Genetic disorders in the growth hormone-IGF-I axis
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General discussion

Genetic disorders in the GH-IGF-I axis in mouse and man

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Abstract

Animal knockout experiments have offered the opportunity to study genes that play a role in growth and development. In the last few years, reports of patients with genetic defects in the GH-IGF-I axis have greatly increased our knowledge of genetically determined causes of short stature. In the various chapters of this thesis, patients are described with a genetic defect in the GHRH receptor, the GH signaling pathway, IGF-I or the IGF-I receptor, all of which are components of the GH-IGF-I axis. In this final chapter the animal data and human reports of genetic disorders in the GH-IGF-I axis will be reviewed, in order to describe the role of the GH-IGF-I axis in intrauterine and postnatal growth. In addition, the effects of the GH-IGF-I axis on development and function of different organ systems as brain, inner ear, eye, skeleton, glucose homeostasis, gonadal function and the immune system will be discussed. The number of patients with genetic defects in the GH-IGF-I axis is small and a systematic diagnostic approach and selective genetic analysis in a patient with short stature are essential to identify more patients. Finally, the implications of a genetic defect in the GH-IGF-I axis for the patient and the therapeutic options will be discussed.

Introduction

Reports of patients with genetic defects in the growth hormone – Insulin-like Growth Factor-I (GH-IGF-I) axis, in addition to animal knockout experiments, have increased our knowledge on the role of the GH-IGF-I axis in growth and development throughout life considerably. By means of these reports we will describe the factors playing a role in intrauterine as well as postnatal growth and the effect of the GH-IGF-I axis on the development and function of different organs. Finally, the tools for the diagnostic approach for the physician and the clinical implications for the patients will be discussed.

Intrauterine growth

The first evidence that intrauterine growth is determined by GH-independent IGF-I secretion, comes from animal experiments. Mice with severe GH deficiency due to spontaneous mutations in the genes encoding GHRHR (little mouse), Pit 1 (Snell dwarf) and Prop-1 (Ames dwarf) have a normal birthweight (1). The GH insensitive Laron mouse is born with a normal body size and weight (2). In contrast, IGF-I and IGF1R knockout mice have birthweights of 60% and 45% of normal, respectively. From these observations it was concluded that IGF-I is a growth determinant factor for intrauterine growth, independently of GH (3-5). The effects of IGF-I are mediated through the IGF-I receceptor (IGF1R), which is in line with the observation that the IGF1R -/- mice and the IGF1R-/- IGF-I-/- mice are equally growth retarded at birth (45 % of normal) (3). IGF1R-/- knockout mice die within minutes after birth due to respiratory failure (3). IGF1R+/- mice are phenotypically normal (3). Disruptions in the IGF-I signaling pathway as described in mice deficient in Akt1, insulin receptor substrate-1 (IRS-1) or IRS-2 result in reduced intrauterine growth (6-9).

In the human, GH deficiency and insensivity result in a normal birth size (10-12). In contrast, an IGF-I gene deletion or mutation results in severe intrauterine growth retardation as is demonstrated in the patient described by Woods *et al.* (birth weight and length –3.9 SDS and –5.4 SDS, respectively), Walenkamp *et al.* (birth weight and length –3.9 SDS and –4.3 SDS, respectively) and Bonapace *et al.* (birth weight and length –4 SDS and –6.5 SDS, respectively) (13-15). The finding

that genetically determined low IGF-I levels, due to polymorphisms in the IGF-I promoter region, result in a reduced birth weight and length support the role of IGF-I in fetal growth (16, 17).

There are also indications of an IGF-I dose effect on intrauterine growth. IGF-I heterozygous mouse are 10-20% smaller at birth (4) and in the human we found that carriers of an IGF-I missense mutation have a 10% lower birth weight than non-carriers (14).

In contrast to the observations in mice, heterozygous mutations of the IGF1R result in intrauterine growth retardation in the human (18-22). The degree of IUGR varies between -1.5 SDS and -3.5 SDS for birth weight and between -0.3 SDS and -5.8 SDS for birth length. The different mutations result in a variable degree of remaining IGF-I signaling, which may explain the wide range of birth size. Another hypothesis is that maternal IGF-I resistance during pregnancy in the mothers with a heterozygous IGF1R mutation contribute to more severe growth retardation. This is supported by the finding that children with an affected mother are more growth retarded at birth than children with an apparently non-affected mother (22).

IGF1R haploinsufficiency is associated with a variable degree of intrauterine growth retardation (-1.8 SDS to –5.6 SDS) as is demonstrated in patients with a terminal 15q deletion, including the IGF1R gene (23-30). The report of a patient with three copies of the IGF1R gene supports the concept that a gene dose effect plays a role in intrauterine growth. This patient is born at 42 weeks gestational age with a birth weight of 5140 gr, a birth length of 60 cm and a head circumference 38.5 cm (28).

So far, genetic defects of the IGF-I signaling pathway are not described in human. However, in a recent study of Laviola *et al*. the IRS-2 and Akt pathway were down-regulated in human placentas from pregnancies complicated by IUGR, indicating an important role for IGF-I signaling in the appropiate development of the fetopla-cental unit (31).

ALS (acid-labile subunit) forms a ternary complex with IGF-I or IGF-II and with IGFBP-3 or IGFBP-5 in the circulation to increase the half-life of the IGFs. ALS deficiency appears to have no effect on fetal growth in ALS knockout mice (32). Data on intrauterine growth of ALS deficient patients are limited, as the first described patient is adopted and gestational age is not available. At the age of one week

weight was 2500 gr and length was 47 cm, which is considered normal (33). Also from the second patient no data regarding gestation, birth weight and length are available (34). As expression of ALS occurs late in fetal life (35), severe effects on intrauterine growth are unlikely.

Although this review focuses on genetic defects of the GH-IGF-I axis, the importance of the role of IGF-II in intrauterine growth cannot be left unmentioned. IGF-II acts as an important modulator of placental cell proliferation and maturation (36). A positive correlation has been demonstrated between cord blood IGF-II and placental weight (37). However, controversy exists with regard to the relation between fetal serum IGF-II levels and fetal growth. Some studies report reduced cord blood IGF-II levels in IUGR babies (38-40), others report similar levels of IGF-II in IUGR and normal fetuses (41). Human mutations in the gene encoding IGF-II, located on 11p15, have not been identified sofar. However, alterations in imprinting status of the IGF-II gene are associated with severe IUGR as part of the Silver Rusell syndrome (a condition characterized by severe IUGR, postnatal growth failure, dysmorphic facial features and body assymetry). The IGF-II gene is paternally expressed. A maternal duplication of 11p15 or demethylation of the telomeric imprinting centre ICR1 on 11p15, resulting in under-expression of IGF-II is found in 50 % of patients with Silver Russell syndrome (42-44).

Postnatal growth

The first two weeks of postnatal life growth of the Snell dwarf, the Ames dwarf, the little and Laron mouse is indistinguishable from the wild type littermates (1). However, at postnatal day 40, their size is about 50% of normal, which confirms the increasing role of GH in postnatal life. The size of the IGF-I knockout mice decreases progressively from 60% of normal at birth to 30% of normal at 8 weeks. Double knockout mice (GHR-/- and IGF-I-/-) have a postnatal growth pattern of 17% of normal (45). These findings demonstrate that GH dependent IGF-I action is the main determinant of postnatal growth, but that GH and IGF-I have also independent effects (45). Targeted gene deletion of liver-specific IGF-I and ALS (LID/ALSO mouse), resulting in a 85-90% reduction of circulating IGF-I, shows a 20% lower body weight than control mice. This implicates that, while endocrine

IGF-I is important in postnatal growth, tissue IGF-I also plays a role since the total IGF-I-/- mouse is more growth retarded (46).

The classical heterozygous IGF1R-/+ mice are phenotypically normal, with normal expression of IGF1RmRNA, suggesting that the intact wild-type allele is up-regulated and implying that a single functional IGF1R allele is sufficient to assure normal growth (3). However, later experiments inducing reduced availability of the IGF1R (41% less than normal) showed a growth deficit of 13% in males and of 6% in females, implying that a partial reduction in IGF-I signaling reduces the growth potential, at least in the male mouse (47).

In the human, severe isolated GH deficiency leads to a final height ranging from -3.9 to -6.1 SDS (mean -4.7 SDS) (48). GH deficiency due to GHRHR mutations results in a mean final height of –7.2 SDS (49). Patients with GH insensitivity due to a GHR mutation reach a final height varying from -3.2 SDS to -12 SDS (12, 50, 51) and due to a STAT5b mutation from -5.9 SDS to -7.8 SDS (52-54). Complete IGF-I deficiency due to a deletion of the IGF-I gene results in a final height of -6.9 SDS (13). The final height of the patient with an inactivating mutation of the IGF-I gene is -8.5 SDS (14). The height of a patient with a mutation resulting in extremely low IGF-I levels at the age of 19 months is -6.2 SDS (15). These results show that GH deficiency results in a similar growth pattern as primary IGF-I deficiency, which emphasizes the predominant role of GH dependent IGF-I production in postnatal growth. Treating GH deficient patients with GH results in a better growth response than treating GH insensitive patients with recombinant human (rh)IGF-I, suggesting that a direct GH effect is necessary for optimal postnatal growth (55).

GH insensitivity caused by a STAT5b mutation results in severe postnatal growth retardation in males and females. In contrast, the homozygous ablation of STAT5b in mice results in loss of the sexual dimorphism in body growth: the postnatal growth curves of female wild type, female STAT5b-/- and male STAT5b-/- mice show no significant difference (56). STAT5b gene disruption in mice leads to a loss of the sexually dimorphic pituitary pulsatile GH secretion pattern and liver gene expression. It is uncertain how this difference with regard to the interaction between sex steroids and the GH-STAT5b-IGF-I axis in mouse *vs* human is can be explained. One explanation may be that in the mouse the growth promoting effect of the typical male pattern of GH secretion (high GH peaks, low throughs) is trans-

mitted by STAT5B, while the irregular GH profile of female rats, with lower peaks and higher troughs, is primarily transmitted by another signal transduction route. Knocking out STAT5B in the mouse can then be expected to abolish the sexual dimorphism in growth in the rat. In the human, the GH profile is not different between sexes, and is similar to the pattern of GH secretion seen in male rats. A STAT5B defect would then be expected to have a similar effect in both sexes 56, 57)

Patients with IGF-I insensitivity due to IGF1R mutations show more variety in postnatal growth (-0.9 SDS to -4.8 SDS) than patients with IGF-I deficiency. The phenotypical variability in IGF-I insensitivity is most probably caused by differences in remaining IGF-I signaling or by a compensatory mechanism that up-regulates the expression of the normal IGF1R allele. In addition, a gene dose effect seems to play a role in postnatal growth as is demonstrated by the severe postnatal growth failure (-3.5 SDS to –6.3 SDS) of patients with IGF1R haploinsufficiency due to a terminal 15q deletion, including the IGF1R gene (23, 25, 26, 28-30). The finding that trisomy of terminal 15q resulting in duplication of the IGF1R gene is associated with tall stature supports this hypothesis (58).

Two patients with ALS deficiency due to a mutation in the ALS gene have been described (33, 34) and we have recently reported two affected sibs (59). They present with a variable degree of growth retardation: height at 14.6 years of age –2.05 SDS and final height -0.8 SDS in the patient described by Domene *et al*, -2.1 SDS at 15.5 years of age in the patient described by Hwa *et al*, final height was -4.2 SDS in one of the sibs and height was -4.3 SDS at the age of 16.5 years in the other sib. Extremely low IGF-I and IGFBP-3 levels is a common finding in all these patients. A high flux of free IGF-I into the tissues and rapid proteolysis of IGF-I can explain this feature. Another possibility is that paracrine and autocrine IGF-I effects, stimulated by increased GH production, compensate for the deficiency of circulatory IGF-I (34).

Regulation of GH secretion

The regulation of GH secretion is complex. Shortly, GHRH stimulates GH synthesis and determines the amplitude of the pulses, while the intermittent withdrawal of

somatostatin regulates the timing of the GH pulses. The low amplitude spontaneous GH release in patients with a GHRHR mutation supports this hypothesis (60). The autofeedback mechanism of GH and IGF-I suppresses GHRH and stimulates somatostatin expression.

Age, gender, sex hormones and adiposity influence the magnitude of GH secretion (61). Ghrelin, a hormone predominantly produced in the stomach, stimulates GH release and appetite. Ghrelin -/- mouse have a normal phenotype, suggesting that ghrelin itself is not required for growth (62). Human mutations have not yet been described. Ghrelin acts via the GH secretagogue receptor (GHSR). Recently, two families with a GHSR missense mutation resulting in impairment of the constitutive activity of the receptor were identified. The heights of the homozygous probands were −3.7 SDS and −3.2 SDS, supporting the hypothesis that ghrelin signaling via the GHSR plays a role in the GH-IGF-I axis (63).

In patients with GH insensitivity low IGF-I levels result in loss of negative feedback and as a consequence increased GH secretion. IGF-I therapy restores GH secretion to normal, implying that the sensitivity of the feedback mechanism is intact in these patients (64). In addition to the increased GH secretion, prolactin levels are slightly elevated in patients with GHI due to a GHR mutation (65). This may be the result of increased GHRH secretion as a consequence of the diminished negative feedback of IGF-I and GH, which also stimulates the prolactin secretion from somatolactotroph cells in the pituitary. Indeed, the elevated prolactin levels are suppressed upon IGF-I administration, in line with the inhibitory action of IGF-I on hypothalamic GHRH neurons (65). In patients with a STAT5b mutation prolactin levels are even more elevated (52, 54, 66). This can be explained by the obligatory role of STAT5b in mediating the negative feedback action of prolactin on tuberoinfundibular dopamine neurons; in the absence of STAT5b the signal transduction in the hypothalamic dopamine neurons is impaired (67).

Patients with IGF-I insensitivity are characterized by elevated IGF-I levels as a result of increased GH production due to pituitary and hypothalamic IGF1R deficiency. This is supported by the finding that IGF1R-/- mice exhibit signs of somatotropic stimulation in the pituitary (61). However, in patients with poor caloric intake IGF-I can be in the normal range, which should be considered as relatively high for a malnourished infant or child. Restoring the nutritional status increases the IGF-I level above the normal range (22).

Patients with IGF1R haploinsufficiency have normal or elevated IGF-I levels (23, 25, 26, 30). Stimulated GH secretion was normal in all tested patients (24, 26, 28-30). Apparently, IGF1R haploinsufficiency has no major impact on GH and IGF-I secretion.

IGF binding proteins

IGF-I is secreted in the circulation and associated with soluble high-affinity binding proteins, the IGFBPs. IGFBP-3 and IGFBP-5 form a complex with IGF-I and ALS. This ternary complex slows the clearance of IGF-I. Changes in IGFBP expression have an important role in modulating the growth-promoting actions of the IGFs.

In mice, reports of IGFBP knockout models are limited and demonstrate only few phenotypic manifestations (68). Overexpression of IGFBP-1 in transgenic mice result in a modest and transient impairment of fetal growth. Also maternal IGFBP-1 excess is associated with reduced fetal growth indicating placental insufficiency (69). In human there is a striking inverse correlation between maternal and fetal circulating levels of IGFBP-1 and fetal size (39, 44, 70, 71). It is postulated that IGFBP-1 inhibits the growth promoting effect of IGF-I by binding fetal IGF's in IUGR. Indeed, IGFBP-1 levels and mRNA expression are markedly elevated in the umbilical cord blood of babies with profound and prolonged hypoxia, which is considered as a leading cause of IUGR (72). In a recent study the key role for IGFBP-1 in mediating the effects of hypoxia on fetal growth was confirmed in zebrafish, suggesting this is a conserved physiological mechanism to restrict IGF stimulated growth under hypoxic conditions (73).

IGFBP-1 is synthesized in the liver, where its expression is under control of insulin which suppresses its production (74). It has also been suggested that GH directly regulates IGFBP-1 secretion (75). In the patient with the IGF-I deletion IGFBP-1 was low. This patient was severely insulin resistant and a combination of high insulin levels, the absence of IGF-I and increased GH levels probably contributes to the low IGFBP-1 levels. Treatment of this patient with rhIGF-I resulted in beneficial effects on insulin resistance, decreased GH secretion and higher IGFBP-1 levels (76). The patient with the inactivating mutation of the IGF-I gene was not severely

insulin resistant and had also low IGFBP-1 levels, suggesting an important role for a direct suppressive effect of GH on IGFBP-1 (14). The patient with ALS deficiency also had low IGFBP-1 levels, which can also be attributed to stimulated GH levels (33).

IGFBP-2 levels were low in the patient with the IGF-I gene deletion. rhIGF-I treatment increased the IGFBP-2 levels (76). IGFBP-2 in the patient with the IGF-I mutation was -1 SDS (14). The patient with ALS deficiency had low IGFBP-2 levels (33). These data suggest that GH plays a role in regulating IGFBP-2 production.

In the absence of ALS, IGFBP-3 is cleared very fast, resulting in extremely low serum concentrations (33, 77). The transcription of IGFBP-3 is induced by activation of the GH signal transduction pathway, including STAT5b. Therefore, IGFBP-3 is a valuable biochemical parameter in differentiating a GH receptor or postreceptor defect from an IGF-I or IGF1R defect. In the latter IGFBP-3 levels are normal (13, 14, 22), while in patients with a GHR defect or a STAT5b mutation IGFBP-3 is low (51, 52, 54, 77).

The GH-IGF-I axis brain development

IGF-I plays a key role in the development of the central nervous system, stimulating neurogenesis and synaptogenesis, facilitating oligodendrocyte development, promoting neuron and oligodendrocyte survival and stimulating myelination (78). In addition, IGF-I appears to be a potent agent for rescuing neurons from apoptosis. As systemic IGF-I is not readily transported through the blood-brain-barrier local production of IGF-I is considered to be responsible for these effects (79).

Psychomotor development is normal in patients with GH deficiency or insensitivity (80). Patients with primary IGF-I deficiency due to a deletion or mutation of the IGF-I gene are severely mentally retarded and microcephalic with a head circumference at birth of -4.9 SDS (13) and -5.7 SDS (15) and in adulthood of -5.3 SDS (13) and -8 SDS (14), emphasizing the essential role of GH-independent IGF-I production for prenatal brain development. The head circumference of carriers of the inactivating IGF-I mutation is within the normal range. However, carriers have a lower head circumference than non-carriers (-1 SDS vs 0.5 SDS). Microcephaly is

a common feature in patients with IGF-I insensitivity due to a heterozygous IGF1R mutation, however less severe than in complete IGF-I deficiency (-3 SDS to -5.6 SDS)(22). Psychomotor development in these patients varies from retarded with an IQ of 60 to completely normal. Head circumference in patients with IGF1R haploinsufficiency due to a terminal 15q deletion is not well documented. In two patients head circumference was measured: -5.3 SDS (25) and -3 SDS (26). In most cases psychomotor development was delayed, but the possible contribution of other genes in the deleted region makes conclusions on a causal relation difficult.

Hearing

Recently, the role of IGF-I in auditory function was evaluated. Auditory brainstem responses were analized in IGF-I-/- mice, showing an all-frequency involved bilateral sensorineural hearing loss. The delayed response to acoustic stimuli along the auditory pathway indicates the contribution of the central nervous system to the hearing loss in IGF-I deficiency (81). At a cellular level a significant decrease in number and size of auditory neurons, increased apoptosis of cochlear neurons, a significant reduced volume of the cochlea and cochlear ganglion result in abnormal differentiation and maturation of the cochlear ganglion cells and abnormal innervation of the sensory cells in the organ of Corti (82).

Audiograms of the three patients with complete IGF-I deficiency due to a homozygous deletion or mutation of the IGF-I gene demonstrate severe bilateral sensorineural deafness (13-15). This is confirmed by absent brain stem evoked potentials in one of the patients (14). Seven of the 21 family members of the patient with the inactivating IGF-I mutation, including 9 carriers of the inactivating IGF-I mutation, revealhearing abnormalities. However, no significant association with the carriership could be detected (14). In patients with GH deficiency or insensitivity or heterozygous IGF1R receptor mutations hearing problems have not been reported.

In conclusion, IGF-I is a key factor in development and postnatal differentiation and maturation of the inner ear.

Vision

In a recent study, the ocular dimensions of patients with Laron syndrome were compared with reference values. Patients with Laron syndrome have significantly shorter axial length of the eye and shallower anterior chambers. Treatment with IGF-I increases the axial length of the eye (83). The patient with an inactivating IGF-I mutation, has a shallow anterior chamber, suggesting that IGF-I may play a role in ocular growth (14).
Retinal vascularization is significantly reduced in patients with defects of the GHR, IGF-I and IGF1R indicating that IGF-I plays an important role in this phenomenon (84). A strong association has been found between reduced IGF-I levels in preterm children and development of retinopathy of prematurity. Deficient IGF-I levels after premature birth result in initial poor retinal vascular development and a large area of avascular retina (85).

Skeletal features

Limited elbow extensibility is seen in 85% of patients with Laron syndrome over 5 years of age, with increasing severity with age (50). In addition, 6 of 8 patients with GH deficiency due to a PROP1 mutation show symmetrical limitation of elbow extensibility, correlated with age (86). In the patient with the inactivating mutation of the IGF-I gene we also found this feature but it was not described in the other patients with IGF-I defects at 19 months and 15.5 years, suggesting that IGF-I plays a role in elbow extensibility later in life. The mechanism is unknown.

Micrognathia is a striking feature in two patients with IGF-I defects. Diewert *et al.* found that major growth movements and developmental changes in craniofacial tissues take place between 7 and 12 weeks of gestation (87). Significant alterations in growth during this period may produce significant irreversible effects on postnatal craniofacial morphology. One can hypothesize that prenatal IGF-I deficiency may disturb this process and result in micrognathia.

Bone Mineral Density

Studies on skeletal structure in IGF-I -/- mice show a 17% decrease in cortical bone but an increase in trabecular bone (23% in male and 88% in female) (88). This phenomenon is also observed in IGF-I deficient double knockout mice (liver-specific IGF-I and ALS deletion) (89). Yakar *et al.* postulated that the ternary complex (IGF-I, IGFBP-3 and ALS) influences bone acquisition in a compartment-specific manner (i.e.cortical vs trabecular bone). The finding that IGF-I deficient mice exhibit greater impairment in bone accretion than GH deficient mice, implies a GH-independent effect of IGF-I on bone formation during postnatal growth (90). Dual-energy X-ray absorptiometry (DEXA) is a method to assess BMD (in g per cm^2). However, the method does not correct for antero-posterior depth and is therefore greatly influenced by bone size. Bone mineral apparent density (BMAD) calculates volumetric density to minimize the effect of bone size on BMD values (91). This method greatly contributes to the interpretation of BMD in patients with short stature.

Patients with GH deficiency due to GHRHR mutations or GH insensitivity due to GHR mutations have a normal BMAD (92-94). BMAD in the patient with an IGF-I gene deletion is mildly reduced. Treatment with rhIGF-I resulted in a 7% increase of BMAD, compared to a 17% increase of BMD, suggesting that the increase in BMD is partly attributable to an increase in bone size (95). In contrast, severe osteoporosis is demonstrated in the patient with an inactivating IGF-I mutation (14). Two patients with a heterozygous mutation of the IGF1R gene have a normal BMD (18, 22). In one patient with ALS deficiency severe osteoporosis is found at the age of 16 years (BMD at lumbar spine -4.6 SDS), with a partial recovery at 19 years of age (BMD at lumbar spine –2.1 SDS) (96). In the patient we recently described a similar pattern was observed with an increasing BMD from -5.2 to -4.1 and -2.5 SDS at the age of 16, 17 and 19 years, respectively. This suggests that sex steroids can reduce the osteoporotic effects of ALS deficiency during puberty.

Glucose homeostasis

It has been well documented that GH exerts direct effects on insulin secretion as well as indirect effects through increased lipolysis, resulting in elevated free fatty

acid (FFA) levels and impaired insulin sensitivity, the "lipotoxic effect" (97). Acute administration of GH has an insulin-like effect, mediated by the JAK-2 signaling pathway. Activation of insulin receptor substrate (IRS)-1 and IRS-2 leads to recruitment of PI3 kinase and, analogous to the post-receptor events for insulin, results in increased glucose uptake (98). GH therapy is often associated with impaired insulin sensitivity. Prevention of lipolysis by coadministration of GH with FFA regulators can partially prevent the deterioration of insulin sensitivity, indicating that insulin resistance is a consequence of enhanced GH-induced lipolysis (99). In addition, the GH plus FFA regulator combination treatment significantly enhances linear body growth in SGA and control rats. The precise mechanism of this observation remains to be elucidated (100). Chronic excess of GH, as in acromegaly, is associated with insulin resistance and impairment of insulin receptor signal transduction. Treatment of acromegalic patients with somatostatin analogues improves insulin resistance (101). Post receptor cross talk between the insulin receptor and GH receptor signaling pathway is believed to play a key role in this process (102).

IGF-I, which has 48% amino acid sequence identity with proinsulin, enhances insulin sensitivity. Epidemiological studies have demonstrated that lower baseline IGF-I levels are associated with a higher risk of insulin resistance (103). Genetically determined low IGF-I levels are associated with an increased risk of insulin resistance (104). Treatment with rhIGF-I improves insulin sensitivity in normal individuals (105), in patients with insulin resistance (106) and in those with diabetes type I (107) or type II (108). The patient with IGF-I deficiency due to an IGF-I deletion had severe insulin resistance, which improved with rhIGF-I treatment (76). Patients with a heterozygous inactivating mutation of the IGF-I gene had higher fasting insulin levels than non-carriers (14). Two patients with a heterozygous IGF1R mutation had a moderate degree of insulin resistance (22, 109). The first described patient with ALS deficiency was insulin resistant (33). These results demonstrate a role for IGF-I in glucose homeostasis. Whether IGF-I directly affects insulin sensitivity or by regulating endogenous GH levels is a topic of intense research.

Body composition

GH is known to have a lipolytic effect, which is illustrated by the finding that GH treatment in GH reduces adiposity and improves lipid profiles (110). Patients with GH resistance have a markedly decreased ratio of lean mass to fat mass, indicating the lack of the direct antilipolytic effect of GH (50). In the patient with the IGF-I gene deletion, in whom the direct GH effects are intact, a low body fat content was found (19.9%), which is in line with a direct antilipolytic effect of GH (95). Total body fat content increased in this patient after 1 year of treatment with rhIGF-I, which can be explained by the fall in GH levels induced by rhIGF-I. IGF-I has no direct effects on lipolysis or lipogenesis.

Puberty and gonadal function

Puberty is delayed in all conditions associated with IGF-I deficiency: GH deficiency due to a GHRHR mutation (49), GH insensitivity caused by a GHR mutation (50, 111), or a STAT5b mutation (54), IGF-I gene deletion (76) and an inactivating IGF-I mutation (14). One female with a heterozygous IGF1R mutation had menarche at the age of 18 years (22), but one of the patients described by Abbuzahab *et al*. had a normal onset of puberty (18). The first patient with a mutation of the ALS gene had a delayed onset of puberty (112), but a later report showed a normal onset of puberty (34). These findings suggest that IGF-I plays a role in pubertal onset.

The pubertal growth spurt is decreased in patients with GH deficiency or resistance with normal testosterone levels. This was initially interpreted by speculating that testosterone needs the presence of normal GH secretion to exert its full growth promoting effect (113). Later, evidence was provided that the growth stimulating effect of testosterone in puberty may be primarily caused by conversion to estrogens and that estrogens are responsible for the pubertal growth spurt and epiphysial closure (114).

IGF-I-/- mice are infertile. Males have drastically reduced testosterone levels, caused by a significant developmental delay of Leydig cells. Females fail to ovulate and possess an infantile uterus with hypoplastic endometrium (115). It is difficult

to hypothesize on the role of GH and IGF-I in reproductive function in the human, as most reported patients with GH-IGF-I defects are too young. Patients with GHRHR mutations (116) and GHR mutation are reported to be fertile. Gonadal function of a 30 year old patient with STAT5b mutation was normal (54). The 55 year old patient with an inactivating IGF-I gene mutation had a small testicular volume, low inhibin B levels and elevated FSH levels, indicating compromised Sertoli cell function and impaired spermatogenesis (14). The role of IGF-I deficiency in the partial gonadal failure is unclear, as this patient underwent a bilateral inguinal hernia operation, with possible damage to the testicles. Patients with a heterozygous deletion or mutation of the IGF-I (13, 14) or IGF1R gene (22) are fertile. More patients and accurate follow-up at adult age will contribute to unravel the role of IGF-I in human reproduction.

Immune system

It has been well established that GH and IGF-I affect the development and function of the immune system (117). Most defects of the GH-IGF-I axis are not associated with immune disorders, except the STAT5b mutation. STAT proteins are involved in the signaling pathway of cytokine receptors (118) and STAT5b-/- mouse exhibit a severe immunologic phenotype (119). So far, 5 females and 1 male with a STAT5b mutation have been reported (52-54, 66, 77). Five patients have signs of immune deficiency. Clinically this results in lymphoid interstitial pneumonia due to Pneumocystis carinii (52), recurrent pulmonary infections (53, 77), and recurrent infections of skin and respiratory tract, severe chronic lung disease and herpetic keratitis (66). One patient suffers from juvenile idiopathic arthritis (53). The 30 year old male patient has no history or signs of immune deficiency (120). Thus, in the human an intact STAT5b is not obligatory for a normal immune phenotype.

We have described a patient with partial GH insensitivity and severe immune deficiency due to a mutation of I-κB, disturbing the NF-κB signaling pathway (121). In vitro studies have shown that GH binding to the GHR can promote the NF-κB signaling pathway (122). Another patient with severe combined immunodeficiency (SCID) and GH insensitivity has been described (123). This patient has a mutation of the IL2Rγ chain gene, suggesting a common underlying pathogenic mechanism for the endocrinological and immunological problems.

More reports on patients with combined growth and immune disorders are needed to increase our knowledge on the interaction between the immune system and the GH-IGF-I axis.

Longevity

Several lines of evidence suggest an inverse relationship between body size and lifespan in mice (124). For example, Laron mice live up to 55% longer than normal mice (125). Genetic studies in various experimental models suggest that the ageing process is regulated by genes that encode proteins from the GH-IGF-I axis. Exciting studies in IGF1R +/- mice with 50% reduction in receptor level, show that females live 33% longer and males 16% longer. The longer lifespan could not be attributed to other factors and the authors conclude that IGF-I may be a central regulator of mammalian lifespan (126). At a cellular level, fibroblasts with a reduced number of receptors are better able to survive to oxidative stress. By causing damage to DNA, protein, lipids and cellular components oxidative stress is the major determinant of the ageing process.. At a molecular level the intracellular signaling pathway is downregulated. Repressing intracellular signals of insulin and IGF-I is an evolutionarily conserved mechanism for extending lifespan.

Caloric restriction also has a positive effect on lifespan. Mild food restriction in normal and the Ames dwarf mice demonstrates changes in the expression of genes related to insulin and IGF-I signaling pathways (127) . These changes may cause the animals to become more sensitive to insulin. Since insulin sensitive mutant animals live longer, it is suggested that these genes may play a role in aging and life span determination. Studies in the human are limited.

Diagnostic approach

The number of patients with defects in the GH-IGF-I axis is still small, but we believe that they represent a tip of the iceberg. Future studies aimed at detecting known or unknown molecular defects in patients with short stature will undoubtedly make the tip of the iceberg grow.

The diagnostic process to reveal an abnormality in the GH-IGF-I axis begins with an

alert physician, who is not satisfied with the diagnosis of idiopathic short stature. The medical history should include birth weight, length and head circumference, as low values of these parameters for gestational age are features of a genetic defect in IGF-I, the IGF1R or a not yet described IGF-I signaling disorder. It cannot be stressed enough that measurement of length at birth is very important to detect underlying pathology and the fear that stretching the legs could be harmful for the hip joint is unjustified (128). Careful evaluation of milestones in development is necessary to have an impression of the psychomotor development. Family history should include height of other family members and if possible birth data. The presence of hearing abnormalities in a family should alert the physician to consider an IGF-I defect.

After excluding organic and systemic disorders like celiac disease and Turner syndrome the IGF-I and IGFBP-3 level will determine the follow up. We recently presented guidelines for the diagnostic process of patients with severe short stature of unknown origin (129).

The procedure for detailed functional and genetic analysis of a patient with short stature and a possible defect in the GH-IGF-I axis depends on the local setting. In the Leiden University Medical Center the Leiden Growth Genetics Working group, consisting of pediatric and adult endocrinologists, clinical and molecular geneticists, molecular biologists and interested physicians meet to discuss the locally, nationally and internationally referred patients. The referring physician is asked to complete a form (Appendix A) to register all the information that is necessary to make a presumptive diagnosis, including phenotypic features of the patient and his or her family and biochemical measurements (IGF-I, IGFBP-3, GH stimulation test). One of the members of the group assesses the information and can advise on additional testing, for example an IGF-I generation test, measurement of other binding proteins, GH-binding protein or ALS. After presentation of the patient and discussion the decision for the next diagnostic step is made, which can be sequencing a specific gene, Multiplex Ligation-dependent Probe Amplification (MLPA) analysis, Single Nucleotide Polymorphism (SNP) array or detailed functional experiments.

Implications for the patient

Idiopathic short stature is an unsatisfactory diagnosis for the physician as well as for the patient. To find the cause of short stature is important for the patient for several reasons. First, with a definite diagnose the often long-lasting diagnostic process will come to an end. Second, it will be possible to give information on the specific defect and accompanying problems. For example, life style advices in case of higher risk of insulin resistance in patients with a heterozygous IGF1R mutation or prevention of osteoporosis in patients with ALS deficiency . Finally, therapeutic options can be discussed. It has been reported that GH therapy in patients with a heterozygous IGF1R mutation or deletion improves growth and head circumference(18, 30, 109, 130). In the late 1980's trials with rhIGF-I started in patients with GHR defects. Approximately 60 children have been treated with rhIGF-I injections for 2 years or longer. Height SDS increases, although not as much as GH treatment in GH deficient patients (131). The explanation for the modest growth response to rhIGF-I is probably the absence of direct GH effects at the level of the growth plate, including enhancement of local IGF-I production. Recently an rhIGF-I-rhIGFBP-3 complex, with a longer half life, has been developed as therapeutic agent. Trials in patients with GH insensitivity are currently in progress (132).

Future perspectives

Future research should be focused on identifying patients with established defects in the GH-IGF-I axis and to evaluate carefully the clinical and biochemical features. Genetic defects in the GH-IGF-I axis are rare and international collaboration will increase the knowledge on the role of the GH-IGF-I axis in growth and development. Finally, it will be a challenge to find new defects in the GH-IGF-I axis in order to unravel the molecular mechanisms that are responsible for the effects of GH and IGF-I on pre-and postnatal growth and development.

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Appendix A

Application form for genetic analysis of a patient with a growth disorder

LU
MC

Patient details

Name

Birth date

Gender

Referring physician

Name

Address

City   Zip Code

Country

E-mail

Date

Medical history

Neonatal period

Gestational age   weeks   days

Birth weight   gr   =   SDS

Birth length   cm   =   SDS

First length after birth: date   cm   =   SDS

Head circumference at birth   cm   =   SDS

First year

Feeding problems   No ☐   Yes:

Psychomotor development

Deafness   No ☐   Yes: indicate type and severity

Other diseases

Date   Diagnosis

Date   Diagnosis

Date   Diagnosis

Symptoms of immune deficiency   No ☐   Yes:

Medication   No ☐   Yes:

Family history

Are the parents related ?   Yes ☐   No ☐

Father   Genetic background

Height   cm   =   SDS

Sitting height   cm   Head circ.   cm

Puberty

Mother   Genetic background

Height   cm   =   SDS

Sitting height   cm   Head circ.   cm

Puberty   Menarche   yr

Siblings   Grandparents

Sib 1, gender [   ]   Height [   ]   Father's father   yr of birth [   ]   Height [   ] cm

Sib 2, gender [   ]   Height [   ]   Father's mother   yr of birth [   ]   Height [   ] cm

Sib 3, gender [   ]   Height [   ]   Mother's father   yr of birth [   ]   Height [   ] cm

Sib 4, gender [   ]   Height [   ]   Mother's mother yr of birth [   ]   Height [   ] cm

Physical examination

Dysmorphic features   No [ ]   Yes: [   ]

Syndrome ?   No [ ] Yes: [   ]

Date [   ] [   ] [   ]

Height [   ] cm = [   ] SDS   **Radiological examination**

Weight [   ] kg = [   ] SDS   Date [   ] [   ] [   ]

BMI [   ] kg/m2 = [   ] SDS   Bone age [   ] yr

Sitting height [   ] cm   Method [   ]

Sitting height/height [   ] = [   ] SDS   Signs of skeletal dysplasia ?   No [ ]

Head circ. [   ] cm = [   ] SDS   Yes: [   ]

Biochemical results

Date [   ] [   ] [   ]   Date [   ] [   ] [   ]

IGF-I [   ] nmol/L = [   ] SDS   Free T4 [   ] pmol/L

IGF-II [   ] nmol/L = [   ] SDS   TSH [   ] mU/L

IGFBP-3 [   ] mg/L = [   ] SDS   Prolactin [   ] [microgr/L] ref range [   ]

ALS [   ] nmol/L = [   ] SDS   Celiac disease excluded ?   No [ ]   Yes [ ]

GH stimulation tests

Date [   ] [   ] [   ] Type of the test [   ] GH peak [   ] mU/L   Priming ? [   ]

Date [   ] [   ] [   ] Type of the test [   ] GH peak [   ] mU/L   Priming ? [   ]

IGF-I generation test

Date [   ] [   ] [   ] GH dose (mg/m2) [   ] for [   ] days

IGF-I at day [   ] : [   ] nmol/L = [   ] SDS   IGFBP-3 [   ] mg/L = [   ] SDS

IGF-I at day [   ] : [   ] nmol/L = [   ] SDS   IGFBP-3 [   ] mg/L = [   ] SDS

IGF-I at day [   ] : [   ] nmol/L = [   ] SDS   IGFBP-3 [   ] mg/L = [   ] SDS

Date [ ] [ ] [ ] GH dose (mg/m2) [ ] for [ ] days

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

Date [ ] [ ] [ ] GH dose (mg/m2) [ ] for [ ] days

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

IGF-I at day [ ] : [ ] [ ] nmol/L = [ ] SDS IGFBP-3 [ ] mg/L = [ ] SDS

Genetic analysis

Karyogram [ ]

DNA analysis

Which genes have already been analyzed ?

Gene [ ] Method [ ] Result [ ]

Gene [ ] Method [ ] Result [ ]

Gene [ ] Method [ ] Result [ ]

Conclusion and presumptive diagnosis

[ ]

Additional remarks

[ ]

Instructions for sending the form and the material

Save the completed form and send as attachment to: m.walenkamp@lumc.nl
or
Print the completed form   [Print Form]   and send to:
M.J. Walenkamp
Dept. of Pediatrics J6-S
Leiden University Medical Center
PO Box 9600
2300 RC Leiden   Include the growth charts and pedigree !
the Netherlands

Send 2 tubes EDTA blood (7-10 ml), couriered at room temperature or a DNA sample,
complete the separate requisistion form for molecular diagnostic analysis
and send this to:
Center for human and clinical genetics, dept. of molecular genetics (LDGA)
Leiden University Medical Center, Building 2 zone S-06-P
PO Box 9600
2300 RC Leiden
the Netherlands