Biological Determinants of Responsiveness to Growth Hormone: Pharmacogenomics and Personalized Medicine

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Abstract

It is becoming most clear that many genes are involved in controlling the regulation of growth. Ultimately however, at the level of growth hormone (GH), the relevant question may be not whether a patient is GH-deficient, but whether he is GH-responsive. As these disturbances can be divided into two gross categories, namely alterations causing subnormal GH secretion and/or those presenting with subnormal GH sensitivity/responsiveness, the main aim of this review is to focus on genes involved in growth regulation leading to short stature caused by an alteration of GH insensitivity/GH responsiveness; in other words, clinical circumstances where individually adapted GH replacement therapy may help to increase height velocity and eventually final height.

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Introduction

The most fundamental characteristic of infancy as well as childhood is growth. Although the process of growth is multifactorial and complex, the growth pattern of children, if evaluated in the context of normal standards, is rather predictable. In Switzerland for instance, the growth charts of the First Zurich Longitudinal Study of Growth and Development, where data are expressed as SDS, are used and serve the process of identifying abnormal growth well [1]. Height in a population follows approximately a gaussian distribution, similar to many other polygenetic traits. Any deviation from a normal pattern of growth can be the first manifestation of a wide variety of disease processes, including endocrine and non-endocrine disorders and, importantly, may involve any organ system of the human body.

For a considerable period of time, growth disorders were managed on the basis of a growth hormone (GH)-oriented classification system. Nowadays, however,

clinicians are well aware that (a) GH on its own is not the major mediator of skeletal growth, (b) the tests used to diagnose GH deficiency (GHD) have many problems (see Chapter 1), and (c) many genetic defects have been described and have presented important insights into the molecular basis of GHD and non-GHD growth failure [2]. Furthermore, as pediatric endocrinologists we are regularly confronted with children presenting with short stature who are given the label of idiopathic short stature (ISS). ISS is a purely descriptive term referring to a child with a height below the age reference for population and sex, in whom with our diagnostic tools no etiological diagnosis has been made and in whom it may have to be accepted that this is the extreme of the normal distribution. Unlike hypertension there is no firm end-point to measure against so placing a cut-point for height normality becomes difficult.

One of the best known problems is to distinguish ISS from 'partial' GHD. There is no 'gold standard' to assist in GH testing as all parameters have arbitrary cut-off levels and low accuracy [3–6]. In addition, neither serum insulin-like growth factor 1 (IGF-1) nor IGF-binding protein 3 (IGFBP-3) measurements are by themselves of predictive value [7]. However, a sound clinical diagnosis is crucial before any treatment so the decision-making processes must be viewed in the total clinical setting. Here we see false-positively tested children diagnosed as 'partial GHD', whereas the false-negative ones are labeled as ISS. But it is possible that in both cases the short stature arises because of a diminished GH secretion for a given GH sensitivity.

However, as we know that recombinant human GH (rhGH) leads in almost all children to an increase of height velocity in the first year, which tapers off in the following years, we have to assume that in the vast majority of those children either the endogenous GH secretion is suboptimal for normal growth, or that the GH sensitivity/responsiveness can be increased by the administration of either physiological or pharmacological doses of rhGH. Moreover, even at the level of GH, many genes are involved in controlling growth regulation. As these disturbances can be divided into two gross categories, namely alterations causing subnormal GH secretion and/or subnormal GH sensitivity/responsiveness, the main aim of this review is to focus on genes involved in growth regulation leading to short stature caused by an alteration of GH insensitivity/GH responsiveness. In these clinical circumstances, individually adapted GH replacement therapy may help to increase height velocity and eventually final height [8] although as discussed in Chapter 2 the two may not be synonymous.

Pharmacogenomics of Growth

Pharmacogenetics (impact of one gene) and pharmacogenomics (impact of several genes, genome) is the study how a person's gene/genome can influence his/

her response to medication [9]. Based on the research in the field of GHD, we know that GHD is quite heterogeneous in terms of etiology as well as age at diagnosis and that improvement in adult height (over and above that of the untreated state) is the major aim of treating GHD children with rhGH [10]. The final height attained as a result of intervention is influenced, in part, by the dose, injection frequency and duration of rhGH therapy. Despite optimization of these factors, a proportion of GHD patients do not reach their target height [10, 11].

A number of mathematical models for predicting growth and final outcome have been proposed enabling the clinician to ‘personalize’ the growth-promoting therapy on the grounds of efficacy and economy [12] although whether the factors identified really impact on response has not been tested in formal randomized control trials. There are several problems with these types of models:

(1) Although prediction models are useful to give an average effect, they are not individualizable.

(2) They often only focus on one outcome, usually short-term growth, whereas interest may be more centered on final height. The two need not necessarily be related and the factors that influence response in the first year of treatment may differ totally from those that lead to prediction of the individual’s final height.

(3) Very few prediction models have been constructed from an a priori hypothesis and care needs to be taken that there has been no interference from other factors accompanying the disease that might affect prognosis. The problem is that importance can be ascribed to factors that are merely ‘markers’ for other factors of real importance. Examples of this can be seen in models which demonstrate that individuals who are extremely short, growing very poorly and whose heights are subsequently further away from their genetic height respond best to treatment. All these factors are simply a marker of ‘how bad the disease is’ and could perhaps be more easily summarized by a similar single factor that actually describes the severity of the condition.

(4) Rules derived from one data set may reflect associations that have occurred by chance and often result from overfitting of the data.

(5) There is always the possibility that the predictors are idiosyncratic to the population, the setting, to the clinicians or to other aspects of the original study.

The identification of these parameters is understandable as they are easy to measure as opposed underlying genetic and epigenetic factors that might explain this individual variability of GH response. However, with an increased understanding of the factors involved in human growth, these genetic and epigenetic factors may become more important and accessible. For example, based on animal knockouts and human mutational analyses the most factors/genes affecting IGF generation as well as the structure of the growth plate deserve consideration [13].

In future, therefore, the use of specifically designed and personalized prediction models may well facilitate the decision about whether the growth response

to a given therapy (rhGH; rhIGF-1) in an individual child is appropriate or not [14, 15]. Based on modifiable (start of treatment, optimal dose of treatment, etc.) and non-modifiable (start heights SDS, bone age, target height SDS, etc.) variables including genetic/genomic variants as well as phenotype-genotype relationship, the realistic growth potential will be calculated. With regard to GH treatment, pharmacogenomics may play, therefore, a major role in the individual response to therapy, at least in the 'sub'-group of subjects not following the current prediction models.

GH Insensitivity (GHI)

The classic phenotype of severe growth failure associated with elevated serum GH concentrations was first described by Laron et al. [16] and can be classified as primary IGF-1 deficiency [17, 18]. Severe forms of primary IGF-1 deficiency have been observed with molecular defects involving the GH receptor (GHR), the GHR cascade and IGF-1/acid-labile subunit/IGFBP-3 ternary complex as well as the type 1 IGF receptor. Based on molecular data, defects anywhere along the pathway from GH binding to its receptor to the IGF-1 action at the growth plate may contribute to postnatal growth failure [17, 18]. This does not include the complex biology of the growth plate manifest at present only in our understanding of the importance of the fibroblast growth factor receptor-3 in achondroplasia. Although we are accustomed to gene deletion/mutation leading to disease, there is clear evidence that either a gene haploinsufficiency, a common polymorphism and/or partially disturbed signaling pathway may impact on susceptibility to disease or modification of treatment response in a number of ways.

GH Receptor

Growth defects result from rare molecular defects including exon deletions or mutations (nonsense, frameshift, missense) of the GHR [19] are unusual in humans. Three types of *GHR* mutations either affecting expression, activation or signaling are specifically highlighted in this review, but consideration is also given to heterozygous forms of *GHR* mutations as well as polymorphism within *GHR* gene possibly affecting growth as exemplars of what has already been discussed.

Expression Failure [20, 21]

Inherited GHI is a heterogeneous disorder that is often caused by mutations in the coding exons or flanking intronic sequences of the *GHR* gene. In 4 children with GHI, Metherell et al. [21] described a novel point mutation that led to activation

of an intronic pseudoexon resulting in inclusion of an additional 108 nt between exons 6 and 7 in the majority of GHR transcripts. This mutation lies within the pseudoexon [A(-1)→G(-1) at the 5' pseudoexon splice site] and, under in vitro splicing conditions, results in inclusion of the mutant pseudoexon, whereas the wild-type pseudoexon is skipped. The presence of the pseudoexon results in inclusion of an additional 36-amino-acid sequence in a region of the receptor previously alleged to be involved in homodimerization, which may be essential for signal transduction [21]. Based on functional studies, Maamra et al. [20] have shown that this elongated GHR remained trapped around the nucleus and is therefore poorly expressed at the cell membrane, reflecting a trafficking defect and, thus, reduced downstream signaling. These properties may explain the relatively mild phenotype of these subjects.

Activation Failure

The substitution of histidine for aspartate 152 (D152H GHR) has been described in a context of familial GH resistance [22]. Originally, this mutation was suggested to interfere with receptor homodimerization because it was found to abolish homodimerization of GHBPs. But later, Waters et al. [23, 24] showed that GHR is constitutively homodimerized at the cell membrane, which presumably involves contacts between transmembrane or juxtamembrane domains of each receptor chain. The activation process mediated by the ligand is assumed to involve a conformational change such as relative rotation of upper and lower domains of the receptor. Based on this model, D152H GHR may interfere with this conformational change.

Signaling Failure

In a short statured family, Ross et al. [25, 26] documented a heterozygous expression of a severely truncated GHR mutant resulting from a mutation at the splice acceptor site of exon 3. A GHR short of the cytoplasmic domain would be devoid of any signaling capacity and because internalization is also impossible, such truncated receptors accumulate at the membrane and act as dominant-negative forms [25]. Less dramatically truncated, homozygous/compound heterozygous GHR leading to GHI have been reported [27, 28]. The truncations of the GHR were after residue 449 (nonsense sequence of residues 424–449) and 581 (nonsense sequence of residues 560–581), respectively. In both cases, STAT5 activation, a component of subsequent post-GHR signaling, was drastically impaired [29].

Heterozygosities for GHR Mutations

There are several reports presenting data on heterozygous *GHR* gene defects possibly leading to short stature [30–33]. Defects in the *GHR* gene were present at a modest frequency (approx. 30%) in persons who were selected for short

stature (height SDS <-2), low GH binding protein – and IGF-1 concentrations and poor height velocity. The frequency is much lower in short subjects (approx. 2%) presenting with adequate GH concentrations. However, Rosenbloom et al. [34] found minimal or no effect on stature analyzing subjects presenting with heterozygous *GHR* gene mutations. Further, Johnston et al. [35] studied the intracellular signaling domain of the GHR in children with ISS and concluded that ISS is not related to heterozygous and/or dominant-negative *GHR* variants. Interestingly, in the study by Woods et al. [33] focusing on phenotype-genotype relationships the mean adult heights of both mothers and fathers were reduced when compared to British standards from 1958. These standards, however, are not ideal for comparison in view of the diverse ethnic origin of the patients, but this finding suggests the possibility of a heterozygote effect. Furthermore, among the 19 families in whom a homozygous *GHR* defect in the affected children was found, the height deficit between the two parents was generally not uniform, suggesting that other genes may be influencing the magnitude of heterozygote effect in each individual. Further, in this same report, certain *GHR* gene mutations producing the GHBP-positive phenotype, in which GH binding is normal, were described. These forms are more likely to act in a semi-dominant manner. This is because dimeric GH binding is a prerequisite for GHR activation so that mutant receptors that bind GH normally could dimerize with wild-type GHR, reducing the number of active wild-type homodimers. This hypothesis was tested, but the expected decrease in mean parental height SDS between the GHBP-positive group when compared with those in the GHBP-negative group could not be found [33].

Polymorphism of the GHR

While a mutation can change the amino acid sequence and influence the transcript function, a polymorphism is not expected to cause a major change in protein function. A polymorphism is defined as a DNA sequence variant that occurs in at least 1% of the population [36]. Polymorphisms of the *GHR* gene have been reported in the general population and have been described in exons 3, 6 and 10 [31]. While the latter two are classical single nucleotide polymorphisms, the polymorphism in exon 3 is an unusual one, leading to retention (full-length, fl; GHRfl) or deletion of exon 3 (d3; GHRd3), which encodes a 22-amino-acid residue sequence in the extracellular domain [37, 38]. GHRd3 has recently been associated with the degree of height increase in response to GH replacement in children born short for gestational age (SGA), in those with ISS, and in a GHD population [39, 40]. Patients with at least one GHRd3 allele (GHRfl/GHRd3; GHRd3/GHRd3) had a significantly better first year response leading to an improved adult height on rhGH treatment than patients with homozygosity for GHRfl [39]. However, reported studies are not all consistent which may reflect

differing populations and conditions and the high probability of false-positive results arising from post-hoc analysis particularly in small sample size populations [41–49].

In a recent study we analyzed in a total of 186 subjects the impact of GHR genotypes (GHRd3/d3; GHRd3/fl; GHRfl/fl) on growth response to rhGH replacement therapy in two groups of patients (group A: mean rhGH dose: 26.5 µg/kg/day, n = 104; group B: 36.5 µg/kg/day, n = 82) suffering from severe idiopathic isolated GHD (mean maximal GH peak on GH stimulation tests: 0.6 and 1.3 ng/ml, respectively) [12]. Importantly, these patients were followed up to final height and were individually analyzed for the first 4 years on rhGH replacement therapy. In contrast to previous reports, the subjects with the GHRd3/d3 and GHRd3/fl were not pooled but analyzed separately [39, 45, 47]. Overall, in the subjects presenting with either the GHRd3/d3 or GHRd3/fl genotype, a significantly better response (height velocity) to the replacement therapy during the first 2 years of therapy was noted, although in the third and fourth year of therapy, this improved height velocity was observed in those patients with the GHRfl/fl genotype. Further, during the first 2 years on therapy a GHRd3 allele-dependent effect on height was found in study A ($r = 0.82$), which could not be reported in study B, where a higher rhGH dose was used. However, at final height the effect of rhGH treatment was identical irrespective of the specific GHR genotype. Comparing the difference between final adult height SDS and the midparental target height SDS in our severely GHD patients with the large cohort of Caucasian GHD children recruited from the KIGS database, no difference was noted [50]. Similar data were previously published by the Genentech Growth Study Group underlining the effectiveness of the rhGH treatment used in our studies [10]. From these data reporting final height it can be concluded that in severe GHD subjects, the presence or absence of the GHRd3 allele has no impact on either baseline phenotype or final height, although a difference in response (height velocity a different parameter) to rhGH replacement therapy during the first years may be observed depending on the genotype.

When comparing these findings obtained from patients with severe GHD with the previous studies focusing on GHR allele genotype and response to rhGH replacement therapy, the patients, the individual conditions, their related growth disorder as well as the rhGH doses used have to be carefully analyzed [41–49]. In the first report, for instance, Dos Santos et al. [40] studied patients with either SGA or ISS so that in effect patients with normal GH secretion were treated with supraphysiological rhGH doses. Besides the various conditions studied it is also possible that the differences between the studies reported so far represent the problems of sample size. False-positive findings are more likely with small samples sizes and for quantitative trait loci phenotypic variations tend to be overestimated with small sample sizes [51, 52]. Only large-scale

studies of well-defined conditions or pooling the data sets will help to resolve these statistical issues.

Bearing in mind that rhGH replacement and/or therapy in any subject does not result in a constant increase of height velocity over the whole duration of treatment underlines the fact that neither GH responsiveness nor GH sensitivity is constant. Dose-response relationship may vary in every specific condition with or without any underlying growth disorder. Even for a specific condition and dose-response curve, an increase of rhGH dose may well itself affect GHR sensitivity, signaling and thus response to treatment. This fact is well established in any rhGH-treated child whose response changes after the first years on treatment [12]. Moreover, the dose response of rhGH differs according to the condition that is treated. For instance, children with GHD, Turner syndrome, SGA or ISS respond differently (and the rhGH dose is adapted accordingly) but a change in GH sensitivity with treatment remains a variable. Thus, the positive effect resulting from the GHRd3 genotype may well be downregulated and/or altered when supraphysiological doses of rhGH are given. This hypothesis could explain why severe GHD subjects might present a rhGH dose-dependent GHR genotype-related effect on growth response with an apparent plateau at around 26 $\mu\text{g/kg/day}$ (16 IU/m²/week), whereas this effect disappears with higher rhGH doses that lie further up on the dose-response curve for this condition. Similarly, SGA children treated with higher doses of rhGH showed no difference in growth response according to the GHR exon-3 genotype in contrast to SGA children treated with lower doses [40, 41]. Duration of GH treatment also seems to play a major role in defining the sensitivity to the GHR genotype, in addition to the etiology of the short stature and its severity.

In summary, focusing on patients with severe IGHD, we observed a GHRd3 allele dose-dependent effect in subjects treated with rhGH during the first 2 years, with significantly better responses depending on GHRd3/d3:GHRd3/fl genotype, although no difference was observed at final height. Taking all the studies focusing on isolated GHD into account it becomes clear that the final impact of the GHR genotypes on the rhGH response is minimal [39, 43, 45, 47]. The same finding seems to be true for children treated suffering from SGA [48, 53]. Given the importance of the response to attainment of final height it may, nevertheless, well be an additional variable having some impact on growth and GH sensitivity, at least at the beginning of rhGH treatment. These findings are also supported by a clinical case report of a child with GHI syndrome, caused by a compound heterozygosity of *GHR* gene mutation. Father (mutation in exon 4 leading to a stop codon) and mother (mutation in exon 3 leading to a stop codon) carrying either GHRfl (father) or GHRd3 (mother) were of normal stature. Therefore the authors concluded that a single copy of either GHRfl or GHRd3 is sufficient for normal growth [38].

Altered GHR Signaling

Defects in the GHR signaling pathway deserve further consideration given the critical role played in rodent growth. Each subunit of the dimeric GHR associates non-covalently (through its box one motif) with a molecule of cytosolic Janus-family tyrosine kinase 2 (JAK2). Following binding of one GH molecule, the dimeric GHR undergoes conformational changes that induce transphosphorylation of JAK2 and initiation of GHR signaling. Ligand-activated JAK2 phosphorylates multiple tyrosines on the intracellular domain of the GHR, which then serve as docking sites for cytosolic components of at least three distinct signaling pathways: the signal transducer and activator of transcription (STAT), the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways. A proline-rich region in the intracellular N-terminal domain (ND; residues 279–286 in the box one motif) seems to be required for JAK2 and MAPK activation, as well as for phosphorylation of STAT1 and STAT3; tyrosines in the C-terminal portion of the intracellular domain are essential for STAT5 activation. These signaling cascades culminate in the regulation of multiple genes [24, 29, 54]. Our understanding of each pathway for GH-promoted functions has been based predominantly on studies employing rodent models and reconstitution systems. Valuable insights can be gained from identification of defective intracellular components of GH signaling in human disorders but the pleiotropic effects of such defects often complicate characterization. To date, only a limited number of mutations of intracellular GH signaling components have been demonstrated to be convincingly associated with growth retardation.

STAT5b

The identification of, to date, 10 subjects suffering from severe growth failure associated with homozygosity for mutations of the *STAT5b* gene has confirmed the central role of STAT5b in the GH-induced IGF-1 expression and in mammalian postnatal growth [29]. Therefore, in this circumstance GHI is most severe.

Other Defects Leading to GHI Syndromes

So far, no reports of growth failure caused by *JAK2* gene alterations have been published. Further, impaired STAT3 activation has been reported in ISS [55]. However, no mutations were identified, but following GH therapy increased IGF-1 levels in addition to an increase in height velocity was observed.

PTPN11-Gene

Noonan syndrome (OMIM: #163950) is an autosomal dominant dysmorphic syndrome characterized by hypertelorism, a downward eyeslant, and low-set posteriorly rotated ears. Other features include short stature, a short neck with webbing

or redundancy of skin, cardiac anomalies, epicanthic folds, deafness, motor delay, and a bleeding diathesis. Approximately 50% of cases have been associated with gain-of-function mutations of *PTPN11*, the gene encoding the non-receptor-type protein tyrosine phosphatase src homology region 2-domain phosphatase-2 (SHP-2) [56]. This tyrosine phosphatase is involved in intracellular signaling for a variety of hormones, growth factors and cytokines. Activated SHP-2 has been implicated as a negative regulator of GH signaling, and mutations of the SHP-2-binding site in the GHR prolongs GH-promoted tyrosyl phosphorylation of the GHR, JAK2 and STAT5b [57, 58].

Recent reports have indicated more severe stature impairment in Noonan patients with mutations in *PTPN11*, as well as lower serum IGF-1 and IGFBP-3 concentrations, higher GH concentrations and a more modest response to GH therapy, consistent with mild GH resistance [59, 60]. Heterozygous mutations in the *KRAS* gene, downstream effector of SHP-2, were also recently reported to be associated with Noonan syndrome but the impact of such mutations on components of the GHR signaling pathway is not clear [61]. Another potential site for molecular defects of GHR signaling is suppressor of cytokine signaling-2 (*SOCS2*), which is involved in the negative regulation of cytokine action through the inhibition of JAKs and STATs [62, 63].

Growth Responsiveness to GH Therapy, the Impact of the Growth Plate

Studies are already in progress to assess both proteomic and genomic biomarkers for the evaluation and management of short stature and for the assessment of responsiveness to GH therapy. While mutations and polymorphisms of known genes involved in the GH/IGF-1 axis have been identified in various forms of short stature, attention should also be drawn to non-GH/IGF-related factors such as *SHOX* (see below), fibroblast growth factor receptor-3 [64], the C-type natriuretic peptide receptor 2 (NPR2) [65] known to affect linear growth as well as growth plate structure and impact, therefore, on the effect of GH/IGF axis as well. In addition to other factors yet to be identified, it is also very likely that epigenetic factors will provide important insights into the evaluation of short stature.

Longitudinal bone growth occurs rapidly in early life and slows down and eventually ceases at the end of puberty. This decline in growth rate is due primarily to a decrease in the rate of chondrocyte proliferation and is accompanied by a structural change in growth plate cartilage. Although in humans the age-dependent decline in growth rate is interrupted by a brief period of growth acceleration, which peaks during early to mid puberty and may partly be induced by estrogen increasing the activity of GH/IGF-1 axis [66, 67], this programmed senescence appears not to be caused by a systemic mechanism but rather by a mechanism intrinsic to the

growth plate itself. Based on data focusing on growth plate senescence and hormonal impact it becomes clear that the enhanced growth responsiveness of GH at the young age is unlikely to be mediated by IGF-1, but appears to reside within the growth plate itself [68–70]. Any disorder at the level of growth plate is bound to result in short stature and may present with different growth responsiveness and, therefore, a given GH therapy has to be correspondingly personalized [71]. In addition, for these non-GHD conditions, GH is used in a pharmacological manner rather than as physiologic replacement, and, therefore, the GH dose is rather high in order to improve height velocity and increase final height [72].

SHOX (Short Stature HOmeoboX Containing Gene; OMIM: #312865)

The *SHOX* gene was discovered in 1997 during the search for genes underlying the short stature of Turner syndrome and is located in the pseudoautosomal regions at the distal ends of the X and Y chromosome, at positions Xp22.3 and Yp11.3. The gene encodes a homeodomain transcription factor responsible for a significant proportion of long bone growth [73]. In addition to its role in explaining the growth deficit in Turner syndrome, *SHOX* haploinsufficiency is also the primary cause of short stature in 50–70% of individuals who have Leri-Weill dyschondrosteosis (LWD, OMIM: #127300) and in about 2–15% of ISS [72, 74, 75].

Clinically, *SHOX* deficiency is associated with a broad spectrum of phenotypic effects, ranging from short stature without dysmorphic signs to profound mesomelic skeletal dysplasia, a form of short stature characterized by disproportionate shortening of the middle (mesial) segments of the upper as well as lower limbs [76]. There is strong evidence that *SHOX* is a major mediator of linear growth; first, it is expressed in the developing skeleton during fetal life and is specifically expressed in bone marrow fibroblasts and proliferating hypertrophic chondrocytes; second, deficiency of *SHOX* at the growth plate is associated with marked disorganization of chondrocyte proliferation [77], and third, there is a dose-dependent association between the number of active copies of the *SHOX* gene and height [78].

Conclusions

The concept that genetic variation contributes in general to variability in disease phenotypes and therefore in drug responses is widely accepted and validated in many research settings. Therefore, the identification of an association between a clinical phenotype, such as short stature, and a genetic variant, for instance within the *GHR* gene, or a set of genetic variants is an increasing theme also in the field of pediatric endocrinology. Although many such associations were not reproduced in subsequent studies and there are many challenges to overcome in

the implementation of pharmacogenetic as well as pharmacogenomic vision in clinical practice, potential solutions are also evolving rapidly and may help to individualize GH treatment based upon well-defined GH responsiveness and careful genetic analysis.

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