Genetic disorders in the growth hormone-IGF-I axis

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General introduction

Growth is a complex process leading to an increase in size. On a cellular level growth is determined by an equilibrium between hyperplasia (increase in cell number), hypertrophy (increase in cell size), and apoptosis (programmed cell death). These cellular processes are regulated by multiple factors. External factors, including nutrition, psychosocial factors and physical environment interact with internal factors as genetic make-up, hormones and growth factors (1). Despite this complexity, most children grow in a remarkably predictable manner. Deviation from the normal growth pattern can be one of the first manifestations of a disruption of this growth process due to an underlying disorder. Accurate assessment of growth and knowledge of normal growth is therefore a prerequisite for optimal care of children (2).

Stages of growth

Four distinct stages of growth can be considered: fetal, infant, childhood and puberty.

With respect to fetal growth, the first trimester is characterized by forming of the organ systems, coordinated by the expression of various developmental genes. Major cellular hyperplasia takes place in the second trimester, in which peak growth velocity is reached (approximately 62 cm/year) (3). The third trimester is dominated by maturation of the organs and further body growth. The intrauterine environment, determined by maternal factors and placental function, has a large impact on fetal growth throughout gestation. The poor correlation between birth size (weight and length) and parental size reflects the dominant influence of this intrauterine environment over the genotype (3). Fetal factors associated with poor intrauterine growth consist of chromosomal abnormalities as trisomy 21, Turner syndrome and Cornelia de Lange syndrome. Endocrine factors that have been identified to play a role in intrauterine growth are IGF-I, IGF-II, and insulin.

In infancy (the first year of life) children grow rapidly (25 cm/year), but at a decelerating rate. Besides nutritional input the GH-IGF-I system, as well as genetic factors play a role in this stage. In the first two to three years the child establishes its own growth channel, which is highly correlated with target height (gender-corrected mid-parental height). By four years of age average growth velocity is 7 cm/year. At this stage GH, in addition to thyroid hormone, is the major hormonal determi-

nant of growth. Puberty is the last growth phase, characterized by a growth spurt followed by a rapid decrease of growth velocity due to fusion of the growth plate. Besides GH and IGF-I, estrogen is the main determinant of pubertal growth and epiphysial fusion in boys and girls (1).

As discussed above, various known and unknown factors play a role in the process of growth and development in different stages of life. This thesis will focus on the consequences of genetic defects in the GH-IGF-I axis on this complex process.

The GH-IGF-I axis – the historical perspective

Sixty years ago a method for measuring growth hormone activity in human plasma still had to be discovered. At present, the molecular mechanisms underlying GH and IGF-I action are topics of intense research. In the next paragraph the milestones in the history of the GH-IGF-I axis that lead to our current knowledge will be described (4). With this knowledge we were able to identify new genetic defects in patients with short stature, that were previously diagnosed as idiopathic short stature. Consequently, these patients have helped us to further unravel the role of the GH-IGF-I axis in growth and development.

Until 1956, GH activity could only be measured with the "tibia test": administration of GH increases the thickness of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats (5, 6). In 1957 Salmon and Daughaday measured the uptake of radioactive sulphate into costal cartilage in hypophysectomized rats and discovered that, if 10% normal rat plasma was added, there was a 200-300% increase in sulfate uptake. With the administration of increasing doses of GH, however, the sulphate uptake was only slightly increased (7). This laid the basis for their hypothesis that a GH dependent factor, which they termed sulfation factor (SF), was responsible for the stimulation of sulfate uptake. They found low levels of SF activity in patients with hypopituitarism, while patients with acromegaly had high levels of activity. Further proof came from administration of purified human GH to patients with hypopituitarism, which resulted in an increased serum SF level (8), while GH administered to a patient with Laron dwarfism failed to increase the low serum sulfation factor concentration (9). The findings that not only sulphate

uptake, but also protein and DNA synthesis was stimulated in a GH dependent way, and the observation that SF was active in muscle as well, led to the introduction of the more general term: somatomedin, which reflected the expanding scope of SF action (10). The original somatomedin hypothesis was formulated, proposing that GH stimulates somatomedin synthesis and release from the liver and that somatomedin reaches the main target organs via the circulation to act as an endocrine agent (Fig. 1, left panel) (10). In the meantime, another research field showed that non-suppressible insulin-like activity (NSILA) fractions demonstrated somatomedin activity, when added to hypophysectomized rats. On the other hand somatomedin had NSILA action. This raised the suspicion that somatomedin and NSILA were identical. The primary structure of two components of NSILA was published in 1978, which were termed Insulin-like Growth Factor-I and –II (IGF-I and IGF-II) (11, 12). In 1983, Klapper, and colleagues demonstrated that somatomedin-C was identical to IGF-I (13).

In the seventies, the IGF binding proteins (IGFBP's) were discovered (14, 15). After isolation of IGFBP-1 (16) Furlanetto *et al.* showed that the major IGF-BP complex in serum was composed of three elements: somatomedin, an acid stable and an acid-labile subunit (17). The latter two components were IGFBP-3 and ALS. The

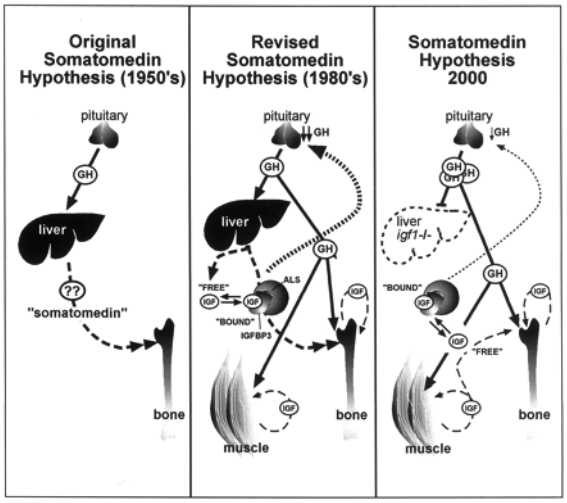

Figure 1. Evolving concepts of the somatomedin hypothesis (with permission from (21) copyright 2001, The Endocrine Society).

binding proteins appeared to act as carrier proteins, prolonging the half life of the IGF's by protecting them from proteolytic degradation, regulating the local action of IGF's and modulating IGF-I receptor activation. In addition, they seemed to regulate cell activity in various ways (18).

In the 1980's molecular biology allowed to determine that IGF-I was expressed in multiple tissues throughout embryonic and postnatal development and adult life, indicating that IGF-I also acts in a paracrine manner (19, 20). A revised version of the somatomedin hypothesis postulated that both endocrine and locally produced IGF-I are responsive to GH and therefore responsible for the effects of GH (Fig. 1, middle panel) (21). In addition, strong indications were found that GH also had a

Table 1. Characterization of genes in the GH-IGF-I axis and the first clinical report on a genetic defect.

Gene	Characterization	First clinical report on genetic defect
GH1	1979, Martial *et al*. Human growth hormone: complementary DNA cloning and expression in bacteria (31)	1981, Phillips *et al*. Molecular basis for familial isolated growth hormone deficiency. (32)
GHR	1987, Leung *et al*. Growth hormone receptor and serum binding protein: purification, cloning and expression (33)	1989, Godowski *et al*. Characterization of the Human Growth Hormone Receptor Gene and Demonstration of a Partial Gene Deletion in Two Patients with Laron-Type Dwarfism (34)
GHRH	1992, Mayo *et al*. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone (35)	1996, Wajnrajch *et al*. Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse (36)
IGF-I	1983, Jansen *et al*. Sequence of cDNA encoding human insulin-like growth factor I precursor (37)	1996, Woods *et al*. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene (38)
IGF1R	1992, Abbott *et al*. Insulin-like growth factor I receptor gene structure (39)	2003, Abuzzahab *et al*. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation (40)
STAT5b	1996, Silva *et al*. Characterization and cloning of STAT5 from IM-9 cells and its activation by growth hormone (41)	2003, Kofoed *et al*. Growth hormone insensitivity associated with a STAT5b mutation (42)
ALS	1988, Baxter RC. Characterization of the acid-labile subunit of the growth hormone-dependent insulin-like growth factor binding protein complex (44)	2004, Domene HM *et al*. Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene (45)
GHSR	1996, Howard *et al*. A receptor in pituitary and hypothalamus that functions in GH release (30)	2006, Pantel *et al*. Loss of constitutive activity of the GHSR in familial short stature (43)

direct effect on the epiphyseal growth plate (22). Experiments with IGF-I knockout mice, exhibiting a birth weight of only 60% of normal, indicated a direct, GH-independent effect of IGF-I on prenatal growth (23-25).

Tissue specific gene deletion experiments in mice resulted in the most recent, but undoubtedly not the final, revision of the somatomedin hypothesis, incorporating the role for IGF-I in glucose homeostasis and bone modeling (Fig. 1, right panel) (26). Mice with liver-specific IGF-I gene-deletion (LID) and consequently markedly reduced circulating IGF-I levels develop insulin resistance (27). In addition, these LID mice show a significant decrease in cortical bone volume (27).

Genes encoding the different components of the GH-IGF-I axis have now been identified and in the last few years mutations and deletions in these genes have been described in the human. Table 1 shows the original reports on the characterization of the genes involved in the GH-IGF-I axis and the first clinical description of the genetic defect.

The GH-IGF-I axis – present view

GH secretion is regulated by the hypothalamic factors GH releasing hormone (GHRH) and somatostatin. The pulsatile fashion of GH secretion is regulated by an interaction between these hormones. The release of GH is controlled by a wide range of other neurotransmitters and neuropeptides (28). The most potent GH secretagogue is ghrelin, a hormone predominantly produced by the stomach (29) whose plasma levels fluctuate with food intake. Ghrelin acts via the growth hormone secretagogue receptor (GHSR), which is highly expressed in the brain and in the pituitary (30).
The biological actions of GH are mediated by the transmembrane GH receptor (GHR). The GHR is a cytokine receptor, subject to various modifications during synthesis of which the generation of a soluble GH binding protein (GHBP), consisting of the extracellular domain of the GHR, is the most significant. The GHR uses the JAK-STAT signal transduction pathway (Fig. 2). Activation of the receptor ultimately results in transcription of target genes, including IGF-I, IGFBP-3, and ALS. Binding of IGF-I to the IGF-I receptor type I results in activation of this tyrosine kinase receptor leading to the physiological actions of IGF-I (Fig. 3).

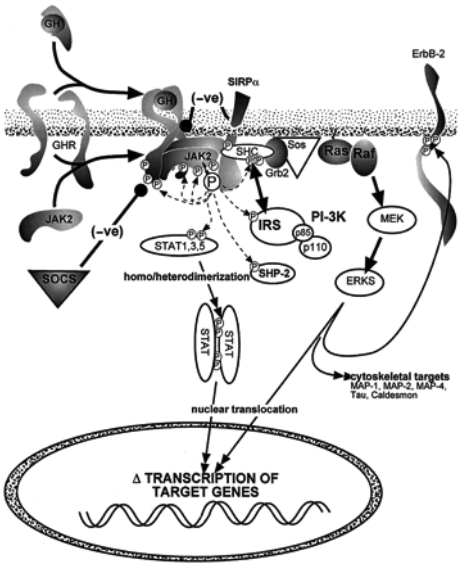

Figure 2. GH signal transduction pathway (with permission from (21) copyright 2001, The Endocrine Society).

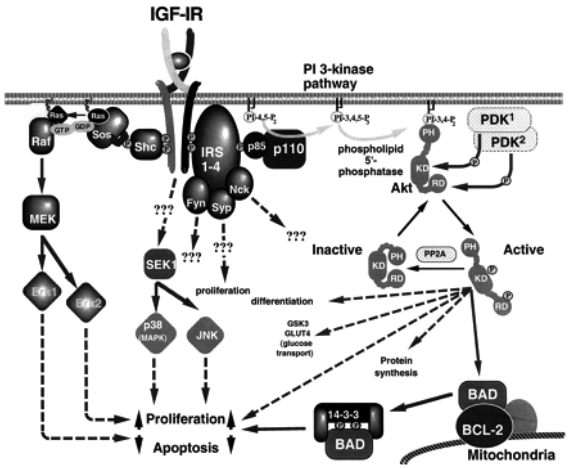

Figure 3. IGF-I signal transduction pathway (with permission from (21) copyright 2001, The Endocrine Society).

Outline of this thesis

Alert physicians, collaborating with geneticists and molecular biologists have presented many reports on patients with genetically determined causes of short stature. This thesis, describing the phenotypical and molecular characteristics of patients with genetic defects in various components of the GH-IGF-I axis is the result of such collaboration. The aim of this thesis was to study the genotype-phenotype relationship in these patients and to unravel the role of the GH-IGF-I axis in the complex process of growth and development throughout life.

Chapter 1 offers a general introduction and is followed by a review on genetic disorders in the GH-IGF-I axis, including a proposal for the diagnostic evaluation of patients with severe short stature in *chapter 2*.

Classical GH deficiency can be the result of mutations in the GHRH receptor gene, a defect in one of the genes involved in pituitary development or a mutation or deletion in the GH1 gene. *Chapter 3* describes two sibs with a GHRHR mutation and this report is focused on the positive effect of the combined treatment of GH and GnRH analogue on final height.

GH insensitivity is caused by a genetic defect of the GHR (Laron syndrome) or a post GHR signaling defect. The first male patient with GH insensitivity caused by a homozygous STAT5b mutation is described in *chapters 4 and 5*: the clinical and biochemical features in *chapter 4* and a detailed description of the growth hormone secretion pattern and immunological function in *chapter 5*.

The first patient with a homozygous missense mutation of the IGF-I gene, resulting in a bioinactive IGF-I protein is described in *chapter 6*, followed by the structural and functional analysis of the mutant IGF-I in *chapter 7*.

IGF-I resistance can be the result of genetic defect of the IGF-I receptor. In *chapter 8* a mother and daughter with a heterozygous missense mutation in the intracellular part of the IGF1R is described. A positive effect of GH treatment in a patient with a heterozygous terminal 15q deletion, including the IGF1R receptor, shows the clinical implications of this defect in *chapter 9*.

In *chapter 10* the significance of the findings is discussed, followed by a summary in *chapter 11*.

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