



Universiteit
Leiden
The Netherlands

Acromegaly : irreversible clinical consequences

Wassenaar, M.J.E.

Citation

Wassenaar, M. J. E. (2010, June 10). *Acromegaly : irreversible clinical consequences*. Retrieved from <https://hdl.handle.net/1887/15664>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/15664>

Note: To cite this publication please use the final published version (if applicable).

Chapter 12.

**THE EFFECT OF THE EXON-3 DELETED
GROWTH HORMONE RECEPTOR
POLYMORPHISM IN VARIOUS CLINICAL
CONDITIONS: A SYSTEMATIC REVIEW.**

M.J.E.Wassenaar, N.R.Biermasz, A.M.Pereira, J.A.Romijn.

Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center,
Leiden, The Netherlands.

Article submitted

ABSTRACT

The GHR is the first key molecule mediating growth hormone (GH) action. In 2004 Dos Santos *et al.* were the first to link a common GH receptor (GHR) polymorphism, the exon 3 deleted GHR (d3GHR), to increased responsiveness to recombinant human GH (rhGH) treatment in children with short stature. Since then, this polymorphism has been under extensive investigation regarding its influence to the response on rhGH therapy in different clinical populations. This review focuses on studies evaluating the effect of the polymorphism on respectively growth, GH-insulin-like growth factor I (IGF-I) axis, metabolism, cardiovascular disease risk, and survival.

There are discrepant results on the effects of the d3GHR on the GH-IGF-I axis between studies in GHD and acromegaly. However, some of the few studies investigating the effect of this polymorphism on the GH-IGF-I axis indicate a positive effect of the GHR_{d3} genotype (GHR_{d3-d3} or GHR_{wt-d3}) on IGF-I concentrations. The GHR_{d3} genotype seems to affect metabolism, since in patients with type 1 and 2 diabetes mellitus (DM) and in patients with impaired glucose tolerance (IGT) the frequency of the GHR_{d3} genotype is significantly lower than in the normal population. In addition, in a cohort of healthy Caucasian children and adolescents, higher insulin secretion in the presence of the d3GHR was demonstrated. These findings suggest that increased bioactivity of this allele confers a protection function against type 2 DM. The GHR_{d3-d3} isoform is more frequently present in patients suffering from starvation, indicating that both the elevated GH gene expression and increased GHR-mediated GH responsiveness may constitute adaptive responses to the effects of persistent malnutrition, since increased circulating GH appears to form part of a physiological response to nutrition deprivation. The prevalence of the GHR_{d3} isoform in well-fed populations is currently unknown. But in patients with type 2 DM and IGT, the GHR_{d3} isoform is associated with more severe obesity than in patients with the other isoforms. In 2 cohorts studied, there was no association between cardiovascular risk factors like hypertension and stroke and the d3GHR polymorphism.

We conclude that the effects of the d3GHR polymorphism are not limited to subtle variations in growth parameters in (non)GHD children treated with rhGH, but includes many pathophysiological processes in which the GH-IGF-I axis is involved. The implications of these genotype-phenotype relationships of the GHR for routine clinical practice seem to be limited.

- I. Introduction
 - A. The GHR gene structure
 - B. Impaired growth due to mutations of the GHR
 - C. GH resistance syndromes caused by post-GHR defects
 - D. Polymorphisms of the GHR

- II. Effects of d3GHR on the GH/IGF-I axis
 - A. Pathogenesis
 - B. Growth hormone deficiency
 - 1. Growth hormone deficiency in children
 - 2. Growth hormone deficiency in adults
 - C. Active and controlled acromegaly
 - D. Conclusion

- III. Effect of d3GHR on growth
 - A. Pathogenesis
 - B. Children with short stature due to growth hormone deficiency
 - C. Children with short stature not due to growth hormone deficiency
 - 1. Small for gestational age
 - 2. Idiopathic short stature
 - D. Turner syndrome
 - E. The effect of d3GHR genotypes on basal height
 - F. Conclusion

- IV. Effects of the d3GHR on other endocrine disorders
 - A. Pathogenesis
 - B. Growth hormone deficiency in adults
 - C. Acromegaly
 - 1. Active acromegaly
 - 2. Controlled acromegaly
 - 3. Response to treatment
 - D. Conclusion

- V. Effect of d3GHR on metabolism
 - A. Pathogenesis
 - B. Diabetes Mellitus
 - 1. Type 1 Diabetes Mellitus
 - 2. Type 2 Diabetes Mellitus
 - C. Impaired glucose tolerance
 - D. Conclusion

- VI. Effect of d3GHR on cardiovascular diseases
 - A. Pathogenesis
 - B. Hypertension
 - C. Stroke
 - D. Conclusion

- VII. Effect of d3GHR on survival
 - A. Pathogenesis
 - B. Obesity and starvation
 - C. Conclusion

- VIII. Summary and conclusions

I. Introduction

Human growth hormone (GH) promotes post-natal growth of the skeleton and soft-tissue as well as acts as an important regulator of bone turnover, muscle mass, and immune function. In addition, the GH-insulin-like growth factor type I (IGF-I) axis is important for metabolic control¹. GH has several effects via the growth hormone receptor (GHR) on metabolism. Some of these effects are IGF-I-dependent, such as on glucose uptake and protein metabolism, whereas other effects are IGF-I-independent, including stimulation of insulin secretion, lipolysis, and gluconeogenesis¹.

The biological actions of GH are mediated by the activation of its cell-surface receptor, the GHR. The composition of this dimeric GHR depends on the assortments of coding polymorphisms of the GHR gene. Three variants of the GHR, that differ in the presence or absence of exon-3 ($\text{GHR}_{\text{wt-wt}}$, $\text{GHR}_{\text{wt-d3}}$, and $\text{GHR}_{\text{d3-d3}}$), are commonly seen². In mammals, the variation causing the isoforms may occur at either the genomic³ or mRNA splicing level². The function of exon 3 is unknown, although this exon, and thus the deletion, is in close proximity to the GH binding site⁴. The loss of exon 3 appears to have little effect on the receptor, as $\text{GHR}_{\text{wt-d3}}$ and $\text{GHR}_{\text{d3-d3}}$ are stable and functional receptors without apparent differences in binding activity or internalization compared with $\text{GHR}_{\text{wt-wt}}$. While either allele alone is sufficient for normal growth³, the presence of at least one deleted allele is thought to confer an increased growth response to GH therapy⁵.

The presently known functional consequences of the exon 3 deleted GHR polymorphism in various clinical conditions and physiological processes are the subjects of this review.

I A. The GHR gene structure

The GHR is a member of the class I hematopoietic cytokine receptor superfamily, which currently includes more than 40 members⁶. The GHR gene is located on the short arm of chromosome 5 (p13.1-p12) and has 9 exons that encode the receptor and several additional exons in the 5-prime untranslated region. The coding exons span at least 87kb. The GHR consists of an extracellular domain of 246 amino acids (aa), a single transmembrane domain of 24 aa, and a cytoplasmic domain of 350 aa⁷. Exon 2 encodes an 18-amino-acid signal sequence. Exons 3 to

7 encode the extracellular domain. Exon 8 encodes the transmembrane domain, and exon 9 and 10 encode the cytoplasmic, signal-transduction domain and the 3-prime untranslated region. The active form of the GHR is a receptor homodimer^{8,9}. The first step in GHR activation is the binding of GH to the GHR. GH binding to preformed dimers of GHRs located at the cell membrane surface induces rotation of the intracytoplasmatic subunits of the dimerized receptors, resulting in the activation of associated kinases like the Janus tyrosine kinase (JAK) 2, the mitogen-activated protein (MAP) kinase, and the phosphatidylinositol-3 (PI-3) kinase. The pathways involving these three kinases are assumed to account for the most signaling events triggered by the GHR¹⁰ and are also referred to as the JAK/STAT (signal transducers and activators of transcription) pathway. Detailed description of the features of the receptor is beyond the scope of this review article, but numerous reviews have been published on this subject¹⁰⁻¹⁶.

I B. Impaired growth due to mutations of the GHR

In a small number of humans, growth defects result from rare mutations of the GHR. Mutations have been found to alter various intrinsic features of the GHR that are required for proper activation of the receptor itself, or of some components of the GHR signaling cascades. A clinical consequence of inactivating mutations of the GHR is the GH insensitivity syndrome, first described by Laron in 1966. Laron syndrome is an autosomal, fully penetrant recessive disease resulting from GH resistance. Phenotypic characteristics are severe growth retardation, acromicria, small gonads and genitalia, abdominal adiposity, and high GH concentrations in combination with very low circulating IGF-I concentrations. The pathogenesis of this syndrome is explained by various molecular defects mainly affecting the GHR, including exon deletion and mutations (nonsense, frameshift, missense)¹⁷. Most of these mutations affect the extracellular domain of the receptor, which results in the absence of circulating GH binding protein (GHBP). Normal or high GHBP levels in GH resistance syndromes denote that the mutation resides within transmembrane or cytoplasmic domains of the GHR or affects post-receptor components. At present three types of mutations of the GHR have been described, resulting in different severities of the phenotypic consequences, i.e. expression failure¹⁸, activation failure¹⁹⁻²¹, and signaling failure²²⁻²⁴.

I C. GH resistance syndromes caused by post-GHR defects

Recently, two mutations have been described in STAT5B. First, a homozygous mutation in STAT5B was associated with GH insensitivity²⁵. The mutated Stat5 was inactive and blocked all cellular events downstream in the JAK2-STAT5 cascade, including IGF-I deficiency. Second, a mutation was described leading to the total absence of detectable mature protein due to early termination. This mutation combines severe GH insensitivity with immunodeficiency^{26;27}. In addition, mutations of IGF-I²⁸ or of the IGF-I receptor²⁹ leading to intrauterine and postnatal growth retardation have also been documented as causes of GH resistance syndromes. Both the post-growth hormone receptor mutations as the IGF-I (receptor) mutations are not the focus of this review.

I D. Polymorphisms of the GHR

Most of the GHR polymorphisms are single nucleotide polymorphisms or microdeletions. The functional role of most of these polymorphisms at the molecular level and the way they can modify GHR function at the physiologic level have not been elucidated. Polymorphisms of the GHR have been described in exon 3, 6 and 10³⁰. One of these polymorphisms, the deletion of exon 3, has been studied more extensively because of its relatively high prevalence. The expression of the different d3GHR isoforms is consistent with simple mendelian inheritance of 2 different alleles in which exon 3 is spliced in an 'all or none' fashion. The alternative splicing of exon 3 in GHR transcripts is the result of an unusual polymorphism that significantly alters splicing of the transcript. The relatively high prevalence (~ 40% and ~10% for heterozygosity and homozygosity in the population, respectively) for the allele producing transcripts lacking exon 3 suggests the possibility that it may play a role in polygenically determent events, i.e. may have selective advantage under certain circumstances. A genetic polymorphism resulting in deletion of an entire exon from mRNA without compromising structure or function of the resultant protein is very unusual³¹. It was initially thought that the isoform lacking exon 3 was tissue dependent³²⁻³⁴. However, it became apparent that the exon 3 deletion was not tissue dependent but individual dependent³⁵.

The effect of the deletion of exon 3 has been investigated in several clinical conditions, some studies focusing on the single or double deletion of exon 3 as one genotype (GHR_{wt} vs. GHR_{d3}), and other studies regarding the single or double deletion of exon 3 as two different

genotypes ($\text{GHR}_{\text{wt-wt}}$ vs. $\text{GHR}_{\text{wt-d3}}$ and $\text{GHR}_{\text{d3-d3}}$).

II. Effects of d3GHR on GH/IGF-I axis

II A. Pathogenesis

Biologically active GH binds its transmembrane receptor (GHR), which dimerizes to activate an intracellular signal transduction pathway, resulting in complex effects including synthesis and secretion of IGF-I. IGF-I is responsible for most anabolic and mitogenic actions of GH, including growth promotion and development of organs and tissues during all growth stages³⁶. IGF binding protein 3 (IGFBP3) is the main of several proteins which increases IGF-I half-life, transports it to the target cells and guides its interaction with membrane receptors. Serum IGF-I and IGFBP3 are usually reduced in patients with GHD.

The prevalence of the different isoforms of the GHR is comparable between GHD, acromegaly, and healthy subjects, which excludes a role of GH in regulating GHR isoform expression patterns including the d3GHR. The question is whether the different d3GHR isoforms explain some of the individual differences in GH and IGF-I concentrations in patients with disturbances in the GH-IGF-I axis. For the purpose of this review we separately analyze the effects of the d3GHR on diseases of the GH-IGF-I axis, i.e. in children with GHD, adults with GHD, patients with active and controlled acromegaly.

II B. Growth hormone deficiency

GHD is a clinical condition in children, in whom GH production is insufficient for normal height gain and development of bone and muscle. GHD is a clinical condition in adults, in whom GH production is inadequate for the maintaining of muscle- and bone mass and other GH dependent body functions. There are several causes for GHD including congenital and acquired pituitary lesions.

II B1. *Growth hormone deficiency in children*

GHD in children is responsible for impaired height gain, bone and muscle development. The effect of the GHD will only develop some months after birth and, if left unadjusted, is responsible for late puberty. Clinical signs are impaired growth, immature face, and (a slightly) obese

stature.

Five studies in GHD children have investigated the effects of d3GHR genotype on the GH-IGF-I axis³⁷⁻⁴¹. The effect of d3GHR genotype on baseline IGF-I concentrations in GHD was studied by Blum *et al.*, who reported lower IGF-I concentrations in GHR_{wt-wt}³⁷. Another study demonstrated genotype specific differences in IGF-I concentrations in response to (short-term) rhGH treatment, with the lowest response in GHR_{wt-wt} despite equal GH and IGFBP3 concentrations in the different genotypes³⁹. This is in accordance with the theory proposed by Dos Santos⁵, of enhanced growth in GHR_{d3} in children who are small for gestational age (SGA) or have idiopathic short stature (ISS). In fact, they provided evidence for increased bioactivity of GHR_{d3} *in vitro* in response to various GH concentrations compared with GHR_{wt-wt}⁵. In addition, a slightly higher GH sensitivity regarding short-term IGF-I generation during rhGH therapy was seen in ISS patients with the GHR_{d3} genotype⁴². The three remaining studies in GHD children were unable to demonstrate any effect on IGF-I^{38;40;41}. Studies in Turner's syndrome of Cushing's disease did not observe any effect on IGF-I either^{43;44}.

In conclusion, two of the seven studies providing data on the effect of the d3GHR genotype on the GH-IGF-I axis in GHD demonstrated a genotype specific difference. This effect was uniform in both studies, indicating higher IGF-I concentrations in the GHR_{d3} genotype, compared with GHR_{wt-wt}³⁷⁻⁴¹. There were no genotype-phenotype relationships with respect to GH or IGFBP3 concentrations. Additional studies are required to establish whether the subtle clinical effect of the GHR_{d3} genotype is reflected in circulating IGF-I concentrations.

II B2. Growth hormone deficiency in adults

The syndrome of GHD is different in adults compared with children. Although GH requirements decrease after puberty, the percentage of prepubertal GHD subjects who have insufficient GH reserve during retesting in adulthood, varies considerably among the different studies with a frequency of persistence of GHD between 20 and 87%⁴⁵. In adulthood, the metabolic actions of the GH/IGF-I axis take place at plasma levels which are markedly lower than those needed to promote longitudinal growth. IGF-I concentrations in adults are positively related to GH secretion patterns and are able to detect part of the adult patients with suspected GHD. Although many adult patients with GHD have normal IGF-I levels, there is agreement that IGF-I is the best parameter during follow-up of rhGH replacement therapy⁴⁶.

Van der Klaauw *et al.* showed that the GHR_{d3} genotype was associated with differen-

ces in effects of rhGH during short-term, but not during long-term, individually adjusted rhGH replacement therapy in adults with GHD⁴⁷. In accordance to children with GHD^{37;39}, a higher increase in IGF-I levels in GHR_{d3} patients compared with GHR_{wt-wt} patients was demonstrated after a comparable dose of rhGH⁴⁷. Meyer *et al.* demonstrated in a German population of adult GHD patients on 1 year of rhGH therapy that GHR_{d3} patients required 25% less GH for the same IGF-I levels compared with GHR_{wt-wt} patients⁴⁸. Finally, Moyes *et al.* studied a large cohort of, at baseline comparable, adults with GHD on an identical dose of rhGH for 1 year. After 1 year of rhGH treatment, patients with GHR_{d3-d3} demonstrated a significantly larger increase in IGF-I concentrations compared with patients with GHR_{wt-wt}. However, when GHR_{d3-d3} and GHR_{wt-d3} patients were analyzed together this difference with GHR_{wt-wt} was not present⁴⁹. In contrast to these 3 studies, a Swedish study in GHD adults treated for 1 year with individually titrated rhGH dose did not demonstrate any effect of the GHR_{d3} on IGF-I levels⁹⁶.

In conclusion, four studies focused on the effect of d3GHR on the GH-IGF-I axis in response to rhGH in GHD adults, with contrasting short-term results. However, the differences between the genotypes in effect on the GH-IGF-I axis were subtle, or only demonstrable in GHR_{d3-d3} *vs.* GHR_{wt-wt}. When patients with GHR_{wt-d3} and GHR_{d3-d3} are analyzed together, the effect vanished. Moreover, most of the above mentioned studies included relatively low numbers of patients, resulting in low statistical power. Only prospective and large studies with large numbers of all genotypes will have the required statistical power to identify a potential, additional contribution of d3GHR polymorphism to the GH-IGF-I axis in response to GH therapy.

II C. Active and controlled acromegaly

Acromegaly is a chronic, slowly progressive disease caused by a growth hormone-secreting pituitary adenoma leading to increased circulating GH levels and increased IGF-I secretion by peripheral tissues, especially the liver. The GHR_{d3} genotype has been associated with increased IGF-I levels^{37;39;47}. Several studies assessed the effects of the d3GHR polymorphism on the disease course in acromegaly. An underlying concept was that the d3GHR polymorphism may have caused a stimulation of the GH-IGF-I axis and therefore rendered acromegalic patients with the GHR_{d3} genotype more susceptible for the effects of GH.

Only a few studies have focused on the effect on biochemical parameters of the d3GHR genotype in active acromegaly patients. One study in patients with newly diagnosed

active acromegaly revealed that patients carrying a GHR_{d3} allele had lower GH concentrations, but comparable serum IGF-I concentrations, than patients without this genotype, in accordance with the notion of a more active GH signal⁵⁰. However, Kamenicky *et al.* was not able to show an effect of the GHR_{d3} genotype on GH-IGF-I relationships in 105 patients with active acromegaly⁵¹. Another study, studied 152 patients at baseline and 1 to 5 years post-treatment. Although these authors observed no effects on GH and IGF-I concentrations at baseline in GHR_{d3} patients, these patients were less likely to achieve normal IGF-I concentrations during treatment for acromegaly¹⁰. However, the pathophysiological mechanism behind these unconfirmed observations is not completely understood.

Circulating lymphoid cells express GHR variants, and, therefore, peripheral lymphocytes may be useful for evaluating GHR physiology. Ochoa *et al.* compared expression patterns of the GHR isoforms in lymphocytes between healthy individuals and patients with active acromegaly⁵². In healthy subjects a predominance of the $\text{GHR}_{\text{wt-wt}}$ isoform was demonstrated. This is in accordance with the theory that the absence of exon 3 does not impair GHR physiology⁵³. In addition, $\text{GHR}_{\text{wt-wt}}$ was negatively correlated with IGF-I levels in healthy individuals. In contrast, acromegalic patients demonstrated similar amounts of the $\text{GHR}_{\text{wt-wt}}$ and GHR_{d3} isoforms, and these isoforms neither correlated with circulating GH levels nor with IGF-I levels. This suggests that the interaction between GH and its receptor may be altered in acromegaly.

In conclusion, there are large discrepancies between the results of the few studies in acromegaly on the effect of the d3GHR isoforms on the GH-IGF-I axis. However, since the main objectives of these three studies were different, additional larger studies are required in both active and (long-term) controlled acromegalic patients to clarify the effects of the d3GHR genotypes on the GH-IGF-I axis in acromegaly and to confirm the interesting observation that the d3GHR polymorphism may be associated with an adverse biochemical treatment result.

II D. Conclusion

There are discrepancies between the studies in both GHD and acromegaly on the effect of the d3GHR on the GH-IGF-I axis. Nonetheless, the majority of these studies indicate a positive effect of the GHR_{d3} genotype on IGF-I concentrations^{37;39;47;52}. However, considering the relatively small number of studies, which in general include relatively low numbers of subjects, it is hard to draw firm conclusions at present.

III. Effect of d3GHR on growth

III A. Pathogenesis

Under normal physiological circumstances, linear growth is a variable trait, individually different and dependent on numerous factors, both genetical and environmental. Just as spontaneous linear growth, stimulated growth in response to rhGH treatment varies extensively amongst individuals, also resulting from both genetic and non-genetic effects. One of the genetic factors considered responsible for this variation in stimulated growth is the expression of exon 3 of the GHR. The loss or retention of exon 3 is thought to affect receptor expression or function, specifically by affecting the binding of human growth hormone, receptor processing, transport, stability, binding to other ligands, dimerization of GHR monomers, or signal transduction as described by Dos Santos *et al.* in 2004⁵. They demonstrated that GHRd3 induced a higher transcriptional activity of the reporter construct in response to various GH concentrations than GHR_{wt-wt}⁵. Potential physiological variations in GH sensitivity due to GHR differences in short children with an otherwise normal GH-IGF-I axis can be compensated by adaptations of endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on basal growth rate. In addition, there is no evidence that the d3GHR influences normal adult height variation⁵⁴. However, in patients with GHD this compensatory effect of the GH-IGF-I axis is deficient.

III B. Children with short stature due to growth hormone deficiency

GHD is a condition of inadequate production of GH. Deficiency of GH produces significantly different problems at various ages. GHD can be congenital or acquired in childhood or adult life. It can be partial or complete. It is usually permanent, but sometimes transient. It may be an isolated deficiency or occur in association with deficiencies of other pituitary hormones. In newborn infants the primary manifestations may be hypoglycemia. In later infancy and childhood, growth failure may be the major effect.

GH deficiency is treated by rhGH. A fixed rhGH dose, calculated according to weight and body surface area, accelerates growth from a pre-treatment rate of 3-4 cm/year to 6-12 cm/year in the first year of therapy in children⁵⁵. This therapeutic effect can be further improved by earlier treatment, larger rhGH doses, individualized doses based on IGF-I concentrations⁵⁶, and

more frequent administrations of rhGH⁵⁵. Since this therapeutic efficacy of rhGH shows high inter-individual variation, there is possibly a role for the complex cascade of numerous genes by which GH regulates growth velocity, including the d3GHR polymorphism.

Several studies have been published on the effect of the exon 3 deleted GHR polymorphism with some reporting positive and others negative results with respect to the influence of this polymorphism on growth parameters in GHD children. It is important to note that those studies were performed in different populations, harboring variations in their responses to GH therapy. In addition, there are differences between studies in growth parameters, the dosages of rhGH, and durations of follow-up. These studies are summarized below in *Table 1*. Jorge *et al.* were the first to extend the findings, described by Dos Santos *et al.*, of altered GHR function by the deletion of exon 3 in GHD children^{5,57}. Both increased growth velocity in the first year of treatment with rhGH and increased final height were achieved in children with non-isolated GHD with the GHR_{d3} genotype. In addition, R  z *et al.* also demonstrated an increased first and second year growth velocity for both the GHR_{wt-d3} and GHR_{d3-d3} genotypes in comparison with the GHR_{wt-wt} in children with isolated GHD, but they were unable to replicate the finding of Jorge *et al.* on increased final height^{41,54,57}.

In contrast, other studies reported negative results on these pharmacogenetic effects of rhGH. Blum *et al.* were unable to confirm a stimulating effect of the GHR_{d3} genotype on growth parameters in 107 children with severe GH deficiency after 1 year of treatment with rhGH in a study in which GHR_{wt-d3} and GHR_{d3-d3} patients were pooled (GHR_{d3})³⁷. This was in keeping with de Graaff *et al.* and Pilotta *et al.*, although the latter published not enough data to be represented in *Table 1*^{38,40}. Higher IGF-I levels have been attributed to the GHR_{d3} genotype, although this did not result in increased height gain or growth velocity^{37,39}.

Most studies focussed on the short-term results of rhGH treatment, i.e. growth velocity or height gain in the first 1 or 2 years of treatment. To date, only 2 studies reported on the effect of d3GHR on final height after treatment with rhGH. Jorge *et al.* demonstrated a stimulating effect for both the GHR_{d3} isoform as for the GHR_{d3-d3} isoform, which could not be explained by pre-treatment characteristics or differences in rhGH dose⁵⁷. Raz *et al.* demonstrated a stimulating effect of the GHR_{d3} isoform in the short-term after rhGH treatment in a large cohort of GHD children, but were unable to demonstrate this effect on final height⁴¹. Of all studies on the effect of the d3GHR on growth in GHD children, Raz *et al.* were able to report a difference in basal growth rates prior to treatment as reflected by a difference in

baseline height between the different genotypes⁴¹. However, in a recent meta-analysis of studies reporting baseline height, we showed that there is indeed a subtle difference between GHR_{d3} and GHR_{wt-wt} genotypes in children with GHD⁵⁸. The discrepancies in the effect of the d3GHR polymorphism on the response to (all individualized) doses of rhGH treatment seen in studies on GHD in children can possibly be explained by variations in underlying conditions, individual conditions of the patients studied, severity of GHD, stringency on the criteria used for diagnosis of GHD, duration of rhGH treatment, and the relatively small numbers of subjects included in the studies. In addition, Herishanu *et al.* were unable to demonstrate differences in growth delay in children with Cushing's disease according to the different variants of the d3GHR⁴³.

In conclusion, in most individual studies and in a recent meta-analysis, the growth response during the first year of rhGH treatment, both expressed as height SDS gain and as growth velocity, is slightly, but significantly increased in pre-pubertal short children with the GHR_{d3} genotype, albeit with a small increase of approximately 0.5cm/yr, in comparison with the GHR_{wt-wt} genotype⁵⁸. This polymorphism therefore explains only a small part of the relatively large inter-individual differences in linear growth response. Therefore, there may be other, up till now unknown, genetic factors including the GHR c.1319 G>T polymorphism⁵⁹, that influence growth velocity in GHD patients after GH therapy.

III C. Children with short stature not due to growth hormone deficiency

The cause of growth failure in the majority of short children who do not have GHD is unknown. These otherwise normal children with idiopathic short stature (ISS) or born small for gestational age (SGA) secrete normal amounts of GH in response to pharmacologic stimulation³⁰. The importance of the GHR in modulating the growth promoting action of GH is demonstrated by the abnormal growth of children with complete insensitivity to GH due to inactivating mutations in the gene for the GHR (Laron dwarfism)³⁶. These non-GHD short children may secrete GH normally and yet have defects in the ability of target cells to respond to GH. Such defects could occur either at the GHR or the intracellular mediators of GH signaling. In addition, serum concentrations of the GHBP in children with ISS have shown to be low, suggestive for abnormalities in the GHR gene^{60;61}.

Since 2001 and 2003, the US Food and drug Administration (US FDA) and the European regulatory agency, respectively, allowed the treatment of short children born SGA

with rhGH. In 2003, rhGH was approved by the US FDA to stimulate growth in children with ISS, and soon after it was also approved in Europe. Children with a height of 2.25-3.00 SD below the mean for their ages are now eligible for rhGH prescription. Although most children with SGA/ISS respond to treatment with rhGH with increases in their linear growth rates, their responses are more limited than those of children with GHD who are treated similarly. In accordance to the observations in GHD, in non-GHD children there are relatively large intra-individual variations in response to rhGH treatment. Indicators for a good response on rhGH treatment in this non-GHD patient group include young age, low baseline height, low baseline IGF-I levels, and high rhGH dose. The GHR_{d3} genotype is thought to influence the growth response in non-GHD children, since Dos Santos *et al.* reported on increased growth velocity in SGA and ISS children with the GHR_{d3} genotype and treated with rhGH⁵. They further demonstrated that the GHR_{d3} genotype was not primary related to the genesis of short stature and it had no effect on growth rates or hormonal parameters before start of rhGH therapy⁵. In addition, GHR isoforms are not involved in the etiology of short stature⁶². For the purpose of this review, SGA and ISS are discussed separately.

III C1. *Small for gestational age*

Children SGA have a birth weight below the 10th percentile for that gestational age, usually due to intrauterine growth restriction (IUGR). The status of being born SGA can be a consequence of fetal, maternal, or environmental factors, but the exact (multifactorial) etiology remains usually unknown. The majority of infants born SGA (85%) experience spontaneous catch-up growth in the first few months and up to 2 years, followed by a normal pattern of development. Those 15% failing to experience catch-up growth do not reach their target height and remain short throughout life^{63;64}. The mechanisms involved in catch-up growth remain unclear. The finding of higher basal GH levels suggest GH hypersecretion as a factor for early catch-up growth⁶⁵. On the other hand, birth weight, birth length, gestational age, and mid-parental height have also been identified as factors influencing catch-up growth^{63;65}. Although the majority of these children is non-GHD, rhGH treatment improves average linear growth in these children⁵. This effect is largest during the first 2 years of treatment and diminishes after 4-6 years of continuation of rhGH⁶⁶. When rhGH is discontinued catch-down in growth velocity may occur⁶⁶.

Dos Santos *et al.* treated pre-pubertal patients born SGA with normal GH secre-

tion with supraphysiological rhGH replacement doses and demonstrated a strong relationship between GHR_{d3} genotype and growth velocity. Shortly after, these findings were confirmed by two other groups^{67,68} despite the fact that SGA children are heterogeneous regarding the origin of the short stature⁶⁹. In accordance, increased spontaneous post-natal growth velocity in the GHR_{d3} genotype compared with the GHR_{wt-wt} genotype was reported by Jensen *et al.*⁷⁰. In addition, de Graaff *et al.* demonstrated a higher frequency of GHR_{d3} in children with GHD born SGA compared with GHD born appropriate for gestational age³⁸.

Conflicting findings were reported by four Spanish studies of the same group who were unable to demonstrate an effect of the GHR_{d3} genotype on pre- or post-pubertal growth after rhGH treatment in varying doses^{71,72} or without rhGH treatment⁷³. These contrasting observations can be caused by the relatively heterogeneous age distribution of the cohorts studied (large proportions post-pubertal children), the genotype assignment techniques used, and/or the exclusion of patients with a minor increase in height (<0.5 SDS)⁷⁴.

A single unconfirmed study studied the effect of the GHR_{d3} genotype in pre-natal growth in SGA and showed decreased fetal growth velocity in this genotype⁷⁰. However, fetal growth is generally considered independent of fetal GH levels, whereas fetal IGF-I levels are associated with fetal size⁷⁵⁻⁷⁹.

Only one study assessed the long-term effect of d3GHR polymorphism on near final height (adolescence), demonstrating increased height in patients with the GHR_{d3} isoform⁴¹.

III C2. Idiopathic short stature

ISS refers to extreme short stature (<3 SD below the mean), despite minimal 2 of the following: normal size for gestational age at birth, normal body proportion, no evidence for endocrine dysfunction, no chronic organic or psychiatric disease, no emotional disturbances, and normal food intake. In ISS the short stature is due to decreased growth rate without a diagnostic explanation after an ordinary growth evaluation⁵. Only 5-9% of ISS children reach an adult height lower than 2 SD below the mean⁸⁰ and rhGH treatment is recommended to increase height in these non-GHD children.

In accordance to SGA children, Dos Santos *et al.* reproduced their finding of a strong relationship between GHR_{d3} genotype and growth velocity in pre-pubertal children with ISS, treated with supraphysiological rhGH replacement doses⁵. Toyoshima *et al.* demonstrated that children with ISS carrying the GHR_{d3} allele presented higher GH sensitivity regarding short-

term IGF-I generation during rhGH treatment⁴². This finding supports the rhGH pharmacogenetic findings in relation to GHR_{d3} genotype, described by Dos Santos *et al.*⁵. In addition, although the GHR_{d3} genotype was less frequent in Japanese (20%)⁸¹ and Koreans (17-22%)⁸² than in Caucasians (25-44%), in Korean pre-pubertal ISS children the GHR_{d3} genotype significantly affected growth velocity in short-term rhGH treatment. In contrast, the Spanish study by Carrascosa *et al.*, like in SGA, was unable to demonstrate an effect of d3GHR genotypes on height gain or height velocity during 1 to 2 years of rhGH treatment in a large cohort of pre-pubertal Spanish children with ISS⁷⁴. Again, these authors excluded patients with poor growth response (<0.5 SD in 2 years) to rule out the main genotype abnormalities of the GH-IGF-I axis^{29,30}. This could have created a selection bias and possibly they were therefore not able to show a stimulating effect of GHR_{d3} genotype on growth velocity. At present, there are no data available on the effect of the d3GHR polymorphism on final height in ISS.

In conclusion, 13 studies have been published on the effect of d3GHR on stimulated growth in non-GHD. Although the different studies are conflicting, a meta-analysis of the data in prepubertal children with short stature indicates that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD⁵⁸. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment and this effect is more pronounced at lower doses of rhGH and higher age⁵⁸.

III D. Turner syndrome

Turner syndrome is a chromosomal disorder affecting females in whom all or part of one of the X-chromosome is absent. Short stature, a prominent feature of patients with Turner's syndrome, occurs in 100% of patients with 45,X0 karyotype and in 95.2% of patients with non-45, X karyotype. The cause of growth failure in Turner's syndrome is currently unknown, but theories exist about a dysfunctional GH response resulting in partial GH insensitivity on the one hand and a primary bone defect on the other hand⁸³. Treatment during childhood consists of early GH therapy in supraphysiological doses and estrogen replacement around the normal age of puberty. An average gain of 10 cm (range 3.9–24.8 cm) in final height may be achieved by the early introduction of growth-promoting therapies⁸³. Inter-individual variations in response on rhGH treatment are possibly explained by differences in parental height, age at start of GH treatment, and dose of rhGH⁸³.

Turner syndrome is a more specific and homogenous disorder of linear growth failure

and of different severity and origin than short stature due to GHD, SGA, or ISS. Girls with Turner syndrome with the GHR_{d3-d3} genotype on a high dose of rhGH for 1 year demonstrated a significantly higher growth velocity than their GHR_{wt-wt} and GHR_{wt-d3} counterparts and exceeded their growth prediction by far^{29;67}. Accordingly, the GHR_{wt-wt} Turner girls failed to reach their height prediction, and GHR_{wt-d3} showed intermediate responsiveness to rhGH. The effect of the GHR_{d3} genotype was far more evident in Turner girls than in patients born SGA⁶⁷. When the total period of (a low dose of) rhGH administered in Turner girls was taken into account, GHR_{d3-d3} genotypes were associated with enhanced growth velocity for a longer time period and a height gain almost twice as great when compared with the other two genotypes, despite comparable levels of rhGH admitted and independent of an effect on serum levels IGF-I⁸⁴. In a Korean population with a known relatively low allelic prevalence of the d3GHR, there was no effect of this polymorphism on linear growth in 115 prepubertal patients with Turner's syndrome⁴⁴.

In conclusion, only three studies have focused on the effect of the d3GHR polymorphism on stimulated growth in Turner syndrome, two of them (both of the same group) reporting increased height gain and growth velocity in the short term and increased final height in favor of GHR_{d3} when compared with GHR_{wt-wt} . This clear effect of the GHR_{d3} genotype is possibly due to the homogeneity of the population studied.

III E. The effect of d3GHR genotypes on basal height

Data on the effect of the d3GHR polymorphism on spontaneous growth in non-GHD children are scarce, but conflicting. Only 2 studies were performed both in SGA^{70;85}. Jensen *et al.* demonstrated a positive effect of the GHR_{d3} isoform, whereas Audi *et al.* could not find an effect of the GHR_{d3} isoform on spontaneous growth^{70;85}. We meta-analyzed these findings and we were unable to demonstrate a difference in baseline growth between the different genotypes in non-GHD children⁵⁴.

III F. Conclusion

The different studies on growth in response to rhGH treatment in different clinical conditions demonstrate a variety of results regardless of the cause of shortness (GHD or non-GHD). In addition to the factors held responsible for intra-study variation (*vide supra*), it is likely that differences between studies are at least in part caused by small sample sizes and heterogeneous

characteristics of the patients studied. False-positive findings are more likely with small sample sizes and for quantitative trait loci phenotypical variations tend to be overestimated with small sample sizes⁴¹. A recent meta-analysis in prepubertal children with short stature supports this finding, indicating that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD children.

The GHR_{d3} genotype is not expected to be associated with physiologic variations in human growth or to be a primary cause of short-stature in humans, since potential variations in growth hormone sensitivity due to the GHR differences in non-GHD short children can be compensated by endogenous pituitary growth hormone secretion, which masks the effects of GHR polymorphisms on basal growth rate⁵. When this compensation is impossible, however, like in GHD children, the d3GHR genotype affects the growth response (spontaneous or stimulated). We demonstrated this hypothesis in a recent meta-analysis, indicating that the baseline growth in GHD children with de GHR_{d3} isoform is significantly increased⁵⁸.

IV Effect of d3GHR on other endocrine disorders

IV A. Pathogenesis

Deficient or excessive GH secretion induces typical clinical syndromes, with changes in many tissues, organs and organ functions, and altered body composition. In addition to physical symptoms and co-morbid diseases, patients with GH diseases experience a large range of complaints, i.e. functional limitations, diminished work-related productivity, social isolation, fatigue, poor health-related quality of life, etcetera^{85;91}. However, there are major individual differences in the expression and severity of (co-)morbidity. Even after (long-term) control of the altered GH status (in acromegaly) strong individual differences in clinical outcome are reported⁸⁶. We discuss several studies suggesting a possible effect of the d3GHR polymorphism on disease presentation in patients with active or biochemically well-controlled GHD or GH excess, respectively. In these patients natural feedback regulation within the GH-IGF-I axis is absent, implying that a possible effect of the different d3GHR isoforms should be demonstrable.

IV B. Growth hormone deficiency in adults

In adult patients with GHD, three groups of GHD can be identified: 1) childhood onset GHD with GH replacement in childhood and with normal stature, 2) GHD without GH replacement in adulthood with short stature, and 3) patients with adult-onset GHD due to different causes but mainly related to (treatment for) pituitary adenomas. Other causes for adult onset GHD are traumatic brain injury, sub-arachnoidal hemorrhage, or subdural haematoma. Therefore, GHD in adults is a heterogeneous syndrome. In contrast to the clear cut efficacy parameters of rhGH treatment in children (height gain, growth velocity, and final height), the effects of rhGH treatment in GHD adults are more subtle, like improvement in quality of life, muscle strength, lipid metabolism, body composition, and bone mineral density^{92,93}. Several studies have assessed whether the large inter-individual variability in response to rhGH replacement therapy in GHD adults might be partly attributed to the d3GHR polymorphism in addition to more obvious causes such as differences in cause and severity of GHD.

GH and IGF-I affect lipogenesis and HDL cholesterol expression^{94,95}. Van der Klaauw *et al.* demonstrated that the GHR_{d3} genotype contributed to the beneficial changes induced by rhGH in lipid metabolism⁴⁷. However, there was no significant effect of the polymorphism on clinical parameters including BMI, total cholesterol, LDL cholesterol, and blood pressure⁴⁷. In accordance, a recent Swedish study did not find a pharmacogenetic difference in response to rhGH between the genotypes in body composition⁹⁶.

In conclusion, two studies focused on the effect of d3GHR on clinical outcome in GHD adults after rhGH treatment, with contrasting short-term results. The number of participating patients, age, rhGH dose, and distribution of d3GHR isoforms were comparable in both studies. In contrast to short children, in whom the primary efficacy of GH therapy is easily evaluated by growth velocity and final height, the end-points to define responsiveness to rhGH therapy in adults are more subtle and diverse. Larger prospective studies with appropriate statistical power are necessary to clarify the additional contribution of d3GHR in the responses to rhGH therapy.

IV C. Acromegaly

The clinical severity of acromegaly varies considerably between individuals both during active disease and after long-term remission of acromegaly, most likely related to the severity and duration of active disease. However, since the GHR_{d3} genotype has been reported to increase

growth velocity in children with GHD during rhGH therapy^{5,57,97} and since in GHD the GHR_{d3} genotype has been associated with increased IGF-I levels^{37,39,47}, assumptions have been made regarding an effect of the d3GHR polymorphism on the disease course in acromegaly. It has been hard to prove the theory that the d3GHR polymorphism may have caused a change in the GH-IGF-I axis and therefore rendered acromegalic patients with the GHR_{d3} genotype more susceptible for the effects of GH, since only few studies on this topic have been conducted showing different results (*vide supra*). However, On the other hand, the limited pharmacogenetic effect of enhanced signal transduction in GHR_{d3}, patients can also be overruled by the excess of GH. We therefore discuss studies in active and controlled acromegaly focusing on the clinical consequences of the disease.

IV C1. Active acromegaly

Several studies focused on the effect of clinical parameters in patients with active acromegaly. Mercado *et al.* observed that GHR_{d3} patients with active acromegaly had an increased prevalence of type 2 DM (odds ratio 2.0). However, this was not confirmed by others^{43,51,98,99}. In addition, Mercado *et al.* could not demonstrate an effect of the d3GHR polymorphism on gender, height, BMI, percentage of macroadenomas, and arterial hypertension between the GHR genotypes in active acromegaly, although patients with the GHR_{d3} genotype were younger at diagnosis⁵¹. The late clinical outcome of acromegalic patients after successful treatment in relation to these GHR isoforms needs to be confirmed. Montefusco *et al.* studied 76 patients with active acromegaly retrospectively and reported a significantly lower BMI in GHR_{d3} compared with GHR_{wt-wt} and equal prevalences of type 2 DM and hypertension in all genotypes, although GHR_{d3} carriers had more frequently a normal glucose tolerance⁹⁹. Thus, these data indicate that the GHRd3 genotype is associated with glucose metabolism and body weight in patients with active acromegaly.

IV C2. Controlled acromegaly

Recently, our group reported the late clinical outcome of patients with acromegaly in relation to the different GHR genotypes¹⁰⁰. In 86 patients, the GHR_{d3} genotype was associated with adverse clinical outcome with respect to osteoarthritis, dolichocolon, and adenomatous colonic polyps but not with other co-morbidities such as cardiovascular risk factors. These observations await confirmation by other groups.

IV C3. Response to treatment

With respect to treatment response, Bianchi *et al.* recently demonstrated that in acromegalic patients with active disease after unsuccessful neurosurgery and somatostatin analog treatment, GHR_{d3} patients required significantly lower doses of Pegvisomant for a shorter period of time to achieve normalized IGF-I levels, when compared with GHR_{wt-wt} patients¹⁰¹. This is compatible with a higher sensitivity of the GHR_{d3} genotype. However, the exact mechanism underlying this observation is not yet understood. This might include more efficient binding of Pegvisomant to the receptor in GHR_{d3} patients, or increased affinity for pegvisomant of the GHR_{d3}. Bernabeu *et al.* confirmed these results showing that the GHR_{d3} genotype predicted an improved response to pegvisomant therapy in acromegaly¹⁰².

In conclusion, in acromegalic patients the GHR_{d3} genotype has shown to positively affect glucose metabolism and body weight and negatively affect osteoarthritis, dolichocolon, and adenomatous polyps. The therapeutic response to somatostatin analogs or pervisomant was better in the GHR_{d3} variant. However, this is based on few studies with varying inclusion criteria and different study outcomes.

IV D. Conclusion

In the different studies on the effect of the d3GHR on the GH-IGF-I axis in both GHD and acromegalic subjects effects of the d3GHR were either absent or rather subtle. In prepubertal children with short stature the GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of approximately 0.5 cm during the first year of rhGH treatment. In acromegaly, there may be an effect of the d3GHR on clinical outcome, i.e. osteoarthritis, dolichocolon, and adenomatous polyps. Moreover, the therapeutic responses to somatostatin analogs or pervisomant were better in the GHR_{d3} variant in the patients with active acromegaly. Up till now we can not simply explain these findings by the theory that GHR_{d3} patients are more susceptible to the effects of GH, based on the finding of Dos Santos *et al.* that there is an increased bioactivity of GHR_{d3} *in vitro* in response to various GH concentrations. Large, prospective studies are needed to confirm these differences in clinical outcome in GHR_{d3} patients compared with GHR_{wt-wt} patients.

V. Effect of d3GHR on metabolism

V A. Pathogenesis

Blood glucose levels are closely regulated in health. The principal organ of glucose homeostasis is the liver, which absorbs and stores glucose (as glycogen) in the post-absorptive state and releases it into the circulation between meals to match the rate of glucose utilization by peripheral tissues.

The GH-IGF-I axis is important for metabolic control^{11:39:47}. GH has many metabolic effects, including IGF-I dependent effects, such as glucose uptake and protein metabolism, and IGF-I independent effects such as the stimulation of insulin secretion and pancreatic beta cell proliferation¹⁰³ and the decrease of peripheral insulin sensitivity through inhibition of glucose disposal in skeletal muscle¹⁰⁴. The question rises whether the increased bioactivity of the GHR_{d3} genotype affects metabolism.

V B. Diabetes Mellitus

V B1. *Type 1 diabetes mellitus*

Type 1 DM is a disease resulting from insulin deficiency. Diabetic nephropathy is one of the complications associated with DM. Recent evidence has suggested that the glomerular podocyte is a target for GH action, and, therefore, the GH-IGF-I axis may play a role in the development of diabetic nephropathy^{105:106}. Another recent study has demonstrated that deficiency of GHR in mice causes a reduction in systolic blood pressure and plasma renin levels, as well as an increase in aortic endothelial NO synthase levels¹⁰⁷. Gu *et al.* therefore investigated whether the d3GHR polymorphism was involved in the pathogenesis of diabetic nephropathy in patients with type 1 DM¹⁰⁸. However, they were unable to associate the d3GHR genotypes and diabetic nephropathy in these patients, although the allele frequency of GHR_{d3} was low, and not in Hardy Weinberg equilibrium, and comparable to the frequency found in Swedish patients with type 2 DM (*vide infra*)¹⁰⁹.

In conclusion, in patients with type I DM, the allele frequency of the d3GHR is not in accordance to HWE. No effect of the d3GHR was demonstrable on diabetic nephropathy.

V B2. Type 2 diabetes mellitus

Type 2 DM is a multi-genetic disease and an increasing problem worldwide. The disease may be present in the sub-clinical form for years before diagnosis, demonstrable by impaired glucose tolerance (IGT) (*vide infra*). Since the GH-IGF-I axis is important for metabolic control¹, there might be an effect of the d3GHR polymorphism on metabolic diseases. It was recently reported that a polymorphism of the IGF-I promoter caused lower IGF-I levels and an increased risk of type 2 DM^{110,111}.

The question was raised whether the GHR_{d3-d3} genotype with increased GHR sensitivity potentially leading to increased IGF-I levels would protect against type 2 DM. Strawbridge *et al.* demonstrated that the allele frequencies of the d3GHR in type 2 DM patients disagree with the Hardy Weinberg equilibrium, due to a lower proportion of GHR_{d3-d3}. These findings suggest that increased bioactivity of this allele confers a protection function against type 2 DM¹⁰⁹ due to increased IGF-I levels as a result of increased GH sensitivity which promotes glucose uptake¹⁰⁹ and decreased insulin sensitivity. This effect has also been demonstrated in centenarians¹¹². However, if other factors outweigh this protective effect of GHR_{d3-d3} genotype, the resulting type 2 DM in subjects with GHR_{d3-d3} genotype seems to have a more severe phenotype associated with increased age-adjusted IGF-I and BMI¹⁰⁹, which is possibly due to an increase in GHR_{d3} activity.

In conclusion, the only study focusing on the effect of the d3GHR genotype on type 2 DM shows a lower allele frequency of GHR_{d3} in patients with type 2 DM¹⁰⁹. This was in accordance to the allele frequency demonstrated in type 1 DM¹⁰⁸. However, in GHR_{d3} patients with type 2 DM, the disease seems to be more severe than in the other genotype.

V C. Impaired glucose tolerance

IGT is a pre-diabetic state, which is associated with insulin resistance and increased risk of cardiovascular pathology. IGT may precede type 2 DM by many years. IGT is also a risk factor for mortality. Obesity and a lack of exercise make progression to overt DM more likely.

In accordance with the findings in patients with type 1 and 2 DM, the d3GHR genotype does not meet the Hardy Weinberg equilibrium in patients with IGT¹⁰⁹. In addition, a recent study in 145 healthy Caucasian children and adolescents higher insulin secretion during an oral glucose tolerance test was found in children who carry at least one d3GHR allele, when adjusted for the degree of insulin sensitivity¹¹³.

In conclusion, the GHR_{d3-d3} genotype may positively influence pancreatic beta cell compensatory capacity and therefore protects against IGT. This effect is comparable to patients with type 2 DM.

V D. Conclusion

There are discrepancies between the several studies in GHD and acromegaly on the effect of the d3GHR on the GH-IGF-I axis. However, the majority of studies indicate a positive effect of the GHR_{d3} genotype on the IGF-I concentrations^{37;39;47;52}, but regarding the small number of studies it is hard to draw firm conclusions. Moreover, since GH and IGFBP3 concentrations are not different for the different genotypes, one might assume that an increase in IGF-I secretion is caused by enhanced receptor expression or function, like binding of GH, receptor processing, transport, stability, and signal transduction, resulting in an increase in IGF-I concentrations. Uncovering the mechanism will promote our understanding of the GH-IGF-I axis. Therefore prospective studies are needed.

VI. Effect of d3GHR on cardiovascular diseases

VI A. Pathogenesis

Genetic factors are known to play a major role in hypertension¹¹⁴. A combination of genome wide linkage scans and candidate gene analysis has identified a variety of genetic loci that contribute to blood pressure variation and risk of hypertension¹¹⁵. Arterial hypertension itself is an important risk factor for stroke, largely because hypertension itself is associated with the formation of atheromatous deposits, which can narrow or obstruct cerebral arteries. The etiology of cerebrovascular stroke is heterogenous, however, with a strong genetic component¹¹⁶.

Short stature has long been known to be associated with an increased risk of atherosclerosis¹¹⁷ and coronary heart disease¹¹⁸. Short stature is also associated with elevated pulse pressure, a surrogate marker of arterial stiffness¹¹⁹. Since the risk of both hypertension and stroke appears to be inversely correlated with adult height, individual variation in the genes encoding GH and its receptor (GHR) might be associated with hypertension and stroke. The question rises whether the d3GHR polymorphism is one of the genetical factors influencing the risk on hypertension and stroke.

VI B. Hypertension

Hypertension can be classified either essential (primary) or secondary. Essential hypertension indicates that no specific medical cause can be found to explain a patient's condition. Secondary hypertension indicates that the high blood pressure is a result of (*i.e.*, secondary to) another condition, such as kidney disease or tumours (pheochromocytoma and paraganglioma). Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure. Even moderate elevation of arterial blood pressure leads to shortened life expectancy.

Only one study has been performed regarding the effect of d3GHR on the risk for development of hypertension. A borderline non-significant association between females with hypertension and the GHR_{d3} phenotype was demonstrated, however the population studied was small¹²⁰.

In conclusion, with regard to the current literature, no relationship exists between d3GHR genotype and the risk for development of hypertension. More research in larger populations is required.

VI C. Stroke

Stroke is the rapid loss of brain functions due to a disturbance in the blood vessels supplying the brain. This can be due to ischemia caused by thrombosis or embolism or due to a hemorrhage. As a result, the affected area of the brain is unable to function, leading to inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or inability to see one side of the visual field. A stroke can cause permanent neurological damage, complications, and death. It is the number two cause of death worldwide and may soon become the leading cause of death worldwide. Risk factors for stroke include advanced age, hypertension (high blood pressure), previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, cigarette smoking, and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke.

The same authors investigating the effect of d3GHR on developing hypertension, investigated the effect of d3GHR on the risk for stroke, but failed to demonstrate any effect¹²⁰.

In conclusion, no effect of the d3GHR genotype on the risk for stroke was demonstrable. However, more studies in larger populations are needed.

VI D. Conclusion

Hypertension and stroke are major causes of morbidity and mortality. The risk for both hypertension and stroke appears inversely related with adult height, but the inter-individual variation in the d3GHR is not associated with stroke and hypertension. The association between stature and hypertension and stroke could not be exerted solely via a genetic influence upon stature. In addition, final height in the normal population, in contrast to GHD of small patients treated with rhGH, is not determined by variation in the d3GHR, but by other genetic and environmental factors like adult height, nutrition, etc. The risk on hypertension and stroke seems not to be mediated by the d3GHR polymorphism. However, only 2 populations have been studied, so it is hard to draw firm conclusions. Studies in other genetic populations might add new information to this topic.

VII. Effect of d3GHR on survival

VII A. Pathogenesis

The deletion of exon 3 was shown to be a genomic event that occurred in the several steps during the evolutionary lineage from Old World monkeys genome to Pongina and Hominina genomes^{120;121}. Following the long-lived integration of proviral sequences in the vicinity of exon 3 of the GHR gene, a homologues recombination seems to have occurred in humans, resulting in the excision of exon 3 in one of our ancestors. It is unknown how this polymorphism has become as frequent as up to 50% of Caucasian people. Because of its current frequency it is clear that the homologues recombination is a very ancient event. Is it thought that the exon 3 deletion had played a favorable role, fitting with the Darwinian evolution of our species and favoring its propagation in given human subgroups¹²², but up till now, no functional role could have been identified that substantiates this speculation. Focusing on extreme populations might contribute to the identification of the role of the GHR_{d3} genotype.

VII B. Obesity and starvation

Obese patients are at risk of early death, mainly from diabetes, coronary heart disease and cerebrovascular disease. The greater the obesity; the higher the morbidity and mortality rates.

In contrast, in many areas of the world, people are on the verge of malnutrition. Al-

though the basic condition of protein-energy metabolism (PEM) is the same in all parts of the world from whatever cause, malnutrition resulting from long periods of near-total starvation produces unique clinical appearances in children virtually never seen in the West.

Only few studies have investigated the role of the d3GHR polymorphism in obesity or starvation. Strawbridge *et al.* demonstrated that in patients with type 2 DM and IGT the prevalence of the GHR_{d3-d3} isoform was significantly lower in a non-diabetic control population¹⁰⁹. In addition, in this small proportion of type 2 DM and IGT patients with the GHR_{d3-d3} genotype the phenotype was associated more with severe obesity and increased IGF-I levels¹⁰⁹.

In contrast, in a West-African population with persistent under-nutrition a high prevalence (70%) of GHR_{d3} genotypes was reported¹²³. Starvation increases GH pulse frequency and amplitude resulting in an increase in circulating GH levels¹²⁴. Coupled with reduced insulin secretion, this leads to increased lipolysis and ketogenesis, insulin resistance and conservation of glucose and protein¹²⁵. The maintenance of a high level of circulating GH in malnourished individuals may therefore have been of selective value. However, increased lipolysis gives rise to elevated levels of circulating free fatty acids which have been shown, at least *in vitro*, to decrease the expression of GHR by approximately 50%¹²⁶. Decreased amounts of GHR on the cell surface would be expected to lead to reduced secretion of IGF-I. The presence of at least one GHR_{d3} allele, demonstrated in 70% of the West-African population, may serve to ameliorate these effects of decreased expression of the GHR by virtue of its increased responsiveness to GH, and IGF-I levels may consequently be near normal.

VII C. Conclusion

In conclusion, the GHR_{d3-d3} isoform is more frequent in patients suffering from starvation, indicating that both the elevated GH gene expression and increased GHR-mediated GH responsiveness may constitute adaptive responses to the effects of persistent malnutrition, since increased circulating GH appears to form part of a physiological response to nutrition deprivation. The prevalence of the GHR_{d3} isoform in well-fed populations is currently unknown. But in patients with type II DM and IGT, with the scarcely prevalent GHR_{d3} isoform, this isoform is associated with more severe obesity than in patients with the other isoforms. Further research on this interesting topic is required.

VIII. Summary and Conclusions

This review focused on the presently known functional consequences of the exon 3 deleted GHR polymorphism in various clinical conditions and physiological processes. We conclude that the effects of the d3GHR polymorphism are not limited to subtle variations in growth parameters in (non)GHD children with short stature treated with rhGH and to phenotype-genotype correlations and pharmacogenetic responses to therapy in patients with acromegaly. The effects also seems to affect many pathophysiological processes in which the GH-IGF-I axis is involved.

Two major implications of this review emerge. First, most studies included relatively low numbers of subjects, resulting in a low statistical power and explaining at least in part conflicting results between studies. In addition, most studies did not included independent additional cohorts for confirmation of their results. Second, the implications of these genotype-phenotype relationships of the GHR for routine clinical practice seem to be limited.

REFERENCES

- (1) Dominici FP, Argentino DP, Munoz MC, Miquet JG, Sotelo AI, Turyn D. Influence of the crosstalk between growth hormone and insulin signalling on the modulation of insulin sensitivity. *Growth Horm IGF Res* 2005; 15(5):324-336.
- (2) Urbanek M, Russell JE, Cooke NE, Liebhaber SA. Functional characterization of the alternatively spliced, placental human growth hormone receptor. *J Biol Chem* 1993; 268(25):19025-19032.
- (3) Pantel J, Grulich-Henn J, Bettendorf M, Strasburger CJ, Heinrich U, Amselem S. Heterozygous nonsense mutation in exon 3 of the growth hormone receptor (GHR) in severe GH insensitivity (Laron syndrome) and the issue of the origin and function of the GHRd3 isoform. *J Clin Endocrinol Metab* 2003; 88(4):1705-1710.
- (4) Kratzsch J, Wu Z, Kiess W, Dehmel B, Bosse-Henck A, Reuter W *et al.* The exon 3-retaining and the exon 3-deleted forms of the growth hormone-binding protein (GHBP) in human serum are regulated differently. *Clin Endocrinol (Oxf)* 2001; 54(1):61-68.
- (5) Dos SC, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 2004; 36(7):720-724.
- (6) Kelly PA, Djiane J, Postel-Vinay MC, Edery M. The prolactin/growth hormone receptor family. *Endocr Rev* 1991; 12(3):235-251.
- (7) Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ *et al.* Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature* 1987; 330(6148):537-543.
- (8) de Vos AM, Ultsch M, Kossiakoff AA. Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science* 1992; 255(5042):306-312.
- (9) Brown RJ, Adams JJ, Pelekanos RA, Wan Y, McKinsty WJ, Palethorpe K *et al.* Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nat Struct Mol Biol* 2005; 12(9):814-821.
- (10) Herrington J, Carter-Su C. Signaling pathways activated by the growth hormone receptor. *Trends Endocrinol Metab* 2001; 12(6):252-257.
- (11) Strous GJ, dos Santos CA, Gent J, Govers R, Sachse M, Schantl J *et al.* Ubiquitin system-dependent regulation of growth hormone receptor signal transduction. *Curr Top Microbiol Immunol* 2004; 286:81-118.
- (12) Finidori J. Regulators of growth hormone signaling. *Vitam Horm* 2000; 59:71-97.
- (13) Moutoussamy S, Kelly PA, Finidori J. Growth-hormone-receptor and cytokine-receptor-family signaling. *Eur J Biochem* 1998; 255(1):1-11.
- (14) Kelly PA, Ali S, Rozakis M, Goujon L, Nagano M, Pellegrini I *et al.* The growth hormone/prolactin receptor family. *Recent Prog Horm Res* 1993; 48:123-64.:123-164.
- (15) Carter-Su C, Smit LS. Signaling via JAK tyrosine kinases: growth hormone receptor as a model system. *Recent Prog Horm Res* 1998; 53:61-82; discussion 82-3.:61-82.
- (16) Argetsinger LS, Carter-Su C. Mechanism of signaling by growth hormone receptor. *Physiol Rev* 1996; 76(4):1089-1107.

- (17) Laron Z. Natural history of the classical form of primary growth hormone (GH) resistance (Laron syndrome). *J Pediatr Endocrinol Metab* 1999; 12 Suppl 1:231-49.:231-249.
- (18) Maamra M, Milward A, Esfahani HZ, Abbott LP, Metherell LA, Savage MO *et al.* A 36 residues insertion in the dimerization domain of the growth hormone receptor results in defective trafficking rather than impaired signaling. *J Endocrinol* 2006; 188(2):251-261.
- (19) Duquesnoy P, Sobrier ML, Duriez B, Dastor F, Buchanan CR, Savage MO *et al.* A single amino acid substitution in the exoplasmic domain of the human growth hormone (GH) receptor confers familial GH resistance (Laron syndrome) with positive GH-binding activity by abolishing receptor homodimerization. *EMBO J* 1994; 13(6):1386-1395.
- (20) Bernat B, Pal G, Sun M, Kosiakoff AA. Determination of the energetics governing the regulatory step in growth hormone-induced receptor homodimerization. *Proc Natl Acad Sci U S A* 2003; 100(3):952-957.
- (21) Waters MJ, Hoang HN, Fairlie DP, Pelekanos RA, Brown RJ. New insights into growth hormone action. *J Mol Endocrinol* 2006; 36(1):1-7.
- (22) Ayling RM, Ross R, Townner P, Von LS, Finidori J, Moutoussamy S *et al.* A dominant-negative mutation of the growth hormone receptor causes familial short stature. *Nat Genet* 1997; 16(1):13-14.
- (23) Milward A, Metherell L, Maamra M, Barahona MJ, Wilkinson IR, Camacho-Hubner C *et al.* Growth hormone (GH) insensitivity syndrome due to a GH receptor truncated after Box1, resulting in isolated failure of STAT 5 signal transduction. *J Clin Endocrinol Metab* 2004; 89(3):1259-1266.
- (24) Tiulpakov A, Rubtsov P, Dedov I, Peterkova V, Bezlepkina O, Chrousos GP *et al.* A novel C-terminal growth hormone receptor (GHR) mutation results in impaired GHR-STAT5 but normal STAT-3 signaling. *J Clin Endocrinol Metab* 2005; 90(1):542-547.
- (25) Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J *et al.* Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med* 2003; 349(12):1139-1147.
- (26) Hwa V, Little B, Adiyaman P, Kofoed EM, Pratt KL, Ocal G *et al.* Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab* 2005; 90(7):4260-4266.
- (27) Rosenfeld RG, Kofoed E, Buckway C, Little B, Woods KA, Tsubaki J *et al.* Identification of the first patient with a confirmed mutation of the JAK-STAT system. *Pediatr Nephrol* 2005; 20(3):303-305.
- (28) Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 1996; 335(18):1363-1367.
- (29) Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E *et al.* IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003; 349(23):2211-2222.
- (30) Goddard AD, Covello R, Luoh SM, Clackson T, Attie KM, Gesundheit N *et al.* Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group. *N Engl J Med* 1995; 333(17):1093-1098.
- (31) Stallings-Mann ML, Ludwiczak RL, Klinger KW, Rottman F. Alternative splicing of exon 3 of the human growth hormone receptor is the result of an unusual genetic polymorphism. *Proc Natl Acad Sci U S A* 1996; 93(22):12394-12399.

- (32) Urbanek M, MacLeod JN, Cooke NE, Liebhaber SA. Expression of a human growth hormone (hGH) receptor isoform is predicted by tissue-specific alternative splicing of exon 3 of the hGH receptor gene transcript. *Mol Endocrinol* 1992; 6(2):279-287.
- (33) Esposito N, Paterlini P, Kelly PA, Postel-Vinay MC, Finidori J. Expression of two isoforms of the human growth hormone receptor in normal liver and hepatocarcinoma. *Mol Cell Endocrinol* 1994; 103(1-2):13-20.
- (34) Mercado M, DaVila N, McLeod JF, Baumann G. Distribution of growth hormone receptor messenger ribonucleic acid containing and lacking exon 3 in human tissues. *J Clin Endocrinol Metab* 1994; 78(3):731-735.
- (35) Wickelgren RB, Landin KL, Ohlsson C, Carlsson LM. Expression of exon 3-retaining and exon 3-excluding isoforms of the human growth hormone-receptor is regulated in an interindividual, rather than a tissue-specific, manner. *J Clin Endocrinol Metab* 1995; 80(7):2154-2157.
- (36) Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev* 1994; 15(3):369-390.
- (37) Blum WF, Machinis K, Shavrikova EP, Keller A, Stobbe H, Pfaeffle RW *et al*. The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. *J Clin Endocrinol Metab* 2006; 91(10):4171-4174.
- (38) de Graaff LC, Meyer S, Els C, Hokken-Koelega AC. GH receptor d3 polymorphism in Dutch patients with MPHD and IGHD born small or appropriate for gestational age. *Clin Endocrinol (Oxf)* 2008; 68(6):930-934.
- (39) Marchisotti FG, Jorge AA, Montenegro LR, Berger K, Carvalho LR, Mendonca BB *et al*. Comparison between weight-based and IGF-I-based growth hormone (GH) dosing in the treatment of children with GH deficiency and influence of exon 3 deleted GH receptor variant. *Growth Horm IGF Res* 2008.
- (40) Pilotta A, Mella P, Filisetti M, Felappi B, Prandi E, Parrinello G *et al*. Common polymorphisms of the growth hormone (GH) receptor do not correlate with the growth response to exogenous recombinant human GH in GH-deficient children. *J Clin Endocrinol Metab* 2006; 91(3):1178-1180.
- (41) Raz B, Janner M, Petkovic V, Lochmatter D, Eble A, Dattani MT *et al*. Influence of growth hormone (GH) receptor deletion of exon 3 and full-length isoforms on GH response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab* 2008; 93(3):974-980.
- (42) Toyoshima MT, Castroneves LA, Costalonga EF, Mendonca BB, Arnhold IJ, Jorge AA. Exon 3-deleted genotype of growth hormone receptor (GHRd3) positively influences IGF-I increase at generation test in children with idiopathic short stature. *Clin Endocrinol (Oxf)* 2007; 67(4):500-504.
- (43) Drori-Herishanu L, Lodish M, Verma S, Bimpaki E, Keil MF, Horvath A *et al*. The Growth Hormone Receptor (GHR) Polymorphism in Growth-retarded Children with Cushing Disease: Lack of Association with Growth and Measures of the Somatotrophic Axis. *Horm Metab Res* 2009.
- (44) Ko JM, Kim JM, Kim GH, Lee BH, Yoo HW. Influence of parental origin of the X-chromosome on physical phenotypes and growth hormone responsiveness of patients with Turner syndrome. *Clin Endocrinol (Oxf)* 2010.
- (45) Toogood AA, Shalet SM. Diagnosis of severe growth hormone (GH) deficiency in young adults who received GH replacement therapy during childhood. *Acta Paediatr Suppl* 1997; 423:117-20.:117-120.

- (46) Aimaretti G, Baldelli R, Corneli G, Croce C, Rovere S, Baffoni C *et al.* IGFs and IGFBPs in adult growth hormone deficiency. *Endocr Dev* 2005; 9:76-88.:76-88.
- (47) van der Klaauw AA, van der ST, Baak-Pablo R, Biermasz NR, Guchelaar HJ, Pereira AM *et al.* Influence of the d3-growth hormone (GH) receptor isoform on short-term and long-term treatment response to GH replacement in GH-deficient adults. *J Clin Endocrinol Metab* 2008; 93(7):2828-2834.
- (48) Meyer S, Schaefer S, Stolk L, Arp P, Uitterlinden AG, Plockinger U *et al.* Association of the exon 3 deleted/full-length GHR polymorphism with recombinant growth hormone dose in growth hormone-deficient adults. *Pharmacogenomics* 2009; 10(10):1599-1608.
- (49) Moyes VJ, Walker DM, Owusu-Antwi S, Maher KT, Metherell L, Akker SA *et al.* d3-GHR Genotype Does Not Explain Heterogeneity In GH Responsiveness In Hypopituitary Adults. *Clin Endocrinol (Oxf)* 2009.
- (50) Schmid C, Krayenbuehl PA, Bernays RL, Zwimpfer C, Maly FE, Wiesli P. Growth hormone (GH) receptor isoform in acromegaly: lower concentrations of GH but not insulin-like growth factor-1 in patients with a genomic deletion of exon 3 in the GH receptor gene. *Clin Chem* 2007; 53(8):1484-1488.
- (51) Kamenicky P, Dos SC, Espinosa C, Salenave S, Galland F, Le BY *et al.* D3 GH receptor polymorphism is not associated with IGF1 levels in untreated acromegaly. *Eur J Endocrinol* 2009; 161(2):231-235.
- (52) Ochoa R, Zarate A, Cerbon MA, Salcedo M, Lopez R, Galvan RE *et al.* Expression of growth hormone receptor isoform exon-3-excluding and exon-3-retaining messenger RNAs in peripheral lymphocytes from normal and acromegalic subjects. *Horm Res* 2003; 60(2):68-72.
- (53) Urbanek M, Russell JE, Cooke NE, Liebhaber SA. Functional characterization of the alternatively spliced, placental human growth hormone receptor. *J Biol Chem* 1993; 268(25):19025-19032.
- (54) Wassenaar MJ, Dekkers OM, Pereira AM, Wit JM, Smit JW, Biermasz NR *et al.* Impact of the exon 3-deleted GH receptor polymorphism on baseline height and the growth response to recombinant human growth hormone therapy in growth hormone deficient (GHD) and non-GHD children with short stature: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2009.
- (55) van der Lely AJ, Janssen JM, Lamberts SW. Adult vs childhood onset GHD: is there a real clinical difference? *Growth Horm IGF Res* 1998; 8 Suppl A:21-3.:21-23.
- (56) Cohen P, Bright GM, Rogol AD, Kappelgaard AM, Rosenfeld RG. Effects of dose and gender on the growth and growth factor response to GH in GH-deficient children: implications for efficacy and safety. *J Clin Endocrinol Metab* 2002; 87(1):90-98.
- (57) Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab* 2006; 91(3):1076-1080.
- (58) Wassenaar MJ, Dekkers OM, Pereira AM, Wit JM, Smit JW, Biermasz NR *et al.* Impact of the exon 3-deleted growth hormone (GH) receptor polymorphism on baseline height and the growth response to recombinant human GH therapy in GH-deficient (GHD) and non-GHD children with short stature: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2009; 94(10):3721-3730.

- (59) Wan L, Chen WC, Tsai Y, Kao YT, Hsieh YY, Lee CC *et al.* Growth Hormone (GH) receptor C.1319 G>T polymorphism, but not exon 3 retention or deletion is associated with better first-year growth response to GH therapy in patients with GH deficiency. *Pediatr Res* 2007; 62(6):735-740.
- (60) Carlsson LM, Attie KM, Compton PG, Vitangcol RV, Merimee TJ. Reduced concentration of serum growth hormone-binding protein in children with idiopathic short stature. National Cooperative Growth Study. *J Clin Endocrinol Metab* 1994; 78(6):1325-1330.
- (61) Mauras N, Carlsson LM, Murphy S, Merimee TJ. Growth hormone-binding protein levels: studies of children with short stature. *Metabolism* 1994; 43(3):357-359.
- (62) Hujerir Y, Hess O, Shalev S, Tenenbaum-Rakover Y. Growth hormone receptor sequence changes do not play a role in determining height in children with idiopathic short stature. *Horm Res* 2006; 65(4):210-216.
- (63) Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den HH, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res* 1995; 38(2):267-271.
- (64) Karlberg J, bertsson-Wikland K, Kwan CW, Chan FY. Early spontaneous catch-up growth. *J Pediatr Endocrinol Metab* 2002; 15 Suppl 5:1243-55.:1243-1255.
- (65) Leger J, Limoni C, Collin D, Czernichow P. Prediction factors in the determination of final height in subjects born small for gestational age. *Pediatr Res* 1998; 43(6):808-812.
- (66) de ZF, Butenandt O, Chatelain P, bertsson-Wikland K, Jonsson B, Lofstrom A *et al.* Growth hormone treatment of short children born small for gestational age: reappraisal of the rate of bone maturation over 2 years and metanalysis of height gain over 4 years. *Acta Paediatr Suppl* 1997; 423:207-12.:207-212.
- (67) Binder G, Baur F, Schweizer R, Ranke MB. The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children. *J Clin Endocrinol Metab* 2006; 91(2):659-664.
- (68) Tauber M, Ester W, Auriol F, Molinas C, Fauvel J, Caliebe J *et al.* GH responsiveness in a large multinational cohort of SGA children with short stature (NESTEGG) is related to the exon 3 GHR polymorphism. *Clin Endocrinol (Oxf)* 2007; 67(3):457-461.
- (69) Johnston LB, Savage MO. Should recombinant human growth hormone therapy be used in short small for gestational age children? *Arch Dis Child* 2004; 89(8):740-744.
- (70) Jensen RB, Vielwerth S, Larsen T, Greisen G, Leffers H, Juul A. The presence of the d3-growth hormone receptor polymorphism is negatively associated with fetal growth but positively associated with postnatal growth in healthy subjects. *J Clin Endocrinol Metab* 2007; 92(7):2758-2763.
- (71) Carrascosa A, Esteban C, Espadero R, Fernandez-Cancio M, Andaluz P, Clemente M *et al.* The d3/fl-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational-age children: results from a two-year controlled prospective study in 170 Spanish patients. *J Clin Endocrinol Metab* 2006; 91(9):3281-3286.
- (72) Carrascosa A, Audi L, Esteban C, Fernandez-Cancio M, Andaluz P, Gussinye M *et al.* Growth hormone (GH) dose, but not exon 3-deleted/full-length GH receptor polymorphism genotypes, influences growth response to two-year GH Therapy in Short Small-for-Gestational-Age Children. *J Clin Endocrinol Metab* 2008; 93(1):147-153.

- (73) Audi L, Carrascosa A, Esteban C, Fernandez-Cancio M, Andaluz P, Yeste D *et al.* The exon 3-deleted/full-length growth hormone receptor polymorphism does not influence the effect of puberty or growth hormone therapy on glucose homeostasis in short non-growth hormone-deficient small-for-gestational-age children: results from a two-year controlled prospective study. *J Clin Endocrinol Metab* 2008; 93(7):2709-2715.
- (74) Carrascosa A, Audi L, Fernandez-Cancio M, Esteban C, Andaluz P, Vilaro E *et al.* The exon 3-deleted/full-length growth hormone receptor polymorphism did not influence growth response to growth hormone therapy over two years in prepubertal short children born at term with adequate weight and length for gestational age. *J Clin Endocrinol Metab* 2008; 93(3):764-770.
- (75) Bang P, Westgren M, Schwander J, Blum WF, Rosenfeld RG, Stangenberg M. Ontogeny of insulin-like growth factor-binding protein-1, -2, and -3: quantitative measurements by radioimmunoassay in human fetal serum. *Pediatr Res* 1994; 36(4):528-536.
- (76) Bocconi L, Mauro F, Maddalena SE, De IC, Tirelli AS, Pace E *et al.* Insulinlike growth factor 1 in controls and growth-retarded fetuses. *Fetal Diagn Ther* 1998; 13(3):192-196.
- (77) Langford K, Nicolaides K, Miell JP. Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. *Hum Reprod* 1998; 13(5):1389-1393.
- (78) Leger J, Noel M, Limal JM, Czernichow P. Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. *Pediatr Res* 1996; 40(1):101-107.
- (79) Ostlund E, Bang P, Hagenas L, Fried G. Insulin-like growth factor I in fetal serum obtained by cordocentesis is correlated with intrauterine growth retardation. *Hum Reprod* 1997; 12(4):840-844.
- (80) Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, bertsson-Wikland K *et al.* Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. KIGS International Board. Kabi Pharmacia International Growth Study. *J Clin Endocrinol Metab* 1999; 84(4):1174-1183.
- (81) Iida K, Takahashi Y, Kaji H, Okimura Y, Nose O, Chihara K. Difference between Japanese and Caucasian populations in the allelic frequency of growth hormone receptor polymorphisms. *J Pediatr Endocrinol Metab* 2009; 22(1):41-46.
- (82) Ko JM, Park JY, Yoo HW. The common exon 3 polymorphism of the growth hormone receptor (GHR) gene and effect of growth hormone therapy on growth in Korean children with idiopathic short stature. *Clin Endocrinol (Oxf)* 2008.
- (83) Elsheikh M, Dunger DB, Conway GS, Wass JA. Turner's syndrome in adulthood. *Endocr Rev* 2002; 23(1):120-140.
- (84) Binder G, Trebar B, Baur F, Schweizer R, Ranke MB. Homozygosity of the d3-growth hormone receptor polymorphism is associated with a high total effect of GH on growth and a low BMI in girls with Turner syndrome. *Clin Endocrinol (Oxf)* 2008; 68(4):567-572.

- (85) Audi L, Esteban C, Carrascosa A, Espadero R, Perez-Arroyo A, Arjona R *et al.* Exon 3-deleted/full-length growth hormone receptor polymorphism genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n = 247) and in an adult control population (n = 289) show increased fl/fl in short SGA. *J Clin Endocrinol Metab* 2006; 91(12):5038-5043.
- (86) Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. *J Clin Endocrinol Metab* 2005; 90(5):2731-2739.
- (87) Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di SC *et al.* High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. *J Clin Endocrinol Metab* 2003; 88(7):3196-3201.
- (88) Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H *et al.* Increased prevalence of regurgitant valvular heart disease in acromegaly. *J Clin Endocrinol Metab* 2004; 89(1):71-75.
- (89) van der Klaauw AA, Biermasz NR, Hoftijzer HC, Pereira AM, Romijn JA. Previous radiotherapy negatively influences quality of life during 4 years of follow-up in patients cured from acromegaly. *Clin Endocrinol (Oxf)* 2008; 69(1):123-128.
- (90) van der Klaauw AA, Bax JJ, Smit JW, Holman ER, Delgado V, Bleeker GB *et al.* Increased aortic root diameters in patients with acromegaly. *Eur J Endocrinol* 2008; 159(2):97-103.
- (91) Wassenaar MJ, Biermasz NR, van DN, van der Klaauw AA, Pereira AM, Roelfsema F *et al.* High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. *Eur J Endocrinol* 2009; 160(3):357-365.
- (92) de BH, Blok GJ, Van d, V. Clinical aspects of growth hormone deficiency in adults. *Endocr Rev* 1995; 16(1):63-86.
- (93) Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004; 89(5):2192-2199.
- (94) Lind S, Rudling M, Ericsson S, Olivecrona H, Eriksson M, Borgstrom B *et al.* Growth hormone induces low-density lipoprotein clearance but not bile acid synthesis in humans. *Arterioscler Thromb Vasc Biol* 2004; 24(2):349-356.
- (95) Rudling M, Parini P, Angelin B. Growth hormone and bile acid synthesis. Key role for the activity of hepatic microsomal cholesterol 7 α -hydroxylase in the rat. *J Clin Invest* 1997; 99(9):2239-2245.
- (96) Barbosa EJ, Palming J, Glad CA, Filipsson H, Koranyi J, Bengtsson BA *et al.* Influence of the exon 3-deleted/full-length growth hormone (GH) receptor polymorphism on the response to GH replacement therapy in adults with severe GH deficiency. *J Clin Endocrinol Metab* 2009; 94(2):639-644.
- (97) Sobrier ML, Duquesnoy P, Duriez B, Amselem S, Goossens M. Expression and binding properties of two isoforms of the human growth hormone receptor. *FEBS Lett* 1993; 319(1-2):16-20.
- (98) Mercado M, Gonzalez B, Sandoval C, Esquenazi Y, Mier F, Vargas G *et al.* Clinical and Biochemical impact of the d3 (fl) Growth Hormone Receptor genotype in Acromegaly. *J Clin Endocrinol Metab* 2008.
- (99) Montefusco L, Filopanti M, Ronchi CL, Olgati L, La-Porta C, Losa M *et al.* d3-growth hormone receptor polymorphism in acromegaly: effects on metabolic phenotype. *Clin Endocrinol (Oxf)* 2009.

- (100) Wassenaar MJ, Biermasz NR, Pereira AM, van der Klaauw AA, Smit JW, Roelfsema F *et al.* The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. *J Clin Endocrinol Metab* 2009; 94(12):4671-4678.
- (101) Bianchi A, Giustina A, Cimino V, Pola R, Angelini F, Pontecorvi A *et al.* Influence of Growth Hormone Receptor d3 and full-length isoforms on biochemical treatment outcomes in acromegaly. *J Clin Endocrinol Metab* 2009.
- (102) Bernabeu I, varez-Escola C, Quinteiro C, Lucas T, Puig-Domingo M, Luque-Ramirez M *et al.* The exon 3-deleted growth hormone receptor is associated with better response to pegvisomant therapy in acromegaly. *J Clin Endocrinol Metab* 2010; 95(1):222-229.
- (103) Nielsen JH, Linde S, Welinder BS, Billestrup N, Madsen OD. Growth hormone is a growth factor for the differentiated pancreatic beta-cell. *Mol Endocrinol* 1989; 3(1):165-173.
- (104) Jorgensen JO, Moller L, Krag M, Billestrup N, Christiansen JS. Effects of growth hormone on glucose and fat metabolism in human subjects. *Endocrinol Metab Clin North Am* 2007; 36(1):75-87.
- (105) Reddy GR, Pushpanathan MJ, Ransom RF, Holzman LB, Brosius FC, III, Diakonova M *et al.* Identification of the glomerular podocyte as a target for growth hormone action. *Endocrinology* 2007; 148(5):2045-2055.
- (106) Flyvbjerg A. The role of growth hormone in the pathogenesis of diabetic kidney disease. *Pediatr Endocrinol Rev* 2004; 1 Suppl 3:525-9.:525-529.
- (107) Egecioglu E, Andersson IJ, Bollano E, Palsdottir V, Gabrielsson BG, Kopchick JJ *et al.* Growth hormone receptor deficiency in mice results in reduced systolic blood pressure and plasma renin, increased aortic eNOS expression, and altered cardiovascular structure and function. *Am J Physiol Endocrinol Metab* 2007; 292(5):E1418-E1425.
- (108) Gu HF, Efendic S, Brismar K. Lack of an association between GHR exon 3 polymorphism and diabetic nephropathy in the Genetics of Kidneys in Diabetes (GoKinD) population. *Diabetologia* 2008; 51(12):2333-2334.
- (109) Strawbridge RJ, Karvestedt L, Li C, Efendic S, Ostenson CG, Gu HF *et al.* GHR exon 3 polymorphism: association with type 2 diabetes mellitus and metabolic disorder. *Growth Horm IGF Res* 2007; 17(5):392-398.
- (110) Wallander M, Brismar K, Ohrvik J, Ryden L, Norhammar A. Insulin-like growth factor I: a predictor of long-term glucose abnormalities in patients with acute myocardial infarction. *Diabetologia* 2006; 49(10):2247-2255.
- (111) Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A *et al.* A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes* 2001; 50(3):637-642.
- (112) Barbieri M, Gambardella A, Paolisso G, Varricchio M. Metabolic aspects of the extreme longevity. *Exp Gerontol* 2008; 43(2):74-78.
- (113) Sorensen K, Aksglaede L, Munch-Andersen T, achmann-Andersen NJ, Leffers H, Helge JW *et al.* Impact of the growth hormone receptor exon 3 deletion gene polymorphism on glucose metabolism, lipids, and insulin-like growth factor-I levels during puberty. *J Clin Endocrinol Metab* 2009; 94(8):2966-2969.

- (114) Tanira MO, Al Balushi KA. Genetic variations related to hypertension: a review. *J Hum Hypertens* 2005; 19(1):7-19.
- (115) Caulfield M, Munroe P, Pembroke J, Samani N, Dominiczak A, Brown M *et al.* Genome-wide mapping of human loci for essential hypertension. *Lancet* 2003; 361(9375):2118-2123.
- (116) Schulz UG, Flossmann E, Rothwell PM. Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. *Stroke* 2004; 35(4):819-824.
- (117) Reed DM, Resch JA, Hayashi T, MacLean C, Yano K. A prospective study of cerebral artery atherosclerosis. *Stroke* 1988; 19(7):820-825.
- (118) Krahn AD, Manfreda J, Tate RB, Mathewson FA, Cuddy TE. Evidence that height is an independent risk factor for coronary artery disease (the Manitoba Follow-Up Study). *Am J Cardiol* 1994; 74(4):398-399.
- (119) Langenberg C, Hardy R, Kuh D, Wadsworth ME. Influence of height, leg and trunk length on pulse pressure, systolic and diastolic blood pressure. *J Hypertens* 2003; 21(3):537-543.
- (120) Horan M, Newsway V, Yasmin, Lewis MD, Easter TE, Rees DA *et al.* Genetic variation at the growth hormone (GH1) and growth hormone receptor (GHR) loci as a risk factor for hypertension and stroke. *Hum Genet* 2006; 119(5):527-540.
- (121) Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amsellem S. Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. *J Biol Chem* 2000; 275(25):18664-18669.
- (122) Bougneres P, Goffin V. The growth hormone receptor in growth. *Endocrinol Metab Clin North Am* 2007; 36(1):1-16.
- (123) Millar DS, Lewis MD, Horan M, Newsway V, Rees DA, Easter TE *et al.* Growth hormone (GH1) gene variation and the growth hormone receptor (GHR) exon 3 deletion polymorphism in a West-African population. *Mol Cell Endocrinol* 2008; 296(1-2):18-25.
- (124) Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberty KG, Samojlik E *et al.* Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *J Clin Endocrinol Metab* 1992; 74(4):757-765.
- (125) Norrelund H. The metabolic role of growth hormone in humans with particular reference to fasting. *Growth Horm IGF Res* 2005; 15(2):95-122.
- (126) Thimmarayappa J, Sun J, Schultz LE, Deikhamron P, Lu C, Giallongo A *et al.* Inhibition of growth hormone receptor gene expression by saturated fatty acids: role of Kruppel-like zinc finger factor, ZBP-89. *Mol Endocrinol* 2006; 20(11):2747-2760.

