Isolated Growth Hormone Deficiency (GHD) in Childhood and Adolescence: Recent Advances

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The diagnosis of GH deficiency (GHD) in childhood is a multistep process involving clinical history, examination with detailed auxology, biochemical testing, and pituitary imaging, with an increasing contribution from genetics in patients with congenital GHD. Our increasing understanding of the factors involved in the development of somatotropes and the dynamic function of the somatotrope network may explain, at least in part, the development and progression of childhood GHD in different age groups. With respect to the genetic etiology of isolated GHD (IGHD), mutations in known genes such as those encoding GH (*GH1*), GHRH receptor (*GHRHR*), or transcription factors involved in pituitary development, are identified in a relatively small percentage of patients suggesting the involvement of other, yet unidentified, factors. Genome-wide association studies point toward an increasing number of genes involved in the control of growth, but their role in the etiology of IGHD remains unknown. Despite the many years of research in the area of GHD, there are still controversies on the etiology, diagnosis, and management of IGHD in children. Recent data suggest that childhood IGHD may have a wider impact on the health and neurodevelopment of children, but it is yet unknown to what extent treatment with recombinant human GH can reverse this effect. Finally, the safety of recombinant human GH is currently the subject of much debate and research, and it is clear that long-term controlled studies are needed to clarify the consequences of childhood IGHD and the long-term safety of its treatment. **(*Endocrine Reviews* 35: 376–432, 2014)**

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Abbreviations: ACP, adamantinomatous craniopharyngioma; aPAI-1, active plasminogen activator inhibitor type 1; BMAD, bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; BSA, body surface area; CI, confidence interval; CNS, central nervous system; CXCL12, chemokine ligand 12; CXCR4,chemokine receptor 4; D2R, dopamine D2 receptor; Dlk1, Δ-like protein 1; E15.5, embryonic day 15.5; FGF, fibroblast growth factor; FSIQ, Full-Scale IQ; GABA, γ-aminobutyric acid; GC, glucocorticoid; GHR, GH receptor; GHRHR, GHRH receptor; GR, GC receptor; HDL, high-density lipoprotein; IGFBP3, IGF binding protein 3; IGHD, isolated GH deficiency; HMG, high-mobility group; ITT, insulin tolerance test; IVS, intervening sequence; LBM, lean body mass; LDL, low-density lipoprotein; LV, left ventricular; MRI, magnetic resonance imaging; NmU, neuromedin-U; PIT1, pituitary specific transcription factor 1; rhGH, recombinant human GH; SDS, SD score; SGA, small for gestational age; shRNA, short hairpin RNA; SIR, standard incidence ratio; SMR, standardized mortality ratio; TBI, traumatic brain injury; T2DM, type 2 diabetes mellitus.

I. Introduction

Isolated GH deficiency (IGHD), either congenital or acquired, is the commonest pituitary hormone deficiency, and in children, its main manifestation prompting investigation is growth failure (1). GH is a 191-amino-acid peptide, synthesized and secreted by the anterior pituitary somatotropes, with multiple feedback signals and neurotransmitters regulating and modulating its secretion throughout life, either directly or indirectly, principally through hypothalamic GHRH and/or somatostatin (2). The last 2 decades have witnessed an explosion in our understanding of GH deficiency (GHD) and, in particular, its etiology, diagnosis, and management. We have significantly advanced our knowledge of the development of the anterior pituitary gland as a highly complex process during which the sequential temporal and spatial expression of early (*Hesx1*, *Sox2*, *Sox3*, *Lhx3*, *Lhx4*, *Ptx1*, *Ptx2*, and *Otx2*) and late (*Prop1* and *Pou1f1*) transcription factors and signaling molecules (of the bone morphogenetic protein and fibroblast growth factor [FGF] families, wingless and sonic hedgehog signaling) results in shaping of the gland, cell lineage commitment, and the specification of hormone-producing cells, including somatotropes (3). In this context, our understanding of the genetic etiology of GHD, either in isolation or in combination with other pituitary hormone deficiencies, is expanded. In addition, advances in studies of the development and maturation of somatotropes not only shed light on the functional organization of the somatotrope network but also highlighted the role of autocrine and paracrine factors in the development and function of somatototropes, with possible implications for the etiology, pathophysiology, and disease progression of both congenital and acquired GHD.

In childhood, the diagnosis of GHD is a multistep process involving clinical history and examination with detailed auxology, biochemical testing, and pituitary imaging, with an increasing contribution from genetics in patients with congenital GHD (1, 4–6). However, this diagnosis remains a subject of considerable controversy with remarkable variability in clinical practice internationally. Despite the significant accumulated experience in the use of recombinant human GH (rhGH) in children, there is still great variability in the responsiveness to treatment, with no satisfactory way to quantify and predict this response and uncertainty with respect to the best dosing regimen. Children diagnosed with GHD may require rhGH treatment for a variable period, ranging from a few years to lifelong, and in this respect, the surveillance of the long-term safety of rhGH treatment is a particularly important and controversial subject. Furthermore, there is now evidence that, apart from height, IGHD may have an impact on diverse systems and functions from cardiovascular health to brain structure and cognition, with these effects being evident even in childhood.

Over the last 2 decades, an ever-increasing number of studies and reviews have dealt with particular aspects of GHD, ranging from genetic etiology to pathophysiology, diagnosis, and treatment. In this review, we will assume this previous knowledge and focus on recent (over the last 6 years) advances in the field of childhood GHD.

II. Specification, Organization, and Maturation of Somatotropes

In the developing murine anterior pituitary, the expression of *Gh* is considered a hallmark of the differentiation of somatotropes that appear by embryonic day 15.5 (E15.5) in the anterolateral wings of the developing gland, following the appearance of thyrotropes and corticotropes (7). A critical step in this process is the onset of expression of the transcriptional activator *PROP1* by E12.5, which is required for the emergence of the *POU1F1* lineage (somatotropes, lactotropes, and thyrotropes) and its maintenance until E15.5. In turn, *POU1F1* is required for the expression of *Ghrhr*, the production of GH, and the postnatal expansion of GH-producing cells. Its expression is detected by E13.5, reaches its peak in the differentiated somatotropes by E16, and is maintained in adulthood (3, 8). The appearance of differentiated somatotropes is followed by a dramatic increase in their number and their migration by E18.5 throughout the central and lateral parts of the anterior lobe. There is increasing evidence that in addition to the role of the cell-type-specific factors (*PROP1* and *POU1F1*), other extracellular factors (hormones, neuropeptides, and signaling molecules), diverse molecular pathways and the developing pituitary capillary network are also required for the establishment, proliferation, maintenance, and organization of somatotropes. In this respect, the development of transgenic murine models

Table 1. Murine Models With Defects Specific to the Somatotrope Axis

Murine Strain	Characteristics	Pituitary Phenotype	Other	Ref.
Direct effect on GH cells				
rGH-DT-A	Ablation of somatotropes with diphtheria toxin	Reduction in GH and, to a lesser extent, PRL-producing cells	Normal birth weight, reduction in size after 2 weeks, dwarfism	471
GHTK	Transgenic mice expressing the herpes virus-1 thymidine kinase (HSV1-TK) in somatotropes	Inducible destruction of somatotropes with fialuridine treatment; treated GHTK animals developed dwarfism, small AP, and severely reduced GH and PRL cells	Animals allowed period of recovery before euthanasia showed repopulation of the pituitary with mature GH cells; first evidence for the regeneration potential of pituitary cells	75
$^{\Delta exon3}$hGH	Overexpression of the 17.5-kDa isoform of GH	Growth retardation depending on dosage of Δ*exon3* allele, somatotrope loss, macrophage invasion, and APH	PRL, TSH, and LH deficiency	73
GH-M2	Different severity of ablation of GH cells (GH-M2^{low}, GH-M2^{med}, GH-M2^{high})	Severity depending on number of transgene; GH-M2^{high} severe dwarfism, undetectable pituitary GH	GH-M2^{med}: GH, PRL, and TSH deficiencies; GH-M2^{high}: all hormones affected	14
GHCre$^{+/-}$/iDTR$^{+/-}$	Induced GHD; transgenic animals express the inducible diphtheria-toxin receptor (iDTR) only in somatotropes; model of adult-onset IGHD	Adult Cre$^{+/-}$/iDTR$^{+/-}$ mice treated with DT have reduction in the number of somatotropes and dramatic reduction in the size of AP	No other pituitary hormones affected; improved whole body insulin sensitivity independent of diet, no change in NEFA and TGs	472
GHCre$^{+/-}$/iDTR$^{+/-}$	Higher dose and multiple ip injections of DT, compared with above model, leading to maximum destruction of somatotropes	Early (d 11) after injury, there is almost 90% reduction of somatotropes; expansion of the Sox2^{+} stem/progenitor cells after DT-induced somatotrope injury	At 5 months post injury somatotropes are partly regenerated, with almost 50% compared with controls	82
Pit1-Cre/ Egr2$^{GFP(DT)/+}$	Selective ablation of somatotropes by ablation of pituitary Egr2^{+} cells; novel murine model of IGHD	Affected postnatal expansion of somatotropes: partially depleted at P10, almost disappear at P50; decrease in GH mRNA, circulating GH and IGF-1; other hormones not affected	Atypical metabolic effects: hypoglycemia, increased insulin sensitivity, and glucose clearance	473
GHRHR and GHRH				
Little (lit) mouse	Identification *of Ghrhr* mutation (aspartic acid to glycine, D60G) in the *little* mouse	By 3 wk of age, homozygous animals are smaller than WT with defects in GH and IGF-1, reduced pituitary GH and GH mRNA content	Defect in Ghrh-induced cAMP signaling	474
GHRHKO	Disruption of GHRH locus and peptide	APH, somatotrope hypoplasia, GHD, severe postnatal growth retardation	None	74
GHRH-M2	Targeted, possibly reversible, ablation of hypothalamic GHRH neurons	Marked APH, somatotrope cell hypoplasia, GHD, dwarfism; GH response to GHRH	PRL deficiency	13
Gsh1$^{-/-}$	Loss of GHRH neurons as a result of *Gsh1* loss	Increased perinatal and postnatal mortality; surviving Gsh1$^{-/-}$ animals: postnatal dwarfism, 25% of normal weight by 8 wk of age, APH, low circulating GH	Reduction in PRL and LH; ACTH, TSH, and FSH not affected	475

(*Continued*)

Table 1. Continued

Murine Strain	Characteristics	Pituitary Phenotype	Other	Ref.
Nestin-G$\alpha_{q/11}$-deficient	Lack of Gnaq/Gna11 alleles in neuronal and glial precursor cells	In mice with only one intact Gna11 allele: reduced number of somatotropes resulting from decrease in GHRH. Inhibition of ghrelin-induced GH release	Administration of GHRH restores normal postnatal proliferation of somatotropes	476
D2R$^{-/-}$	Knockout of the D2R	Growth retardation, reduction in GH concentration, significant decrease in the population of somatotropes, reduced GHRHR expression	Reduced release of hypothalamic GHRH related to the lack of D2R	57, 58
Other				
Br-M3-KO	Lack of M3 muscarinic achetylcholine receptor in brain	Dwarfism, anterior pituitary hypoplasia, GHD	PRL deficiency; rescue by treatment with GHRH analog	478
Ghsr$^{-/-}$	Knockout of the GH secretagogue receptor (*GHSR*)	Only modest weight reduction; subsequently reported low IGF-1, reduction in pituitary GH mRNA content		479, 480
Transgenic mice with liver-specific expression of hIGFBP1	Transgenes with α1-antitrypsin promoter fused to hIGFBP1 cDNA	Dwarfism and somatotrope hypoplasia; reduced numbers of PRL cells; possible association with alteration of hypothalamic control	Pituitary GH/total protein ratio similar in transgenic and WT animals; no difference in plasma GH concentration	481

Abbreviations: AP, anterior pituitary; APH, AP hypoplasia; DT, diphtheria toxin; PRL, prolactin; WT, wild-type.

with defects at various levels of the hypothalamo-pituitary-somatotrope axis, or specific somatotrope ablation, allow the study of the factors that control somatotrope development (Table 1). More recently, the development of mice with fluorescently tagged GH in association with advanced techniques for in vivo imaging at the cellular level has further aided the elucidation of the development, functional maturation, and control of somatotrope function and GH secretion, thereby furthering our understanding of the pathogenesis of GHD (Table 2). We will review the recent advances in this field and show how improved understanding of these developmental processes may lead to incremental advances in our knowledge of the causes and consequences of GHD in childhood and adolescence.

A. Organization of somatotropes during development and throughout postnatal life

Recent birthdating studies challenge the notion of the temporally discrete differentiation of the anterior pituitary cell types and suggest that the first waves of somatotropes are in fact specified earlier than expression of terminal differentiation markers would suggest, between E11.5 and E13.5, almost concurrent with the differentiation of other anterior cell types, with only a small pro-

Table 2. Murine Models for Studying Somatotrope Function In Vivo

Strain	Characteristics	Use	Ref.
GH-eGFP	Express construct containing the signal peptide and the first 22 residues of hGH in frame with eGFP	Direct in vivo imaging and tracking of somatotrope cells; visualization of spontaneous and secretagogue-induced activity in identified GH cells	10
171hGH/Cs-TG	Intact *hGH1* and LCR in 171-kb fragment of human chromosome 17	Study of the effect of treatment with various factors on GH production in situ and in primary pituitary cell cultures	39
GHRH-eGFP	Express eGFP targeted to the secretory vesicles in GHRH neurons	Characterization of properties of individual GHRH neurons and structural and functional connections of the GHRH neuron network	482
1.6GHRHR/Luc	Transgene created by cloning the 1.6-kb proximal promoter of rat GHRHR into a pA3 luciferase vector	Luciferase expression in GH cells; in vivo study of GHRHR-specific expression and factors affecting control and maturation of somatotropes	483

Abbreviation: eGFP, enhanced green fluorescent protein.

portion of GH-positive cells dividing at E15.5 (9). The generation of transgenic mice expressing green fluorescent protein (GFP) in somatotropes (GH-eGFP) (10), together with advanced imaging and reconstitution techniques that allow the direct in vivo 3-dimensional visualization of the pituitary at the cellular level, have provided new insights into their development, organization, and function. Live imaging of GH-eGFP tagged fetal pituitaries demonstrates that somatotropes first appear as small isolated cells that aggregate rapidly to form organized strands of GH-eGFP cells that are observed as early as E15.5. In the next 24 hours, their number increases dramatically, and there is a continuum of interconnected GH cells in the parenchyma, which coincides with an increase in GH synthesis and secretion and expression of GH secretagogue receptors (11). By E18.5, somatotropes form strands of cells that crisscross the soma of the developing anterior lobe and the lateral wings. This preferential distribution of somatotropes in the central and lateral areas of the lobe does not seem to depend on the time of exit from the cell cycle (9) and results from a dynamic process during which somatotropes migrate to form homotypic 3-dimensional networks (12). Recent experiments using overnight time-lapse multiphoton imaging have tracked their migration, revealing that at E18.5, although some cells remain mainly static, a large proportion of granular GH-secreting somatotropes display motilities of various speeds and directions (12). After birth, the organization of somatotropes into a cellular continuum is maintained, and in murine adult pituitaries, they form clusters interconnected by numerous intercrossing strands of single GH cells, with the larger clusters positioned at the intersection of the strands (11).

However, the mechanism that triggers and maintains their network organization remains unknown. It is likely that this process is, at least in part, driven by GHRH; in GHRH-M2 transgenic mice, where GHRH neurons are ablated by the targeted expression of the M2 viral channel (13), the residual somatotropes fail to form clusters in the adult pituitary. This does not seem to simply result from the reduced number of somatotropes, because transgenic mice with a primary reduction in the number of somatotropes (GH-M2^{med}) still exhibit clusters of somatotropes (14) (Figure 1). In addition, molecules involved in cell-cell interactions, components of adherens and gap junctions such as cadherin and integrin complexes, may have a role both in the development of the pituitary gland and the structural and functional organization of GH-producing cells. Chauvet et al (15) demonstrated that different endocrine cells in the anterior pituitary have a specific and dynamic expression profile of cadherins, depending on the developmental stage. At E15.5, somatotropes are stained for E-cadherin, and by E18.5, there is coexpression of N-cadherin with gradual loss of E-cadherin. The expression of N-cadherin in somatotropes that are organized in clusters suggests a role in the formation and connectivity of the network. Postnatally, the expression of cadherin-18 (*Cdh18*) increases coincident with the increase in GH demand, suggesting that it may be important for the function and adaptation of the somatotrope network (15).

B. The functional role of the somatotrope network

There is now compelling evidence that the organization of the endocrine cells of the anterior pituitary in 3-dimensional homotypic networks is not merely topographic but ensures the coordinated response to various inputs and the plasticity of the gland throughout life (16–18). In the case of somatotropes, their network organization ensures the coordinated response to GHRH and other inputs and the generation of GH pulses despite the difference in the timing of individual cell stimulation. However, the architecture and function of this network is plastic throughout life and is modified in response to altered demand for GH (11, 16).

The relationship between somatotrope organization and functional output is demonstrated at the time of sexual maturation in male mice, when there is a transient increase in the density of the somatotrope clusters that coincides with the increased activity of the GH axis; castration in prepubertal mice results in remodeling of the GH network and absence of large GH cell clusters in the lateral zones (11). Female mice do not exhibit these transient changes at the time of sexual maturation, so in pubertal and young sexually mature animals, there is a sexual dimorphism in the organization of somatotropes, although there is no persistent structural difference between males and females with respect to the architecture of the GH cell network during adulthood (19). Functionally, mice with differences in somatotrope organization exhibit sexually dimorphic GH network responses to the same GHRH stimulus (19). This has been demonstrated by monitoring the calcium responses to identical GHRH stimuli in GH cell slices taken from young adult male and female GH-eGFP pituitaries. In males, almost 60% of GH cells in the lateral regions of the gland respond to GHRH with a coordinated increase in cell activity, which outlasts the duration of the stimulus and generates high-amplitude GH pulses. In contrast in females, only 35% of GH cells respond to the same input by mounting a transient but coordinated response, irrespective of the localization of GH cells. This sexually dimorphic response requires the presence of an intact GH network, because it is not observed in cells of transgenic mice with a severely disrupted network (13, 19) or in isolated cells (19). In addition, a change in the sex steroid milieu by gonadectomy in both male and female animals induces changes in the somatotrope net-

Figure 1.

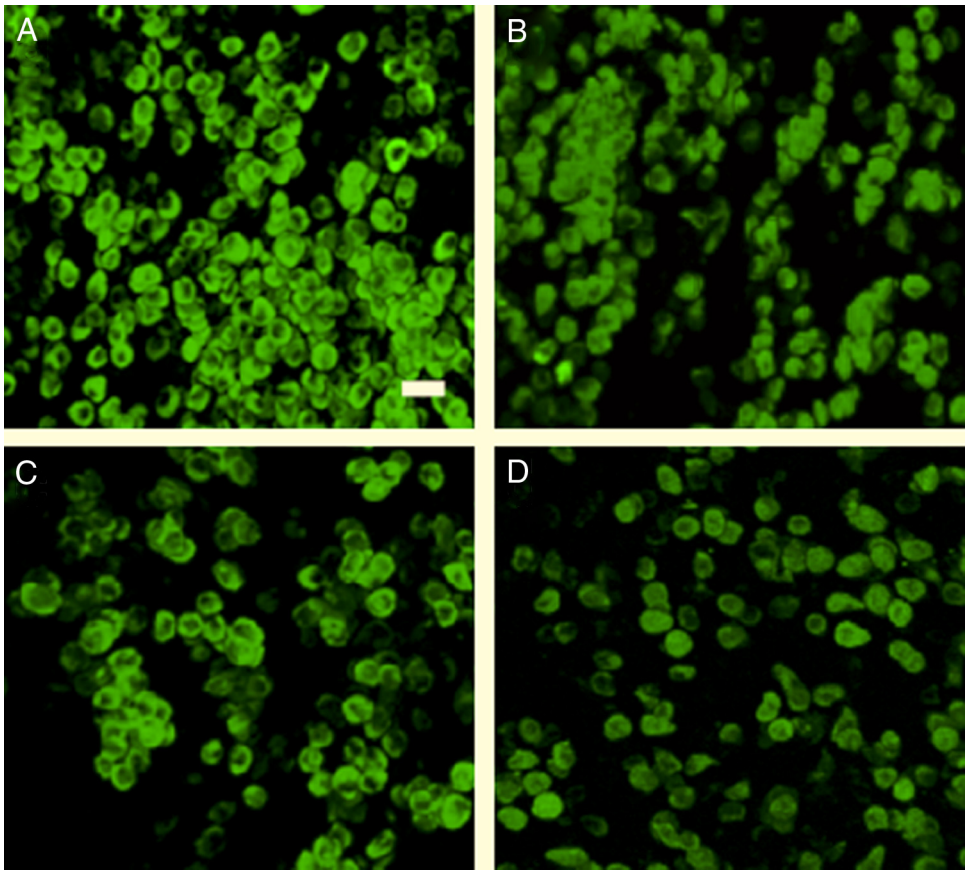

Figure 1. Somatotropes are organized into a network characterized by clusters, which are dependent on GHRH. A, Two-photon imaging of GH-eGFP transgenic mice shows that cells are organized into a network with clusters of cells. B–D, In mice with varying degrees of somatotrope ablation (B, low; C, medium), the reduced number of GH cells still form clusters, whereas in mice with GHRH neuron ablation (D), somatotropes are no longer in clusters. Scale-bar, 20 μm. Images are courtesy of E. Waite, I. C. A. F. Robinson, C. Lafont, and P. Mollard.

work, which can be reversed by appropriate sex steroid replacement (19).

Therefore, it seems that the plasticity of somatotrope function in response to the high demands in puberty does not depend only on the effects of sex steroids at the hypothalamic level that affect the intrinsic properties of GHRH neurons (20, 21), but there is also a direct effect on the properties and organization of the GH cell network at the pituitary level (19). By using time-lapse microscopy to image acute pituitary slices prepared from female GH-eGFP mice, Schaeffer et al (22) demonstrated that ovariectomy enhances, transiently and acutely, the motility of GH cells, whereas exogenous administration of estrogen suppresses these events; this highlights the ability of estrogens to remodel the GH cell network both structurally and functionally. However, the exact mechanism by which sex hormones modify the network responsiveness to GHRH in vivo is still to be determined. In addition, it seems that when somatotropes are functioning in an integrated network, only a proportion of cells is required to support the full sexually dimorphic GH output. It is possible that the remaining nonresponsive cells may serve as a functional reserve that could generate GH output in case of a reduction of cell numbers, provided that the network connectivity of the subgroup is intact. This may be extrapolated to explain, at least in part, the wide differences in the degree of GHD observed in different cases of congenital or acquired insults to somatotropes.

Recent studies of the functional and structural organization of prolactin cells in response to a state of high demand has shown that pituitary endocrine cell networks can undergo persistent structural and gap junctional reorganization that increases the long-distance cell-to-cell connectivity and coordinated activity (18). Whether this response, which may constitute a type of endocrine memory, applies to the somatotrope network is still unknown.

In addition to the organization of the somatotrope network, recent studies reveal the emerging role of the rela-

tionship of pituitary cell networks with the microcirculation and environment. In the normal adult pituitary, somatotropes are near the vascular network, with clusters and strands of cells being surrounded by capillaries, whereas during pituitary development, there is an interplay between the development of the capillary network and the emergence of the hormone-producing cells. In pituitaries of Prop1$^{-/-}$ mice, the failure of the normal cell differentiation occurs in parallel with a reduction in the normal vascularization of the pituitary and an abnormal pattern of expression of vascular endothelial factors (23). At a functional level in the adult, cellular in vivo imaging and sensor studies reveal that at basal conditions, the partial oxygen tension is maintained relatively stable in the pituitary microcirculation. However, administration of GHRH induces a GHRH-dependent increase in the local pituitary blood flow rate and oxygen consumption and recurrent bursting patterns of electrical spikes in somatotropes that are associated with intense network activity and the generation of GH pulses in vivo (17).

III. Other Factors Involved in the Proliferation and Maturation of Somatotropes

The secretory capacity of anterior pituitary cells and of somatotropes in particular is controlled by a number of paracrine and autocrine factors and feedback signal loops with somatostatin being the archetypal hypothalamic inhibitory factor (24). Although most of these signaling molecules are neuropeptides (ie, galanin, ghrelin, somatostatin, and neuromedin U) or neurotransmitters (ie, γ-aminobutyric acid [GABA] and acetylcholine), there is an expanding list of hormones, cytokines and their receptors, growth factors (25) (IGFs, FGFs, and TGF) and tissue factors (adiponectin, resistin) as well as retinoic acid and derivatives of purines, arginine and fatty acids that are implicated in the cell-to-cell interactions within the anterior pituitary (26).

It is now recognized that an expanding number of pituitary paracrine factors are implicated in the control of secretion of GH and the other anterior pituitary hormones (24, 26); these factors have roles in the development of the central nervous system (CNS) and of the anterior pituitary including maturation of specific cell lineages (27, 28). In this respect, they may be important for the determination, maturation, migration, and proliferation of somatotropes, either directly or indirectly via their effect on other cells including folliculostellate cells (29, 30). A common theme in this process is that although neuropeptides and other molecules are ubiquitously present during the development of the anterior pituitary, there seems to be a specificity regarding the time during which they colocalize with the different pituitary cell types. For example, neuromedin-U (NmU), the endogenous ligand of the G protein-coupled receptors NMUR1 and NMUR2 (31) is a 23-amino-acid peptide widely distributed in the CNS and is implicated in the hypothalamic control of various functions such as appetite or ACTH secretion (31, 32); it may also play a role in the development of somatotropes. In the rat anterior pituitary, immunoreactivity for NmU first appears in lactotropes by E16, and by E20, its presence is detected in an increasing percentage of somatotropes. At around the same time that coincides with the differentiation of somatotropes, almost a quarter of the cells that have positive immunostaining for galanin are in fact somatotropes, but this fraction is reduced considerably at E21 to almost 10% (27). Immunohistochemical studies looking at the role of neuropeptides in human fetal pituitaries are sparse. In a recent study, GH-immunoreactive cells were detected by the ninth week of gestation, colocalizing with immunoreactive angiotensin-II. The specific angiotensin-II immunoreactivity only in the GH-immunoreactive cells seems to persist until the last week of gestation, supporting a possible role for angiotensin-II in the control of GH secretion (33). By the 23rd week of gestation, immunoreactivity for galanin is present in somatotropes, as well as gonadotropes, thyrotropes, and lactotropes, and persists to birth (33). Another example for the role of different factors in the development of somatotropes comes from the observation that mutant mice heterozygous for the lack of hypothalamic IGF-1 receptor (bIGF1RKO$^{+/-}$) are born small and exhibit dysfunction of the hypothalamo-pituitary axis, namely low expression of *Pou1f1* and *Ghrh*, small pituitaries with low GH content, low systemic IGF-1 and acid labile subunit, and manifestation of the metabolic consequences of GHD (25). This suggests a role for IGF-1 feedback at the hypothalamic level in the regulation of the GHRH–GH–IGF-1 axis and in the development and plasticity of somatotropes.

Glucocorticoids, thyroid hormones, components of the AMP-activated protein kinase pathway, hypothalamic acetylcholine signaling via neuronal M3 receptors, other neuropeptides, and retinoic acid have all been shown to be involved in the maturation of somatotropes (34), but the exact mechanisms of action and extent of their contribution in vivo remains to be established. A detailed discussion of the role of paracrine factors in the control of anterior pituitary function is beyond the scope of this manuscript and has been extensively reviewed in recent publications (24, 26, 35, 36). In the next sections, we will focus on the role of selected factors in the development and maturation of somatotropes and their possible implications for the etiology of childhood GHD.

A. The role of glucocorticoids in the development and maturation of somatotropes

The well-established role of glucocorticoids (GCs) on the control of GH secretion and their long- and short-term effects on growth have been reviewed recently (37). With regard to their role on the development of somatotropes, there is evidence from studies on embryonic chick, rat, and murine pituitaries that GCs are implicated in their differentiation (38), proliferation, and functional maturation, as well as the expression of GH (34, 39, 40). Differentiation of somatotropes can be induced in vitro with adrenal GCs (41, 42). More recently, Suga et al (43) elegantly demonstrated the generation in vitro of functional anterior pituitary tissue from embryonic stem cells; in this setting, intermediate Pou1f1^{+} precursors differentiated into GH cells when cultured in media containing GCs, whereas culture in media containing estradiol enhanced their differentiation to prolactin-producing cells.

In vivo studies in chick embryos have demonstrated that there is a steady increase in the plasma concentration of corticosterone (the main GC in avian species) from E11 to E17, which correlates with an increase in the number of somatotropes. In addition, exogenous administration of ACTH in chick embryos at E9 to E11, a time when endogenous ACTH and corticosterone concentrations are low, leads to a significant and premature increase in the number of somatotropes by E14 (41); however, in cultures of anterior pituitary cells from E11 chick embryos, it is the administration of corticosterone and not ACTH that significantly increases the number of somatotropes, suggesting that the induction of GH cells by ACTH occurs indirectly via the increase of corticosterone and its action on the GC receptor (GR) (41). Although there is a suggestion that in chick embryos the action of GCs on the differentiation of somatotropes and GH gene expression may involve both the GC and mineralocorticoid receptor (44), there are no data supporting this role of the mineralocorticoid receptor in other species. However, the role of the GR is further supported by the effect of its selective ablation in the brain and pituitary of transgenic mice (GR^{CaMKCre}); these animals are born phenotypically normal but develop severe growth retardation, reduced weight, and early postnatal lethality by postnatal days 6 to 10 (45).

The mechanism of GC action on the differentiation of somatotropes and the control of *GH* expression is unclear. Despite the presence of GR binding sites in the proximal promoter upstream of the transcription initiation site (46) and the first intron of human *GH* (47), there is no conclusive evidence that the effect of GCs on the regulation of GH gene expression is direct. There is, however, a suggestion that their effect may be indirect via the transcriptional activation of genes encoding proteins of the RAS and Erk1/2 signaling pathway resulting in the phosphorylation and activation of ETS/Elk-1 transcription factors (48) that in turn interact with POU1F1 and facilitate its binding to the GH locus control region (49). The role of other, yet unidentified, factors cannot be excluded. For instance, in embryonic chick pituitaries, *Ras-dva*, a component of the FGF signaling pathway with homologs identified mainly in nonmammalian vertebrates, has been identified as a GC-regulated gene expressed in cells of the Pou1f1 lineage that may have a role in mediating the effects of GCs in the developing anterior pituitary (50, 51).

Although the mechanism by which GCs control the differentiation and maturation of somatotropes is not fully elucidated, their emerging role may account for the recent clinical reports of GHD in infants with a genetically proven defect leading to isolated GC deficiency (52) or resistance (53). In these infants, however, there was no evidence of anterior pituitary hypoplasia on magnetic resonance imaging (MRI); in addition, in one of these reports, the GHD was transient (52), whereas in the other, there is no information on long-term follow-up to confirm the persistence of GHD (53).

B. The dopaminergic system is involved in the proliferation of somatotropes

The dopamine D2 receptor (D2R), which is coupled to inhibitory Gi/Go proteins (54), is the predominant subtype of the dopamine receptor in the anterior pituitary, with a well-established role in inhibiting the proliferation of lactotropes and the synthesis and release of prolactin (55, 56). Over the last years, there is increasing evidence that D2R is also important for the control of the population of somatotropes, the hypothalamic control of GH secretion, and normal body growth. One of the first indications came from mice with targeted loss of the D2R (D2R$^{-/-}$), which are born with normal weight but develop growth failure within the first 2 months. This is more evident in male D2R$^{-/-}$ animals, which exhibit an almost 15% decrease in body weight, whereas females show catch-up growth in the next 3 to 4 months. Interestingly, in the first month of life, there is a significant decrease in serum GH concentration, and in vitro, cells from pituitaries of the D2R knockdown mice show an impaired basal and stimulated GH release in response to GHRH (57). Since this report, the D2R-knockout dwarf mouse has been a useful model for studying the role of D2R on GHRH-GH regulation; in fact, it was shown that in the D2R-knockout mouse, the observed reduction in GH release in vitro is related to a decrease in the number of somatotropes. The population of somatotropes in the pituitaries of the adult male D2R$^{-/-}$ mice is significantly decreased, representing only about 25% of all cells com-

pared with about 40% in pituitaries from wild-type animals (58). However, adult animals still exhibit almost normal serum GH concentration, suggesting the presence of compensatory mechanisms in vivo. The involvement of the D2R in the control of the population and function of somatotropes seems to be mainly at the level of the hypothalamus. The hypothesis is that the loss of dopaminergic signaling via hypothalamic D2R at a critical age results in reduced release of GHRH from hypophysiotropic neurons and subsequent defects in the clonal expansion of somatotropes (56).

This interaction between the dopaminergic neurons, D2R, and GHRH-releasing neurons is further supported by the anatomic juxtaposition of the catecholaminergic system and GHRH neurons in mice (59). Colocalization studies in mice indicated that almost 70% of the population of the GHRH neurons contain tyrosine hydroxylase, a rate-limiting enzyme for the biosynthesis of catecholamines, including dopamine (59). More recently, elegant studies have elucidated the distribution and morphology of the catecholaminergic neurons in the human hypothalamus (60–63). Stereoscopic images of the diencephalon demonstrated that the cell bodies of dopaminergic and GHRH-immunoreactive neurons overlap primarily at the basal part of the infundibulum; the density of the dopaminergic fibers on the cell bodies of the GHRH-releasing neurons suggest that these anatomical juxtapositions may in fact be functional synapses with a direct role in the regulation of GHRH secretion (63). This close association between the catecholaminergic system and GHRH-releasing neurons may be the basis of the regulatory role of neurotransmitters on the secretion of GH. It is also possible that this may be the pathophysiological basis of the long-described effect of stress on the control of GH secretion and the GHD observed in children with psychosocial deprivation, which is associated with a decreased but reversible GH secretion (64, 65).

C. Emerging role of chemokines and other factors in the proliferation and development of somatotropes

Chemokines are a family of peptides, usually 60 to100 amino acids long, that signal through G protein-coupled receptors and have a well-established role in the control of migration and chemotactic activity of leukocytes. There is now increasing recognition that some chemokines and their receptors are expressed in the CNS and hypothalamus and may function as neurotransmitters or modulators of neuroendocrine interactions with a role in the modulation of response to stress, regulation of anterior pituitary hormone secretion, and water balance at the hypothalamic level, via the autocrine control of arginine vasopressin neurons (66). With regard to the function of somatotropes, there has been early recognition that chemokines (such as the cytokine-induced neutrophil chemoattractant) stimulate the secretion of GH, as well as prolactin, in a concentration-dependent manner (67).

There is now evidence that, in addition to a role in regulating secretion, some chemokines, through their corresponding receptor activation, are also implicated in the proliferation of somatotropes. This was first suggested by the detection of chemokine receptor 4 (CXCR4) mRNA in a GH-releasing cell line of rat pituitary adenoma (GH4C1) and the observation that its ligand, stromal cell-derived factor 1/chemokine ligand 12 (CXCL12), causes both proliferation of somatotropes and release of GH (68). This supports the notion that the activation of CXCR4 may be a regulatory mechanism for GH secretion and pituitary cell proliferation via multiple intracellular pathways involving activation of ERK1/2 and Pyk2, a calcium-dependent, cytosolic tyrosine kinase (36).

More recently, double-immunofluorescent staining of rat anterior pituitaries for CXCR4 and GH or prolactin demonstrated that CXCR4 is specifically expressed in the somatotropes and has a role in GH release and proliferation of somatotropes (29). Although in primary rat anterior pituitary cells, stromal cell-derived factor 1/CXCL12 increases the transcription and release of GH, there is no synergistic effect with GHRH administration, suggesting that signaling via the CXCL12/CXCR4 and GHRH-mediated pathways may use similar intracellular mediators or even interfere with each other (29). In the rat anterior pituitary, CXCL12 is secreted by folliculostellate cells and, via the interaction with its receptor, has an autocrine role in the directional extension and interconnection of their cytoplasmic processes. In this context, it is possible that the CXCL12/CXCR4 axis, under the influence of components of the extracellular matrix, may also have a functional role in the determination and maintenance of the cellular arrangements in the anterior pituitary, including somatotropes (30).

Another factor with a possible role in the development of somatotropes is Δ-like protein 1 (Dlk1), also known as preadipocyte factor 1 (Pref-1). This is a transmembrane protein characterized by epidermal growth factor-like repeats, which is expressed in pituitary somatotropes and has been shown to reduce GH expression by repressing Pou1f1 in vitro (69). Dlk1 is a noncanonical Notch ligand and acts as a modulator of the Notch signaling pathway, which is in turn implicated in maintaining the proliferation of progenitor cells during pituitary development (3). Dlk1-knockout mice exhibit pre- and postnatal growth retardation, increased adiposity (70), increased circulating leptin, and a reduced number of pituitary GH-immunoreactive cells compared with wild-type animals, but no

difference in the expression of GH mRNA (71). To explain these apparently contrasting results, it has been suggested that, during development, Dlk1, expressed in somatotropes or neighboring cells, induces the proliferation of somatotropes in an autocrine or paracrine manner, respectively, and maintains the proliferation of progenitors via Notch. In the Dlk1-knockout animals, the smaller number of GH-producing cells may be expressing more GH mRNA as a result of enhanced Pou1f1 transcriptional activity and the effect of increased leptin signaling stimulating somatotropes (71). Our group has since reported that loss of Dlk1 has in fact an effect on all pituitary hormones, although the phenotypes are mild without an overt impact on normal function, whereas the associated reduction in size is unlikely to be caused by the reduction in GH secretion (72).

D. Effect of loss of somatotropes

The organization and interplay of somatotropes in the pituitary microenvironment raises the question of what would be the consequence of their loss both on the organization of the GH cell network and on other hormone-producing cells. These effects were studied in a transgenic murine strain (GH-M2) with somatotrope-specific expression of a modified influenza virus ion channel (^{H37}AM2), leading to different degrees of ablation of somatotropes depending on the copy number of the transgene (GH-M2$^{low\text{-}to\text{-}high}$). Interestingly, the GH-M2^{med} mice exhibited not only a disruption of the somatotrope network and a reduction in β-catenin staining in somatotropes but also a reduction of β-catenin staining in non-somatotrope cells, although less severe. It is, therefore, suggested that the normal cellular organization of somatotropes may be required for the formation of adherent junctions and subsequently the organization and communication of other endocrine cells within the pituitary (14). In addition, the GH-M2^{med} and GH-M2^{high} strains exhibit deficits in some (TSH and prolactin) or all of the hormone-producing cell lines, respectively, which may be a secondary result of the loss of somatotropes after their differentiation. These findings are reminiscent of those observed in pituitaries of the $^{\Delta exon3}$hGH transgenic mice, where abnormal packaging of GH results in loss of somatotropes, inflammatory macrophage invasion, and loss of other pituitary cells as a result of bystander damage (73).

The secondary loss of somatotropes, as is observed in the GHRH-knockout mice with disruption of the *Ghrh* locus, does not seem to affect the other endocrine cell types; the GHRH-knockout animals appear normal at birth and manifest significant growth retardation by 3 weeks of age with IGHD and hypoplasia of the anterior pituitary (74). Interestingly, transgenic animals with ablation of GHRH neurons (GHRH-M2), in addition to the hypoplasia of somatotropes and GHD, also exhibit prolactin deficiency (13). In this case, it is possible that the disruption of the GHRH neurons in the arcuate nucleus also disrupts hypophysiotropic peptides that are produced along with GHRH from the same precursor and affects the production of prolactin and GH.

Most interestingly, it is now becoming clear that the pituitary gland has the potential for regeneration and recovery in response to a somatotrope-specific insult. In one of the earliest studies, Borrelli et al (75) generated transgenic mice that expressed the herpes virus-1 thymidine kinase specifically in somatotropes (GHKT), which, therefore, were susceptible to treatment with synthetic nucleotides such as fialuridine. GHKT animals were treated with fialuridine for different lengths of time, either pre- or postnatally; one of the main observations was that in animals treated for 8 weeks and allowed a period of recovery before being killed, there was a repopulation of their pituitaries with mature GH cells. One of the major advances in our understanding of the response to somatotrope loss came from the identification of the pituitary progenitor/stem cells and their emerging role in both pituitary development and pathology (76–78). In the adult murine pituitary, there persists a small population of progenitor/stem cells that express Sox2 (79), an early transcription factor important for the maintenance of a multipotent phenotype (80, 81). These cells persist mostly in a narrow zone lining the pituitary cleft, a remnant of Rathke's pouch, but are also scattered throughout the pituitary and have the ability to form pituispheres in culture and to differentiate in vitro to all of the hormone-producing cells as well as to folliculostellate cells (79). Recently, Fu et al (82) used a transgenic murine model of induced damage to somatotropes (GHCre/iDTR) that expresses the inducible receptor for diphtheria toxin (iDTR) in somatotropes under the control of the rat GH promoter. Animals injected with diphtheria toxin for 3 consecutive days showed a significant reduction in the number of somatotropes, without a change in the numbers of ACTH or α-glycoprotein subunit-positive cells. Although within 11 days from the insult there was an almost 90% reduction in the number of GH cells, by 4 to 5 months, there was a partial restoration in their number to almost 50% compared with wild-type animals. Further examination of their pituitaries showed that 4 days after injury, there was an almost 2-fold increase in the number of the Sox2$^+$ progenitor/stem cells predominantly in the marginal area, but also in the parenchyma of the gland, which could be due to an increase in proliferation rather than a decrease in apoptosis. More specifically, soon after the insult on somatotropes, there were cells staining positive both for Sox2 and

GH, indicating that new somatotropes derived from this stem cell/progenitor pool and not from proliferation of remaining somatotropes or transdifferentiation of lactotropes (82) (Summary Box 1).

IV. Genetic Causes of Isolated GHD

The incidence of congenital IGHD varies between 1 in 4000 to 1 in 10 000 live births, and although it is most commonly sporadic, depending on the cohort screened, between 3% and 30% of cases are familial, implicating genetic factors. Classically familial IGHD has been classified into 4 types, depending on the inheritance pattern, as autosomal recessive (types IA and IB), dominant (type II), or X-linked (type III) (83). However, the expanding knowledge on the molecular defects leading to IGHD and the emerging patterns of phenotype/genotype correlations may challenge this classification. The commonest genes implicated in the genetic etiology of IGHD are those encoding GH (*GH1*) and the receptor for GHRH (*GHRHR*), whereas IGHD may be the only or first presentation of mutations in genes encoding early (*OTX2*, *HESX1*, *SOX2*, and *SOX3*) or late (*PROP1* and *POU1F1*) transcription factors. Depending on the cohort studied and the criteria used for defining GHD, mutations in known genes are identified in almost 11% of GHD patients, with a higher prevalence in familial compared with sporadic IGHD (34% vs 4%, respectively) (84).

V. *GH1* Mutations Causing GHD in Childhood

GH1 is located on the long arm of chromosome 17 (17q22–24) within a cluster of 5 homologous genes that encompass a region of almost 65 kb: *CSH-1* (chorionic somatomammotropin hormone), *CSH-2* and *CSHP* (chorionic somatomammotropin pseudogene), and *GH2* (a variant expressed in the placenta). The pituitary-specific expression of GH is regulated by the highly polymorphic proximal promoter (85) and a locus control region 15 to 32 kb upstream of *GH1* (49, 86, 87). The correct splicing of this 5-exon gene results in the generation of the mature, full-length 22-kDa peptide, which constitutes almost 75% of the total circulating GH, whereas the 20- and 17.5-kDa splice variants correspond, respectively, to 5% to 10% and 1% to 5% of transcripts (88).

Autosomal recessive IGHD type IA was first described in pedigrees with homozygous *GH1* deletions, ranging in size from 6.5 to 45 kb, the most frequent being the 6.7-kb deletion (89) arising from unequal recombination and crossing over within the GH cluster during meiosis (88, 90). Patients present with severe growth failure (height SD score [SDS] <-4.5) by the first 6 months of age with undetectable GH concentrations and tend to develop antibodies on treatment, although the latter is not a constant finding even in patients with the same molecular defect (91). In addition to *GH1* deletions, compound heterozygous mutations that result in frameshift (92, 93) or homozygous nonsense mutations affecting the signal peptide (89, 94) can result in a severely truncated or absent GH molecule. On the other hand, children with autosomal recessive *GH1* mutations have a low but detectable GH concentration and a good response to rhGH with no antibody formation. Type IB GHD may be caused by splice-site, frameshift, and nonsense *GH1* mutations in patients from consanguineous pedigrees or specific ethnic backgrounds (84, 90, 94–102) (Table 3).

A. Heterozygous *GH1* mutations affecting splicing of exon 3

Perhaps the most extensively studied form of congenital GHD is the autosomal dominant type II GHD caused by heterozygous *GH1* mutations, most of which affect the splicing of the *GH-1* gene by different mechanisms (Figure 2). Single base mutations within the first 6 nucleotides of intron −3 (intervening sequence [IVS]-3) result in skipping of exon 3 and production of the 17.5-kDa isoform that exerts a dominant-negative effect on the secretion of the 22-kDa molecule (73, 103). The 17.5-kDa isoform lacks amino acids 32 to 71 and, hence, the loop that connects helix 1 and helix 2 in the tertiary structure of GH. Exon 3 skipping can also result from mutations in an exonic splice enhancer motif (ESE1, GAAGAAG) that strengthens the use of the upstream weak 3′-splice site and suppresses a downstream cryptic splice site

Box 1. Maturation and organization of somatotropes
Apart from known transcription factors involved in the development of somatotropes, paracrine and autocrine factors are important for their development and maturation.
These include neuropeptides, the dopaminergic system, GCs, or chemokines.
Somatotropes are organized in functional three dimensional networks, ensuring the coordinated response to various inputs and the plasticity of the system to demand.
Studies on murine models with a targeted somatotrope insult reveal that the pituitary gland has the potential for regeneration and recovery.

Table 3. Mutations in *GH1* Causing GHD

GH1 Mutation	Location	GHD Type	Comments	Ref.
Gross gene deletions				
6.7 kb	*GH1*	IA, HM	With/without antibodies	96, 89
7.0 kb	*GH1*	IA, HM	With/without antibodies	97, 98
7.6 kb	*GH1*	IA, HM	With/without antibodies	91, 99
45 kb	*GH1*, *GH2*, *CSH*, *CSHL1*	IA, HM	No antibodies; different recombination sites	102
Double deletions/complex chromosomal rearrangements				
40 kb (total)	*GH1*, *GH2*, *CSH1*, *CSH2*	IA	Antibodies	100
Deletions/insertions within coding region				
c.50delC	Exon 2; codon −11	IA, CHT	CHT with 6.5-kb deletion; frameshift within the SP; antibodies present	101
c.243–244delAG	Exon 3	IA, CHT	CHT with 6.5-kb deletion; frameshift; termination codon in exon 4; no antibodies	92
c.270–285del16	Ex3	CHT	CHT with IVS3+4, A→T; in silico prediction of frameshift nonsense0mediated decay; unaffected parent carrier	484
c.64–65Ins26	Exon 2	IA, HM	Frameshift; introduces 86 novel aa before a stop codon; no antibodies	93
Single bp mutations within the coding region and in ESEs				
p.L-11P	Exon 2; SP	II, HT	Signal peptide	109
p.W-7X	Exon 2; SP	IA, HM	No mature GH; antibodies present	94
p.E-4X	Exon 2; SP	IA, HM	No mature GH; no antibodies	89
p.E32X; E3+1,G→T	Exon 3; c.172G→T	II, HT	ESE1: loss of exon3 (aa 32–71)	106
p.E32K; E3+1,G→A	Exon 3; c.172G→A	II, HT	ESE1.	107
p.E32A; E3+2,A→C	Exon 3; c.173A→C	II, HT	ESE1: 17.5 kDa (68%), 20 kDa (22%)	108
p.E33G; E3+5,A→G	Exon 3; c.176A→G	II, HT	ESE1: 17.5 kDa (62%), 20 kDa (27%)	105
p. K41X; E3+28, A→T	Exon 3; c199A→T	II, HT	In silico prediction of truncated product; possible effect on splicing	485
p.K41R	Exon 3; c.200A→G	II, HT	ESE2: 20% exon skipping	109
p.C53S	Exon 3; c.236G→C	HM	Bio-inactive GH; reduced affinity for GHR	486
p.P59S	Exon 3;c.253C→T	II, HT	Partial bio-inactive GH; lower affinity for binding to GHR and activation of Jak2/Stat5 signaling	125
p.P59 liter	Exon 3; c.254C→T	II, HT	Partial bio-inactive GH; lower affinity for binding to GHR and activation of Jak2/Stat5 signaling	126
p.R77C	Exon 4; c.307C→T	II, HT	Partial GH insensitivity	116, 120
p.P89 liter	Exon 4; c.344C→T	II, HT	Affects trafficking in the secretory pathway	123
p.S108C	Exon 4; c.400A→T	HT, NA	Reduced secretion.	109
p.S108R	Exon 4; c. 400A→C	II, HT	Reduced signal transduction	109
p.V110F	Exon 4; c.406G→T	II, HT	Likely to result in steric hindrance	130
p.D112G	Exon 4; c.413A→G	II, HT	Bioinactive GH; prevents GHR dimerisation	118
p.G120V	Exon 4; c.437G→T	IB, HM	No in vitro studies; likely antagonistic action	84
p.T175A	Exon 5; c.601A→G	II, HT	Reduced signal transduction	109
p.R178H	Exon 5; c.611G→A	II, HT	Affects GH secretion, binding, and signaling	84, 124
p.I179M	Exon 5; c.615C→G	HT	Bioinactive GH; reduced ERK activation	119
p.R183H	Exon 5; c.626G→A	II, HT	Impaired GH secretion	117, 121
p.C182X	Exon 5; c.624C→A	IB, HM	Truncated protein; disulfide bond disruption	84
Splice site mutations				
IVS2–1,G→A	Intron 2	II, HT	3′-Acceptor splice site	109
IVS2–2,A→T	Intron 2	II, HT	3′-Acceptor splice site	487
IVS3+1,G→A	Intron 3	II, HT	Skipping of exon3, del32–71GH	488
IVS3+1,G→C	Intron 3	II, HT	Skipping of exon3, del32–71GH	489
IVS3+2,T→C	Intron 3	II, HT	Skipping of exon3, del32–71GH	487
IVS3+4,A→T	Intron 3	II, HT	In silico prediction of exon 3 skipping	84
IVS3+5,G→A	Intron 3	II, HT	Skipping of exon3, del32–71GH	490
IVS3+5,G→C	Intron 3	II, HT	Skipping of exon3, del32–71GH	491

(*Continued*)

Table 3. Continued

GH1 Mutation	Location	GHD Type	Comments	Ref.
IVS3+6,T→C	Intron 3	II, HT	Skipping of exon3, del32–71 GH	492
IVS3+6,T→G	Intron 3	II, HT	Skipping of exon3, del32–71 GH	493
IVS4+1,G→C	Intron 4	IB, HM	Loss of aa 103–126 in exon 4; frameshift in 5	94
IVS4+1,G→T	Intron 4	IB, HM	Loss of aa 103–126 in exon 4; frameshift in 5	90
IVS4+5,G→C	Intron 4	IB, HM	Loss of aa 103–126 in exon 4; frameshift in 5	95
IVS4−1,G→A	c.456G→A	II, HT	No aa change; assumed to affect splicing	209
Mutations affecting ISEs or size of intron				
IVS3del+28_45	Intron 3, ISEm2	II, HT	18-bp deletion; skipping of exon 3	111, 112
IVS3del+56_77	Intron 3	II, HT	Removes BPS in intron 3; skipping of exon3	113
IVS3+28,G→A	Intron 3, ISEm1	II, HT	Abnormal splicing	111, 112

Abbreviations: aa, amino acid; BPS, branching point site; ESE, exon splice enhancer; CHT, compound heterozygous; GHBP, GH binding protein; HM, homozygous; HT, heterozygous; ISE, intronic splice enhancer element; NA, inheritance pattern not reported; SP, signal peptide; c. denotes nucleotide position on cDNA, with the A of the translation start site (ATG) of the cDNA numbered +1.

(104). The first mutation in ESE1 was reported by Moseley et al (105) in the fifth nucleotide of exon 3 (E3+5nt, A→G); although it resulted in an amino acid change from glutamate to glycine (E33G), its mechanism of action was via its effect on splicing. Transient expression assays demonstrated that, in addition to complete exon skipping, the mutation resulted in the activation of the downstream cryptic splice site at nucleotide 45 of the exon (E3+45), causing loss of amino acids 32 to 46 of the GH molecule (20 kDa). The 22-kDa isoform represented only 11% of transcripts, whereas the majority consisted of the 17.5-kDa (62%) and 20-kDa (27%) products. In fact, mutations in any of the bases of ESE1 lead to either complete or partial exon 3 skipping and the generation of the 17.5- and 20-kDa isoforms at various concentrations (35%–68% and 20%–37%, respectively) (105–108). More importantly, ESE1 mutations result in abnormal splicing even if there is no amino acid change, which underlines the observation that even translationally silent mutations can affect splicing and lead to phenotypic manifestations (104, 105). ESE2 is a second splice enhancer within exon 3, 12 nucleotides upstream of the cryptic splice site. The heterozygous missense mutation (K41R) in ESE2 leads to exon skipping in about 20% of transcripts with pleiotropic effects on the phenotype, ranging from normal to short stature (109, 110). Disruption of sequences in intron-3, downstream of the consensus splicing sites, that affect intronic splice enhancers (111, 112) or the branching point site (113) can also result in skipping of exon 3, supporting the notion that the size of intron-3 is important for the integrity of the splicing mechanism (110, 113).

Independent of the mechanism underlying its expression, both in cell cultures and in transgenic murine models, the 17.5-kDa isoform has a dominant-negative effect on the secretion of the 22-kDa molecule (73, 103) and transgenic mice overexpressing the 17.5-kDa isoform show a defect in the maturation of the GH secretory vesicles and anterior pituitary hypoplasia. More importantly, mouse lines expressing multiple copies of the exon-3–deleted allele had a more severe phenotype, almost undetectable GH, and profound pituitary hypoplasia with loss of somatotropes and macrophage invasion in comparison with mice with the lower copy number. Unexpectedly, the most severely affected animals developed, in addition, TSH, prolactin, and LH deficiency. It is therefore possible that the activated macrophages accelerate the loss of somatotropes and that other cell lines may be destroyed by bystander damage (73).

At the cellular level, the 17.5-kDa isoform lacks the protein linker domain between the first 2 helices of GH and a cysteine residue (C53) that is involved in the formation of a disulfide bond between helix 1 and helix 2 and is retained in the endoplasmic reticulum. This triggers a misfolded protein response and disrupts the secretory pathway and trafficking of GH and other hormones, including ACTH (73, 114). The amount of the 17.5-kDa product has to reach a critical threshold to exert its dosage-dependent effect, with increasing amounts leading to reduced cell proliferation and apoptosis of somatotropes (110, 115).

B. Heterozygous *GH1* mutations affecting GH action and bioactivity

Heterozygous missense mutations affect GH secretion and/or action by diverse mechanisms (116–120). The p.R183H mutation results in defects in exocytosis of secretory granules (121, 122), whereas the p.P89L may cause a more profound and early disturbance in the secretory pathway by altering the orientation of the GH helices and affecting the correct folding of the molecule (123). In other cases, the mechanism is more complex, as is illustrated by the p.R178H mutation that, in addition to

Figure 2.

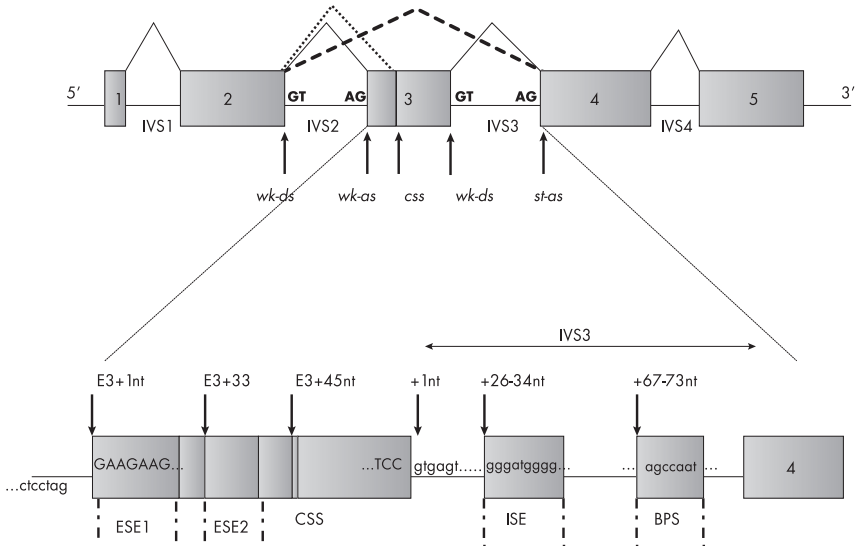

Figure 2. Schematic presentation of *GH1*, with the 5 exons and IVSs. Exon 3 is flanked by weak acceptor (wk-as) and weak donor (wk-ds) splice sites, compared with the strong acceptor site (st-as) in exon 4. Normal splicing, with inclusion of all 5 exons, results in the generation of the 22-kDa protein (black lines). Use of the cryptic splice site (css) in exon 3 will result in the 20-kDa isoform that lacks amino acids 32 to 46 (dotted line), whereas complete skipping of exon 3 (bold dotted line) results in the generation of the 17.5-kDa product that lacks amino acids 32 to 72. Focus on the area of exon 3 and IVS3 highlights the elements that are important for the correct splicing of *GH1*. They include the exonic splice enhancers (ESE1 and ESE2) and cryptic splice site (CSS) within exon 3 and intronic elements such as the first 6 nucleotides of intron 3, the intronic splice enhancer (ISE), and branching point site (BPS). Arrows indicate the nucleotide position of these elements.

the dimerization of the GH molecule, also affects its binding affinity for the GH receptor (GHR), resulting in reduced activation of the downstream signaling pathway (124). Other mechanisms may result in reduced binding affinity for the GHR and decreased activation of the downstream signaling pathway, as in the heterozygous missense mutations p.P59L and p.P59S (125, 126). In addition, the heterozygous p.R77C substitution has been reported in a patient with growth retardation and delayed pubertal development, who showed normal catch-up growth with rhGH replacement therapy. However, there is no clear phenotype-genotype correlation because the same mutation has also been identified in family members of normal height. Patients do not always have short stature; they may have normal or slightly increased GH secretion and low IGF-1 and GH binding protein concentrations. Functional studies did not show any difference between the mutant and wild-type GH molecule in terms of binding to the GHR and activation of the downstream Jak2/Stat5 pathway. However, it is possible that the mutation results in a reduced capability to induce the *GHR/GHBP* gene transcription compared with the wild-type molecule (120).

C. Evolving phenotype of patients with heterozygous *GH1* mutations

Patients with autosomal dominant IGHD have significant variation in the severity of GHD; they present with low but detectable GH and variable height deficit, with or without anterior pituitary hypoplasia on MRI (38%–50%) (84, 127, 128). Pedigrees harboring the p.R183H or E3+1,G→A mutations highlight the fact that patients with the same mutation can present with variable height deficit (<−4 SDS to normal) and even attain normal adult height without treatment (128, 129). Although it has been suggested that patients with splice-site mutations are more severely affected compared with those with missense mutations (130), this may not always be the case. It has been shown that patients with the IVS3+1/+2 splice site or the p.P89L mutation may develop additional pituitary hormone deficiencies including ACTH, prolactin, TSH, or gonadotropin deficiency (123, 131, 132). This evolving phenotype is unpredictable, dictating the need for life-long follow-up. In these patients, the lack of negative feedback on GHRH may increase the stimulatory drive on somatotropes and the production of GH both from the normal and mutant allele, creating a vicious circle of increased 17.5-kDa isoform that further accelerates damage to the anterior pituitary (115, 133). Another intriguing observation is that, even in patients with a genetic cause (p.E32A), GHD may appear to reverse when they are retested in the transition period. This may be due to the fact that treatment with rhGH removes the endogenous drive by GHRH and leads to recovery of somatotropes. However, this is a temporary effect observed in patients tested

at the time of transition, who should continue to be followed up for surveillance of GHD (108).

D. Toward a genetic treatment for GHD type II

Attempts for a genetic approach to treatment of some forms of autosomal dominant IGHD stemmed from the observation that interventions aiming to control the deleterious, increased levels of the 17.5-kDa isoform may, at least in part, restore GH secretion and hence the phenotype. At the cellular level, small interfering RNA constructs that specifically target the exon-3–deleted transcripts can reduce the production of the 17.5-kDa isoform by almost 90% (110). Exposure of peripheral blood monocytes from patients with type II GHD to pharmacologic agents has shown that the ratio of the 17.5- to 22-kDa molecule may be decreased (sodium butyrate) or increased (dexamethasone and digoxin) depending on the type of agent and exposure (134). Recently, Shariat et al (135) rescued the pituitary function of a murine model of type II GHD by mating them with transgenic animals expressing short hairpin RNAs (shRNAs) that target specifically the transcripts encoding the 17.5-kDa isoform; the degradation of the incorrectly spliced transcripts in vivo reversed the IGHD phenotype and restored the morphology of the anterior pituitary.

More recently, Lochmatter et al (136) developed an exogenous delivery system involving lentiviral vectors that express a human micro-RNA-30–based shRNA (shRNAmir) to target the anomalous junction between exons 2 and 4 of *GH1* (shRNAmir-Δ3), which is specific for only the mis-spliced mRNA lacking exon 3. Using rat pituitary tumor GCs that expressed both the wild-type and Δexon3 GH, they demonstrated that the shRNAmir reduced the expression of mutant protein, leading to an increase of wild-type GH secretion, without affecting the viability of the transduced cells. Although we are still far from an in vivo application, this approach has the potential of introducing a genetic rescue to the population of somatotropes, especially given our recent understanding that the somatotrope population has the potential for regeneration and repair.

VI. IGHD Caused by *GHRHR* Mutations

GHRH, which is important for proliferation of somatotropes and GH secretion (137), acts by binding to the G protein-coupled receptor GHRHR on the cell surface of somatotropes. This 423-amino-acid peptide consists of an N-terminal extracellular domain, 7 transmembrane domains, and an intracellular C-terminal domain; the encoding 13-exon gene (*GHRHR*) is on chromosome 7p14, and its expression requires the presence of Pit-1 (POU1F1).

The implication of GHRHR in the etiology of IGHD stemmed from observations on a spontaneously occurring dwarf mouse model (*little*) that has a homozygous missense mutation (D60G) in the extracellular domain of the receptor (*Ghrhr*). Although in this animal model the development of the anterior pituitary is not affected, there is severe anterior pituitary hypoplasia, with an almost 10-fold decrease in the number of somatotropes and decrease in the pituitary GH content, leading to the suggestion that GHRH signaling is required for somatotrope proliferation. Subsequently, Wajnrajch et al (138) described the first homozygous loss-of-function *GHRHR* mutation (p.E72X) resulting in a truncated protein missing the whole of the transmembrane and the intracellular domain in 2 patients from a consanguineous Indian family with extreme short stature, frontal bossing, truncal obesity, and severe GHD with failure to respond to standard GH stimulation tests or to repetitive GHRH stimulation. Since then, more than 20 mutations have been reported in *GHRHR*, including nonsense (138–140), missense (84, 141–145), and splice-site (140, 141, 146–148) mutations, in addition to deletions (142, 149, 150) or regulatory mutations affecting the *POU1F1* binding site in the promoter region (151) (Table 4).

Homozygous or compound heterozygous *GHRHR* mutations are identified in 10% of patients with familial recessive IGHD (143) and in about 3% of our cohort of patients with IGHD (84). Although most patients are from consanguineous pedigrees or from certain ethnic backgrounds of the Indian subcontinent and Brazil (152–155), *GHRHR* mutations have also been reported in patients from nonconsanguineous pedigrees (84, 140, 141) and from diverse ethnic backgrounds such as Somalia, Spain, Japan, and China (84, 141, 143, 147, 148, 156). Interestingly, there are recent reports of *GHRHR* mutations even in patients with sporadic GHD (139, 157); however, in these cases, extensive family history and auxological data for parents have been incomplete and the GH status of carriers has not been fully investigated. Recently, Godi et al (139) reported 3 unrelated patients of Italian origin with sporadic IGHD who were heterozygous for the p.V10G *GHRHR* change, resulting in failure of the receptor to localize to the cell membrane. In one case, the change was also present in an unaffected parent and sibling, and it has also been identified in one of the normal-stature controls. Although the authors suggested that the p.V10G may represent a novel form of IGHD caused by a dominant *GHRHR* mutation with variable penetrance, it cannot be ex-

Table 4. Mutations in *GHRHR* Causing Isolated GHD

Mutation	Type	Location	Familial	MRI	Function	Ref.
c.−124, A→C	CHT, regulatory	Promoter	Y	APH	Affects Pit-1 binding site, reduced promoter activity	151
p.V10G	HT, missense	SP exon 1	N	APH/normal	Reduced cell surface expression	139
p.Q43X	CHT, nonsense	ECD exon 2	Y (NC)	APH	Truncated protein, lack of	140
p.E72X	HM, nonsense	ECD exon 3	Y (C)	APH	functional GHRHR	138
p.R94 liter	HM, missense	ECD exon 4	Y	APH	Predicted to affect ligand binding	84
p.G136V	CHT, missense	TD exon 5	Y (NC)	Normal	Reduced signal transduction	141
p.H137 liter	CHT, missense	TD, exon 5	Y (NC)	NA	Reduced cAMP response to GHRHR, normal cell surface expression	142
p.L144H	HM/CT missense	TD exon 5	Y (NC)	APH	Reduced cAMP response to GHRHR, normal cell surface expression	143
p.R161W	CHT missense	TD, exon 6	Y (C)	APH	Predicted to affect cAMP response	84
p.A176V	HM, missense	TD, exon 6	Y (C)	APH	Reduced cAMP response to	144
p.A222E	HM, missense	TD, exon 7	Y (C)	NA	GHRHR, normal cell surface	143
p.F242C	CHT missense	TD, exon 7	Y (NC)	NA	expression	143
p.W273S	HM missense	TD, exon 9	Y (C)	APH	Predicted to affect cAMP response	84
p.K329E	CHT missense	TD exon 11	Y	APH	Reduced cAMP response to GHRHR, normal cell surface expression	151
p.R357C	HM, missense	TD exon 11	Y, (C)	Normal/APH	Inactive mutant receptor in vitro	145
p.E382E	HM or CHT	Exon 12	Y, (CN) N	Normal	Skipping of exon 12 in in vitro splicing assay	156, 157
c.340delG	HM, deletion	ECD exon 4	Y (C)	APH	Frameshift; stop codon 85 bp downstream of mutation	149
c.1121–1124 del 4	HM, deletion	ID, exon 12	NA	APH	Heterozygous relatives unaffected	150
c.1140–1144 del 5	CHT, deletion	ID, exon 12	Y (NC)	NA	Reduced cAMP response to GHRH	142
IVS1+1,G→A	HM, splice site	Intron 1	Y (C)	NA	Aberrant splicing products,truncated protein	152
IVS1+2,T→G	HM, splice site	Intron 1	Y (C)	APH	Aberrant splicing products, truncated protein	146
IVS2+3,A→G	CHT, splice site	Int 2	Y (NC)	Normal	Aberrant splicing products	141
IVS3+1,G→A	CHT, splice site	Intron 3	Y (NC)	APH	In-frame stop codon in intron 3; truncated protein	140
IVS4–2, A→G	CHT Splice site	Int4.	N	APH	Predicted to disrupt the normal acceptor splice site with retention of intron 4	157, 347
IVS7–1,G→A	HM	Intron 7	Y (C)	NA	In silico prediction of loss of splice acceptor site	157
IVS7+1,G→C	HM, splice site	Intron 7	Y	APH	Aberrant splicing, truncated protein	477
IVS8+1,G→A	HM, splice site	Intron 8	Y (NC)	APH	In-frame stop codon in intron 8; truncated protein	147
IVS12+2,T→A	HM, splice site	Intron 13	Y (C)	Normal/APH	Predicted aberrant splicing products	148

Abbreviations: APH, anterior pituitary hypoplasia; C, consanguineous; CHT, compound heterozygous; ECD, extracellular domain; HM homozygous; HT, heterozygous; ID, intracellular domain; N, no; NA, not available; NC, nonconsanguineous; SP signal peptide; TD, transmembrane domain; Y, yes.

cluded that a digenic or oligogenic effect may account for the phenotype.

Patients with *GHRHR* mutations classically present with early and severe growth failure (height SDS up to −7.4), have a blunted GH response to different provocation tests and low IGF-1 and IGF binding protein 3 (IGFBP3) concentrations, and respond well to rhGH treatment. In untreated individuals, the mean adult height is reported in the range of 130 ± 10.6 and 113.5 ± 0.7 cm for males and females, respectively (154). The phenotype seems to be less severe compared with children with recessive *GH1* mutations, because neonatal hypoglycemia, midfacial hypoplasia, and microphallus are uncommon. The reason for this difference is not clear, but it is not solely

due to the severity of GHD because in both cases, the stimulated peak GH concentration can be below 3 μg/L. It is possible that in these patients, although the number of somatotropes is markedly reduced, their differentiation and development is not grossly disrupted and they maintain the ability to respond to stimuli perhaps independently of the GHRH. This is supported by the observation that patients without functional GHRHR may have detectable GH pulses and show a rise in the concentration of GH after simulation with GH-releasing peptide-2 (158). The presence of anterior pituitary hypoplasia on MRI has been considered an almost invariable finding because of the effect of GHRH on the proliferation of somatotropes. However, even patients with the same mutation may have variable anterior pituitary imaging, especially in childhood, with a hypoplastic or normal anterior pituitary (141, 148, 157). A possible explanation may be the variability in the age of presentation and the lack of uniformly used age-matched reference standards for assessing pituitary MRI. In these patients, it would be interesting to review the results, if any, of their repeat pituitary imaging when they reach late adolescence or early adulthood.

With the possible exception of the reported p.V10G, heterozygosity for *GHRHR* mutations does not seem to affect height or bone health but has an effect on body composition with a lower weight and increased insulin sensitivity compared with age-matched controls (159, 160).

With regard to the mechanism of action of missense mutations, in vitro functional studies of cells transiently transfected with plasmid constructs of mutant *GHRHR* (ie, H137L, p.G136V, L144H, A176V, A222E, F242C, and K329E) showed reduced cAMP production after GHRH stimulation with normal cell-surface localization of the receptor, suggesting a defect in ligand binding (141, 161). To date, only one missense mutation (p.V10G) has been shown to have a different mechanism of action in vitro, as it interferes with cleavage of the signal peptide resulting in retention of the receptor in the endoplasmic reticulum and failure to translocate to the cell membrane (139). However, there is still no robust evidence for a dominant-negative effect on the wild-type allele (139, 151). On the other hand, nonsense and splice-site mutations are predicted (146–148) or have been shown (141, 156) to produce an abnormal or truncated receptor. Interestingly, Inoue et al (156) reported that the efficiency of splicing may be affected even by a synonymous homozygous *GHRHR* change (p.E382E) resulting from a single nucleotide substitution (c.1146G→A) located in a complex exonic splice enhancer in exon 12; RT-PCR analysis of COS-7 cells transfected with a minigene construct containing either wild-type or mutant exon 12 revealed that the wild-type construct generated 2 splice products, with and without inclusion of exon 12, whereas the mutant construct produced only 1 with skipping of exon 12 (156). However, in this case, the functional consequences on the downstream signaling of the receptor have not been studied. In addition, in cases of *GHRHR* mutations that may affect splicing, it is uncertain to what extent the alternative splicing products are produced in vivo and what would be their biological activity. Recent studies have demonstrated an effect of genetic variation in both *GH1* and *GHRHR* on height in normal populations. Recent linkage association studies (162, 163) reveal that GHRHR haplotypes contribute to the determination of normal height variation. However, rather than single markers, analysis of extended-marker haplotypes is needed to detect a strong association, thus accounting for 1.8% of the variation and a decrease in height of 1.2 to 3.8 cm, depending on the population (163). In a Spanish population, single-nucleotide polymorphisms (SNPs) in *GHRHR* (rs4988498; p.Glu121Asp) along with four single-nucleotide polymorphism genotypes in *GH1* contribute up to 6.2% of the variation in height SDS, and this contribution of *GHRHR* gene variation to height seems to depend on the population, especially for the extremes of height SDS (162)

VII. GHD Caused by Mutations in Early and Later Transcription Factors

In children, GHD may result from mutations in early transcription factors in association with other developmental abnormalities, including variants of septo-optic dysplasia and/or ocular defects (*HESX1*, *OTX2*, *SOX2*, and *SOX3*) (3, 164–167), skeletal defects (*LHX3* and *PITX2*) (168, 169), and intellectual impairment (*SOX3* and *SOX2*) (165, 170) with or without other pituitary hormone deficiencies.

X-linked GHD in association with agammaglobulinemia has long been recognized as a distinct entity, but although the *Btk* (Bruton tyrosine kinase) gene, a key regulator of B cell development, has been associated with this condition, its genetic etiology remains unknown (171). *SOX3*, a member of the SOX (SRY-related high-mobility group [HMG] box) family of transcription factors, has been implicated in the etiology of X-linked hypopituitarism with a highly variable phenotype. Patients with over- or underdosage of *SOX3* present with IGHD or combined pituitary hormone deficiencies, with or without mental retardation or learning difficulties and an ectopic/undescended posterior pituitary on MRI (165). In different pedigrees, large (3.9–13 Mb) or submicroscopic (685.6 kb) duplications encompassing *SOX3* have been associ-

ated with the phenotype. In addition, expansion of the first polyalanine tract of *SOX3* by 7 or 11 residues or an in-frame deletion resulting in the loss of 6 alanine residues have been reported in association with variable phenotypes (IGHD or combined pituitary hormone deficiencies). The mechanism involves reduced transcriptional activation of target genes due to retention of the mutant protein in the cytoplasm with failure of the protein to translocate to the nucleus (165). Conversely, polyalanine tract deletions result in increased transcriptional activation in vitro, which may be comparable to increased dosage of the gene (172). In any case, the variability in the size of the polyalanine tract in *SOX3* is an uncommon cause of IGHD or hypopituitarism, and to date, there have been no reported point mutations in *SOX3* leading to functional compromise.

On rare occasions, heterozygous *HESX1* mutations (p.E149K, p.S170L, or p.T181A) (167, 173) may be associated with IGHD. These patients exhibit a relatively milder phenotype compared with the severe manifestations of septo-optic dysplasia, with or without optic nerve hypoplasia, whereas the occurrence of an ectopic/undescended posterior pituitary on MRI and anterior pituitary hypoplasia is common but not always present (p.S170L) (167).

OTX2 (orthodentic homeobox 2) is another early transcription factor, important for the formation of anterior structures and the forebrain. To date, more than 20 heterozygous mutations have been reported in patients with variable ocular malformations, and the reported pituitary phenotype ranges from partial to complete GHD or hypopituitarism with or without an ectopic posterior pituitary on MRI (174), or even without an ocular phenotype (p.N233S) (166). The heterozygous p.R90S mutation in *OTX2* was identified in a patient with IGHD, unilateral anophthalmia, and learning difficulties in association with anterior pituitary hypoplasia and an ectopic posterior pituitary (175). A similar phenotype has been reported in a patient with a 15-bp deletion leading to frameshift (c.221_236del15) (166). On the other hand, a heterozygous whole-gene deletion has been described in a patient with IGHD, bilateral ocular defects, and anterior pituitary hypoplasia but with a normally positioned posterior pituitary on MRI. Due to lack of follow-up data, it is not clear whether in these subjects the manifested GHD will remain as the only endocrine deficit or whether additional pituitary hormone deficiencies will develop over time (166, 175). Although mutations in other early transcription factors (*LHX3* and *LXH3*) manifest with growth failure and GHD, this is not in isolation but in combination with multiple pituitary deficits (169, 176). To date, there is one reported case of an individual with a heterozygous *LHX4* mutation (p.A210P) with partial GHD and normal adult height, whereas his offspring who carried the same mutation, had combined GH, ACTH, TSH, and gonadotropin deficiency (169).

GHD may also be part of the endocrine phenotype in patients with heterozygous *SOX2* mutations in association with bilateral anophthalmia or severe microphthalmia with or without other developmental defects (esophageal atresia, genitourinary tract abnormalities, spastic diplegia, developmental delay, or sensorineural hearing loss) (164, 170). The endocrine deficit in patients with *SOX2* haploinsufficiency was first shown to be restricted to gonadotropes, but recent evidence from transgenic animal models may explain why at least some patients with *SOX2* mutations manifest GHD in addition to hypogonadotropic hypogonadism. Jayakody et al (177) generated a transgenic murine model with selective absence of *Sox2* in the developing Rathke's pouch and demonstrated the role of *Sox2* in the development of somatotropes. Although these embryos survived to birth, they had significant anterior pituitary hypoplasia detectable from E12.5 to E14.5, with a marked reduction in the expression of *POU1F1* and disruption of the differentiation of somatotropes and thyrotropes. In this model, the reduced proliferation of perilumenal progenitors probably resulted in a reduced pool of undifferentiated precursors, which is insufficient to allow the subsequent differentiation of enough numbers of hormone-producing cells at late gestation, leading to reduced numbers of somatotropes and severe pituitary hypoplasia.

GHD may also be the first or only manifestation of genetic defects in factors involved in later stages of the differentiation of the cells of the somatotrope lineage. Homozygous *PROP1* mutations are typically associated with GH, TSH, prolactin, and gonadotropin deficiency, although the timing and extent of these deficits vary and the full phenotype may not be evident from the outset. For instance, patients homozygous for the p.R120C mutation in *PROP1* may first present in childhood with GHD before the later development of TSH, prolactin, and gonadotropin deficiency (178). Rarely, the function of somatotropes may be retained and patients may even attain normal final height without GH replacement (179). On the other hand, autosomal recessive and dominant *POU1F1* mutations are associated with GHD, manifesting as a severe growth deficit in the first years of life, prolactin and variable TSH deficiency and a hypoplastic anterior pituitary. However, we have reported on an adult patient with IGHD and a hypoplastic anterior pituitary who, although homozygous for the p.E230K *POU1F1* mutation, did not develop other pituitary hormone deficiencies (180).

VIII. Other Genetic Factors of GHD in Childhood

Because mutations in known genes are identified in a relatively small percentage of patients with IGHD, other yet unidentified factors may account for its etiology. However, although *GHRH* was an obvious candidate, a multicenter study did not identify any functionally significant changes in patients with IGHD (181). Another candidate factor that is involved in the control of GH secretion is the GH secretagogue receptor (GHSR), which is expressed in the hypothalamus and pituitary and mediates the action of ghrelin on the regulation of GH release (182, 183). Recessive and dominant *GHSR* mutations (W2X, R237W, and A204E) have been reported in association with variable phenotypes, ranging from normal to partial GHD or IGHD (184, 185). Although there is some evidence that mutations may result in impaired constitutive activity of the receptor, whereas its ability to respond to ghrelin is maintained (184), there are contradictory data from animal studies, because murine models with targeted deletion of the receptor ($ghsr^{-/-}$) have a near-normal phenotype (186). Because central cholinergic stimulation positively regulates GH release, the gene encoding the muscarinic cholinergic receptor (*mAchR*) was also studied in families with autosomal recessive GHD, but no functional changes were identified (187).

On the other hand, results from genome-wide association studies identify an ever-increasing number of loci, currently at 180, that account for more than 10% of normal height variation (188, 189). Among them, the association with height of *HMGA2*, which determines chromatin structure and transcriptional regulation (190); cyclin-dependent protein kinase 6 (*CDK6*); hedgehog-interacting protein (*HHIP*); and the zinc finger transcriptional activator *ZBTB38* have been confirmed in independent meta-analyses (189, 191). In a recent screen of 105 hypopituitary patients for mutations in *HMGA2* and *CDK6*, apart from known variations, a novel heterozygous 20-bp deletion was identified 9 bases before the start of the fourth *HMGA2* exon (192). This de novo change was identified in a patient with severe IGHD (low peak GH to arginine and IGF-1 −6 SDS), who presented at the age of 3 years with a height of −4.8 SDS, micropenis, bone age delay, and structural pituitary defects on MRI (ectopic posterior pituitary and severe anterior pituitary hypoplasia) and attained normal adult height after rhGH treatment. Analysis of the patient's fibroblasts showed a significant decrease in the expression of 2 of the HMGA isoforms compared with controls, but without a change in the only known target of HMGA2, namely IGFBP2 (192). Although the function of these isoforms is unknown, one cannot definitely exclude the possibility that their reduced concentration may account for the phenotype; the pigmy phenotype of the $Hmga2^{-/-}$ mice (193) further supports its role in the control of growth.

IX. Acquired GHD in Childhood

A number of conditions that affect the hypothalamo-pituitary axis, ranging from tumors to infection, vascular causes, infiltrative diseases, or damage secondary to trauma, surgery, or irradiation (Table 5 and Figure 3), may lead to acquired GHD in children. For example, in case of tumors of the hypothalamo-pituitary area, growth failure may be the first presenting symptom in almost a third of patients before the development of overt neuroophthalmic signs, leading to delayed diagnosis (194). In other cases, the development of GHD may be insidious and may occur years after the initial insult as in the case of traumatic brain injury (TBI) (195, 196). In this section, we will focus on how recent research on the development and

Table 5. Acquired Insults to the Hypothalamo-Pituitary Axis That May Result in GHD

Cause	Examples
Tumors in/around pituitary area	Craniopharyngioma, germinoma, optic glioma, dysgerminoma, ependymoma, pituitary adenoma, meningioma, chordoma
Metastatic tumors (rare)	Childhood Hodgkin's, nasopharyngeal carcinoma
Cystic lesions	Rathke's cleft cyst, arachnoid cyst, dermoid cyst
Radiotherapy	Cranial irradiation for CNS tumors, hematologic malignancies, conditioning for bone marrow transplant
Brain trauma	TBI: after road traffic accident, child abuse, accidental After neurosurgery Subarachnoid hemorrhage (pituitary apoplexy, vascular causes)
Inflammation/infection	Meningitis, encephalitis, pituitary abscess, sarcoidosis, tuberculosis, autoimmune processes, lymphocytic hypophysitis
Infiltration	Langerhans cell histiocytosis Iron overload: hemochromatosis, thalassemia and diseases requiring chronic transfusions
Psychosocial deprivation	

Figure 3.

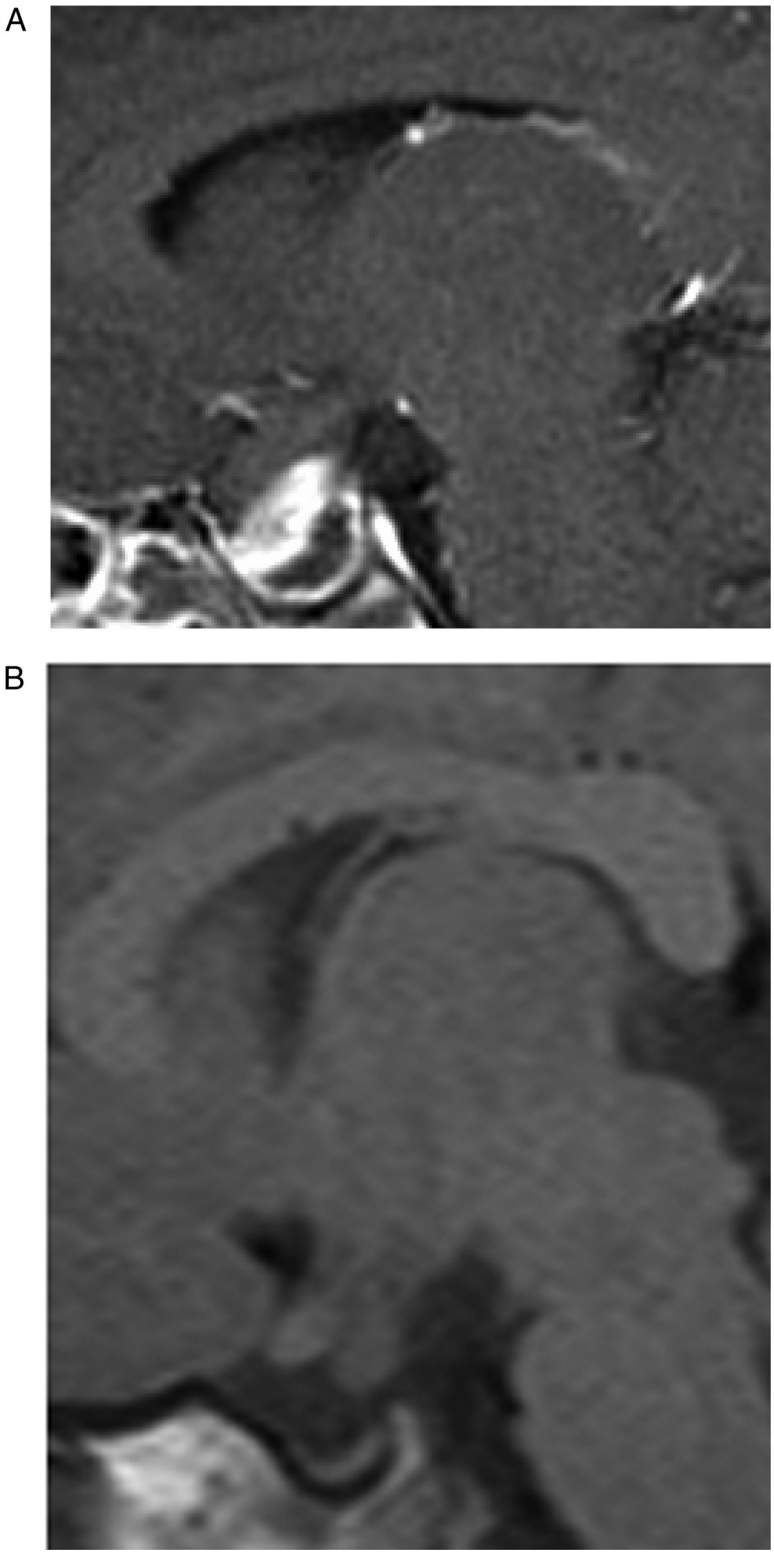

Figure 3. A and B, Pituitary MRIs of children with acquired GHD due to craniopharyngioma (A), shown as an enhancing solid mass with a cystic component that occupies the sella turcica and Langerhans cell histiocytosis (B); the pituitary stalk is thickened, with anterior pituitary hypoplasia and absence of the bright spot of the posterior pituitary.

function of somatotropes has furthered our understanding of the etiology of acquired GHD in children.

One of the recent breakthroughs came from the understanding of the molecular pathogenesis of adamantinomatous craniopharyngiomas (ACPs); these rare sporadic tumors of embryonal origin are derived from remnants of Rathke's pouch and account for almost 80% of tumors of the hypothalamo-pituitary area in children (197). Activating mutations in the gene encoding β-catenin (*CTNNB1*), a component of the Wnt signaling pathway, have long been identified in ACPs (198, 199). Recently Gaston-Massuet et al (200) developed a murine model ($Hesx1^{Cre/+}$;$Ctnnb1^{+/lox(ex3)}$) that expresses a degradation-resistant mutant form of β-catenin in early progenitors of Rathke's pouch. Although most mutant mice die perinatally by 4 weeks of age, those who survive exhibit pituitary hyperplasia, marked hypopituitarism, and extreme growth failure with severe disruption of the differentiation of the *POU1F1* lineage, with only a few scattered Pou1f1-positive cells identified in sections of $Hesx1^{Cre/+}$; $Ctnnb1^{+/lox(ex3)}$ pituitaries. Ultimately, they all develop lethal pituitary tumors that closely resemble human ACPs; this tumorigenic effect of the activated mutant β-catenin is observed only when it is expressed in undifferentiated progenitors and not when committed or differentiated cells are targeted to express the protein (200). Previously, Olson et al (201) demonstrated that β-catenin is essential for the differentiation of the *Pou1f1* lineage, with the effect of the lack of β-catenin being first observed by E15.5, with a reduction of *Pou1f1* expression and defects in cell lineage differentiation. Therefore, in addition to this role for normal differentiation of the *Pou1f1* lineage, β-catenin also has a critical role in

the control of cell proliferation of embryonic and postnatal pituitary progenitors (200).

Somatotropes are particularly vulnerable to damage after serious TBI; although the prevalence of permanent hypopituitarism after TBI in adults ranges between 23% and 70% in different studies (202–204), there is agreement that the GH axis is the most commonly affected (10%–33%), followed by the gonadal (8%–23%), adrenal (5%–23%), and thyroid (2%–22%) axes, with a much lower prevalence of permanent diabetes insipidus (0%–6%) (196, 202–204). This susceptibility of somatotropes may be explained by their location in the wings of the pituitary gland, receiving their vascular supply from portal vessels, whereas ACTH- and TSH-secreting cells are located in the medial part of the pituitary and receive their blood supply from both portal vessels and the anterior pituitary artery.

In children and adolescents, the incidence of hypopituitarism after TBI is reported in the range of 10% to 60% (196, 205, 206). Despite these reports, the number of children receiving rhGH for TBI-induced GHD, as registered in a large international database, remains very small compared with those with idiopathic GHD (141 vs 23–722) (207). In the study by Aimaretti et al (196), 23 patients aged 16 to 25 years were followed up at 3 and 12 months after the event. Although hypopituitarism was present in 35% of patients at 3 months, retesting of these patients at 1 year showed that hormone deficits persisted in 30%, consisting of mainly GH and gonadotropin deficiencies. A subsequent study of 48 pediatric patients after moderate to severe head trauma, who were evaluated either prospectively (n = 26) or retrospectively (n = 22), showed evidence of hypothalamo-pituitary dysfunction in 10.4% (n = 5) at 6 months to 7 years; 2 patients had IGHD, 1 ACTH deficiency, 1 gonadotropin deficiency, and 1 combined GH, ACTH, TSH, and gonadotropin deficiency (205). In contrast to the above-mentioned studies, Niederland et al (206) reported that 61% of children with a history of TBI had pituitary dysfunction almost 3 years after the event, and 42% (11 of 26) had GHD. Although complete auxological data were missing, it seemed that this subgroup did not show any slowing of the growth rate before diagnosis compared with children who had sufficient GH response, but not all studies agree with this observation (207, 208). What becomes evident is that there is a wide variation in factors such as the number of patients included in each study, the definition of severity of TBI, time of testing, and the definition of GHD and tests used for its diagnosis, all of which may affect the accurate estimation of the prevalence of permanent GHD in childhood after TBI. In a recent study of 32 children and adolescents after TBI (208), GHD was diagnosed if patients had a peak GH on overnight testing of less than 5 μg/L and a peak GH on arginine/glucagon stimulation of less than 7 μg/L. Based on these criteria, 16% (n = 5) had GHD, whereas an additional 19% (n = 6) had an abnormal response in one of these tests. The authors found no correlation between GHD and either the severity of TBI, as defined by the Glasgow Coma Scale score, or the IGF-1 concentration, suggesting that both factors are poor predictors for the incidence of GHD. However, the diagnosis of GHD was affected by the time of testing, because the interval between the incident and testing was significantly shorter in patients diagnosed with GHD (0.7 ± 0.6 vs 3.4 ± 3.8 years, $P < .05$) (208). It is not known whether these patients would still remain GHD if they were retested at a later stage. The issue of permanence of GHD after TBI is of particular importance in view of evidence that the pituitary gland has the potential for regeneration and recovery in response to a somatotrope-specific insult (82).

GHD may be the result of autoimmune mechanisms as part of the spectrum of endocrine deficits in autoimmune hypophysitis (210) or in patients with autoimmune polyglandular syndrome who develop antipituitary antibodies (211). Although pituitary autoantibodies recognize variable autoantigens (212), their role in the development of endocrinopathies remains highly debatable. In children, apart from isolated case reports (213, 214), there are not much data for a link between autoimmune factors and GHD. However, De Bellis et al (215) demonstrated that antipituitary antibodies specific to somatotropes can be detected in up to 27% of prepubertal children (7 of 26) with idiopathic IGHD, suggesting a possible involvement of autoimmune factors in the development of GHD. Acquired insults specific to the somatotrope lineage may also result from autoimmunity to PIT1 (pituitary specific transcription factor 1, now known as POU1F1), as was recently demonstrated for adults with GH, TSH, and prolactin deficiency, in association with positive serum anti-PIT1 antibodies and histologically a marked reduction of pituitary somatotropes with infiltration by lymphocytes and plasma cells (216). The mechanism leading to the development of autoimmunity to a cell-specific nuclear transcription factor is yet unknown, because PIT1-reactive T-cell lymphocytes have not yet been isolated. It is yet to be identified to what extent pituitary autoantibodies to various autoantigens could be causative for GHD in children and adolescents with an autoimmune endocrinopathy or those considered to have idiopathic IGHD.

X. Diagnosis of Childhood GHD

The diagnosis of GHD is a multistep process that includes clinical and auxological assessment, biochemical testing (GH stimulation tests and measurement of IGF-1/IGFBP3), and pituitary MRI with contribution from results of genetic testing (1, 217) (Figure 4). The biochemical

Figure 4.

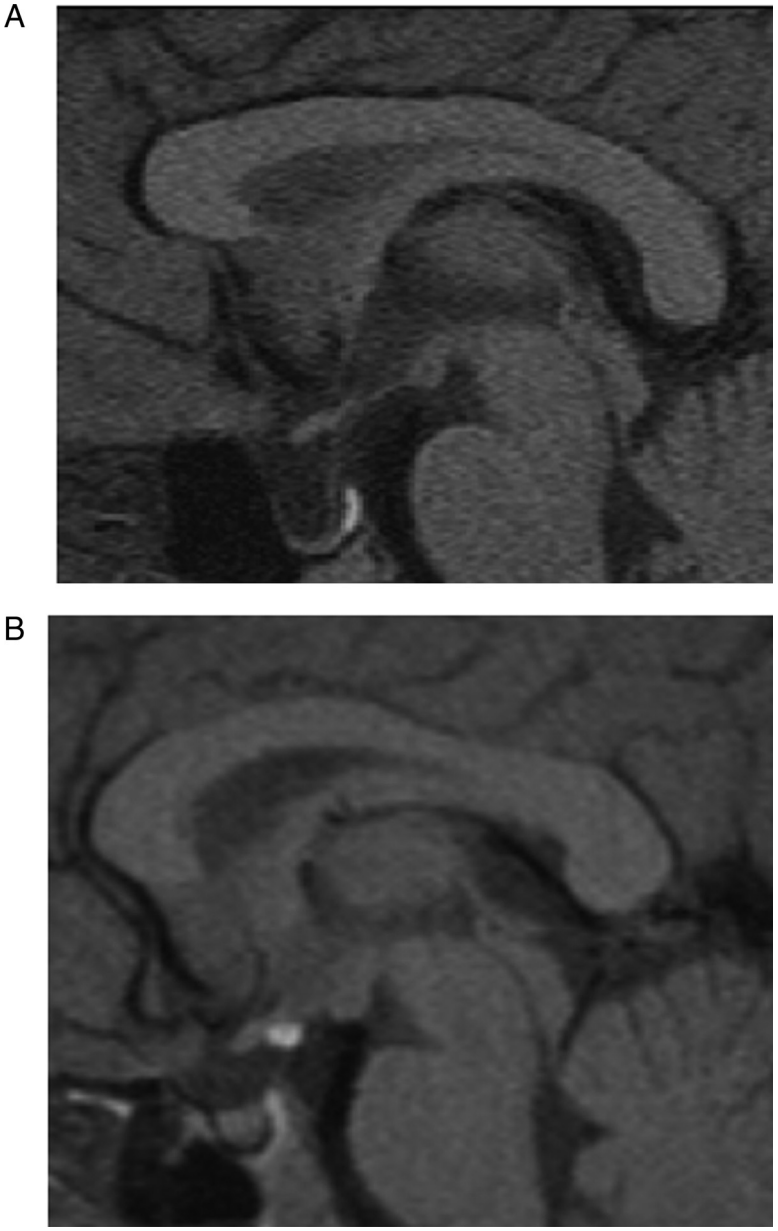

Figure 4. MRIs of patients with IGHD. A, Severe anterior hypoplasia, with only a rim of pituitary tissue and a eutopic posterior pituitary located within the sella, in a patient homozygous for a *GH1* mutation (IGHD type IB). B, MRI of a patient with IGHD showing anterior pituitary hypoplasia and an ectopic bright spot of the posterior pituitary, with normal corpus callosum. In this case, the genetic etiology remains unknown, because screening of known genes did not identify any mutations. [The image in A has been adapted from K. S. Alatzoglou et al.: Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. *J Clin Endocrinol Metab* 94:3191, 2009 (84), with permission. © The Endocrine Society.]

diagnosis of childhood GHD remains controversial, with a number of GH-stimulation protocols (218) that test the GH–IGF-1 axis in a nonphysiological manner, with agents of different strengths in terms of stimulus to axis, acting at variable levels, that may have serious side effects and are contraindicated in infants and have poor reproducibility. Perhaps the most challenging aspect is that there is little evidence to support the classically suggested peak GH cutoff value of less than 10 μg/L that has been set rather arbitrarily based on competitive polyclonal immunoassays and used widely without adjusting for factors that affect GH secretion throughout childhood such as age, sex, pubertal status, adiposity, and lean body mass (LBM) or the assay used for measurement of GH concentration (4). This may lead to misdiagnosis of GHD, because up to 85% of short prepubertal children (28 of 33) who were diagnosed with GHD (peak GH $<$10 μg/L in 2 provocation tests) had a normal GH response when retested 1 to 6 months later (219). Other studies suggest that, depending on the series and the cutoff used for diagnosis, almost 60% to 85% of patients diagnosed with GHD in childhood will have adequate GH secretion when retested in late adolescence or adulthood (220–225), although this may, at least in part, represent a maturation of the pituitary gland.

With respect to the variability in GH measurements in up to 27% of patients, the diagnosis of GHD may be dependent on the assay (226). Apart from factors intrinsic to the assay, this is due to the heterogeneity of the GH molecule (different isoforms, circulating in hetero- and homodimers), circulating GH binding protein and the standard preparation used for calibration (227, 228). The international standard IS 88/624 (recombinant 22-kDa GH), which was available at the time of the 2000 consensus statement on diagnosis of childhood GHD, has been replaced by the international standard IS 98/574, the implementation of which has been recommended by the recent consensus statement on the standardization and evaluation of GH and IGF assays (229). These recommendations and the current use of monoclonal assays make it

imperative to review the accepted GH cutoff used for diagnosis of GHD in children. Some pediatric endocrine societies have already suggested a decrease in the cutoff from 10 to 8 μg/mL (230) or 6 μg/mL (231), or even debate the need for a GH cutoff based on stimulation testing (232, 233). This variation in practice makes it difficult to compare clinical results and outcomes and highlights the need for a review of the consensus on diagnosis.

A. Diagnosis of GHD in different age groups

Particular to pediatric endocrinology, the diagnosis of GHD is even more challenging due to the considerable variation of normal GH secretion from the neonatal period through to adolescence. In neonates, GHD will not present with classical changes in growth velocity but will be suspected in the presence of suggestive signs/symptoms (ie, persistent hypoglycemia) or evidence of other defects/pituitary hormones affected and MRI findings. Because stimulation testing is contraindicated in this age group, a random GH of less than 20 μg/L has been suggested (1), given the increased serum GH concentration in the first days of life (234). Binder et al (235) reported that, using a highly sensitive human GH ELISA, the median GH concentration in dry blood spot samples of healthy neonates (n = 269) was 16.4 μg/L (95% confidence interval [CI], 7.0–39.4 μg/L), whereas the median serum GH concentration of 9 newborns diagnosed with hypopituitarism (age 4–28 days) was 2.1 μg/L (maximum 5.5 μg/L), with no significant overlap between the groups. Based on these groups, the authors concluded that a single cutoff value of 7 μg/L, when measured with the same assay, would have high sensitivity (100%) and specificity (98%) for the diagnosis of neonatal GHD.

In peripubertal children, the biochemical diagnosis of GHD with or without previous sex steroid priming is still a controversial issue. Since the seminal study by Marin et al (236) showing that in normal-stature children, the administration of a short course of ethinyl estradiol for 2 days before the provocation test increases the peak GH response, other studies confirmed that 20% (237) to more than 50% (238) of short normal children would have been diagnosed as GHD if tested without priming, whereas others report that up to 70% of children with an initial low GH response would have a peak of more than 10 μg/L after priming with sex steroids (239). However, there is still no consensus on the patient selection (based on age, bone age, or pubertal status) the type, dose, and duration of sex steroid priming (238, 240, 241); and importantly, the need for it. One of the arguments against is that the effect of sex steroids would be transient, leading to false-negative results and withholding of rhGH treatment with possibly compromised adult height. However, short children (n = 50) who failed an unprimed stimulation test and had a normal GH response after sex steroid priming attained an adult height within their target ranges (mean height SDS, −1.27 ± 0.72) (240).

B. Diagnosis of GHD in overweight children

Among the factors affecting GH secretion and its response to stimuli, adiposity and increasing weight are emerging as significant confounding factors, especially in view of the prevalence of childhood overweight and obesity in developed countries (2%–30% depending on the definition and references used) (242) and the expected increase in their worldwide prevalence from an estimate of 6.7% in 2010 to 9% in 2020 (243).

There is a well-established negative correlation between visceral adiposity and body mass index (BMI) SDS with spontaneous GH secretion (244–246). Not only do obese children and adolescents have reduced endogenous GH secretion, but even in normal-weight individuals, there is a decrease in 24-hour GH secretion and pulse amplitude with increasing adiposity; this negative association seems to be significant for girls rather than boys and pubertal rather than prepubertal children (245–247). In this setting, the problem is to quantify the impact of BMI on the interpretation of GH stimulation tests for the biochemical diagnosis of GHD and to address whether there is a need for BMI-adjusted cutoff values depending on the GH provocation test. However, when interpreting the results of various studies, we should take into account that BMI may be only a surrogate of visceral abdominal adiposity, which in turn correlates with GH secretion (248), whereas data on body composition and LBM would be important in assessing the dynamics of GH secretion in response to stimulation.

In adults, there is evidence that even moderate changes in BMI have an impact on the GH response to stimulation testing. The percentage of normal male adults with a peak GH response less than 9 μg/L to GHRH-arginine stimulation increases from 5%, for those with BMI less than 25 kg/m^2, to 13% and 64% for those with a BMI between 25 and 26.9 kg/m^2 and greater than 30 kg/m^2, respectively, which would have led to a misdiagnosis of GHD in this subgroup (249). To determine BMI-dependent cutoff values for the diagnosis of adult GHD, Corneli et al (250) studied the peak GH response to GHRH-arginine stimulation in 322 patients with hypopituitarism and 318 controls and suggested a peak GH cutoff of 11.5 μg/L for subjects with BMI <25 kg/m^2 (98.7% sensitivity and 83.7% specificity), 8.0 μg/L for overweight patients (BMI 25–30 kg/m^2; 96.7% sensitivity and 75.5% specificity), and 4.2 μg/L for obese subjects (BMI >30 kg/m^2; 93.5% sensitivity and 78.3% specificity). These cutoffs for

GHRH-arginine stimulation testing have been endorsed in the 2007 consensus guidelines (251). The confounding effect of increasing BMI on the interpretation of GH response seems to be consistent, although not proportional, for all provocation tests (252), including those using ghrelin-type GH secretagogues. Recently, Gasco et al (253) studied the GH response to acetylated ghrelin (1 μg/kg iv, with sampling every 15 minutes for 120 minutes) in 78 adult patients who have been diagnosed with GHD after an insulin tolerance test (ITT) and/or GHRH-arginine provocation. The authors suggested a cutoff of 7.3 μg/L in subjects with normal weight (88.2% sensitivity, 90.9% specificity), 2.9 μg/L for overweight patients (sensitivity 92.6%, specificity 100%), and 0.6 μg/L in obese patients (sensitivity 50%, specificity 100%). The diagnostic accuracy of the test was low in obese patients (62.5%), but it was suggested that it could be used in the diagnosis of GHD in overweight (94.1%) and normal-weight patients (89.3%).

However, there is sparse information on the impact of adiposity and BMI on stimulated peak GH and the biochemical diagnosis of GHD in children and adolescents. Misra et al (248) compared the stimulated GH response to GHRH-arginine in 15 overweight adolescent girls (BMI >95th centile) and 30 normal-weight individuals matched for bone age and Tanner stage. Although the overweight adolescents had lower basal and stimulated peak GH concentration compared with controls (*ln* peak GH 1.55 ± 0.28 vs 1.28 ± 0.33 ng/mL, $P = .03$), the fasting IGF-1 concentration and overnight GH secretion were not different between the 2 groups and were not predicted by BMI, suggesting that in overweight adolescents, the maximal capacity to secrete GH may be decreased before the physiological secretory capacity is affected (248).

To address the question of the impact of BMI on the peak stimulated GH, Stanley et al (254) studied retrospectively the results of GH provocation testing in 116 children between 2 and 18 years of age, most of whom were prepubertal (76%), with an average height SDS of −2.4 ± 0.6 and a mean BMI SDS of −0.2 ± 0.9, who had been investigated for short stature. Testing was performed according to various protocols using 4 different secretagogues, without sex steroid priming. For this study, the authors included patients whom they assumed were less likely to have GHD (normal MRI, no other hormone deficiencies, and no organic acquired causes of GHD). Yet 50% of patients with BMI SDS >1 had a peak GH less than 7 μg/L, and 70% of them had a peak GH less than 10 μg/L. None of the lean subjects with BMI SDS <−1 had a peak GH of less than 7 μg/L, but 29% had a peak of less than 10 μg/L (254). Although one cannot be certain that, despite the authors' criteria, patients with true GHD were actually included in this cohort, the study provides evidence that increasing BMI, even within the normal distribution, has an impact on stimulated peak GH and may lead to misdiagnosis of GHD, especially if a higher GH cutoff value is used. However, a larger study cohort is required to assess the impact of BMI on a range of provocation tests. Subsequent studies have confirmed the negative correlation between BMI and peak GH to different provocation tests (255, 256) and estimate that BMI accounts for almost 20% of the variability in peak GH concentration (256). In contrast to these results, in a study that included 17 prepubertal children diagnosed with GHD and 9 young adults with childhood-onset GHD at the time of retesting, Maghnie et al (257) found no correlation between BMI and the peak GH after ghrelin administration ($r = 0.013$, $P = .95$). In this case, the lack of an observed correlation may be due to the small number of patients included, rather than the type of test, because a similar study in adults did not confirm this finding (253). However, one cannot exclude a potential differential effect between children and adults, and a larger study using the same secretagogue would be needed to confirm or refute this hypothesis. Rather surprisingly, Stanley et al (254) reported that the negative correlation between BMI SDS and the peak GH to GHRH-arginine was significant only in prepubertal children ($r = -0.35$, $P = .001$), although this may also be due to the relatively small number of pubertal patients included. Subsequently, in a study of 202 children (64% prepubertal) who underwent a clonidine stimulation test without sex steroid priming, Loche et al (256) reported that the negative correlation between BMI SDS and peak GH was significant for both prepubertal ($r = -0.32$, $P < .001$) and pubertal ($r = -0.28$, $P = .02$) patients.

Different factors contribute to the impaired GH secretion with increasing adiposity, such as an effect of elevated free fatty acids, hyperinsulinemia, and the increase in somatostatin tone (258). Therefore, it is tempting to hypothesize that protocols using arginine or other agents that inhibit somatostatin secretion may alleviate the confounding effect of increasing BMI. However, so far, there is no evidence for such an advantage and the question of how to approach the biochemical diagnosis of GHD in children and adolescents in the context of increasing BMI and visceral adiposity remains unresolved. In these cases, the concurrent measurement of IGF-1 concentration does not seem to help in firmly establishing the diagnosis because there is an inverse relationship between IGF-1 and BMI and total fat mass in adolescents (259). Larger studies are needed to confirm the observed effects and determine the sensitivity and specificity of BMI and provocation-test-dependent cutoff values (Summary Box 2).

C. Predictors for persistence of GHD and the role of pituitary imaging

The vast majority of patients diagnosed with GHD in childhood and with no structural pituitary abnormality on MRI will have adequate GH secretion when retested in late adolescence or adulthood (66%–85% depending on the test and cutoff used) (220–222, 224).

To define persistence of GHD, the Growth Hormone Research Society 2007 consensus (251) adopted a peak GH of less than 6 μg/L after an ITT, because it had a higher specificity (100%) and sensitivity (96%) in detecting permanent GHD in a cohort of 26 patients with childhood-onset GHD (260). To validate the accuracy of this cutoff, Secco et al (223) studied a larger cohort of 79 patients with childhood-onset GHD (peak GH <10 μg/L), of whom the majority (53 of 79) had IGHD, and who were classified as high or low likelihood for persistence according to established criteria (261). Among the patients with IGHD (n = 53), 45 were classified as low likelihood based on normal appearance of the pituitary (n = 26) or anterior pituitary hypoplasia (n = 19) on MRI, whereas 8 of the IGHD patients had structural pituitary abnormalities and hence were classified as high likelihood for persistence. All patients in the cohort had an ITT and measurement of 12-hour spontaneous GH secretion and IGF-1. For the whole group, a GH peak to ITT of 5.6 μg/L classified correctly 87.3% of patients (sensitivity 77.4%, specificity 93.8%), whereas for IGF-1, a cutoff of −2.83 SDS had the best diagnostic accuracy (89.7%) and a specificity of 95.7% (223). With regard to predictors for persisting GHD, severe childhood GHD at diagnosis (peak GH <3 μg/L) accurately predicted persistence in 79.8% of patients (sensitivity 87.1%, specificity 75%) (223), although it could be argued that in this study, the number of patients was relatively small to allow a firm conclusion.

In addition, the presence of structural abnormalities, such as an ectopic posterior pituitary, is predictive for the development of severe GHD, irrespective of the cutoff values used during the initial provocation tests (262). This is consistent with the result of our cohort where the risk of hypopituitarism was 27.2 times greater in patients with an undescended posterior pituitary (95% CI, 3.6–205.1; $P < .001$) (263). The higher location of an ectopic posterior pituitary is also a discriminator of persistence; in most patients with persistent severe GHD (GH peak <5 μg/L), the posterior pituitary is located at the median eminence (93%), whereas in almost 80% of subjects with a GH peak of at least 5 μg/L at reassessment, it is visible along the stalk (220).

The presence of an ectopic posterior pituitary does not always predict the persistence of GHD. Léger et al (264) reassessed 18 young adults with an ectopic posterior pituitary who were diagnosed with GHD in childhood (peak GH <10 μg/L, 50% had IGHD). However, despite the presence of structural pituitary abnormalities, almost 40% of the cohort had a peak GH >5 μg/L at retesting, whereas peak GH was more than 10 μg/L in 22% (4 of 18). There was a relatively small number of patients with IGHD and an ectopic posterior pituitary at diagnosis (n = 9), and only 1 remained GHD with a peak GH of 3.3 μg/L (11%), whereas 1 had already developed additional pituitary deficiencies at the time of reassessment (264). Consistent with this report, up to 50% of patients (4 of 8) with IGHD and an ectopic posterior pituitary may have discordant results with an adequate peak GH >5.6 μg/L (range 7.8–21.9 μg/L) but with a low IGF-1 concentration between −2.9 and −3.5 SDS (223). Despite the normal GH peak, this cohort of patients should be monitored and, if needed, reassessed for development of severe GHD, derangement of metabolic parameters, or even evolving pituitary hormone deficiencies (262, 220).

Box 2. Biochemical Diagnosis of GHD in childhood

The international standard IS 98/574 has been recommended by the recent international consensus statement on the standardization of GH assays.

These recommendations and the use of monoclonal assays make it imperative to review the accepted GH cutoff for the diagnosis of GHD in children.

In neonates, a single GH measurement of less than 7 μg/L measured by a highly sensitive human GH ELISA in dry blood spot samples would have high sensitivity and specificity for the diagnosis of GHD in this age group.

In peripubertal children, the biochemical diagnosis of GHD, with or without sex steroid priming, remains controversial.

An increasing BMI in children, even within the normal distribution, has an impact on stimulated peak GH; the type of secretagogue and strength of the stimulus are also to be taken into account.

There is a need for BMI and provocation-test-dependent cutoff values.

XI. Treatment of GHD in Childhood

A. Assessing and optimizing responsiveness to rhGH

Despite the long experience in the use of rhGH, there is uncertainty regarding the best dosing regimen, its calculation based either on weight or body surface area (BSA), and a satisfactory way to quantify and pre-

dict responsiveness to treatment. It is generally accepted that a daily dose of 0.025 to 0.035 mg/kg/d is sufficient to increase growth velocity to more than 10 cm/y in children with severe GHD and the adult height in treated patients ranges from −1.5 to −0.8 SDS depending on the study (265–268). Girls (269) and adolescents (270, 271) may require higher doses; however, there is no consistent evidence that an increase in the dose of rhGH peripubertally has a beneficial effect with respect to adult height (272–274).

Mathematical growth prediction models have been developed to identify and account for factors contributing to the variability in responsiveness to rhGH, with the aim to individualize and optimize treatment (275–277). However, they explain at best 40% to 60% of the observed variability and are limited by the lack of validation in long-term randomized trials and the fact that they do not take into account the genetic make-up of the individual (278). In this context, 2 large studies have addressed the issue of responsiveness to rhGH with individualization of rhGH dose based either on a target IGF-1 SDS (279) or growth prediction (280).

In a 2-year multicenter randomized controlled trial, Cohen et al (279) studied the treatment outcome in 172 short prepubertal children (height SDS <-2) with low IGF-1 (mean IGF-I SDS of −3.56); however, almost half had a peak GH concentration more than 10 μg/L and therefore might not be classified as GHD. Children were allocated to have frequent adjustments of the dose of rhGH to achieve an IGF-I near the mean (IGF-1 SDS of −0.5 to +0.5; IGF-low group, n = 70), or in the upper normal range (IGF1 SDS of 1.5 to +2.5; IGF-high group, n = 68), compared with a conventional standard rhGH dose of 0.04 mg/kg/d (n = 34). In this respect, the dose of rhGH was changed by 20% for each SD unit between the measured and target IGF-I. This approach resulted in a significantly higher mean daily rhGH dose of 0.1 mg/kg/d (range 0.02–0.346 mg/kg/d) in the IGF-high group compared with the IGF-low group of 0.033 mg/kg/d. Interestingly, patients in the IGF-low group showed remarkable variation in the dose of rhGH required, which ranged from 0.009 to 0.114 mg/kg/d; almost half of patients in this group had less than 0.025 mg/kg/d, whereas a fifth required more than double the dose to achieve the same IGF-1 target, further demonstrating the variability in the responsiveness. During the study period, the change in height SDS was 1.58 in the IGF-high group, 1.08 in the IGF-low group, and 1.0 in the weight-based group, with a direct and positive correlation between the change in height SDS and the change in IGF-1 SDS ($r = 0.43$, $P < .001$) as well as the cumulative rhGH dose ($r = 0.43$, $P < .001$). Although dose titration to achieve higher IGF-I targets resulted in a higher growth response in the 2-year term and usually at higher rhGH doses, it is not possible to conclude that this dosing strategy will result in improved adult height outcome. In addition, although during the treatment period the incidence and severity of adverse events were comparable in the 3 groups, there is no information on the long-term safety of this approach.

In the second titration study, Kriström et al (280) conducted a 2-year multicenter trial of 153 short prepubertal children with GHD (n = 110) and idiopathic short stature (n = 43) who were randomized to receive either a standard (0.043 mg/kg/d) or an individualized (0.017–0.10 mg/kg/d) dose of rhGH within a set dose range according to a prediction model that estimated individual GH responsiveness (275). In the individualized group, the range of the difference from mid-parental height SDS was reduced by 30% compared with the standard dosing group, whereas the mean mid-parental height SDS was equal (−0.42 ± 0.46 vs −0.48 ± 0.67, respectively). The mean individualized dose for GHD was 40 μg/kg/d, and their mean gain in height SDS was comparable to children with idiopathic short stature (1.31 ± 0.47 vs 1.36 ± 0.47, respectively). Although in the individualized treatment group there were 9 children with an IGF-1 concentration more than 3 SDS, the mean IGF-I concentration was not significantly different between the 2 randomization groups (1.58 ± 1.29 vs 1.53 ± 1.11 SDS), and there were no short-term dose-related adverse effects. It is not clear whether different diagnostic criteria and inclusion of more patients with severe IGHD would have altered these short-term results, and there is no indication on longer-term outcomes, with respect to complications, attainment of adult height or the overall cost-effectiveness of treatment.

Another way for early identification and management of poor responsiveness would be to assess individual responses against age-, sex-, and condition-specific first-year height velocity targets (281), but there is still no large study to validate this approach in children with GHD. Despite the limitations of the various approaches, it has been suggested that growth prediction may be used as a guide for decisions about treatment in patients with severe GHD (peak GH <5 μg/L) who have a poor response after the first year of treatment (difference in height SDS <0.4) (282, 283). In addition, an increasing number of recent studies have investigated the use of proteomic (284–289) or genomic (290–292) biomarkers as surrogate indicators to assess responsiveness to rhGH; however, there are, to date, no robust data supporting their use in monitoring treatment in children and adolescents with GHD.

Apart from all of the above, poor adherence to treatment is perhaps the major factor that limits the optimal response to rhGH and impairs adult height (293). A review

of recent studies revealed that anything from 5% up to 82% of pediatric patients miss at least some rhGH doses, depending on the method used for assessing compliance (294), and this seems to be unrelated to sex, age, or underlying diagnosis (293).

B. Pharmacogenomics in children with GHD

The etiology of the well-observed variability in the responsiveness to rhGH even in children with severe or congenital GHD is largely unknown, but it may, at least in part, be explained by genetic factors related to the interaction of human GH with its receptor (GHR), components of the postreceptor signaling pathway (Jak2/STAT generation and availability of IGF-1), and the epiphyseal responsiveness to GH and IGF-1 (295).

A common polymorphism in *GHR* leading to deletion of exon 3 (GHRdel3) was the first to be associated with a better first-year response in children with idiopathic short stature or those born small for gestational age (SGA) and who had at least 1 GHRdel3 allele compared with those who were homozygous for the full-length allele (296). The allele frequency varies in different ethnic groups, with 25% to 34% of GHR alleles in Caucasians being GHRdel3 (296–298), and results of subsequent studies on the influence of the GHRdel3 polymorphism have been contradictory. This was probably due to the heterogeneous nature of the study groups including various ethnic backgrounds and including patients with diverse disorders such as IGHD or multiple pituitary hormone deficiencies or even non-GHD children. The patients were also treated with variable doses of rhGH, and the cohorts included relatively small individual numbers, which made the achievement of statistical power challenging (299–301).

When it comes to treatment of children with GHD, either isolated or in combination with other pituitary hormone deficiencies, Jorge et al (299) reported a significantly greater first-year growth velocity (12.3 ± 2.6 vs 10.6 ± 2.3 cm/y) and greater adult height SDS (−0.8 ± 1.1 vs −1.7 ± 1.2) in patients who carry at least 1 GHRdel3 allele compared with those who are homozygous for the full-length allele. Results of subsequent studies were contradictory, showing that in children with IGHD, the response to treatment is independent of the GHRdel3 polymorphism, either alone (297, 300, 302–304) (Table 6) or in combination with other *GHR* polymorphisms (c.504 A→G, c.1576A→C) (300). In the short term, the GHRdel3 genotype is associated with an up to 0.5cm/y higher growth rate in the first year of treatment in children with short stature due to either GHD or other causes (305). With regard to longer-term effects, patients with severe IGHD (stimulated peak GH ≤2 μg/L), who have at least 1 copy of the GHRdel3 allele, may have a better response to rhGH over the first 2 years of treatment but without an impact on adult height (306). There are now data to support a possible role for another *GHR* polymorphism (c.1319G→T), which may have an independent effect on the first-year response to treatment, with the homozygous (TT) genotype resulting in higher STAT-5 phosphorylation in vitro, but in this case as well, there is no long-term follow-up (302).

When looking at other components of the GH/IGF axis in prepubertal children with severe GHD, the −202 A/C polymorphism in the IGFBP3 promoter has also been associated with an increased IGFBP3 concentration and a greater height velocity in the first year of treatment in patients homozygous for the AA (13.0 ± 2.1 cm/yr) compared with those with the AC (11.4 ± 2.5 cm/yr) or CC (10.8 ± 1.9 cm/yr) genotypes (307). Despite this early effect, the −202A/C IGFBP3 polymorphism does not seem

Table 6. Effect of the del3-GHR Polymorphism in GHD Children Treated With rhGH

Study	n	Diagnosis	Mean rhGH (mg/kg/d)	GHR Genotype, %			Difference in the Effect of *del3-GHR* vs *fl-GHR*: First-Year GV and Final Height SDS
				fl/fl	*fl/d3*	*d3/d3*	
Jorget et al (299)	58	IGHD and MPHD	0.033	47	41	12	Significant difference in first-year GV (+1.7 cm/y) and FH SDS (+0.9 SDS)
Blum et al (297)	107	IGHD	0.028	55	42	3	No difference in GV, FH not studied
Pilotta et al (300)	54	IGHD	0.033	52		48	No difference in GV, FH not studied
Wan et al (302)	154	IGHD	0.028	51	36	13	No difference in GV, FH not studied
Marchisotti et al (303)	28	IGHD and MPHD	0.033 or 0.05	50	39	11	No difference in GV, FH not studied
De Graaff et al (304)	144	IGHD and MPHD	NA	43	47	9	No difference in GV, FH not studied
Raz et al (306)	181	IGHD	0.0315 or 0.027	50	39	11	Significant difference in GV for first and second year; no difference in FH
Carrascosa et al (301)	65	IGHD	0.0317	47	41	12	No difference in GV, FH not studied

Abbreviations: d3, exon 3-deleted allele; FH, final height; fl, full-length GHR; GV, growth velocity; MPHD, multiple pituitary hormone deficiencies; NA, not available.

to have an impact on adult height (307, 308). More recently, Costalonga et al (309) reported that in patients with GHD, either isolated or combined with other pituitary hormone deficiencies, almost 4% of the variability in growth velocity during the first year of treatment can be explained by the variable length of polymorphic cytosine-adenosine repeats $(CA)_{10-24}$ in the IGF-1 promoter, with a better response in patients who have at least 1 non-19CA allele. The effect of the *IGF1*, *IGFBP3*, and *GHR* genotypes seems to be synergistic, at least in the first years, because patients with a combination of 2 favorable genotypes have on average a higher growth velocity by 3.2 cm/y compared with patients with unfavorable combined genotypes. In the long term, patients homozygous for the 19CA allele of the IGF-1 promoter attained a lower adult height SDS compared with those with a non-19CA genotype (-0.6 ± 1.2 vs 0.4 ± 1.1; 95% CI, 0.15–1.9), whereas those with a combination of 2 favorable genotypes had an adult height of 1.5SD greater than those with unfavorable combined genotypes (309). However, this effect of the $(CA)_n$ IGF-1 polymorphism on adult height was not observed in another study including a higher number of patients diagnosed with severe IGHD (308). Polymorphisms in other genes, including the leptin (310) or vitamin D (311) receptors, do not have an impact on responsiveness to treatment in terms of growth velocity or adult height.

In the era of genome-wide associations and next-generation sequencing, there is still a distinct lack of studies with enough statistical power to demonstrate which, if any, combination of genotypes may explain the variability in the response to rhGH treatment and affect its impact on adult height in patients with GHD of various etiologies. This approach might help to establish pharmacogenomic markers and challenge the traditional dosing regimens for a more personalized treatment.

XII. Consequences of GHD in Children Beyond Height and the Effect of Treatment

A. Effect of childhood GHD on brain and cognition

Components of the GH–IGF-1 axis make an important contribution to the development, function, regeneration, and neuroprotection of areas of the CNS, with established roles in neurogenesis (312, 313); axonal elongation and formation of oligodendrocytes, astrocytes, and glial cells (314); angiogenesis, synaptogenesis, and synaptic transmission (315, 316); and prevention of neuronal apoptosis and complex interaction with neurotrophic factors (312, 317). In the CNS, expression of IGF receptors is detected from an early embryonic stage through to adulthood, although with different patterns and intensity, in specific cell types (neuronal stem cells, neurons, and glial cells) and regions (choroid plexus, hippocampus, amygdala, meninges, and vascular sheaths) (317). Conversely, expression of the GHR is detected in the hypothalamus and choroid plexus (318, 319), associated with a possible role in the autoregulation of GH secretion and the receptor-mediated transport of GH via the blood-brain barrier (320), respectively, as well as in the hippocampus and frontal cortex (321, 322), areas involved in memory and cognition. Consistent with these observations, GHD adults with either childhood (323–325) or adult-onset (326–329) GHD show not only impairment in psychological well-being but also defects in attention, memory, and executive function, with evidence of improvement in cognitive function after GH replacement (326, 328, 330, 331).

The impact of GHD and its treatment on cognitive function and behavior would be predicted to be of major importance during childhood and adolescence, yet, until recently, it has been poorly investigated. In this section, we will first summarize results from recent studies on experimental animal models of GHD before focusing on childhood GHD.

1. Animal models of GHD and the role of GH in neuronal function

Early studies of animal models with congenital GHD reported evidence of hypomyelination and poor neuronal growth and synaptogenesis in both the Snell (*Pou1f1* deficient) (332) and *little (Ghrhr*-deficient) mice (333), which were restored with postnatal GH administration. However, subsequent studies of *little* mice did not confirm these findings (334). Surprisingly, the Ames (*Prop1*-deficient) dwarf mice exhibit an increase in the number of hippocampal neurons (335), suggesting that a compensatory upregulated production of local IGF in the hippocampal area may exert an effect independent from the hypothalamo-pituitary axis.

Despite these contrasting results regarding brain structure, experimental animal models with GHD exhibit deficits in cognitive behavior and memory. The spontaneous dwarf rats (SDR), a naturally occurring animal model of somatotrope hypoplasia and GHD due to a point mutation in *Gh* leading to abnormal splicing (336), have deficits in spatial learning and short- and long-term memory compared with normal animals and exhibit reduction of cholinergic neurons in the basal forebrain and an imbalance in the glutamatergic and GABAergic synapses in the hippocampus (337). However, dwarf rats treated with GH between postnatal weeks 4 and 14, therefore being GH-replete in puberty but deficient as adults, had a spatial learning performance comparable to heterozygous ani-

mals, whereas supplementation for longer than 14 weeks did not confer an additional benefit (338). This suggests that, in this animal model, GH supplementation during the transition period can reverse the adverse effects of GHD on memory and learning. On the other hand, animals that were hypophysectomized (339) or subjected to hypoxic stress (340) also exhibit defects in spatial memory that improve if GH is administered at an early stage after injury.

Although it is possible that these effects are the result of the indirect action of GH on the production of IGF, the relationship between circulating and brain IGF-1 remains unclear. Recently, Yan et al (341) demonstrated that adolescent GHD dwarf rats (*dw/dw*) have a low hippocampal IGF-1 concentration that is restored after exogenous GH replacement and increase of circulating IGF-1, although the concentration of IGF-1 in the cerebrospinal fluid remains low. In this case, microarray and RT-PCR analysis of gene expression in the hippocampus of GH-treated dwarf rats showed differential expression of genes that may be involved in microvascular structure and synaptic function, such as *Alox15* (arachidonate-15-lipoxygenase), *Camkk2* (calcium/calmodulin-dependent protein kinase kinase 2), and Hbα- and β-globin genes, but not *Igf1* or its receptor (*Igf1R*) (341). On the other hand, there is evidence that GH may have a direct action in the brain via its interaction with the GHR (342). Aberg et al (342) demonstrated that the addition of GH in cultures of adult hippocampal progenitor cells in medium with low insulin and no FGF2 resulted in increased proliferation indicated by the incorporation of [^{3}H]thymidine, thus suggesting a direct effect of GH on these cells. In the same study, in vivo treatment of hypophysectomized animals with GH for 6 days resulted in a 2-fold increase in bromodeoxyuridine-positive cells in the dentate gyrus of the hippocampus and almost all regions of the brain apart from the corpus callosum and the subventricular zone, the latter exhibiting increased neurogenesis only with prolonged treatment. Apart from an effect on neurogenesis, other mechanisms may include local regulation of expression and/or function of molecules involved in memory and learning, such as the GABAB (343) and *N*-methy-D-aspartate receptors (315, 339, 344, 345).

In fact, Grönbladh et al (346) recently demonstrated that male rats injected with rhGH twice daily for 7 days show a significant dose-dependent upregulation of the density and functionality of the GABAB receptors in certain areas of the brain important for cognitive function, the primary motor cortex and caudate putamen, further supporting the hypothesis that at least some of the actions of rhGH are mediated by the GABAB receptor system. In addition, mice with streptozotocin-induced diabetes have an altered expression of GHRs in the prefrontal cortex and exhibit specific defects in memory and learning processes (322). Treatment with rhGH for 10 days can reverse the cognitive impairment in these animals (348), suggesting a role of rhGH treatment in diseases associated with reduced cognitive performance.

2. Impact of childhood GHD on cognition and brain structure

Although studies report a beneficial effect on psychosocial and cognitive outcome in children treated with rhGH for indications including Prader-Willi syndrome (349, 350), SGA (351, 352), or cancer survivors (353), there is a small number of studies on the cognitive impact of IGHD in children and the effect of its treatment.

Early studies of children with idiopathic IGHD confirmed that IGHD children have an intelligence quotient (IQ) comparable to short-stature controls (354), although this crude measurement could not detect subtle alterations in different aspects of performance and behavior. Subsequently, GHD children with low peak GH to stimulation or with a disturbance in nocturnal GH secretion were shown to have reduced performance as evaluated in a visual motor psychological test (355). Recently, a collaborative Spanish study (356) reported on the baseline cognitive assessment of 79 children with GHD (peak GH <10 μg/L in 2 stimulation tests), with a mean age of 10.2 ± 2.9 (range 3–15) years. It appears that the majority had idiopathic IGHD, although not clearly stated, and children were reassessed at 1 and 3 years after treatment. The overall IQ score at baseline was within the normal range, with higher verbal than performance IQ (102.7 vs. 96.3). Although this study did not have a control group of age- and sex-matched short children for comparison, IQ scores as well as measures of adjustment capacity and social relationships were within the normal range. However, the authors reported that GHD children who were older than 7 years at first assessment (n = 68) had a mean performance IQ score of -0.2 ± 1.2 SDS and mean overall IQ score of -0.1 ± 1.2 SDS, which were considered as slightly below average.

Recently, we studied in detail the cognitive function of 15 prepubertal children with IGHD (peak GH < 6.7 μg/L) and compared them with 14 children with idiopathic short stature (peak GH >10 μg/L) before starting rhGH treatment (357). This consisted of detailed cognitive (Wechsler Intelligence Scales for Children IV, Full-Scale IQ [FSIQ], Verbal Comprehension Index, Perceptual Reasoning Index, and Working Memory and Processing Speed Indices) and motor assessment (Movement-Assessment Battery for Children, ABC test) as well as detailed MRI. When compared with controls, children with IGHD had significantly

lower FSIQ ($P < .02$) and Verbal Comprehension ($P < .006$) and Processing Speed ($P < .05$) index scores, although the mean FSIQ and Verbal Comprehension Index for both groups were within the normal average Wechsler Intelligence Scales for Children IV range. In IGHD children, the Verbal Comprehension Index scores correlated significantly with both IGF-1 SDS ($r = 0.7$, $P < .03$) and IGFBP3 SDS ($r = 0.7$, $P < .02$), with the concentrations of IGF-1 and IGFBP3 explaining approximately 49% of the variance in Verbal Comprehension Index. On the other hand, the FSIQ correlated significantly only with IGFBP3 SDS ($r = 0.6$, $P < .03$). With regard to fine motor skills, children with IGHD had significantly lower scores for manual dexterity ($P < .03$), balance ($P < .008$), and total scores ($P < .008$) of the Movement ABC2 test, suggesting that GHD has an impact on some aspects of development (357).

To examine in detail the structure of the white matter in these children, we used diffusion tensor MRI, an advanced MRI technique that provides quantitative indices of the white matter microstructure (mean diffusivity and fractional anisotropy) that are affected by the axonal diameter, fiber density, and degree of myelination. Low fractional anisotropy (high mean diffusivity) reflects lower structural integrity of the white matter (358). Using volumetric imaging, we showed that although children with IGHD do not have a global reduction in brain volume compared with controls, specific structures are affected, with significantly lower volumes of the splenium of the corpus callosum ($P < .02$), right pallidum ($P < .007$), right hippocampus ($P < .01$), and left thalamus ($P < .01$), suggesting that they may be more vulnerable to variations in the GH–IGF-1 axis. The volume of the right hippocampus in IGHD children correlated significantly with the IGFBP3 SDS ($P < 0.04$, $r = 0.63$), whereas the other neural volumes did not correlate with either the IGF-1 or IGFBP-3 SDS (357). IGHD children also exhibited defects in white matter microstructure in specific areas, with significantly lower fractional anisotropy, thus lower structural integrity, in the corpus callosum ($P < 0.05$) and bilateral corticospinal tracts (right $P < 0.05$, left $P < 0.045$). In this relatively small study population of IGHD children, there was no significant correlation between IGF-1 and IGFBP3 SDS and either fractional anisotropy or mean diffusivity. However, there was a correlation between reduction in cognitive and motor skills scores and the structural white matter abnormalities (fractional anisotropy) of the corticospinal tract (processing speed index and components of the Movement-Assessment Battery for Children) and corpus callosum (processing speed index and FSIQ) (357) (Figure 5).

Therefore, there is now evidence to support the hypothesis that abnormalities in the GH–IGF-1 axis in children affect structural brain development including the volume of brain substructures and the development of the corticospinal tract, leading to reduced fractional anisotropy in GHD children; these structural and white matter tract changes lead to specific impairments in cognition and motor skill function. Early intervention studies are now required to determine whether any of these defects can be reversed by rhGH treatment.

3. Effect of rhGH treatment on cognitive function in children

In one of the earliest reports, Laron et al (359) suggested that children with IGHD had improved intellectual development if treated before the age of 5 years. Subsequently, Stabler et al (354) investigated the impact of 3 years treatment with rhGH in 72 children with IGHD and 59 with idiopathic short stature and reported an improvement in child behavior checklist scores for total behaviors. This was more pronounced for those with IGHD rather than idiopathic short stature, with improvement in attention, anxiety, social competence, and thought problems (354). In the more recent Spanish collaborative study (356), some of the patients of the initial cohort were followed up after 1 and 3 years of rhGH treatment, although the number of children who had cognitive assessment at follow-up were significantly lower, especially in the younger age groups. The authors reported an improvement in mean performance IQ score from the first year of treatment and increasing in subsequent years (from -0.2 ± 1.2 SDS to 0.5 ± 1.2 SDS). After 3 years of treatment, the verbal IQ increased by 0.3 SDS, performance IQ improved by 0.7 SDS, and the FSIQ improved by 0.6 SDS (356). However, in this study, there was no control group for comparison, the SDS scores were still within the normal range, and there was no analysis to indicate whether the observed differences before and after treatment were statistically significant.

Interestingly Arwert et al (360) assessed 13 adults with confirmed childhood-onset GHD on retesting, after at least 3 months of stopping rhGH. Neuropsychological testing and event-related functional MRI scanning demonstrated that patients had subnormal memory speed compared with controls, without impairment of the quality of memory performance. Concomitant functional MRI in these subjects showed an increased activity in the areas of the prefrontal cortex, parietal cortex, and thalamus, suggesting a compensatory recruitment of dorsal prefrontal brain regions (360). In a subsequent study by the same group, patients were randomized to either rhGH treatment or placebo for 6 months with repeat neuropsychological assessment and functional MRI (330). In this case, rhGH treatment resulted in significant improvement in

Figure 5.

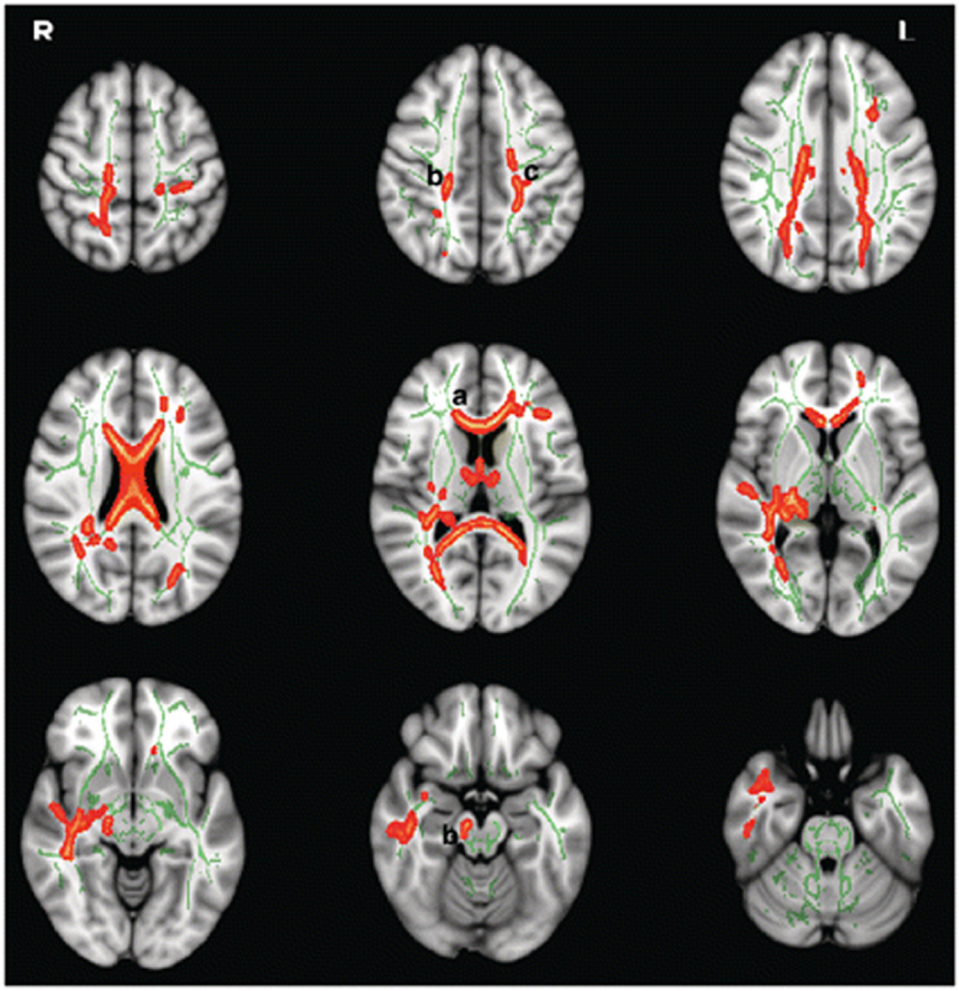

Figure 5. Functional MRI and tract-based spatial statistics analysis comparing fractional anisotropy in children with IGHD and short-stature controls. Areas of the mean fractional anisotropy skeleton in green represent regions where there were no significant differences in fractional anisotropy values; areas in red/yellow are regions where the fractional anisotropy was significantly lower in the IGHD group, as observed in the corpus callosum (a) and right and left corticospinal tract (b and c, respectively). [Reproduced from E. Webb et al.: Effect of growth hormone deficiency on brain structure, motor function and cognition. *Brain*. 135:216, 2012 (357), with permission. © Oxford University Press.]

long-term memory and an increased speed of working memory performance with activation in the parietal and prefrontal areas.

In view of the recently demonstrated effects of GHD on both brain structure and cognition, well-designed studies of both cognitive function and advanced MRI are needed to quantify the effects of rhGH treatment and the possible implications for the cognitive development of GHD children (Summary Box 3).

B. Cardiovascular parameters

It is established that adults with untreated GHD have an adverse metabolic profile and increase in atherogenic factors, with reduced insulin sensitivity, increased proinflammatory markers, unfavourable lipid profile, and changes in coagulation with increase in fibrinogen and active plasminogen activator inhibitor type 1 (aPAI-1), all contributing to impairment of endothelial and cardiovascular function (361–363). The impairment of cardiac performance in GHD adults manifests as reduction of the left ventricular (LV) mass, decrease in contractility, or abnormalities in LV diastolic function (364, 365). Conversely, in adolescents with GHD, the discontinuation of treatment at completion of linear growth may be associated with adverse effects on body composition, lipid profile, and cardiac morphology (366–369), which is now reflected in consensus guidelines on recommendation for rhGH treatment in transition (251, 261).

However, in children with IGHD, there are still no robust data on the pathophysiology and natural history of

the effects of GHD on cardiac morphology, function, and atherogenic parameters, the effect of treatment, and the consequence, if any, for long-term cardiovascular health. Recent evidence for the direct role of GH on the pediatric heart comes from biopsies performed in children with severe congenital heart disease before transplant, showing expression of mRNA for receptors of GH and IGF-1 (370). With respect to the effect of GHD on cardiac morphology and function in children, studies are hampered by several factors, such as the small number of patients in individual series, difficulty in finding an appropriate control group (children with idiopathic short stature or BMI- and BSA-matched controls), the variety and limitations of the methods used for cardiac assessment, and the short duration of follow-up during treatment up to 2 years (371–373).

Most studies agree that prepubertal children with GHD have a lower LV mass index adjusted for either BSA (grams per square meter) or height compared with matched controls (371, 374, 375). In a study of 30 prepubertal children with GHD, 27 of whom had IGHD, Salerno et al (374) reported a lower LV mass index compared with controls (50.2 ± 1.7 vs 60.3 ± 2.5 g/m^2) with more evident reduction observed in those with severe rather than partial GHD (46.3 ± 1.6 vs 53.0 ± 2.2 g/m^2), whereas the cardiac function of GHD children was normal and comparable to controls. The effect of rhGH treatment (30 μg/kg/d) on LV mass was mainly seen within the first year when the LV mass index increased significantly (66.3 ± 2.4 g/m^2) and remained stable in the second year with no development of hypertrophy or impairment of cardiac function (374). However, results on the effect of GHD on cardiac morphology are not consistent; in a recent multicenter study comprising 89 prepubertal children with IGHD and 38 children with idiopathic short stature, there was no significant difference in LV mass index or other cardiac parameters at baseline (376). In the same study, treatment with rhGH either with a fixed (43 μg/kg/d) or an individualized (17–100 μg/kg/d) dose based on GH responsiveness resulted in a significant increase in the LV mass index with an increase in ventricular wall thickness and diameter that were evident early within the first 3 months. After 2 years of treatment, myocardial thickness returned to baseline values, but the LV diameter SDS remained increased, without change in cardiac function (376). Although in this study there was no correlation between the dose of rhGH and the LV mass SDS ($r = 0.09$, $P > .429$) in children with GHD, these effects need to be examined in longer-term studies including a larger number of patients, especially if we want to conclude on the safety of the higher rhGH dose.

Apart from the morphological changes, it has been suggested that prepubertal children with GHD may have a subtle, subclinical impairment of the contractility of the left ventricle. Capalbo et al (375) studied 24 children with GHD, of whom 21 had IGHD and 11 were classified as severe GHD (peak GH <5 μg/L); results were compared with 24 healthy controls who had been investigated for short stature and had been matched for height and BSA. In addition to the lower LV mass adjusted for BSA, the GHD children had significantly lower LV end-systolic diameter and end-diastolic volume, but with normal fractional shortening and no apparent impairment in the overall systolic function. However, further assessment of the LV function showed a higher LV end-systolic wall stress (49.2 ± 1.4 vs 45.7 ± 1 g/cm^2, $P < .05$) and an impaired stress-shortening index (0.10 ± 0.02 vs 0.18 ± 0.02, $P < .007$), indicating that although the fraction of shortening was within the normal range, it was deemed insufficient for the afterload. After 1 year of rhGH (30 μg/kg/d), there was significant reduction in the afterload and end-systolic wall stress (43.9 ± 1.4 g/cm^2, $P < .03$) and improved contractility of the LV with change in stress-shortening index (0.19 ± 0.02, $P < .003$), becoming comparable to controls (375). Follow-up during the second year of treatment demonstrated no further changes in cardiac function. Interest-

Box 3. Effect of GHD on brain structure and cognition

Experimental animal models with GHD exhibit defects in memory and cognitive behavior.

The effect of GH may be direct, affecting neurogenesis, microvascular structure, and synaptic function or the expression of molecules involved in memory and learning such as the GABAB and *N*-methy-D-aspartate receptors.

Children with IGHD have lower FSIQ, verbal comprehension index, and processing speed index score compared with controls as well as lower scores for manual dexterity.

Functional MRI and volumetric imaging show that children with IGHD do not have a total reduction in brain volume; however, specific structures such as the splenium of the corpus callosum and the right hippocampus are affected.

These structural brain abnormalities lead to specific impairment in cognition.

In children, GHD affects the development of the corticospinal tract and the white matter tract changes are associated with defects in fine motor skill function.

The effect of rhGH treatment in reversing these changes is yet to be identified.

ingly, there seems to be a relationshipe between the severity of GHD and the impairment of systolic function, because before treatment, the subgroup of children with peak GH <5 μg/L had a more pronounced difference in stress-shortening index (0.08 ± 0.2 vs 0.18 ± 0.03, $P <$ .003) and end-systolic wall stress (50.8 ± 2.1 vs 45.7 ± 1 g/cm^2) compared with controls; these results were not consistent for those defined as partial GHD (peak GH 5–10 μg/L). In this age group, neither GHD nor its treatment with a standard dose of rhGH had an effect on the diastolic function (375). These clinical findings may be supported by experimental data on the cardiac function of the dwarf rat (*dw*/*dw*), a naturally occurring animal model of somatotrope hypoplasia and GHD (377). Dwarf animals with early-onset GHD exhibit reduction in the contractility of the LV and impairment of diastolic function that is correlated with a significant (25%) decrease in the expression of SERCA2, a Ca^{+2} ATPase of the sarcoplasmic reticulum that is an important regulator of calcium uptake in myocytes (378). In this model, early GH substitution, beginning at 4 weeks of age and continuing through the experimental period of 26 weeks, increased and sustained the cardiac concentration of SERCA2 and preserved cardiac morphology and function.

To have an overview of the possible cardiovascular implications of GHD in childhood, factors affecting the endothelial function and the metabolic and atherogenic profile should also be taken into account. It is well established that treatment of GHD is associated with a decrease in fat mass and an increase in LBM, and most (369, 379–381) but not all (382) studies suggest that continuation of treatment can help to achieve normal adult body composition and maintain an improved lipid profile.

In contrast to adults and adolescents with GHD, prepubertal children seem to have no significant difference in the fasting blood glucose, insulin concentration, homeostasis model assessment index and lipid profile compared with age-, sex-, BMI-, and BSA-matched controls, as was shown in a study of 30 prepubertal children, 7 of whom had IGHD (374). Treatment with rhGH resulted in a decrease in total cholesterol, atherogenic index, and the ratio of total cholesterol to high-density lipoprotein (HDL) (3.5 ± 0.2 vs 2.5 ± 0.1) compared with pretreatment; these indices correlated with the increase in serum IGF-1 (374). However, it is uncertain whether this translates into improvement in later cardiovascular health. A previous study looking at the lipid profile, free fatty acids, and atherogenic index of 59 children and adolescents with GHD (mean age 8.3, range 0.4–16.9, years) showed that although for the whole cohort the mean concentrations of these factors were within the normal range, almost a third of patients had an atherogenic index greater than 4.5 and 20% had free fatty acids above the reference range, whereas low-density lipoprotein (LDL) and triglycerides were above the reference range in 14% and 4%, respectively (383). During 3 years of rhGH treatment (0.02 mg/kg/d), there was a significant decrease in the mean atherogenic index, with 11% of patients being above the reference range, as well as a decrease in free fatty acids and LDL and an increase in HDL (383). These subtle changes in lipid metabolism were also reflected in studies in which, although the total and LDL cholesterol of untreated children with idiopathic IGHD are within the normal range for age, they were significantly higher compared with age-, sex-, and BMI-matched controls ($P < .001$) with improvement after treatment for 1 year (rhGH dose 25–35 μg/kg/d).

In children with GHD, there are now a number of studies on additional factors known to be associated with cardiovascular risk. However, in these cases as well, there may be a lack of an appropriate control group for comparison at baseline, and the reported results of treatment are relatively short-term with unknown implications on the long-term cardiovascular health. Among the factors studied are proinflammatory cytokines (IL-4, IL-6, IL-12, and TNF-α) (384–387), high sensitivity C-reactive protein (288, 388), plasma homocysteine (389), adipokine profile (388, 390), and markers of defects in coagulation (391) and endothelial function (288, 392, 393). Despite the shortcomings in most studies, the findings from GHD children at baseline do not seem to be significantly different from controls, in contrast to observations from untreated adults or adolescents. However, some studies detect subtle changes, the significance of which is difficult to establish. For instance, Cañete et al (391) recently reported that prepubertal children with GHD have an increased concentration of fibrinogen and of aPAI-1 compared with controls, although their results are still within the reported normal range; in the short term, 6 months of rhGH treatment results in comparable aPAI-1concentrations, without a change in the concentration of fibrinogen, but it is not known whether this effect is modified in the long term (391). Similar results were reported for plasma homocysteine, increased concentrations of which are an independent risk factor for cardiovascular disease, atherosclerosis, and stroke. Before treatment, GHD children have a significantly higher serum homocysteine concentrations compared with age-, sex-, and BMI-matched controls (8.4 ± 2.9 vs 6.9. ± 2.9 μmol/L, $P < .03$), although these results are still within the normal range for age; within a year of treatment, plasma homocysteine concentrations decreased to levels comparable to controls at baseline (389).

Despite the absence of overt abnormal findings at baseline, it is now suggested that children with GHD, even at

a young age, have subtle changes that may adversely affect their metabolic and atherogenic profile. The extent to which these may place them at a higher risk for cardiovascular disease in later life and the long-term effects of treatment in reversing this remain to be established.

C. Effect on bone architecture and body composition

GHD in childhood is quoted as a cause of low bone mass with an increased incidence of fractures (394). A number of studies confirm that prepubertal children with GHD have an altered bone geometry and low volumetric bone mineral density (BMD) in the lumbar spine (383, 395–397) or femoral neck (395). However, it is difficult to establish whether the reported effects of GHD and its treatment on bone architecture, bone mineral content (BMC), or BMD are primary or secondary to the low muscle mass in untreated GHD (398, 399). Treatment with rhGH increases muscle mass (395, 396, 399), and this in turn induces bone adaptation and changes in cortical bone geometry.

Although childhood-onset GHD has been associated with lower areal BMD (396, 397, 400), this effect is partly caused by decreased height. In fact, adult male patients with untreated severe congenital GHD due to a homozygous *GHRHR* mutation have a low BMD z-score in the lumbar spine (−3.3) and femoral neck (−2.1); however, the calculated volumetric BMD corrected for bone size (bone mineral apparent density, BMAD) is near normal with reported BMAD z-scores of −1.2 and +0.8, respectively (400). In addition, van der Sluis et al (383) reported that, at the time of diagnosis, children with GHD of various etiologies have a reduction in mean LBM SD score (−2.62), lumbar spine BMD (−1.49), and total body BMD (−0.91) and to a lesser degree in lumbar spine BMAD (−0.35). In their cohort of 59 children with GHD, most whom had organic GHD, 36% of children had a lumbar spine BMD lower than −2 SDS, but only 7% had a lumbar spine BMAD less than −2 SDS. Subsequently, it was confirmed that, once appropriate corrections for body size are made, GHD does not seem to affect significantly the bone density. In a study that included 20 prepubertal GHD children and children with idiopathic short stature, Högler et al (395) reported that although GHD children had lower volumetric BMD at the lumbar spine and femoral neck, the size-corrected regional or total body bone data were within the normal range and were not affected by 2 years of treatment with rhGH.

Irrespective of the etiology of GHD, these reports highlight the importance of size correction for dual-energy x-ray absorptiometry measurements in children, which is now obligatory according to the guideline issued by the International Society of Clinical Densitometry (401). Because there is still no consensus on the optimal approach for this correction, the different methods used should also be taken into account when critically reviewing results on the impact of childhood GHD on bone architecture. In addition, because bone density is just one of the several variables that affect the structural bone strength, there is need for a functional approach to bone densitometry that would analyze the muscle-bone unit rather than its individual components (402). Once these factors are taken into consideration, it becomes apparent why it is still challenging to draw firm conclusions on the impact of childhood GHD on bone architecture and the effects of treatment.

To address this issue, Gahlot et al (403) recently studied the size-corrected bone area and bone mineral content in 30 prepubertal children with GHD (peak GH less than 10 μg/L), most whom had IGHD (n = 21, 70%), and compared them with healthy controls (n = 75). The z-score for the lumbar spine size-corrected bone area and BMC was <-2 in 13.3% and 6.6% of GHD patients, respectively, with no significant difference between boys and girls or children diagnosed with IGHD compared with those with multiple pituitary hormone deficiencies. Although children with GHD had a lower mean z-score for LBM for height compared with controls (-0.6 ± 1.7, $P < .01$), 20% were classified as sarcopenic, having a primary muscle defect (low LBM for height), 13% were classified as osteopenic (low BMC for LBM), and no patient had a mixed muscle and bone defect (low LBM for height and low BMC for LBM) (403), suggesting that GHD may not be a cause of osteoporosis.

With respect to fracture risk in children with GHD, there is insufficient evidence to support an increased risk. On one hand, BMD is only one of the factors that may affect risk of fractures and GH may act on other determinants including muscle strength, bone geometry (399), and bone turnover (404). In addition, the reported risk seems to have been extrapolated from studies on adults with GHD of various etiologies, including multiple pituitary hormone defects of organic cause, with a paucity of evidence from studies in children with IGHD (405). Looking specifically at the prevalence of fractures in patients with IGHD, there has been no report of fractures in a small number of patients with severe untreated congenital IGHD (400), and adults with childhood-onset IGHD had a fracture incidence between 3% and 7% (397, 406), comparable with the general population in these studies.

If we focus on the effect of childhood GHD on body composition, numerous studies have confirmed its association with an increase in percentage of body fat and decreased LBM (383, 395, 396, 399) (407). To evaluate the results of studies on the effect of rhGH on body com-

position of children with IGHD, one should take into account the underlying diagnosis (idiopathic, congenital, or syndromic GHD), the inclusion even of a small number of patients with additional pituitary hormone deficiencies in the study population, as well as the methods used for assessment (such as anthropometry, dual-energy x-ray absorptiometry, bioelectrical impedance analysis, and assessment of total body water by isotopic dilutional method). Treatment with rhGH results in an early normalization of percentage of body fat within 6 months (396) and a steady increase in LBM during a 2- to 6-year treatment period (383, 395). In prepubertal GHD children, treatment with a mean daily dose of 30 μg/kg for up to 2 years results in an increase in muscle mass from −2.4 to −1.0 SDS, as measured by peripheral quantitative computer tomography, and this change is observed from the first 6 months (399). There is a continuing accrual of LBM during continuation of rhGH at completion of linear growth, whereas skeletal mass remains static (369), and this effect is sexually dimorphic with females showing an increase in fat mass when treated with a lower adult dose of rhGH (381).

The baseline body composition of GHD children and the short-term effects of rhGH on body composition may be a predictive factor for the response to treatment in terms of height. The early study by Hoos et al (408) showed that the increase in fat-free mass corrected for height (liters per square meter) within 6 weeks of treatment helps to distinguish poor and good responders, with the best result found at cutoff value of 0.9 L/m^2. More recently, these observations were expanded in a cohort of 88 children with GHD who had total body water measured with an isotope dilution method, before and 6 weeks after treatment with rhGH (35 μg/kg/d). In this case, the change in total body water was a predictor of the first-year rhGH response in terms of height (change in height SDS >0.7); a change in total body water of >0.7 L/m^2 identified correctly 73% of good responders to rhGH (409). Apart from the short-term change during treatment, the baseline body composition may also be a predictive factor (410). In 44 children with IGHD, low fat-free mass and total body water at the start of treatment are predictors of a better 6-month response to treatment with a dose of 30 μg/kg. In these studies, different methods have been used for the assessment of body composition, but it is also possible that the differences in the baseline body composition reflect differences in the severity of GHD in these patients. To what extent these observations can be used in a growth-prediction model or in the calculation of rhGH dose, for instance based on fat-free mass rather than total body weight to optimize response, remains to be established.

XIII. Long-term Issues and Safety of rhGH Treatment in GHD

The various indications for rhGH treatment in childhood and its safety profile have been extensively reviewed (232, 407, 411, 412). Information on the safety of rhGH treatment has mainly been obtained from large postmarketing databases including patients with GHD of variable etiology and including non-GHD subjects (413–417). In fact, numerous studies focus on the effects of treatment of specific groups including patients with Turner (418, 419) or Prader-Willi (420–422) syndrome, chronic renal failure (423, 424), SGA (425–428), or idiopathic short stature (429, 430). On the other hand, studies involving the use of rhGH in cancer survivors (431–434) focus on a particular group of patients that require long-term follow-up and address the multiple confounding factors related to the primary diagnosis and treatment modalities.

Although postmarketing databases provide information on an increasing number of patient-years and confirm the overall safe profile, there should be caution when extrapolating their results to patients with GHD of different etiologies, because they have unavoidable shortcomings: inclusion of idiopathic and organic GHD and non-GHD patients, variable diagnostic criteria, absence of untreated control groups, variable dose and duration of rhGH, reliance on physicians for reporting adverse effects over a short period and lack of posttreatment follow-up (435). Here, we will discuss the results of recent studies on the long-term effects of treatment on metabolism, risk of malignancy, and survival, focusing on children with idiopathic or congenital GHD.

A. Risk of diabetes mellitus

When it comes to the long-term metabolic complications, all published studies agree that rhGH does not affect the risk of developing type 1 diabetes mellitus (standard incidence ratio [SIR] of 0.9–1.4). However, the reported overall incidence of type 2 diabetes mellitus (T2DM) ranges from 14 to 34.4 per 100 000 patient-years (413, 436, 437). This reported incidence for T2DM has been calculated for all children enrolled in each database, irrespective of GH status and after a relatively short mean duration of treatment ranging from 1.8 to 4.4 years. The variation in the reported incidence may well reflect the characteristics of the population, differences in the indication for rhGH treatment, or in ascertainment, diagnosis, and reporting of T2DM. The critical evaluation of these results is challenging because there is no appropriate untreated control group and the reported risk would depend on the age group and reference population used for comparison. With these considerations in mind, it seems that

children treated with rhGH have anything from almost similar to an 8-fold increase in the risk of developing T2DM compared with the general population (437–441). Although it is known that reduced insulin sensitivity associated with rhGH treatment is reversible when treatment is discontinued, stopping rhGH may not always reverse T2DM, indicating that preexisting risk factors are important for modulating the risk of developing T2DM (436).

If we examine more closely the results only for children with GHD, it is evident that the risk for developing T2DM is higher in children with organic compared with idiopathic or congenital GHD. In the initial report from the KIGS database of 23 333 children treated for a median duration of 2.9 (range 1.5–4.7) years with a mean dose of 0.19 mg/kg/wk (dose range 0.16–0.23 mg/kg/wk), there were 18 observed cases of T2DM. Six cases were observed among 10 944 children with idiopathic GHD and 6 cases among the 3148 children with organic GHD, giving an estimated incidence of 23 per 100 000 (95% CI, 9–51) and 77 per 100 000 (95% CI, 26–168), respectively (436). More recently, Bell et al (413) published one of the largest recent series of 54 996 children enrolled in the National Cooperative Growth Study over 20 years, of whom 42.5% (n = 23 393) were diagnosed with idiopathic and 15.2% (n = 8351) with organic GHD. T2DM was reported in 0.1% of children with organic GHD, and there were no observed cases among the more than 23 000 children treated for idiopathic GHD. Data from the Genetics and Neuroendocrinology of Short Stature International study (GeNeSIS), an open label observational safety study, showed that irrespective of the underlying diagnosis, there was a more than 8-fold risk for developing T2DM in patients from the United States (range 2.8–19.5) and a 6.5-fold risk for all patients in the cohort (range 3.3–11.7) (437). In this study, Child et al (437) included 11 686 children treated with rhGH, of whom almost 50% had idiopathic GHD (n = 5725) and 15% were classified as organic GHD (n = 1771). The incidence of T2DM in children with idiopathic GHD was 5.6 per 100 000 patient-years (95% CI, 0.1–31.2) with an estimated SIR of 1.2 (95% CI, 0–6.5), whereas for organic GHD, the incidence was remarkably higher compared with the reference population at 74.7 per 100 000 patient-years (95% CI, 27.2–161) giving an SIR of 19.5 (95% CI, 7.2–42.5). The mean age at diagnosis of T2DM was 15.3 (range 12.3–17.4) years, after a relatively short treatment duration of 2.5 (range 1.1–2.9) years, and with a mean dose of 0.22 (range 0.16–0.32) mg/kg/wk. Among the 7 GHD patients who developed T2DM, only 2 had persistent T2DM, and these were patients with organic GHD who had received total body or cranial irradiation for treatment of leukemia (437). Despite the limitation that this was an observational study and thus results may be biased, it supports the recommendation for increased surveillance in patients with preexisting risk factors for impaired glucose homeostasis.

Yet, a number of questions remain to be answered. To what extent is the metabolic risk inherent to the underlying diagnosis or treatment and how can physicians treating children and adolescents estimate the long-term risk during adult life and, if possible, modify it? In a recent study, Luger et al (442) analyzed data from 5143 patients with adult-onset GHD enrolled in the KIMS database and reported an increasing hemoglobin $A1_c$ on a yearly basis and a higher incidence of diabetes mellitus compared with the reference population, with 10.2% of patients developing diabetes over a median period of 1.7 years of treatment. There was no statistically significant association between the incidence of diabetes and the dose of rhGH or the number of additional pituitary hormone deficiencies, but an increased incidence of diabetes was observed in patients with the following predisposing risk factors: higher BMI, waist circumference, triglyceride concentrations, and blood pressure and lower HDL-cholesterol concentration (442). Although patients with childhood-onset GHD were excluded from the analysis, their results further support the case for increased and early surveillance, at least in the subgroup with increased risk factors.

B. Risk of malignancy

A number of recent reviews have focused on the antiapoptotic and proliferative effects of the GH–IGF-1 system and its implication in the development and progression of cancer (443–447). Results of recent surveys conclude that rhGH treatment does not increase the risk of primary malignancy in children and adolescents without preceding risk factors (413, 448). These studies are reported over a relatively short duration of follow-up, there is no assessment of risk after cessation of therapy, and the estimation of the risk for malignancy is performed using established national epidemiological data. Wilton et al (448) followed up a cohort of 56 503 patients without known increased risk factors for malignancy who have been treated with rhGH (mean dose 0.25 ± 0.1 mg/kg/wk) for a mean duration of 3.6 (range 0.08–9.70) years. Almost half of the patients were treated for idiopathic GHD (54%), 5% for congenital and 3% for acquired GHD, with the remaining being treated for other indications. Overall, there were 32 new cases of malignancy compared with the 25.3 expected, giving an incidence of 16.4 per 100 000 patient-years and an SIR of 1.26 (95% CI, 0.86–1.78). Almost half of the cases, however, were reported early within the first 2 years of enrollment, 11 between 2

and 5 years, and 4 between 5 and 10 years. There were 9 reports of CNS tumors, making them the main observed neoplasm with an SIR of 2.24 (95% CI, 1.02–4.25). Looking at the estimate of cancer risk based on the etiology of GHD, there was no increased risk of malignancy in the idiopathic (SIR 1.01 [95% CI, 0.55–1.70]) or congenital GHD (SIR 0.65 [95% CI, 0.01–3.59]) group. Among the 31 690 children with idiopathic GHD, there were 14 cases of malignancy including cranial tumors (n = 7), 2 cases of non-Hodgkin's lymphoma, and single cases of acute myeloid leukemia, seminoma, rhabdomyosarcoma, papillary kidney cancer, and fibromyxoid sarcoma. However, 2 of the cases of CNS tumors were germinomas that were diagnosed early and in patients initially classified as having idiopathic GHD, suggesting that they may well have been the primary cause of GHD rather than the effect of treatment. In the group of the 1703 children with acquired GHD, there were 2 cases of malignancy, vs the 0.2 expected, giving a significantly higher SIR for malignancy of 8.52 (95% CI, 0.96–30.7). The characteristics and underlying diagnoses of children in the acquired GHD group were not presented in detail. However, looking closer at the 2 reported cases of malignancy, there was 1 of acute lymphoblastic leukemia appearing 2.6 years after starting treatment in a patient with GHD postencephalitis, whereas the second was described as an infiltrative mass in the hypothalamic area appearing 13 months after treatment in a patient with GH and antidiuretic hormone deficiencies. So, even in the organic GHD group, the estimated risk of malignancy may have been overestimated by including a case that is most probably not a de novo intracranial tumor but an evolving infiltrative process. Recently, Bell et al (413) also confirmed that rhGH does not increase the risk for new malignancy in children without risk factors but may increase the risk of a second malignancy in previously treated patients. In this study, they reported on 54 996 patients enrolled in the National Cooperative Growth Study database; 42% had idiopathic GHD (n = 23 393) and 15% organic GHD (n = 8351), whereas the remaining patients were treated for other indications. In the entire cohort, the overall risk of malignancy was comparable to the reference population with an SIR of 1.12 (95% CI, 0.75–1.61). Looking specifically at GHD patients, there were no reports of new neoplasms, including CNS tumors or leukemia, in children diagnosed with idiopathic GHD. Among children with organic GHD, the authors reported recurrence of intracranial tumous (1.8%), second neoplasms (0.5%) and new onset malignancies without identifiable risk factors (0.1%). Almost 2500 patients in this database had a primary diagnosis of an intracranial tumor with the main category being craniopharyngiomas (n = 994). Among them, intracranial tumor recurrence was noted in 7.9% (199 of 2500) and in 8.7% of those with craniopharyngiomas. After the exclusion of patients with craniopharyngiomas, the number of second malignancies was approximately 4.6 cases per 1000 patient-years of rhGH exposure, occurring at a mean age of 13.8 years, after a mean of 3 years of rhGH exposure. In this case, the primary malignancy associated more commonly with the development of a second neoplasm was leukemia, whereas previous exposure to radiation was the main risk factor (413). Consistent with previous reports (432, 434), the SIR for new-onset leukemia in patients without risk factors was comparable to the reference population (SIR 0.54; 95% CI, 1.11–1.58) (413). In the previous reports from the Childhood Cancer Survivor Study, the adjusted relative risk for a second neoplasm in patients treated with rhGH compared with nontreated was 3.21 (432) to 2.15 (434) (95% CI, 1.88–5.46 and 1.33–3.47, respectively). Although in their study Bell et al (413) did not calculate the SIR or adjusted relative risk for the development of a second neoplasm, they calculated that these previously reported risks would correspond to approximately 4.3 cases per 1000 patient-years, which would be comparable to their previous report of 4.6 cases per 1000 patient-years. Despite the methodological differences among these studies and the short duration of follow-up, the authors concluded that children with a previous history of malignancy who are treated with rhGH have an increased incidence of secondary tumors, especially if they have been exposed to radiation as part of their treatment for the primary tumor. However, this latter statement remains controversial, and subsequent studies did not confirm an increased risk of recurrent or secondary neoplasms in patients receiving rhGH, thus supporting a good safety profile even after CNS irradiation (433).

C. Effect of GHD on longevity and long-term safety of rhGH treatment

Despite the well-documented adverse effects of GHD on cardiovascular and metabolic factors (449), the question of whether GHD per se is associated with increased or decreased mortality is highly debated. Aging is associated with the process of somatopause, the physiological progressive reduction of GH secretion and serum IGF-1 (450), and there is strong evidence that patients with pituitary disease have increased mortality, mainly due to cardiovascular factors (451, 452). However, most epidemiological studies on this subject include adult patients with hypopituitarism after surgery or radiotherapy for malignancy, with multiple and variable pituitary hormone deficits (452–454), and therefore, it is unclear whether the observed reduced longevity can be attributed to GHD or whether the other confounding factors can account for the

increased early mortality. On the other side of this debate is the paradox that the disruption of the GH/IGF-1 signaling pathway at various levels may result in increased longevity (455, 456). For example, mice with targeted disruption of the GHR/GH binding protein (GHRKO) are GH-resistant, with profoundly suppressed IGF-I and insulin concentration and a markedly increased lifespan (457, 458). Another murine model, the Ames dwarf mice, which exhibit GHD due to a homozygous loss-of-function *Prop1* mutation, live almost 50% longer than nonmutant controls with greater resistance to oxidative stress and decreased or delayed incidence of cancer (459) However, if Ames (*df/df*) mice are injected with GH for 6 weeks, their lifespan becomes comparable to controls and they have comparable resistance to a number of agents causing cellular stress, suggesting that the lower level of GH exposure from early life contributed to their prolonged lifespan and resistance to cellular stress (460).

In humans, centenarians of Ashkenazi-Jewish descent have an overrepresentation of heterozygous nonsynonymous changes in the IGF-1 receptor gene (*IGF1R*) relative to controls that are associated with a higher serum IGF-1 concentration, a reduction of intracellular signaling, and a trend toward shorter height, confirming that genetic alterations in this system can have an effect on lifespan (461). Furthermore, patients with severe GHR (462) and IGF-1 deficiency (463) have reduced incidence of cancer mortality with upregulation of genes involved in pathways protecting against oxidative stress (*SOD2*) and downregulation of pro-aging mediators (*Ras*, *PKA*, and *Tor*) (462). In addition to this protective effect, the GHR-deficient subjects of the Ecuadorian cohort had similar cardiovascular mortality to their relatives with a normal, though not increased, lifespan due to non–age-related causes of death.

Studies looking specifically at the lifespan of untreated subjects with congenital GHD are rare, but they do provide valuable insight on this subject. It has long been reported that untreated patients with dwarfism and multiple pituitary hormone deficiencies due to a *PROP1* mutation, who live on the Krk island of the Adriatic, have an increased lifespan compared with normal individuals in the same population (464). Aguiar-Oliveira et al (465) identified an extended pedigree with familial IGHD residing in the Itabaianinha region of Brazil, who were homozygous for a *GHRHR* mutation (IVS1+1G→A), and studied the longevity of both affected individuals and heterozygous carriers. Interestingly, during childhood and adolescence (age groups 4–20 years), there was a higher frequency of death in GHD females, although the cause of death in this age group was not related to vascular factors and may reflect a possible effect of GHD on the immune system (466, 467); the reason for the sex-observed difference is unclear. However, when looking at untreated GHD subjects of both sexes after the age of 20 years, there was no difference in the lifespan or cause of death between them and their normal-stature siblings or the general population. These results are in contrast with the previous study by Besson et al (468), who looked at the lifespan of untreated GHD patients from 2 isolated areas of Switzerland, who were homozygous for a 6.7-kb *GH1* deletion. In this case, GHD subjects had a significantly shorter median lifespan compared with their unaffected siblings (56 vs 75 years for males; 46 vs 80 years for females), although the cause of death did not vary between the 2 groups (468). The discrepancy between these unique cohorts may be explained by differences in the genetic background, environmental factors, the period of observation, and inherent methodological differences. Most importantly, the contrasting results of the study by Besson et al (468) may be due to the fact that this is the only study focusing on patients with complete absence of GH.

In view of these observations, the question of the overall safety of rhGH with regard to long-term survival of treated patients becomes a highly topical and controversial issue. In the National Cooperative Growth Study (413), 174 deaths were reported among the 54 996 children enrolled (0.3%), of which 19 (11%) were assessed as related to treatment with rhGH, and the majority were caused by tumors. This event occurred at a mean age of 11.6 years with a mean treatment duration of 2.5 years, was rare in patients with idiopathic GHD (0.1%), and was observed in patients with organic GHD (1%) or those treated for other indications (1.7%). This type of study, however, is time-limited and includes patients of variable underlying diagnoses and cannot fully address the issue of the overall safety of rhGH treatment in patients with GHD.

In this respect, the study on the Safety and Appropriateness of GH treatments in Europe (SAGhE) was set up to assess the long-term outcome in 30 000 low-risk children treated with rhGH between 1980 and 1990 in 8 European countries, who had attained 18 years of age by the census date (2009–2010 depending on the country). Patients were classified as low mortality risk if they were diagnosed with idiopathic IGHD, SGA, or idiopathic short stature without genetic syndromes or defects. An early report of the results on the analysis of mortality has been published for the cohort treated in France (469), in what was perhaps one of the most controversial publications on this field. The cohort from France included 6928 low-risk individuals, with a mean follow-up of 17.3 ± 4.1 years from the time of the start of treatment. Surprisingly, there was an increase in the overall standardized mortality ratio (SMR) calculated at 1.33 (95% CI, 1.08–1.64) with

most deaths observed in the group of idiopathic GHD. This represents an excess of 23 deaths in 17 years, or a 33% increase in all-cause mortality, which would have not been detected if the duration of follow-up had been shorter and limited to 5 or even 10 years. This increase in all-cause mortality was associated with a higher rhGH dose of >50 μg/kg/d (SMR 2.94; 95% CI, 1.22–7.07) but not with the severity of GHD or the duration of total GH exposure. However, children with extreme short stature (height SDS <−3) had a higher mortality risk in univariate analysis, raising the question that at least some of these subjects had in fact an undiagnosed syndromic condition and yet were misclassified as low risk and included in the analysis. For the lower mean rhGH doses in the range of 20 to 30 μg/kg/d and 30 to 50 μg/kg/d, the SMR was 0.95 and 1.34, respectively (95% CI, 0.58–1.57 and 0.52–3.43). When looking at the cause of death, there was no increase in the overall risk of cancer-related deaths, although numbers were relatively small. However, there was an almost 5-fold increase of deaths due to bone tumors (SMR 5.0; 95% CI, 1.01–14.63) and an increase in mortality from cardiovascular disease (SMR 3.07; 95% CI, 1.40–5.83) and cerebrovascular hemorrhagic events (SMR 6.6; 95% CI, 1.79–17.05).

The results from the French cohort were in striking contrast with those published concurrently in the joint report from Belgium, The Netherlands, and Sweden participating in the same SAGhE study (470). In fact, the increase in mortality rate was not confirmed, because among the 2534 low-risk patients, there were 21 deaths, the vast majority due to accident (76%) and none attributable to cardiovascular disease, cancer, or high-dose rhGH treatment (high dose range, 0.048–0.054 mg/kg/d), but because of the relative small number of patients, the standardized mortality ratio has not been calculated. In particular, 15 deaths were observed among the 1666 patients with idiopathic IGHD; the majority were accidental and only 4 due to other causes (pneumonia, other endocrine dysfunction, primary cardiomyopathy, and defect in humoral immunity). In these subjects, the duration of treatment ranged from 0.4 to 11.4 years, and the mean rhGH dose from 0.027 to 0.048 mg/kg/d (470).

It is true that both reports have a number of limitations, some of which are difficult to overcome, such as the lack of an appropriate untreated control group for comparison that may lead to overestimation of risk, the relatively small event numbers leading to low statistical power, and the integration of data from different institutes over an extended follow-up period. However, the SAGhE study is an important step in the study of the long-term safety of rhGH in a way that is free of commercial bias. It is possible that differences in genetic background of the population, environmental or confounding factors in assignment, and interpretation of data may account for the difference between these 2 reports. In view of the methodological shortcomings, rather than trying to explain these contrasting results, we should perhaps consider that they are in fact preliminary reports of a larger European consortium and we need to have the full complement of the study before drawing more conclusive results. However, the issue of the long-term safety of rhGH is here to stay, and the recommendation for an international task force with multiple resources and expertise to answer this question (412) is a necessity (Summary Box 4).

Box 4. Longevity and safety of rhGH treatment

Disruption of the GH–IGF-1 axis may result in increased longevity.

Mice with targeted disruption of the GHR or those with GHD due to a homozygous *Prop1* loss-of-function mutation have increased lifespan and resistance to oxidative stress.

In humans, patients with severe IGF-1 deficiency have reduced incidence of cancer mortality and downregulation of proaging mediators.

Studies in unique patient cohorts have shown that patients with GHD have shorter, increased, or similar lifespan compared with controls, depending on the genetic background and the etiology of GHD (*GH1* deletion, *PROP1* mutation, or *GHRHR* mutation, respectively).

The long-term safety outcome of rhGH treatment has been the subject of the SAGhE study.

In this study, the cohort from France showed an increase in the SMR (1.33), with most deaths occurring in the group of idiopathic GHD.

The increase in all-cause mortality was associated with higher rhGH doses of more than 50 μg/kg/d but not with the duration of total rhGH exposure.

There was an increase in mortality from cardiovascular events, cerebrovascular hemorrhage, and bone tumors. There was no increase in the overall risk of cancer-related deaths.

Results have not been reproduced by reports from other countries participating in the study, and the compiled results from all participating countries are yet to be published.

XIV. Conclusion

Despite the many years of research in the area of GHD, controversies on the etiology, diagnosis, and management of IGHD in children still exist. Our understanding of the dynamic function of the somatotrope network may explain at least in part the development and progression of childhood GHD in different age groups. To date, known genetic factors explain only a small proportion of congenital GHD, prompting investigators to look for novel genes implicated in the etiology of IGHD. Despite the emerging correlation between genotype and phenotype in IGHD and its implication for the long-term follow-up of these patients, there is still considerable variability in the manifestation of congenital IGHD, with some patients even having a normal growth velocity. Additionally, apart from growth, childhood GHD may have an impact on the health and neurodevelopment of children. To what extent treatment with rhGH can reverse this effect is yet unknown, and long-term controlled studies are needed to clarify the consequences of childhood IGHD and its treatment through to adult life. In particular, little is known about the long-term safety of rhGH, and surveillance should be mandatory, particularly in light of recent findings in the SAGhE study.

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