Growth and Growth Factors

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Epigenetic alteration in the imprinted 11p15 region, which leads to intrauterine growth retardation and Silver-Russell syndrome, is selected as the mechanism of the year in this chapter. Under new paradigms, we discover that the constitutive activity of the growth hormone secretagogue receptor may have a role in growth, and that maternal ghrelin is important for fetal growth. We also learn new mechanisms of activation of the PRL and GH receptor. Then, we are reassured that estrogen replacement may be administered at a physiological age in girls with Turner syndrome, with no deleterious effect on growth, which is important for clinical practice. Under clinical trials, we discuss the effects of a long-acting analogue of growth hormone-releasing hormone, those of a rhIGF-I/rhIGFBP3 complex and we also learn about the unexpected effect of metformin on growth in girls born small for gestational age with early-normal puberty. Under new mechanisms, we find how proconvertase 4 controls placenta IGF-II production, and how IGF-II interacts with placental transport systems. Two new genes involved in growth are presented: first, mutations in the natriuretic peptide receptor B gene may be a common cause of apparently idiopathic short stature, then mutations in the CUL7 gene are responsible for the 3-M syndrome. Two reviews are selected in this chapter, on negative regulation of GH receptor signaling, a new and expanding topic, and on Marfan’s syndrome. Finally, in the food for thought section, the putative links between growth, inflammation, and cardiovascular disease are emphasized.

Mechanism of the year
Too little IGF-2, too short

Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome
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Background: Silver-Russell syndrome (SRS) is a congenital disorder characterized by severe intrauterine and postnatal growth retardation, dysmorphic facial features and body asymmetry. Maternal uniparental disomy with respect to chromosome 7 has been found in 10% of affected individuals. Another candidate locus could be the 11p15 region, which contains a cluster of imprinted genes, including IGF-2, that are crucial to the control of fetal growth. The authors investigated whether individuals with SRS had epigenetic defects in the 11p15 region.

Methods: Study of the methylation status of KvDMR1 in KCNQ1OT1, the H19 promoter, IGF2 DMR2 (differentially methylated region) and H19-IGF2 ICR1 (imprinting center region) by Southern blot in leukocyte DNA from 9 SRS patients.

Results: An epimutation (demethylation) in the telomeric domain involving the H19 promoter, ICR1 and IGF2 DMR2 in the 11p15 region has been identified in 5 individuals with clinically typical SRS. This methylation defect was associated with relaxation of imprinting and biallelic expression of H19 and downregulation of IGF2.

Conclusions: The 11p15 imprinted region is involved in SRS. The epimutation consists of a partial loss of paternal methylation marks at three different loci in the telomeric imprinted domain. It results in decreased IGF2 expression and fetal growth retardation.
Imprinting is an epigenetic phenomenon characterized by parental allele-specific differences in gene expression. Human chromosome 11p15 includes paternally expressed (maternally imprinted) genes (such as \textit{IGF2} and \textit{KCNQ1OT1}) and maternally expressed (paternally imprinted) genes (such as \textit{CDKN1C} and \textit{H19}). Genetic or epigenetic alterations in the imprinted 11p15 region have been described in Beckwith-Wiedemann syndrome (BWS), characterized by prenatal and postnatal overgrowth, macroGLOSSia, abdominal wall defects, organomegaly, hemihyperplasia, hypoglycemia and an increased risk of childhood tumors: about 10\% of BWS have hypermethylation of the \textit{H19} promoter [1, 2] which is associated with loss of expression of \textit{H19}, hypermethylation of \textit{IGF2 DMR2} and biallelic expression of \textit{IGF2}. At the opposite end, this study shows that an epimutation in the telomeric imprinting center region on chromosome 11p15 is also involved in SRS. It results in the relaxation of imprinting of \textit{H19} and decreased \textit{IGF2} expression (fig. 1). Moreover, this loss of paternal methylation was partial and, together with the well-known body asymmetry that characterizes SRS, it suggests a mosaic distribution of the epimutation. This mechanism may be involved in most of the individuals clinically classified as SRS. Once more, this observation is in agreement with the parental conflict paradigm of imprinting, which suggests that genomic imprinting allows the paternal genome to promote growth of the fetus and the maternal genome to inhibit growth. Whether the affected subjects have low circulating IGF-II is unknown. Future studies will have to determine the mechanism responsible for this loss of paternal methylation of the 11p15 telomeric domain. It is remarkable that monozygotic twins are overrepresented among individuals with BWS. However, pairs of identical twins are almost always discordant for BWS although the twins always have the same epigenetic defect.

\textbf{Fig. 1.} Map of the 11p15 imprinted region. The \textit{H19} and \textit{IGF2} genes compete for a common set of downstream enhancers located 3’ of the \textit{H19} gene. DMR1 (also called ICR1) is located 2 kb upstream of the \textit{H19} gene and regulates the reciprocal imprinted expression of \textit{H19} and \textit{IGF2} gene by functioning as a chromatin boundary element or insulator. On the maternal chromosome, DMR1 is unmethylated, permitting the binding of a zinc finger protein called CTCF. Binding of CTCF blocks access of the \textit{IGF2} promoter to the downstream enhancers. Thus, the maternal copy of \textit{H19} is activated by these enhancers and is transcribed. Methylation of the paternal copy of DMR1 and the \textit{H19} promoter silences the \textit{H19} promoter and prevents binding of the CTCF protein to DMR1. As a result, the \textit{IGF2} promoter can access the downstream enhancers and \textit{H19} is silenced. In SRS, the paternal chromosome is ‘maternalized’, leading to downregulation of IGF-II expression.
New paradigms
Is there a role for the constitutive activity of the growth hormone secretagogue receptor?

Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature
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Background: The growth hormone secretagogue (GHS) receptor (GHSR) displays a constitutive activity whose clinical relevance is unknown. Pharmacological doses of ghrelin, its endogenous ligand, were shown to stimulate GH secretion and appetite, but the physiological importance of the GHSR-dependent pathways are questioned.

Methods: The authors searched for GHSR mutations in 92 subjects with idiopathic growth hormone deficiency or idiopathic short stature.

Results: They identified a GHSR missense mutation that segregates with short stature within two unrelated families. The mutation resulted in decreased cell-surface expression of the receptor and selectively impaired the constitutive activity of the GHSR, while preserving its ability to respond to ghrelin.

Conclusions: Disruption of GHSR constitutive activity could be a cause of short stature in humans.

Ghrelin, an acylated 28 amino acid peptide isolated from human and rat stomach, has been shown to increase appetite, stimulate growth hormone secretion, and promote fat accumulation and neoglucogenesis. It has been suggested that ghrelin may be involved in the control of energy expenditure in situations of energy shortage. The physiological relevance of these properties is unknown. The ghrelin receptor, a 7-transmembrane segment receptor, is known from in vitro studies to signal in the absence of ghrelin at almost 50% of its maximal capacity [3]. Similarly, the physiological relevance of this constitutive activity is unknown. One would expect short stature and obesity as a phenotypic result of disruption of the GHS receptor pathway. However, animal studies in GHSR null mice have shown minimal phenotypic consequences of GHSR inactivation [4]. In the present study, the authors found a C-to-A transversion located within the first GHSR exon (c.611C→A), either homozygous or heterozygous, in 2 unrelated subjects with short stature (heights of –3.7 and –3.2 SDS) and labeled as having idiopathic short stature or idiopathic partial GH deficiency. Familial studies found the heterozygous mutation in 8 additional subjects with heights of –3.7 to +1.0 SDS, indicating that the 9 heterozygous carriers did not all have short stature, an observation that is consistent with incomplete penetrance of this phenotype. It can rather easily be rationalized that loss-of-function mutations in the ghrelin receptor would lead to the development of short stature. In contrast, with respect to the control of appetite and energy expenditure, it would be expected that loss-of-function mutations in the ghrelin receptor would lead to a lean and not an obese phenotype, because ghrelin is a potent hunger signal. Indeed, recent studies in mice have shown that knockout of either the ghrelin hormone or the ghrelin receptor protects against obesity induced by a high-fat diet. In the present report, the subjects in family 1 were overweight, whereas those in family 2 were lean. In vitro studies showed that the mutation was associated with decreased cell surface expression of GHSR (20% of the wild type), and decreased signal transduction (about half of the wild type). In addition, this substitution was not found in 100 unrelated subjects with normal stature. In mutation carriers, ghrelin levels were in the normal range and GH secretion and IGF-I levels were either normal or slightly decreased. Finally, the phenotypic consequences of the genotype were questionable in this work, despite convincing in vitro experiments. Further studies will be needed to ascertain the role of GHSR mutations in subjects with short stature. This could be the second demonstration of the importance of the constitutive activity of the 7 transmembrane segment receptors in humans, after mutations of the melanocortin receptor 4 (MC4R), which abrogate the constitutive activity of the receptor and segregate with obesity [5]. In the meantime we also learn that maternal ghrelin plays an important role in rat fetal development during pregnancy (see below).
Maternal ghrelin plays an important role in rat fetal development during pregnancy

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Background: Ghrelin, an acylated peptide isolated from rat and human stomach, is the endogenous ligand for growth hormone secretagogue receptor (GHS-R). Ghrelin administration increases appetite, stimulates growth hormone secretion, and promotes fat accumulation and neoglucogenesis. Whether ghrelin is implicated in the control of maternal and fetal energy use during gestation is not known.

Methods: The authors studied the expression of GHS-R in fetal tissues and examined the effect of ghrelin administration in pregnant rats.

Results: High levels of ghrelin receptor mRNA were detected in various peripheral fetal tissues beginning at embryonic 14 day (E14) and lasting until birth. Both des-acyl ghrelin and acyl ghrelin bind to fetal tissues. Treatment of mothers with ghrelin showed that it transits easily to the fetal circulation, and resulted in an increase in birth weight in comparison to newborns from saline-treated mothers, even when maternal food intake following ghrelin treatment was restricted by pair-feeding. Conversely, active immunization of mothers against ghrelin decreased fetal body weight during pregnancy. High levels of des-acyl ghrelin were detected in fetal blood and amniotic fluid. Both acylated and des-acyl ghrelin increased 3H-thymidine and BrdU incorporation of cultured fetal skin cells in a dose-dependent manner.

Conclusions: These results indicate that maternal ghrelin regulates fetal development during the late stages of pregnancy.

Fetal growth is mainly influenced by nutrition provided by the mother. It now seems that the fetus may also have a role in trafficking the energy, and that ghrelin may take part in this role. Although the main source of ghrelin is the stomach, it has also been detected in the hypothalamus, pituitary gland, liver, kidney, pancreas and placenta. The functional ghrelin receptor GHS-R-1a, highly conserved from fish to humans, is expressed widely in both central and peripheral organs, including the brain, pituitary gland, and pancreas. The broad distribution of GHS-R-1a suggests that ghrelin, or at least its receptor, may play important roles. In this work, the administration of acyl-ghrelin to mothers (you recall that ghrelin has this unique structure with an acyl epitope) increased the weight of their newborns, an effect independent of fetal GH and IGF-I production. This might result from a direct effect on fetal tissue, since ghrelin was able to cross the placenta to attain the fetal circulation and promote [3H]-thymidine and BrdU incorporation in fetal skin cells. Interestingly, des-acyl ghrelin stimulated proliferation more potently than acyl ghrelin, although des-acyl ghrelin was thought to be inactive, based on its inability to bind and activate the GHS-R-1a. This suggests that fetal skin cells have several types of receptors: one is the classical receptor for acyl ghrelin, GHS-R-1a, and the other is a novel, yet to be identified, receptor for des-acyl ghrelin. Other studies have reported proliferative activities of des-acyl ghrelin in osteoblasts [6], further suggesting that the GHS-R-1a is unlikely to be the only active GHS-R. Studies of the activities of ghrelin and its receptors are becoming more and more complex…
**Friend or foe, the final balance between agonism and antagonism**

**Two wrongs can make a right: dimers of prolactin and growth hormone receptor antagonists behave as agonists**

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**Background:** Prolactin (PRL) and growth hormone (GH) belong to the superfamily of cytokines. They have two distinct receptor-binding sites (site 1 with higher affinity; site 2 with lower affinity) that each interact with a receptor in a sequential manner: the functional receptor dimer that activates signal transduction. The G129R mutation in PRL and the G120R mutation in GH produce PRL receptor antagonists by disrupting the integrity of binding site 2. The aim of the study was to investigate the molecular interactions between ligands and the PRL receptor.

**Methods:** The authors engineered dimeric forms of the ligands (hGH, hPRL) and their antagonists, including homodimers (PRL-PRL, GH-GH) with 4 potentially functional binding sites, heterodimers (PRL-G129R, G129R-PRL) with 3 potential binding sites, and homodimers (G129R-G129R, G120R-G120R) with 2 potential binding sites. They examined their ability to bind to PRL receptors, induce PRL receptors dimerization, activate signaling pathways associated with the PRL receptor (STAT5, Erk1/2 and Akt phosphorylation assays), and mediate cell proliferation in vitro.

**Results:** In contrast to monomeric hPRL-G129R, homodimeric hPRL-G129R induced PRL receptor dimerization; activated JAK2/STAT5, Ras/Raf/MEK/Erk, and PI3-K/Akt signaling, and stimulated Nb2 cell proliferation. Similarly, homodimeric hGH-G120R was able to mediate signaling via the PRLR and to stimulate Nb2 cell proliferation.

**Conclusions:** A ligand with at least two functional binding sites can act as partial agonist and induce receptor dimerization. A second receptor-binding site 1 can replace the need for a site 2. The PRL/GH receptor family exhibits a considerable degree of flexibility in accommodating ligands and transducing their signals.

Prolactin (PRL) and growth hormone (GH) have similar tertiary structures, as do the extracellular regions of the receptors to which they bind. Both hormones have been detected in the circulation as monomers, dimeric and oligomeric forms. They have two separate receptor binding sites with drastically different binding affinities to the receptor extracellular domains (ECD). As a consequence, each binding site interacts with the receptor homodimer that activates signal transduction. Substitution of a Gly residue in the third α-helix with an Arg residue alters the function of site 2, and results in antagonists (hGH-G120R, hPRL-G129R, hPL-G120R) with little or no biological activity. In this paper, the authors show that homodimeric antagonists were able to activate the PRLR signaling pathways. This functional study highlights the plasticity of the PRL/GH receptor family which can accommodate different ligands and transduce their signals. Interestingly, mutations in the site 2 binding site of GH have been described as responsible for biologically inactive GH in humans [7]. This study suggests that the concept of biologically inactive GH can be even more complex, from antagonistic to partially agonistic activities. In addition, the findings may have implications for designing ligand-based therapeutic agents that target this family of receptors, which may be of interest for cancer or growth diseases.
Salutary effects of combining early very low-dose systemic estradiol with growth hormone therapy in girls with Turner syndrome

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Background: Determining the optimal estrogen replacement regimen with growth hormone (GH) therapy remains a challenge in the management of teenage girls with Turner syndrome. The authors postulated that a very low dose of estradiol administered early with GH will preserve growth potential.

Methods: Multicenter randomized study of either early (12.0–12.9 years; n = 7) or late (14.0–14.9 years; n = 7) estrogen treatment in girls with Turner syndrome who began GH before age 12.0 years. Depot estradiol, 0.2 mg/month i.m., was given initially and increased every 6 months to a maximum of 3 mg/month; GH was 0.05 mg/kg daily. Follow-up was at 3.5 years or later. These girls were matched to the National Cooperative Growth Study registry patients who began GH and oral conjugated estrogen at similar ages and were similarly followed to adult or near-adult height.

Results: Patients treated with depot estradiol at low dose reached an adult or near-adult height significantly greater than that predicted at 12 years of age (p < 0.02). Overall height potential was gained in the first 2 years of the study, during which the early group grew 3.5 cm more than the late group, which was receiving GH alone (p < 0.01). The early depot estradiol group also gained 5.9 cm more height after starting estrogen than did the early National Cooperative Growth Study group treated with oral conjugated estrogen (p < 0.05). Although feminization proceeded slowly on the lowest dose of estradiol, it advanced normally after 6 months of treatment.

Conclusions: Very low-dose parenteral estradiol administered early (in the 12th year) with GH preserves height potential, and possibly improves adult height without interfering with GH treatment and permits relatively age-appropriate feminization.

This study confirms once again that the dose, route, form, and timing of estrogen replacement are important determinants of estrogen effects on growth. Whereas this study suffers from all the classical pitfalls of growth hormone (GH) trials, and ‘early estradiol’ at age 12 is not early at all as compared to 10–10.5 years as a mean age of normal pubertal onset, this is the first study to demonstrate that very low-dose systemic estradiol administered at a late physiological age with GH increases height velocity more than GH alone, and is superior or equal to late administration (14th year of age) with regard to height gain and adult height. Two previous randomized studies in girls with Turner syndrome have shown that early estrogen administration could reduce final height by about 3–5 cm [8, 9], contrasting with a third that suggested that the age makes no difference [10]. However, in these studies the oral route was used and/or the estrogen dose was considerably higher than that used here. Conversely, large retrospective studies generally did not find a difference between ‘early’ and ‘late’ estrogen administration on final height of girls with Turner syndrome: however, the cut-off between early and late administration was 14–15 years, the protocols for estrogen as well as GH administration were variable, and it was difficult from these studies to infer that estrogen administration at a physiological age (11–12 years) had no effect on final height [11, 12]. Otherwise, it has been shown that late estrogen administration clearly had a negative impact on quality of life in young adults with Turner syndrome [13]. Whether the route or the dose of estrogen is the most important variable with respect to growth is not well known. Systemically administered estrogens used in the present study do not undergo a first passage through portal circulation and may not impair the expression of the GH receptor or IGF-I generation. The same positive effect on growth has been observed with the use of transdermal compared with oral estrogens [12]. This small study suggests that minimally feminizing, very low-dose estradiol replacement should be started at somewhat younger ages than usual in Turner syndrome without deleterious effects on growth. Further larger studies are needed to confirm this result. Talking about early estrogen, we should keep in mind that girls have low circulating estrogen even during prepuberty, and that replacement of this function is still to be tested.
Prolonged stimulation of growth hormone and IGF-1 secretion by CJC-1295, a long-acting analogue of growth hormone-releasing hormone, in healthy adults

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Background: A limitation for the therapeutic use of growth hormone-releasing hormone (GHRH) is its short half-life. The aim of the study was to examine the pharmacokinetic profile, pharmacodynamic effects, and safety of CJC-1295, a long-acting GHRH analogue.

Methods: Two randomized, placebo-controlled, double-blind, dose-escalating trials were performed in healthy subjects, aged 21–61 years, with durations of 28 and 49 days. Subcutaneous CJC-1295 or placebo were administered in one of four ascending single doses in the first study and in 2–3 weekly or biweekly doses in the second study. Pharmacokinetic parameters of GH, IGF-1, and CJC-1295 were measured.

Results: After a single injection of CJC-1295, the half-life of CJC-1295 was prolonged (5.8–8.1 days). There were dose-dependent increases in mean plasma GH concentrations by 2- to 10-fold for up to 6 days and in mean plasma IGF-1 concentrations by 1.5- to 3-fold for up to 14 days. After multiple CJC-1295 doses, both mean IGF-1 and GH levels remained above baseline for up to 14 days. Adverse events were frequent but no serious adverse reactions were reported.

Conclusions: Subcutaneous administration of CJC-1295 resulted in sustained, dose-dependent increases in GH and IGF-1 levels in healthy adults, was relatively well tolerated, particularly at doses of 30 or 60 μg/kg. This trial utilizing the long-acting GHRH analogue CJC-1295 is important, since it may lead to a therapeutic alternative to daily injections of recombinant human growth hormone or to long-acting GH preparations. One condition for its efficacy is an intact pituitary, which is the case in most children treated for restricted fetal growth, idiopathic short stature, or idiopathic GH deficiency. These data provide hope for a new therapeutic management of short stature. It is particularly interesting to observe that a single dose of CJC-1295 resulted in a half-life of up to 8 days, and sustained and dose-dependent increments of serum GH and IGF-I levels for nearly 2 weeks. The authors concluded that both single and multiple doses of CJC-1295 were safe and generally well tolerated. However, the frequency of the adverse events was unacceptably high (94% in the active group of the single-dose study) with mild to moderate severity. The most frequently reported adverse events were injection site reactions (70% of patients with a single dose but 100% of patients with multiple doses), consisting of irritation, erythema, induration, pain, itching, and sometimes local urticaria. The other adverse events were headache, diarrhea, flushing and hypotension… These adverse events were dose-dependent and more frequently observed in the multiple-dose study, suggesting an additional biological effect rather than a drug reaction only. Further studies evaluating the clinical efficacy of this compound will have to wait until the producers overcome the side effects.

Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I)/rhIGF-binding protein-3 complex administered to patients with growth hormone insensitivity syndrome

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Background: Growth hormone insensitivity syndrome is characterized by short stature, high serum GH and very low serum IGF-I and IGFBP-3 levels associated with GH receptor gene mutations. Recombinant human (rh) IGF-I treatment has been partly effective in promoting growth in these patients.
**Methods:** A newly developed drug rhIGF-I/rhIGFBP-3, a 1:1 molar complex of rhIGF-I and rhIGFBP-3, has been administered to 4 adolescents with GHIS in an open labelled study to determine IGF-I pharmacokinetics and evaluate its safety and tolerability. rhIGF-I/rhIGFBP-3 was administered in a single subcutaneous injection at 0.5 and 1.0 mg/kg/dose (equivalent to 100 and 200 µg/kg of rhIGF-I) after breakfast with a 2-day interval between doses.

**Results:** Peak IGF-I levels were attained between 19 ± 8.3 and 15 ± 6.2 h for the low and high doses, respectively. The IGF-I circulating levels were similar with the low dose and the high dose, although a discrete dose-dependent increase in circulating IGF-I levels was observed. The IGFBP-3 circulating levels were similar with the low dose and the high dose, although a discrete dose-dependent increase in circulating IGFBP-3 levels was estimated to be 21 ± 4 h. No acute adverse events were reported and all blood glucose measurements were normal.

**Conclusion:** rhIGF-I/rhIGFBP-3 complex is effective in increasing levels of circulating total and free IGF-I into the normal range for a 24-hour period after a single subcutaneous administration in patients with GHIS, and is safe and well tolerated.

Previous trials with rhIGF-I alone in subjects with GHIS have resulted in a mean 1.5–2.5 SDS height gain after 5–7.5 years of treatment. Hypoglycemia was frequently observed, due to the high levels of free IGF-I which occurred after rhIGF-I administration. The half-life of IGFBP-3 in the circulation depends on the formation of a ternary complex composed of IGF-I, IGFBP-3, and the acid-labile subunit (ALS). ALS, IGF-I and IGFBP3 productions are dependent on GH and therefore deficient in GHIS. The question of the benefit of rhIGF-I/rhIGFBP-3 complex administration in subjects with GHIS, whose circulating levels of IGF-I, IGFBP-3, and ALS are low, was therefore raised. The pharmacokinetic profile of the rhIGF-I/rhIGFBP-3 complex used here was expected to be more favorable than that of rhIGF-I alone, since it theoretically extends the half-life of circulating IGF-I. This study shows that in subjects with GHIS, the half-life of IGF-I is about 21 h with the rhIGF-I/rhIGFBP-3 complex as compared to 6 h with rhIGF-I alone. Circulating IGF-I levels obtained with the use of the complex were in the normal range, and daily administration in 1 subject indicated stability of the IGF-I concentrations, although IGFBP3 was rapidly cleared from the circulation. Tolerance was good and blood glucose was maintained. Treatment with rhIGF-I/rhIGFBP3 complex should be evaluated, not only in subjects with GHIS, but also in patients with catabolic state and IGF-I deficiency, a condition of acquired severe GH resistance. In addition, IGF-I/IGFBP-3 complex administration has been shown to improve insulin sensitivity, and could be used in patients with severe insulin resistance due to insulin receptor mutations. Trials evaluating the effects of chronic administration of the complex are now warranted.

**Metformin for growth: an unexpected effect**

**Metformin therapy during puberty delays menarche, prolongs pubertal growth, and augments adult height: a randomized study in low-birthweight girls with early-normal onset of puberty**

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**Background:** Low-birthweight (LBW) girls who enter puberty earlier (around 8–9 years) are at risk for early menarche and short adult stature. There is no validated indication for GnRH agonist and/or GH therapy in these subjects. The aim of the study was to evaluate the effects of insulin sensitization with metformin during puberty in these girls.

**Methods:** 22 LBW girls (BW < -1.5 SDS for gestational age) with onset of breast development (B2) at age 8–9 years were randomized to remain untreated (n = 12) or to receive metformin (850 mg/day; n = 10) for 3 years (mean age at start = 9.0 years). Pubertal growth, body composition by absorptiometry, uterine-ovarian size by ultrasound, fasting insulin, glucose, lipids, leptin, IGF-1 and IGFBP-1 were determined.
Results: Metformin treatment resulted in delayed menarche (p < 0.01; median difference +1.0 year), taller near-adult height (p < 0.01) and leaner body composition (p < 0.001). Metformin was also associated with lower insulin resistance, leptin and IGF-1 levels, higher SHBG and IGFBP-1 levels, and with a less atherogenic lipid profile.

Conclusions: Insulin-sensitizing therapy by metformin for 3 years in LBW girls with early-normal puberty normalized the timing of menarche and increased pubertal height gain and adult stature.

It is not new for us that insulin is a growth-promoting hormone, but it is new that an insulin-sensitizing treatment promotes growth. In treated girls, menarche was delayed by about 1 year and the height gain was 3.5 cm after 3.5 years. However, height at the initiation of treatment was close to +2 SDS with a mean bone age advance of 1–1.5 years: girls with these baseline characteristics do not have a high risk for adult short stature. Whether the height gain would be similar in girls whose height is below the mean with advanced bone age (>2 years), i.e. those with a very high risk of adult short stature, will require further studies. Although the mechanisms by which metformin exerts these effects remain to be elucidated, these data support the concept that insulin is a major co-determinant of the pubertal tempo and of pubertal height gain in girls.

New mechanisms Modulating placental IGF-II

Role of pro-IGF-II processing by proprotein convertase 4 in human placental development

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Background: Intrauterine growth restriction (IUGR) is a leading cause of perinatal mortality. IGF-II plays an important role in regulating fetoplacental growth. The mature IGF-II results from posttranslational processing of the biologically inactive pro-IGF-II peptide. The present study investigates the hypothesis that aberrant processing of IGF-II may be a cause of IUGR.

Methods: Study of the expression of proprotein convertase PC4, the secretion of pro- and mature IGF-II, the sites of proteolysis in pro-IGF-II, the biological activity of IGF-II isoforms in trophoblast cell line and human placental tissues (first and third trimesters) and measurement of the level of IGF-II variants in human sera from non-pregnant individuals, normal pregnant women and pregnant women with IUGR.

Results: PC4 is expressed in the human placenta. It cleaves pro-IGF-II to generate the intermediate processed form, IGF-II (1-102) and, subsequently, mature IGF-II (1-67). This processing confers the ability of IGF-II to activate trophoblast cells migration through AKT phosphorylation. Pregnant women carrying IUGR fetuses had higher levels of pro-IGF-II compared with controls.

Conclusions: Abnormal processing of IGF-II by PC4 may represent a mechanism involved in fetal growth restriction. Elevated maternal pro-IGF-II may be a useful clinical marker for risk of IUGR.

Protein convertases (PC) 1, 2 and 5 have clear biological function, and we now learn that PC4 may be involved in fetal growth. Fetal growth is a complex process involving multiple environmental and genetic factors. Over the last decade, it has been recognized that insulin-like growth factors have a critical role in mediating fetal and postnatal growth [14]. The IGF-II gene is genomically imprinted and paternally expressed in the fetus and placenta. IGF-II regulates fetoplacental growth by stimulating extravillous trophoblast migration/invasion and facilitating nutrient supply through the development of placental exchange. Placenta-specific IGF-II knockout mice have a reduced placental and fetal growth [15]. In humans, placental IGF-II may have a role in subjects with Silver-Russell syndrome (see above). However, this genetic disease does not account for all the frequent restrictions in placental and fetal growth. The results of the present study support the role of PC4-mediated IGF-II processing...
in fetoplacental development. Abnormal IGF-II processing may be a common mechanism of placental and fetal growth restriction. Whether abnormal PC4-mediated processing of IGF-II is a primary cause of placental and fetal growth restriction or is secondary to placental dysfunction remains to be determined.

**Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems**


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Background: The mammalian fetus depends on the supply of maternal nutrients through the placenta. The mechanism which adjusts maternal supply and fetal demand for nutrients for healthy growth and development of the fetus is unknown. IGF-II could regulate the pivotal balance between placental nutrient supply and fetal demand. In knockout mice lacking the imprinted placental-specific IGF-II transcript, growth of the placenta is altered from early gestation but fetal growth is normal until late gestation. This suggests functional adaptation of the placenta to meet the fetal demands.

Methods: The activity and expression of key placental supply genes, the System A amino acid transporter genes and the glucose transporter genes GLUT1 and GLUT3, were compared between mice lacking placenta IGF-II expression only from the P0 promoter, where fetal IGF-II production is normal, and mice lacking IGF-II produced from all promoters in which placental and fetal IGF-II are both lacking.

Results: Placental transport of glucose and amino acids are increased in the IGF-II P0(+/−) null and this upregulation of transport occurs, at least in part, through increased expression of the transporter genes GLUT3 and Slc38a4, the imprinted member of the System A amino acid transporter gene family. The removal of fetal IGF-II decreased fetal demand and abolished upregulation of both transport systems.

Conclusion: These results provide evidence that the placenta can respond to fetal demand signals through regulation of expression of specific placental transport systems.

By now you have got the message that placenta and fetal growth were the hot topics this year. The parental conflict hypothesis has already been mentioned in this chapter, but here is another support. This study, using genetic models of altered growth, provides direct evidence that the activity and expression of specific placental nutrient transporters is modulated by the fetal nutrient demands for growth. In mice that lack placenta IGF-II, the small placenta increases its transfer of glucose and amino acids to meet the nutrient demands of the growing fetus in late gestation. In contrast, when the fetal demand was reduced by deleting the IGF-II gene from all fetoplacental tissues, activity of the placental nutrient transport systems was either at wild-type levels or reduced in late gestation and was associated with a decrease in placental efficiency. The nutrient demand signals appear therefore to originate in the fetus. This study suggests that circulating fetal IGF-II is a likely signal, at least, when the fetal nutrient demand for growth exceeds the nutrient transport capacity of the small IGF-II-deficient placenta. Fetal nutrient requirements could be alternatively signaled to the placenta by other fetal-derived growth factors or by fetal-induced changes in the nutritional or endocrine environments of the mother. Further studies are needed to determine these factors.
From natriuresis to short stature

**Heterozygous mutations in natriuretic peptide receptor-B (NPR2) are associated with short stature**

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**Background:** C-type natriuretic peptide (CNP) is a regulator of skeletal growth, which binds to a membrane receptor, the natriuretic peptide receptor-B and activates a guanylyl cyclase, generating cGMP. Loss-of-function mutations in the NPR-B gene (NPR2) cause the autosomal recessive skeletal dysplasia, acromesomelic dysplasia, Maroteaux type. The aim of the study was to determine the phenotypic features of heterozygous carriers of NPR2 mutations.

**Methods:** This case-control study included 39 members of a family in which 1 member has acromesomelic dysplasia, Maroteaux type. The authors determined anthropometric measures of all the subjects. Familial genotyping for the mutation of the proband was performed by restriction fragment length analysis.

**Results:** The proband with acromesomelic dysplasia, Maroteaux type, was homozygous for a deletion of a single thymidine nucleotide in exon 4 of the gene. She had low IGF-I, GH resistance, very high plasma levels of CNP and its amino terminal propeptide. Sixteen family members were heterozygous carriers of the NPR2 mutation. Their mean height z-scores were $-1.8 \pm 1.1$ (mean $\pm$ SD) vs. $-0.4 \pm 0.8$ for the 23 non-carriers ($p < 0.0005$). Levels of IGF-I, IGFBP3, CNP and its amino-terminal propeptide were normal in the heterozygous carriers.

**Conclusions:** Heterozygous NPR2 mutations are associated with short stature with no skeletal dysplasia or body disproportion. Heterozygosis for NPR2 mutations may be a common cause of ‘idiopathic’ short stature in the general population.

Natriuretic peptides (NPs) comprise a group of atrial and brain peptides (ANP, BNP, and CNP, respectively), which principally mediate natriuretic, diuretic, vasorelaxant, and antimitogenic responses largely directed to reduce blood pressure and maintain fluid volume homeostasis. Their putative role in bone physiology has been suggested by the observation that disruption of the CNP gene in mice produced dwarfism with skeletal abnormalities [16], whereas overexpressing BNP exhibited pronounced skeletal overgrowth. Although the involvement of natriuretic peptides in bone physiology was unexpected, these findings provided strong evidence that CNP/natriuretic peptide receptor-B signaling modulate skeletal growth and bone development. Loss-of-function mutations in the NPR-B gene (NPR2) have been shown to cause acromesomelic dysplasia, Maroteaux type, in humans [17]. The mechanism by which NPR2 mutations interfere with bone growth is largely unknown, and will certainly bring valuable progress into bone physiology.

The present study specified the clinical and biological phenotype of heterozygous carriers of a loss-of-function mutation in NPR2 in a single family, which results in a relative genetic homogeneity, but does not exclude a by-product of an additional family trait. The authors showed that the NPR2 mutations carriers have a mean height 1.4 SD score below that of their non-carrier family members, suggesting an association of heterozygous NPR2 mutations with short stature. The prevalence of the homozygous disease was estimated to be 1/2,000,000, which leads to a frequency of heterozygous carriers of 1/700. Therefore, 1 person in 30 with idiopathic short stature could be a carrier of an NPR2 mutation. Further large studies in the wider population of individuals with idiopathic short stature are necessary to confirm this hypothesis. Mutations in unexpected genes may explain a significant fraction of familial short stature.
Identification of mutations in CUL7 in 3-M syndrome

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Background: Maternal, fetal or placental factors may cause placental and fetal growth restriction. However, most cases of intrauterine growth retardation remain unexplained. 3-M syndrome is an autosomal recessive condition characterized by pre- and postnatal growth retardation, a gloomy face, large head circumference and normal intelligence.

Methods: Homozygosity mapping and molecular study in 29 families with 3-M syndrome.

Results: The underlying gene was mapped to chromosome 6p21.1. Twenty-five distinct mutations were found in the gene cullin 7 (CUL7). CUL7 assembles an E3 ubiquitin ligase complex containing Skp1, Fbx29 (also called Fbw8) and ROC1 and promotes ubiquitination. Using deletion analysis, CUL7 was found to use its central region to interact with the Skp1-Fbx29 heterodimer. Functional studies indicated that the 3-M-associated CUL7 nonsense and missense mutations R1445X and H1464P, respectively, render CUL7 deficient in recruiting the ROC1 RING-finger protein.

Conclusion: Impaired ubiquitination may have a role in the pathogenesis of intrauterine growth retardation in humans.

The ubiquitins and their critical role in protein degradation, which received the Nobel Prize in chemistry for 2004, has already been implicated in cerebrovascular diseases and cancer: it has now made its way into pediatric endocrinology. In fact, it has been here ever since we learnt in the late 1980s that ubiquitin is attached to the GH receptor. But here is another angle of ubiquitin and child growth. Homozygosity mapping performed by the authors in consanguineous families determined a 3.84-Mb interval on chromosome 6p21.1, and CUL7 appeared to be a good candidate in the region because CUL7 null mice have intrauterine growth retardation at a late gestational age with a small placenta, vascular anomalies and abnormal lungs with failure in inflation and marked reduction of alveolar space. CUL7 expression was found to be very high in fetal kidney and brain, and in adult skeletal muscle, pancreas, kidney, placenta and heart. CUL7 transcripts were also detected in osteoblasts, chondrocytes and skin fibroblasts. CUL7 belongs to the cullin family, which is involved in several processes including cell-cycle regulation, signal transduction, oxygen regulation and DNA repair. The cullins are believed to have a scaffold role in assembling E3 ubiquitin ligase complexes that target proteins for ubiquitination and subsequent degradation by the 26S proteasome. The CUL7 mutations found in subjects with 3-M syndrome could therefore disrupt the normal ubiquitination process, and subsequent protein degradation. Alternatively, one other possibility is that the mutation-mediated decay of the abnormal transcript or protein is the primary cause of the disease. Although CUL7 null mice had placental vascular anomalies, this is not the case in affected humans. How CUL7 mutations lead to intrauterine growth retardation remains an open question. If that reminds you of the GH receptor, here is another perspective.

Reviews

Negative regulation of growth hormone receptor signaling

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Summary: Growth hormone (GH) is the main regulator of longitudinal growth in mammals. It controls carbohydrate, lipid, nitrogen and mineral metabolism, and stimulates differentiation and mitogenesis in a variety of cell types in different tissues. Responsiveness to GH in target cells is primarily dependent
upon the expression of the GH receptor (GHR), and GHR signalling has been extensively studied in the previous years: signal transduction is mediated by Janus kinase (JAK) 2, transducer and activator of transcription (STATs) family of transcription factors, including STAT5b. Recently, some of the components in the downregulatory mechanism targeting the activated GH receptor have been defined. Downregulation of the GH receptor involves rapid ubiquitin-dependent endocytosis of the receptor, the action of tyrosine phosphatases and degradation by the proteasome. The SOCS protein family, particularly SOCS2, plays an important role in regulating GH actions.

This review provides valuable insight into GHR signaling, specifically its downregulation. Knowledge of these mechanisms can help understand growth-related disease, explain GH resistance and may be used to develop pharmaceuticals that enhance some of the beneficial actions of exogenous or endogenously secreted GH in a tissue-specific manner.

**Marfan’s syndrome**
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Summary: Manifestations of Marfan’s syndrome include proximal aortic aneurysm, dislocation of the ocular lens, and long-bone overgrowth. Substantial morbidity and premature mortality remain associated with this disorder. Marfan’s syndrome is a systemic disorder of connective tissue caused either by mutations in the extracellular matrix protein fibrillin 1, or by altered regulation of transforming growth factor-β (TGF-β), a family of cytokines that affect cellular performance. Therapeutic application for TGF-β antagonists may therefore be hypothesized in subjects with Marfan’s syndrome.

This review summarizes the current knowledge about the musculoskeletal, ocular, and cardiac involvements in Marfan’s disease, its molecular determinants, and its management. In addition to the obvious structural role of fibrillin-1-rich microfibrils, these microfibrils also have a critical role in the regulation of cytokines, including TGF-β cytokines. Marfan’s syndrome manifests postnatally acquired tissue pathology, and this may largely indicate a failed regulatory (as opposed to structural) role of the extracellular matrix: most manifestations of Marfan’s syndrome seem to implicate dysregulation of TGF-β activity and signaling. Drugs interfering with TGF-β activity may be of interest for Marfan’s syndrome treatment.

**Food for thought**
*An alternative to the Barker hypothesis*

**Infection, inflammation, height, and longevity**
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Background: Life expectancy has doubled during the last three centuries. Although all ages have benefited, the increases among the elderly began many decades after the increases at younger ages. The historical increase in life expectancy at the older ages may be due in part to lifelong reduction in exposure to chronic inflammation. Inflammation could also link childhood mortality and adult height.

Methods: Search for statistical associations between early and later mortality using historical data from cohorts born before the 20th century in France, Sweden, England, and Switzerland.

Results: Increasing longevity and declining mortality in the elderly occurred among the same birth cohorts that experienced a reduction in mortality at younger ages. Concurrently, these cohorts also experienced increasing adult height. Both the decline in old-age mortality and the increase in height could be promoted by the reduced burden of infections and inflammation.
Conclusion: Early growth and cardiovascular diseases of old age may share infectious and inflammatory causes rooted in the external environment.

Usually, the decrease of mortality over the last centuries as well as the increase in mean height has been linked to the improvement in nutrition and to a reduction of organ damage due to infections. Improved public health, vaccines, and socioeconomic level are also believed to contribute to this decrease. Here, the authors propose a general model in which the reduction in lifelong levels of infections and inflammation reduced and delayed the atherosclerotic process and mortality due to heart disease and allowed increased height. The role hypothesized for inflammation considers the importance of heart disease as an historical cause of old-age mortality. In highly infectious environments, children are exposed to high inflammation levels, which promote atherogenesis even without exposure to high-fat diets. The relationship between mortality rates at young and old ages within cohorts were stronger than the relationship between mortality rates at young and old ages in the same year. This supports a developmental relationship between childhood infection and old-age mortality, whereas a nutritional explanation would have led to strong relationships between early and late mortality rates the same year. The changes in adult height showed similarities to the early and later mortality trends, thus suggesting that height was also linked to inflammation. Inflammation and infections during early life may link growth, pre- or post-natal, and later cardiovascular disease, an alternative path to the Barker hypothesis, which emphasizes that diet influences development, growth, and cardiovascular diseases.

References