Genetic disorders in the growth hormone-IGF-I axis

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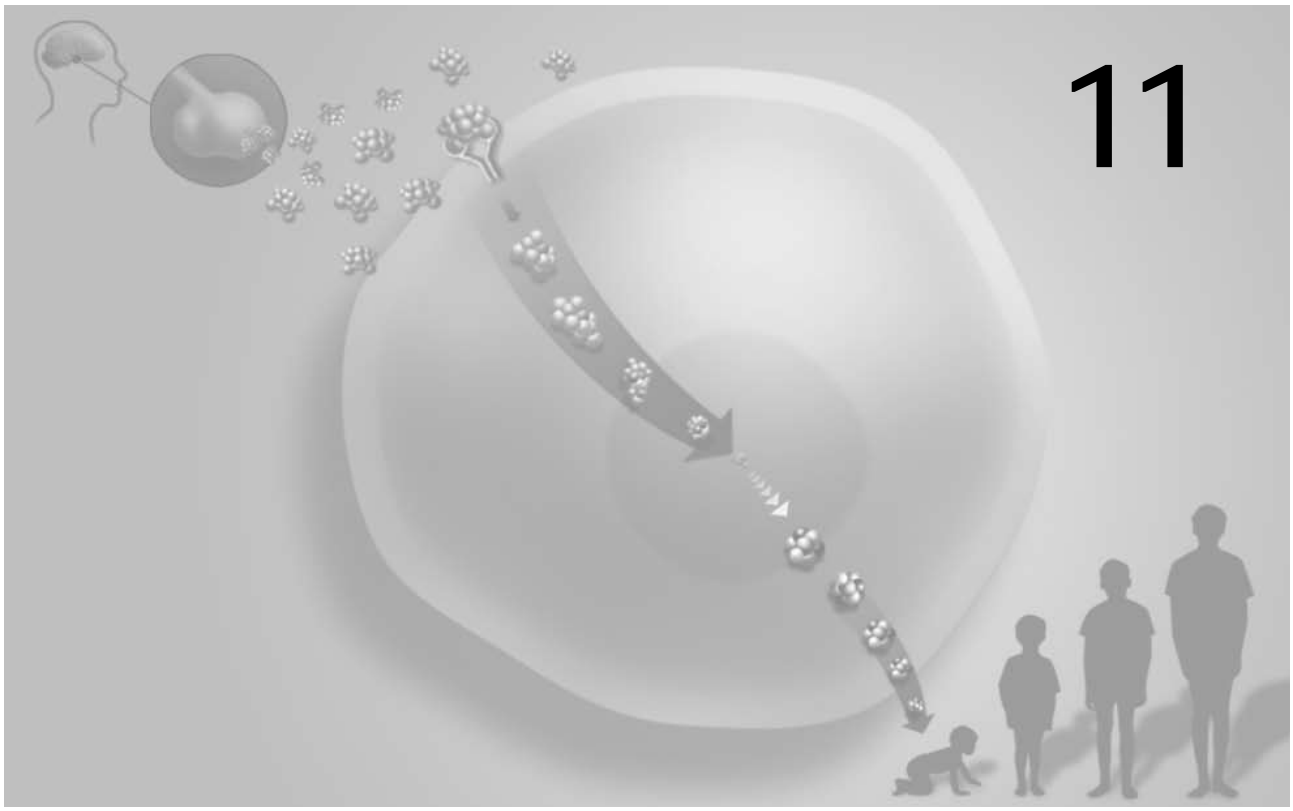
Summary



Growth is a complex process, regulated by multiple external and internal factors. Deviation from the normal growth pattern can be one of the first manifestations of an underlying disorder, disrupting the normal growth process. The GH-IGF-I axis plays a key role in regulating the growth process. This thesis focuses on growth disorders as a result of genetic defects in the GH-IGF-I axis.
The aim of this thesis is to study the genotype-phenotype relationship in patients with a documented genetic defect in a component of the GH-IGF-I axis and to unravel the role of the GH-IGF-I axis in the complex process of growth and development throughout life.

Chapter 1 describes the milestones in the history of the GH-IGF-I axis that lead the basis for our current knowledge. In addition, the present view on the GH-IGF-I axis is summarized.

Chapter 2 gives an overview of the clinical aspects and biochemical parameters of the various genetic defects in the GH-IGF-I axis. Classical GH deficiency, resulting in decreased or absent pituitary GH secretion, can be the result of a mutation in the GH releasing hormone receptor (GHRH-R) gene, a defect in the ontogenesis of the GH producing cells or a mutation or deletion of the GH1 gene. Since the first paper that showed the etiology of Laron syndrome, many mutations in the GH receptor have been identified. Several patients have been described with a STAT5b mutation, which disrupts the GH signal transduction. More recently, genetic defects in the IGF-I gene or the IGF-I receptor gene have been described. All these defects result in proportionate short stature. Therefore, in the workup of a patient with proportionate short stature genetic analysis is essential. However, careful selection of the patients is a prerequisite for optimal results. Therefore, we developed flowcharts, based on the described patients in combination with theoretical considerations. These flowcharts can be used as guidelines in the diagnostic process of patients with idiopathic short stature.

Chapter 3 describes a brother and sister with a mutation of the GHRH-R gene. They presented with a height of -5.8 SDS and -7.6 SDS at an advanced age of 16 and 14.9 years, respectively, and an advanced pubertal stage. Genetic analysis revealed a homozygous single base pair transition at the splice donor site of intron 7. Both patients were treated with GH. In order to combine the growth promoting effect of

GH with postponing puberty and delaying skeletal maturation, GH treatment was combined with the administration of a gonadotropin-releasing hormone analog (GnRHa). This combination treatment was highly effective in increasing final height. Sitting height/height ratio decreased in both patients during treatment. Although this ratio was within the normal population range, one could conclude that combined treatment with GH and GnRHa may result in relatively longer legs. Bone mineral density was in the lower normal range in both patients at adult age. However, both patients developed severe vitamin D deficiency as adults, which confounds the effects of GnRHa treatment on the acquisition of peak bone mass.

GH insensitivity can be caused by defects in the GH receptor (Laron's syndrome) or a defect in the postreceptor signaling pathway. Recently, reports on two female patients with severe postnatal growth retardation, pulmonary problems and immunodeficiency were published. These patients had a homozygous defect in the STAT5b gene. In **chapter 4 and 5** of this thesis the first male patient is described with a homozygous frameshift mutation in the STAT5b gene, resulting in an inactive truncated protein, lacking most of the DNA binding and SH2-domain. The phenotype consisted of severe short stature (final height -5.9 SDS), delayed puberty and high body fat percentage (40%), but no history or signs of pulmonary problems or immunodeficiency. Biochemically extremely low levels of IGF-I (-6.9 SDS), IGFBP-3 (-12 SDS), and ALS (-7.5 SDS) were present. In addition, prolactin levels were elevated. 24-h GH and prolactin secretion characteristics were assessed. The GH secretory parameters were comparable with healthy male controls. However, one could hypothesize that GH secretion is severely suppressed by his visceral adiposity. Prolactin secretion was increased by six-fold. High doses of GH in the IGF-I generation test showed a response of IGF-I to levels approaching reference range values. Evaluation of the monocyte and T-cell function revealed no abnormalities. The heterozygous family members of the patient showed no signs of GH insensitivity.
In conclusion, STAT5b deficiency causes a disruption of the GH signaling pathway, resulting in severe short stature. Although STAT5b plays a role in signaling processes in immune cells, apparently, immunodeficiency is not an obligatory symptom of STAT5b deficiency, whereas hyperprolactinemia appears to be part of the syndrome.

Chapter 6 reports on the first patient with an inactivating homozygous missense mutation of the IGF-I gene. The phenotype consisted of severe intrauterine growth retardation (birth weight -3.9 SDS and birth length -4.3 SDS), reflecting the GH-independent IGF-I secretion *in utero*. Severe mental retardation, microcephaly (head circumference -8 SDS) and sensorineural deafness were observed and considered as a consequence of IGF-I defciency on intrauterine brain development. The postnatal growth pattern was comparable with untreated GH deficient or GH-insensitive patients (final height -8.5 SDS), which is in line with the hypothesis that IGF-I secretion in childhood is mainly GH-dependent. Apparently IGF-I deficiency is well tolerated after adolescence, considering the relative healthy condition of the 55-yr-old patient. Biochemically, the patient had high IGF-I levels (+7.3 SDS) and a stimulated GH secretion in the upper normal range.
In addition, 24 relatives of the patient were studied: nine heterozygous carriers were identified, which had a significantly lower birth weight, final height and head circumference than the noncarriers.

The structural and functional characterization of the IGF-I mutation is described in detail in **chapter 7**. The mutation lead to the expression of IGF-I with an aminoacid substitution of methionine instead of a valine at residue 44 of the protein(val^{44}met IGF-I). Val^{44}met IGF-I exhibited a 90-fold decrease in IGF1R binding compared with wild type IGF-I. An indirect argument for the important role of this part of the molecule is provided by observations that a point mutation in the insulin gene (val^{A3}leu insulin, also termed insulin Wakayama, corresponding with Val^{44} of IGF-I.) result in hyperinsulinemia due to severely defective insulin receptor binding. Activation of downstream signaling by val^{44}met IGF-I was reduced, corresponding with the reduced affinity for the IGF1R. Also, val^{44}met IGF-I was unable to stimulate DNA synthesis. Binding or activation of both insulin receptor isoforms was not detectable. However, val^{44}met IGF-I bound IGFBP-2, IGFBP-3 and IGFBP-6 with equal affinity to IGF-I, suggesting the maintenance of the overall structure. Nuclear magnetic resonance studies confirmed retention of near-native structure with only local side-chain disruptions.

In **chapter 8** we describe a mother and daughter with a heterozygous missense mutation in the intracellular tyrosine kinase domain of the IGF1R. The phenotype of the mother consisted of mild intrauterine growth retardation (birth weight -

2.1 SDS, birth length -0.3 SDS) and progressive postnatal growth failure (final height -4 SDS), microcephaly (head circumference -3 SDS) and failure to thrive. The daughter suffered from severe intrauterine growth retardation (birth weight -3.3 SDS, birth length -4.2 SDS), microcephaly (head circumference -5.2 SDS) and postnatal growth failure. In both patients IGF-I levels were elevated (+1.6 SDS and +2.9 SDS, respectively). Functional characterization revealed normal binding of IGF-I to the IGF1 receptor, but marked reduction of autophosphorylation and activation of the downstream signaling cascade, suggesting inactivation of one copy of the IGF1R gene. A summary of the clinical features of the other patients described in the literature leads to the hypothesis that the degree of intrauterine growth retardation may be determined by the presence or absence of maternal IGF-I resistance.

In **chapter 9** the phenotype of a heterozygous terminal 15q deletion is described, consisting of intrauterine growth retardation, postnatal growth retardation, microcephaly, and elevated IGF-I levels. These phenotypic features are similar to the features found in patients with an inactivating IGF1R mutation and can therefore be ascribed to the loss of one copy of the IGF1R gene. This was diagnosed with a novel genetic technique: multiplex ligation-dependent probe amplification (MLPA). Subsequently, array comparative genomic hybridization was used to define the deleted area, which was 15q26.2->qter. The patient was effectively treated with GH, reaching a final height of -1.8 SDS, which is within the population range, although not in her genetic target range.

Chapter 10 reviews the reports of patients with a genetic defect in the GH-IGF-I axis and summarizes the data of animal knockout experiments. The role of the GH-IGF-I axis in intrauterine and postnatal growth is described. In addition, the effects of the GH-IGF-I axis on the development and function of different organ systems as brain, eye, skeleton, glucose homeostasis, gonadal function and immune system are discussed. In this chapter a systematic diagnostic approach and selective genetic analysis in a patient with short stature is advocated in order to identify more patients with a genetic defect in the GH-IGF-I axis and thereby increasing the knowledge on genes that play a role in the complex process of growth and development.