of the maternal allele. The loss of exon A/B methylation results in suppression of Gs-alpha transcription in the renal cortex leading to PTH resistance. Since the 3-kb deletion was not identified in some AD-PHP-Ib families and many sporadic cases, additional genetic defects may be involved in the development of PHP.