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Physiology and Disorders of the Growth Hormone Receptor (GHR) and GH-GHR Signal Transduction

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GH exerts its actions through binding with two receptor molecules at the cell surface and interaction with Janus kinase and signal transducers and activators of transcription, and other likely effectors to stimulate metabolic effects and IGF synthesis. The circulating GH binding protein is the proteolytic product of the cell surface receptor and serves as a marker of receptor number and function. Thirty-six distinct mutations of the receptor in the extracellular and transmembrane domains cause a clinical picture of severe GH/IGF-I deficiency, whereas two dominant negative mutations of the intracellular domain result in a milder clinical syndrome. These mutations have provided insight into the physiology of the GH receptor. A few patients have been described with what appears to be primary GH insensitivity due to defective signal transduction by the GH-GH-receptor complex. Clinical and biochemical features of primary GH insensitivity are not a function of genotype, with as much variability in a genetically homogeneous population as in a heterogeneous one. Except for those dominant negative mutations where co-transfection of the mutant GH receptor gene with wild-type receptor gene has been informative, evidence for an effect of a single mutant allele remains speculative. Treatment of GH receptor deficiency with recombinant human IGF-I suggests that the absence of a direct effect of GH limits growth response.

Key Words: Laron syndrome; growth hormone receptor; insulin-like growth factor.

Introduction

The physiologic and pharmacologic effects of growth hormone (GH) are the result of binding of GH to the cell surface GH receptor (GHR). The ligand-receptor interaction and subsequent signal transduction can be impeded by molecular defects in the GHR or abnormalities in the signaling pathway [primary GH insensitivity (GHI)], or be secondary to catabolic/disease states (acquired GHI). This review briefly describes GHR activation and GH-GHR signal transduction, discusses the etiology and characteristics of disorders of this system, and considers treatment issues.

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The GH Receptor (GHR) and GH Binding Protein (GHBP)

The recognition that circulating GH binding protein (GHBP) in rabbit serum corresponded to liver cytosolic GHBP *(1)* was followed by the purification, cloning, and sequencing of human GHBP. The human GHBP was found to be structurally identical to the extracellular hormone binding domain of the membrane-bound GHR *(2)*. The entire human GHR gene, localized to the proximal short arm of chromosome 5, was subsequently characterized *(3)*. The gene spans 86 kilobase pairs, and includes 9 exons, numbered 2–10, that encode the receptor and 4 additional exons in the 5' untranslated region. Exon 2 encodes a secretion signal sequence, exons 3 through 7 encode a large extracellular GH binding domain, exon 8 encodes a transmembrane domain, and exons 9 and 10 encode the cytoplasmic domain and 3' untranslated region (**Fig. 1**). The GHR is a member of a family of receptors that includes the receptor for prolactin and numerous cytokine receptors. Members of this family share ligand and receptor structure similarities, in particular the requirement that the ligand bind to two or more receptors, or receptor subunits, and interact with signal transducer proteins to activate tyrosine kinases *(4)*.

GH bound to the soluble GHBP accounts for approx 50% of circulating GH. The sites for immune binding are not affected by the GHBP, so that this bound GH is measurable along with the unbound GH *(4)*. The finding that the GHBP is encoded by the GHR gene makes measurement of GHBP in the circulation a reflection of number and, with rare exceptions that will be discussed, function of the GHR. GHBP is generated by proteolytic cleavage of the full-length membrane-bound receptor molecule in hu-

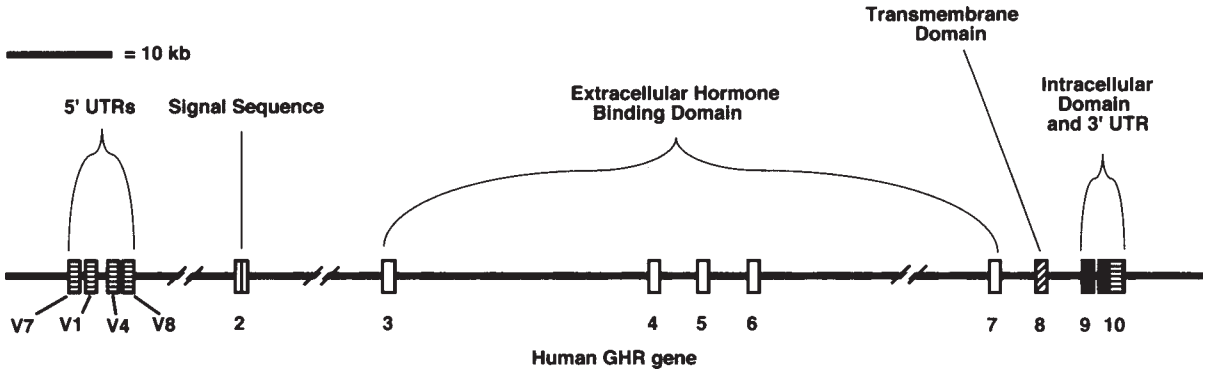

Fig. 1. Representation of the growth hormone receptor gene. The black horizontal line represents intron sequence, diagonal breaks in the lines indicate uncloned portions of the intron and the boxes represent exons, which are enlarged for clarity. Exons with horizontal stripes designate untranslated regions of the transcripts, the vertical striped exon the signal sequence, open exons the hormone binding domain, the diagonal striped exon the transmembrane domain, and the solid exons the intracellular domain. Reproduced with permission from: Edens, A. and Talamantes, F. (1998). *Endocr. Rev.* **19,** 559–582. Copyright The Endocrine Society.

mans and rabbits. In rats and mice, GHBP is formed by alternative splicing in the cytoplasmic domain, forming a truncated isoform *(5)*.

Analysis of the three-dimensional crystal structure of human GHBP in complex with GH revealed a homodimer consisting of one molecule of GH encompassed by two molecules of receptor. Thus, the extracellular domain of the GHR contains two binding sites, one involved in GH binding, the other located close to the membrane spanning region and responsible for homodimerization. The intact receptor lacks tyrosine kinase activity, but is closely associated with JAK2, a member of the Janus kinase family. JAK2 is activated by binding of GH with the GHR dimer, which results in self-phosphorylation of the JAK2 and a cascade of phosphorylation of cellular proteins. Included in this cascade are signal transducers and activators of transcription (STATs), which couple ligand binding to the activation of gene expression, and mitogen activated protein kinases (MAPK). Other effector proteins have also been examined in various systems *(4)*.

Primary Disorders of the GHR and GH-GHR Signal Transduction

Discovery and Premolecular Study of the Condition

Following the initial report *(6)* of three Yemenite Jewish siblings, "with hypoglycemia and other clinical and laboratory signs of growth hormone deficiency, but with abnormally high concentrations of immunoreactive serum growth hormone," 22 patients were reported from Israel, all Oriental Jews, with an apparent autosomal recessive mode of transmission in consanguineous families *(7)*. These reports preceded the recognition of the critical role of cell surface receptors in hormone action and it was postulated that the defect was in the GH molecule that these patients were producing. This impression was substantiated by the observation of free-fatty acid mobilization, nitrogen retention, and growth in patients being administered exogenous GH *(6,7)*. These effects may have been due to other pituitary hormones in the crude extracts administered or to nutritional changes in the investigative setting.

In the first patient reported outside of Israel, in 1968, there was no response to exogenous GH, leading to the hypothesis that the defect was in the GH receptor *(8)*. This hypothesis was substantiated by the failure to demonstrate sulfation factor activation with exogenous GH administration, reported in 1969 *(9)* and reports in 1973 and 1976 that the patients' GH was normal on fractionation, in its binding to antibodies, and in its binding to hepatic cell membranes from normal individuals *(10–13)*. In vitro demonstration of cellular unresponsiveness to GH was demonstrated by the failure of erythroid progenitor cells from the peripheral blood of two patients to respond to exogenous GH *(14)*. The failure of radioiodine labelled GH to bind to liver cell microsomes obtained from biopsy of two patients with Laron syndrome confirmed that the defect was in the GHR *(15)*.

Just before the report that human circulating GHBP was the extracellular domain of the cell surface GHR *(2)*, two reports appeared indicating that GHBP was absent from serum of patients with Laron syndrome *(16,17)*.

Molecular Definition of Abnormalities in the GHR

The report of the characterization of the GHR gene included the first description of a genetic defect of the GHR, a noncontiguous deletion of exons 3, 5, and 6 in two Israeli patients with Laron syndrome *(3)*. The deletion of exon 3 was later identified as an alternatively spliced variant without functional significance, rather than a component of the defect in these patients *(18)*. Only five Israeli patients have been found to have the exon 5,6 deletion defect, out of over 30 subjects with Laron syndrome of Oriental Jewish origin in Israel, emphasizing that, even in this ethnically homog-

enous population, there is heterogeneity for the genetic defects in the GHR *(19)*. No other exon deletions have been described, but 41 additional defects of the GHR gene have been described in association with GHI, including 9 nonsense mutations, 17 missense mutations, 5 frame shift mutations, and 10 splice mutations *(20–37)*. The functional insignificance of exon 3 is emphasized by the fact that no mutations affecting this exon have been associated with GHR deficiency (GHRD).

In **Table 1**, the deletion defect and 35 point mutations associated with typical autosomal recessive GHRD when present as homozygotes or compound heterozygotes are listed, along with two heterozygous mutations demonstrated to have a dominant negative effect *(38,39)*, and four heterozygous mutations thought to be responsible for instances of "idiopathic" short stature accompanied by low serum levels of GHBP and IGF-I with normal or elevated GH responses *(25,37)*.

All but 3 of the 35 defects associated with typical Laron syndrome result in absent or extremely low levels of GHBP (D152H, G223G, R274T). The D152H missense mutation affects the dimerization site, thus permitting the production of the extracellular domain in normal quantities, but failure of dimerization at the cell surface *(30)*. The two defects that are close to (G223G) or within (R274T) the transmembrane domain result in extremely high levels of GHBP *(24,34,36)*. These defects interfere with the normal splicing of exon 8, which encodes the transmembrane domain, with the mature GHR transcript being translated into a truncated protein that retains GH binding activity, but cannot be anchored to the cell surface.

Partial GHI

GH resistance might be expected to occur in an incomplete form, analogous to insulin resistance, androgen insensitivity, or thyroid hormone resistance. Affected children might have growth failure with normal or slightly increased GH secretion, variable but usually decreased GHBP levels, decreased IGF-I concentrations, but not as severely reduced as in GHD or GHRD, and might respond to supraphysiologic doses of GH. It might also be expected, given the need for dimerization of the GHR for signal transduction, that certain mutations could have a dominant negative effect in the heterozygous state.

The intronic mutation present in the heterozygous state in a mother and daughter with relatively mild growth failure (both SDS for height –3.6), and resulting in a dominant negative effect on GHR formation, is not associated with other phenotypic features of GHD. This splice mutation preceding exon 9 results in an extensively attenuated, virtually absent intracellular domain *(38)*. Japanese siblings and their mother have a similar heterozygous point mutation of the donor splice site in intron 9, also resulting in mild growth failure compared to GHRD (SDS for height of siblings, –3.0, –3.5 and of mother –2.0), but with mild phenotypic features of GHD *(39)*. GHBP levels in the Caucasian patients were at the upper limit of normal with a radiolabeled GH binding assay *(38)* and in the Japanese patients twice the upper limit of normal, using a ligand immunofunction assay *(39)*.

These heterozygote GHR mutants transfected into permanent cell lines have demonstrated increased affinity for GH compared to the wild type full-length GHR, with markedly increased production of GHBP. When co-transfected with full-length GHR a dominant negative effect results from overexpression of the mutant GHR and inhibition of GH-induced tyrosine phosphorylation and transcription activation *(38,40)*. Naturally occurring truncated isoforms have also shown this dominant negative effect in vitro *(41–43)*.

Credibility for a heterozygous defect as a cause of short stature requires the demonstration of functional significance, not only by transfection of the mutant allele, but by co-transfection with wild-type GHR gene, to approximate the circumstance in vivo. Goddard et al. *(25)* have identified 6 mutations in 8 children with short stature (SDS for height –5.1 to –2.0) and normal or increased stimulated GH levels. One patient had compound heterozygosity involving a novel mutation in exon 4 (E44K) and a mutation in exon 6 previously associated with GHRD in the homozygous state (R161C). Two other patients were heterozygous for this mutation. The other 5 patients included 2 who were heterozygous for the same novel mutation in exon 7 (R211H), and one each with novel mutations of exon 5 (C122X), exon 7 (E224D), and exon 10 (A478T). The four novel mutations involving the extracellular domain are listed in Table 1 because in vitro expression of these alleles has shown functional effects, although co-transfection studies have not yet been reported. The defect involving exon 10 has not been expressed in vitro. Other defects without demonstrable significance have been described involving exon 10 *(44,45)*. None of these putative partial GHI patients had the clinical phenotype of GHD. Five of the eight patients were treated with GH with variable improvement in growth velocity, from slight to dramatic, in the first year *(25)*. This variable response could be due to GH resistance or to the fact that the patients were not GH/IGF-I deficient.

Whether the distribution of GHBP concentrations in ISS indicates that partial GH resistance is a common cause of short stature remains to be demonstrated. The subjects studied by Goddard et al. *(25)* were selected from the large Genentech National Cooperative Growth Study database. Thus, if heterozygous mutations of the GHR ultimately prove to be one cause of partial GH resistance, this would explain only a very small proportion of idiopathic short stature. This impression is supported by a recent review of 37 patients who had relatively high GH responses to insulin and failure to increase IGF-I concentrations in the serum after several days of GH administration. GHBP concentrations were normal. Only one patient of this group

Table 1
Mutations of the Growth Hormone Receptor Associated with Growth Failure

Type	Defect	Exon	Origin	Reference
Deletion	Exon (3) 5,6	5,6	Oriental Jewish	*3, 19*
Nonsense	C38X	4	N European/Mediterranean	*20*
	R43X	4	Mediterranean/Ecuador/Russia	*20, 21–23*
	Q65X	4	India	*24*
	W80X	5	Germany	*24*
	C122X[g]	5	USA	*25*
	W157X	6	Turkey	*24*
	E183X	6	Mexico	*21*
	R217X	7	African-American/Saudi Arabia	*21, 23, 26*
	G224X[b]	7	Japanese	*27*
Missense	C38S	4	Algeria	*24*
	E44K[a,g]	4	USA	*25*
	S40L	4	Turkey	*24*
	W50R	4	Germany	*24*
	R71K	4	Mediterranean	*23*
	F96S	5	Tunisia	*28*
	V125A	5	European	*23*
	P131Q	6	Vietnamese	*29*
	V144D[c]	6	Mediterranean	*23*
	D152H	6	Pakistani	*30*
	I153T[d]	6	European	*31*
	Q154P	6	Hispanic	*31*
	V155G	6	Saudi Arabian *31*	
	R161C[a,g]	6	Middle East	*23, 37*
	R211G[c]	7	Mediterranean	*23*
	R211H[g]	7	USA	*25*
	E224D[g]	7	USA	*25*
Frameshift	36delC	4	Slovenia	*24*
	46delTT[e]	4	Spain	*21*
	230delTA	7	African	*21*
	282del[d] →	9	European	*31*
	309 delC[b]	10	Japanese	*27*
Splice	intron 2		Turkey	*24*
	intron 4[e]		N. European/Spain/Japanese	*23, 26, 32*
	intron 5		Mediterranean	*23*
	intron 6		Brazil	*26*
	E180 splice	6	Ecuador *Converso*/Oriental Jewish	*33*
	G223G	7	Druse	*24, 34*
	G236 splice	7	Caucasian (Bahamas)	*35*
	R274T	8	Pakistan	*36*
	intron 8[f]		Caucasian (England)	*38*
	intron 9[f]		Japanese	*39*

[a]Compound heterozygote with ISS; R161C also homozygous defect with classic GHRD *(23)*
[b]Compound heterzoygote
[c]Compound heterozygote
[d]Compound heterozygote
[e]Compound heterozygote *(21)*. These mutations also occur as homozygous defects *(26,32)*.
[f]Heterozygous mutation with dominant negative effect
[g]Heterozygous mutations in subjects with idiopathic short stature (ISS) and normal or elevated GH responses

failed to demonstrate a growth response to exogenous GH. The authors concluded that partial growth hormone insensitivity was likely to be a rare cause of unexplained short stature *(46)*.

The possibility of an effect of heterozygosity for a mutation known to cause GHRD in the homozygous state was able to be explored in the unique Ecuadorian cohort with GHRD, which comprises a large population with a single mutation, permitting genotyping of numerous first degree relatives. There were no significant differences in stature between carrier and homozygous normal relatives, indicating a lack of influence of heterozygosity for the E180

splice mutation of the GHR *(45)*. A more general indication of the lack of influence of heterozygosity for GHR mutations involving the extracellular domain on growth comes from studies of the large multicenter European-based GHI study group as well as the Ecuadorian patient population. In these populations the stature of parents and of unaffected siblings does not correlate with statural deviation of affected individuals *(44,45,47)*, while expected high correlation exists between parents and unaffected offspring *(44)*. If the mutations that cause growth failure in the homozygous state also affected growth in heterozygotes, heterozygous parents and predominantly heterozygous siblings would have height SDS values which correlated with those of affected family members. In the Ecuadorian families, there was no difference in height correlations with parents between carriers and homozygous normal offspring *(44)*.

Genetic Anthropology

Ethnic origin is known for 232 of the 250 reported cases of GHRD *(48)*. Of these, nearly 50% are Oriental Jews as described in the original report, or known descendants of Iberian Jews who converted to Catholicism during the Spanish Inquisition. The latter comprise the largest ($n = 70$) and only genetically homogenous patient group. Most of the other defects appear to be highly family specific, with the R43X mutation that is seen in a single Ecuadorian patient, two other nonsense mutations (C38X, R217X), and the intron 4 splice mutation being the only ones, thus far, described which appear in disparate populations, on different genetic backgrounds, indicating mutational hotspots *(20,21,23)*. Since the molecular defect in the GHR has been identified in about one-third of the patients with GHRD outside of Ecuador, it is likely that this list will continue to grow and provide further insight into the function of the GHR.

The finding of a Jewish patient of Moroccan origin with the same mutation as the Ecuadorian patients supports the hypothesis that this mutation was brought to the New World by Spanish conversos (new Christians) fleeing the Inquisition *(26)*. Thus far, there is no explanation for the middle eastern predominance of this condition, although the high frequency of consanguinity in Arab and traditional Jewish populations is certainly a factor. Nearly 90% of patients are either Oriental Jews, Arabs, or other Middle Easterners, from elsewhere in the Mediterranean area, or from the Indian subcontinent. Many of those from other parts of the world may have Middle Eastern Semitic origins. The small numbers of nonMediterranean, nonIndian patients include a genetic isolate of Anglo Saxons from a Bahamian island, five Africans, five Japanese, two siblings from Cambodia, a Vietnamese, and several from northern Europe *(48)*.

GH-GHR Signal Transduction Defect

A post-GHR defect in three siblings of Palestinian Arab origin has been proposed by Laron et al. *(49)*. The children had typical features of severe GHD, but normal GHBP and IGFBP-3 levels with severe IGF-I deficiency. Four GHBP-positive children from two unrelated Pakistani families, who had high GH, low IGF-I, and low IGFBP-3 serum concentrations, appear to have signal transduction defects. In one family, this is associated with the features of severe GHD and the signaling defect is thought to be close to the GHR, preventing activation of both the STAT and MAPK pathways. In the other family there is a less marked phenotype and a defect in activation of MAPK, but not the STAT pathway, demonstrated in studies of cultured fibroblasts from the patients *(50)*.

Acquired Disorders of the GHR and GH-GHR Signal Transduction

The catabolic conditions that have been associated with secondary or acquired GHI, including nutritional deficiency, diabetes mellitus, hepatic insufficiency, and renal failure, do not consistently demonstrate elevated serum GH concentrations, or low levels of IGF-I, or even growth failure. The acquired GH resistance in the GH gene deletion syndrome is due to the development of GH inhibiting antibodies following treatment with GH, and is characterized by very low or unmeasurable serum concentrations of GH *(51)*. Renal disease results in increased IGFBP concentrations with normal or elevated GH and usually normal IGF-I levels *(52)*. Malnutrition and other catabolic states that have been associated with GHI are accompanied by reduced GHBP concentrations and may be teleologically similar to the nonthyroidal illness (sick euthyroid) syndrome *(53)*. The elevated serum concentrations of GH and GHBP, normal IGFBP-3 and decreased IGF-I in Alagille syndrome (chronic intrahepatic cholestasis) likely reflect specific hepatocellular failure to synthesize IGF-I and can be considered as acquired primary IGF-I deficiency rather than acquired GHI *(44,54)*. The more global hepatic defect in cirrhosis is associated with decreased concentrations of IGFBP-3 and GHBP *(55)*.

Clinical Features of IGF-I Deficiency Resulting from GHR Failure (Table 2)

Mortality

The only available report of the effect of GHRD on mortality comes from the Ecuadorian population *(56)*. Because families in the relatively small area from which the Ecuadorian patients originate have intensive experience with this condition, lay diagnosis was considered reliable. Of 79 affected individuals for whom information could be obtained, 15 *(19%)* died under 7 years of age, as opposed to 21 out of 216 of their unaffected siblings (9.7%, $p < 0.05$). The kinds of illnesses resulting in death, such as pneumonia, diarrhea, and meningitis, were no different for affected vs unaffected siblings *(57)*.

Table 2
Clinical Features of Severe IGF-I Deficiency Due to GHD or GHRD

Growth
- Birth weight — normal; birth length — usually normal
- Growth failure, from birth, with velocity 1/2 normal
- Height deviation correlates with (low) serum levels of IGF-I, -II, and IGFBP-3
- Delayed bone age, but advanced for height age
- Small hands or feet

Craniofacial characteristics
- Sparse hair before age 7; frontotemporal hairline recession all ages
- Prominent forehead
- Head size more normal than stature with impression of large head
- "Setting sun sign" (sclera visible above iris at rest) 25% < 10 years of age
- Hypoplastic nasal bridge, shallow orbits
- Decreased vertical dimension of face
- Blue scleras
- Prolonged retention of primary dentition with decay; normal permanent teeth, may be crowded; absent third molars
- Sculpted chin
- Unilateral ptosis, facial asymmetry (15%)

Musculoskeletal/body composition
- Hypomuscularity with delay in walking
- Avascular necrosis of femoral head (25%)
- High pitched voices in all children, most adults
- Thin, prematurely aged skin
- Limited elbow extensibility after 5 years of age
- Children underweight to normal for height, most adults overweight for height; markedly decreased ratio of lean mass to fat mass, compared to normal, at all ages
- Osteopenia indicated by DEXA

Metabolic
- Hypoglycemia (fasting)
- Increased cholesterol and LDL-C
- Decreased sweating

Sexual development
- Small penis in childhood; normal growth with adolescence
- Delayed puberty
- Normal reproduction

The complete lifespan included in the Ecuadorian cohort provided an opportunity to look at adult mortality risk factors. This is of interest because GHD in adults is associated with premature atherosclerosis and increased cardiovascular mortality, with GH replacement therapy improving the risk factors of hyperlipidemia and obesity *(58)*. Twenty-three adults with GHRD had elevated cholesterol levels compared to relatives and nonrelated community controls, normal HDL-cholesterol levels, elevated LDL-cholesterol levels, and normal triglycerides. It was postulated that the effect of IGF-I deficiency due to GHRD was to decrease hepatic clearance of LDL-C, since the triglyceride and HDL-C levels were unaffected. This effect was independent of obesity or of IGFBP-1 levels, which were used as a surrogate for insulinemia *(59)*. The key pathogenic factor was thought to be the absence of GH induction of LDL receptors in the liver *(60)*. Of eight Ecuadorian patients over 50 years of age followed for greater than 7 yr, two died of heart disease, thought to be an uncommon problem in the Andean setting *(57)*. This might suggest comparable increased cardiovascular risk to that seen with GHD in adults.

Growth

The role of IGF-I in normal intrauterine growth is emphasized by the patient with severe intrauterine growth retardation with a proven IGF-I gene defect *(61)*, but this IGF-I synthesis does not appear to be GH-dependent, since many, if not most, GHRD patients have normal intrauterine growth *(62)*. Children with GH gene deletion also have normal intrauterine growth despite total absence of endogenous GH *(51)*. There is rapid postnatal decline in SDS for length (**Fig. 2**) emphasizing the GH-dependency of extrauterine growth. The growth velocity with severe GHD or GHRD is approximately half normal (**Fig. 3**). Occasional periods of normal growth velocity may be related to improved nutrition.

There is generally an absence of a pubertal growth spurt despite normal sexual maturation in the most extensive available data from Israel and Ecuador, emphasizing the importance of IGF-I for this phenomenon *(62,63)*. Among 24 Israeli patients followed from infancy to adulthood, persistent growth beyond the normal time of adolescence was seen only in boys. In the Ecuadorian population girls also showed this phenomenon (Fig. 3). Adult stature in GHRD varies from –12 to –5.3 SDS in Ecuadorian patients and –9 to –3.8 SDS in others in the literature, using the US standards *(62)*. This is a height range of 95 to 124 cm for women and 106 to 141 cm for men in the Ecuadorian population. This wide variation in the effect of GHRD on stature was not only seen within the population but also within affected families, and this intrafamilial variability has also been described with severe GHD due to GH gene deletion *(51)*.

Some patients with GHRD may have an appetite problem in addition to their IGF-I deficiency. Crosnier et al. *(64)* studied a child aged 3 yr 7 mo with GHRD who had severe anorexia. With his usual intake of approx 500 kcal/d he grew at a rate of 2 cm/yr. With moderate hyperalimentation to approx 1300 kcal/d, growth rate increased to 9 cm/yr without significant change in plasma IGF-I level. The hyperalimentation period was associated with an increase in the IGFBP-3 bands on Western ligand blots from total absence in the anorexic period to levels comparable to those seen in GHD . The catch-up growth noted

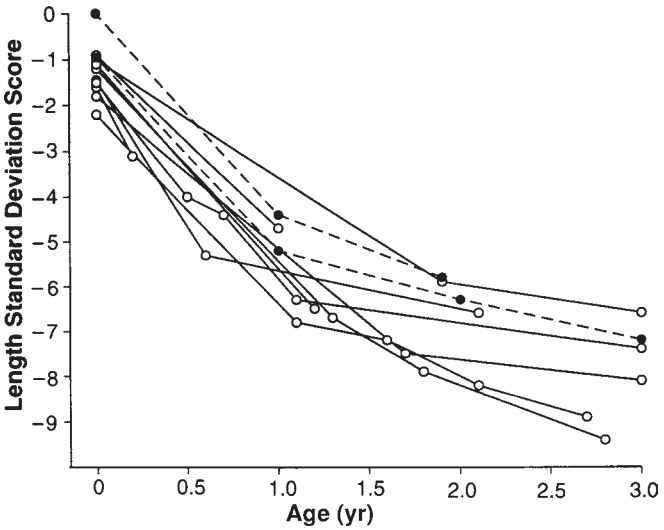

Fig. 2. Length standard deviation scores of nine female Ecuadorian patients (open symbols, solid lines) and two brothers from Russia (solid symbols, dashed lines) with known birth lengths, followed during the first two to three years of life. Reproduced with permission from ref. *62*. Copyright 1994 by Elsevier Science Inc.

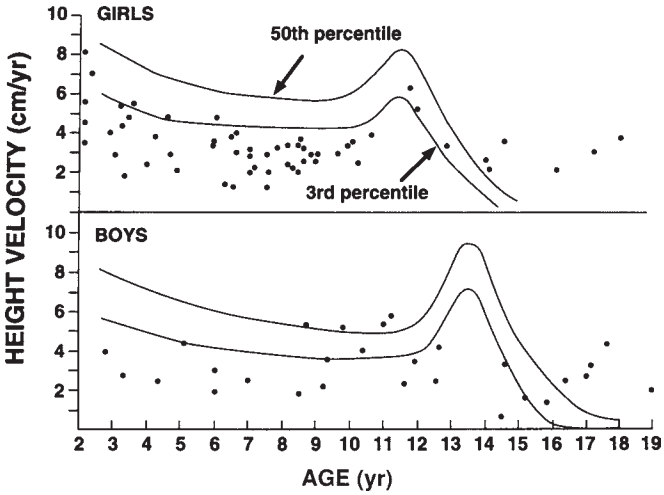

Fig. 3. Growth velocities of 30 Ecuadorian patients (20 female) with GHRD with repeated measures at least 6 mo apart. Third and 50th percentiles from Tanner, J. M. and Davies, P.S.W. (1985). *J. Pediatr.* **107,** 317–329. Reproduced with permission from ref. *62*. Copyright 1994 by Elsevier Science Inc.

could not be explained by hyperinsulinism, which has provided the explanation for accelerated or normal growth in children with GHD and obesity following removal of a craniopharyngioma. There was no appreciable increase in circulating basal or stimulated insulin during the hyperalimentation. In this patient, there was speculation that a nutrition-dependent autocrine/paracrine increase in IGF-I concentration at the cartilage growth plate might have occurred, independent of the GHR. Not considered at the time was the possibility that IGFBP-3 itself might have growth-promoting effects. The importance of adequate nutrition for catch-up growth was emphasized by this study, which also reinforced the notion that normal periods of growth in patients with GHRD without IGF-I replacement therapy, as noted in Fig. 3, might be explained by periods of improved nutrition alone.

Craniofacial Characteristics

Affected children are recognized by knowledgeable family members at birth because of craniofacial characteristics of frontal prominence, depressed nasal bridge, and sparse hair, as well as small hands or feet, and hypoplastic fingernails (**Fig. 4**). Decreased vertical dimension of the face is demonstrable by computer analysis of the relationships between facial landmarks and is present in all patients when compared with their relatives (**Fig. 5**) *(65)*. Blue scleras, originally described in the Ecuadorian population, have now been recognized in other populations with GHRD, as well as in GHD *(66,67)*. This feature is due to decreased thickness of the scleral connective tissue, permitting visualization of the underlying choroid, and is more prominent in younger patients. Unilateral ptosis and facial asymmetry may reflect positional deformity due to decreased muscular activity *in utero*, although mothers do not recognize decreased fetal movement in pregnancies with affected infants *(45,57)*.

Musculoskeletal and Body Composition Characteristics

Hypomuscularity, apparent in roentgenograms, is thought to be responsible for delayed walking despite normal intelligence and time of speech onset *(45)*. Although radiographs of the children suggest osteopenia and dual photon absorptiometry and dual energy X-ray absorptiometry in children and adults confirms this, dynamic bone histomorphometry in adults has been found to be substantially normal, suggesting that some of these findings are artifactual, based on small bone size *(68)*. One fourth of affected children have aseptic necrosis of the capital femoral epiphysis *(45)*.

Limited elbow extensibility seen in most patients over 5 years of age in the Ecuadorian population is an acquired characteristic, absent in younger children and increasing in severity with age *(57,66)*. That this feature is not peculiar to the Ecuadorian population or to IGF-I deficiency due to GHRD has recently been confirmed by finding a Brazilian patient with GHRD with limited elbow extension *(69)* and observing this finding in all but the youngest patient in a family with eight individuals affected by multiple pituitary deficiencies *(67)*. The cause of this contracture is unknown.

Although children appear overweight, they are actually underweight to normal weight for height, while most adults, especially females, are overweight with markedly decreased lean to fat ratios *(45)*.

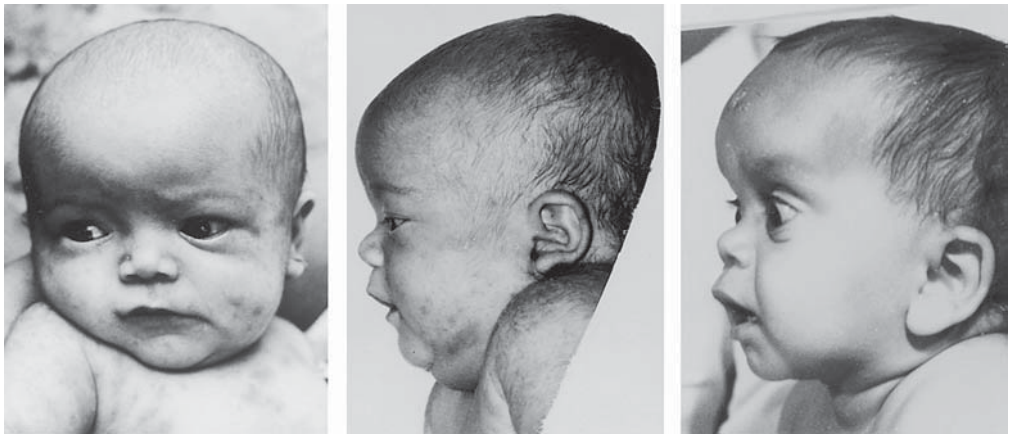

Fig. 4. Front and profile views of one month-old girl, homozygous for the E180 splice mutation of the GHR, demonstrating paucity of hair, prominent forehead, hypoplastic nasal bridge, shallow orbits, and reduced vertical dimension of the face, and profile view of a 3-yr-old patient from the initial report of Laron syndrome *(6)*, demonstrating persistence of these features, and striking similarity with different genetic mutations. Reproduced with permission from ref. *45*. Copyright 1998 by Elsevier Science Inc.

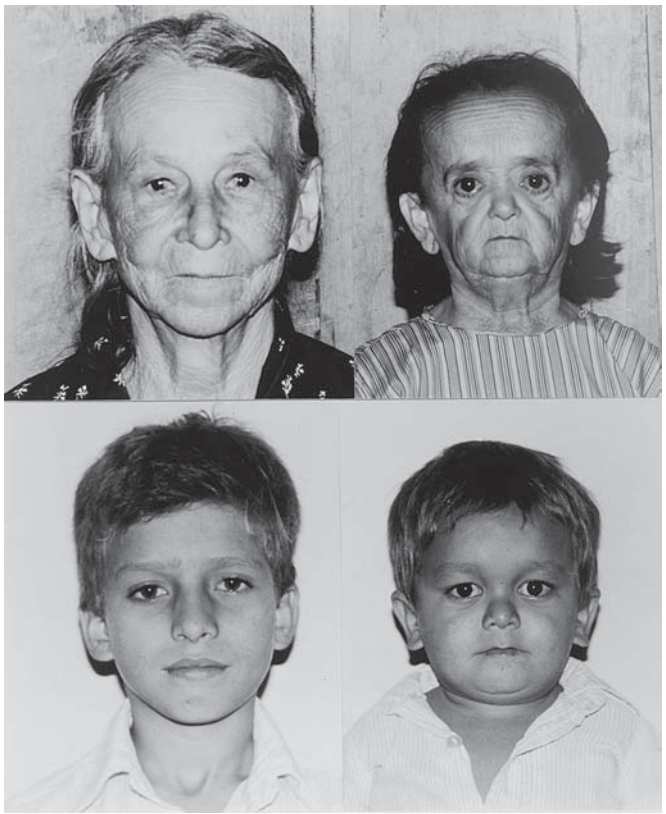

Fig. 5. Comparisons of facial appearance between 52-yr-old patient (right upper panel) and her 76-yr-old mother (left upper panel) and 9-yr-old patient (right lower panel) and his 11-yr-old unaffected brother (left lower panel). Note strong familial similarity but marked difference in facial dimensions. Reproduced with permission from ref. *57*. Copyright Karger AG, Basel Switzerland.

Reproduction

It has long been recognized that severe GHD is associated with small penis size but normal penile growth at adolescence or with testosterone treatment in childhood. This is also true of GHRD. Although puberty may be delayed 3–7 yr in some 50% of individuals, there is normal adult sexual function with documented reproduction by males and females *(56)*. Females require C-section delivery.

Comparison with Other Congenital IGF-I Deficient States

As has been noted, the clinical features described above are indistinguishable between individuals with severe GHD and those with classical GHRD. The recent description of the phenotype of 18 patients with an inactivating mutation of the growth hormone releasing hormone receptor (GHRH-R) notes comparably suppressed IGF-I to severe GHD and GHRD but with normal facies, absence of micropenis in the males, absence of truncal obesity or body segment disproportion, and no evidence of hypoglycemia. Consistent with the severe IGF-I deficiency, these patients have short stature comparable to the other conditions, with height SDS ranging from –6.3 to –8.8 *(70)*.

Intellectual and Social Development

A deleterious effect of GHRD on intellectual development was reported in Israeli patients compared to patients with GHD *(71)*. Among 18 children and adolescents with GHRD administered the Wechsler Intelligence Scale for Children, only three had IQs within the average range (90–110); of the remaining 15 subjects, three were in the low average range (80–89), three in the borderline range (70–79) and nine in the intellectually disabled range (<70). These studies were done without family controls, so that the possibility of other factors related to consanguinity that might affect intellectual development could not be addressed. In a follow-up study 25 yr later, the investigators re-examined eight of the original 18 patients and four new patients with GHRD, excluding five patients with mental disabilities who were in the original study *(72)*. This group had mean verbal and performance IQs of 86 and 92 on the Wechsler scale without evidence of visual motor

integration difficulties that had been noted in the earlier group, but there was a suggestion of deficient short-term memory and attention. The investigators hypothesized that early and prolonged IGF deficiency might impair normal development of the central nervous system, or that hypoglycemia common in younger patients may have had a deleterious effect.

The recent description of intellectual impairment with severe IGF-I deficiency due to partial deletion of the IGF-I gene has added to the concern about potential effects of severe IGF-I deficiency *in utero (61)*. Nonetheless, patients with GH gene deletion and severe IGF-I deficiency have not been intellectually impaired *(51)*, nor have those with severe IGF-I deficiency due to molecular defects in the GHRH-R *(70)*. Sporadic anecdotal reports of patients with GHRD suggested a normal range of intelligence. The collective data from the European IGF-I treatment study group, which includes a wider range of clinical abnormality than either the Ecuadorian or Israeli population, notes a mental retardation rate of 13.5% among 82 patients, but formal testing was not carried out *(47)*. Here again, the high rate of consanguinity was proposed as a possible explanation; hypoglycemia could not be correlated with these findings.

In the Ecuadorian population, exceptional school performance was reported among 51 affected individuals of school age or older who had attended school, with 44 typically in the top three places in their classes and most thought to be as bright or brighter than the smartest of their unaffected siblings *(73)*.

The first controlled documentation of intellectual function in a population with GHRD has been carried out by Kranzler et al. *(74)* in the Ecuadorian patients, focusing on school-age individuals and comparing them to their close relatives and community controls. No significant differences in intellectual ability could be detected among these groups, with the use of nonverbal tests with minimal cultural limitations. It was hypothesized that the exceptional school performance in this population might be related to the lack of social opportunities due to extreme short stature, permitting greater devotion to studies and superior achievement in school for IQ level.

The clinical findings of intellectual impairment with IGF-I gene deletion *(61)* and intellectual normality with GHRD corresponds with gene disruption studies in mice. The IGF-I deleted mouse is neurologically impaired, while the GHRD mouse is behaviorally normal *(75,76)*. Thus, GHD dependent IGF-I production does not appear to be necessary for normal brain development and function.

Biochemical Features of IGF-I Deficiency Resulting from GHR Failure

Growth Hormone

Affected children have random GH levels that are greater than 10 ng/mL and may be as high as 200 ng/mL with enhanced responsiveness to stimulation and paradoxical elevations following oral and intravenous glucose, as is seen in acromegaly *(77)*. The GH levels show normal diurnal fluctuation. Twenty-four hour profiles demonstrate marked GH variability among adult patients with suppression by exogenous recombinant human IGF-I *(78)*. Thus, the normal sensitivity of the GH secretion is preserved, despite elevated levels and lack of feedback suppression from IGF-I.

Postpubertal patients may have normal or elevated basal levels of GH but invariably demonstrate hyperresponsiveness to stimulation, which is all the more impressive with their obesity, which suppresses GH responses in normal individuals. In the Ecuadorian population, mean basal GH level in adults was significantly lower than that in children (11 ± 11 ng/mL vs 32 ± 22 ng/mL, $p < 0.0001$). This is thought to be related to the greater, though still markedly abnormal, IGF-I levels in the adults, resulting in some feedback inhibition of GH secretion *(57)*.

Growth Hormone Binding Protein

It was initially thought that absence of GHBP in the circulation was a requirement for the diagnosis of GHRD, together with the clinical phenotype, very low IGF-I and IGFBP-3 levels, and elevated (in children) or normal to elevated (in adults) GH levels. Early chromatographic analysis, however, showed measurable though reduced GHBP levels in a number of patients. The ligand-mediated immunofunction assay (LIFA) used to measure GHBP serum levels since 1990, uses an antiGH monoclonal antibody to measure the amount of GH bound to GHBP. As a largely functional assay, this should not detect structurally abnormal though expressed GHBP *(77)*.

As noted above, certain genetic defects in the GHR, affecting dimerization or anchoring of the GHBP to the cell membrane, can result in normal or markedly elevated GHBP levels. In the Ecuadorian population, despite in vitro evidence for failure of production of normally spliced receptor, four children and four adults out of 49 patients had serum GHBP levels higher than 40% of the sex-specific lower limit for controls and one adult male had a level in the lower portion of the normal adult male range. The presence or amount of GHBP measured did not relate to stature *(57)*. There were no age-dependent changes, indicating that the difference in IGF values between children and adults was not related to the GHBP levels and the GHBP levels did not correlate with stature or with serum IGF-I levels. Although finding of extremely low or undetectable levels of GHBP serves as an important diagnostic feature, it is not a sine qua non for the diagnosis of GHRD.

Insulin-Like Growth Factors

The lowest serum levels of IGF-I are seen in severe congenital defects in GH synthesis (GH gene deletion, GHRH-

R deficiency), with deletion of the IGF-I gene, and with GHRD. IGF-II is comparably suppressed. In chronic disease states associated with acquired GHI, IGF-I levels are more likely to be reduced than are concentrations of IGF-II and IGFBP-3 *(77)*.

Among 50 Ecuadorian patients homozygous for the E180 splice-site mutation, IGF-I levels were significantly greater in adults 16–67 years of age ($n = 31$, 25 ± 19 mcg/L) than in the 19 subjects under 16 years of age (3 ± 2 mcg/L, $p < 0.0001$), although still markedly below the normal range of 96–270 mcg/L. The children's levels were too low to correlate with stature, but in the adults IGF-I levels correlated inversely with statural SDS with a coefficient of 0.64 ($p < 0.001$). IGF-II levels in adults were also significantly greater than in children (151 ± 75 mcg/L vs 70 ± 42 mcg/L, normal 388 to 792 mcg/L, $p < 0.0001$). The correlation between serum IGF-I and IGF-II levels was highly significant, $r = 0.53$, $p < 0.001$. As noted above, there was no indication of age difference in GHBP levels *(56)*. Therefore, the increased levels of IGF-I and II with adulthood suggest effects on synthesis of these growth factors which are not mediated through the GHR and are presumably under the influence of sex steroids. This hypothesis has been challenged by findings in GHRH-R deficiency discussed below. The correlation of IGF-I levels with stature in adults indicates that, despite the markedly low levels, the influence of IGF-I on stature remains important in these subjects.

IGF Binding Proteins

The principal binding protein for circulating IGFs, IGFBP-3, is considered to serve as a reservoir and delivery mechanism for IGF to tissues. In IGF deficiency states that are the result of GHD or GHRD, IGFBP-3 is reduced and in children and adults with GHRD, this reduction correlates with statural impairment *(79)*. In renal disease, elevated IGFBP-3, as well as IGFBP-1 and IGFBP-2, are thought to prevent the effect of normal levels of IGF-I *(52)*.

Short-term and extended treatment of GHI with IGF-I has failed to result in increases in IGFBP-3 *(78,80–82)*, whereas treatment of GHD with recombinant human GH restores levels to normal. This indicates that IGFBP-3 is under the direct influence of GH.

IGFBP-I is elevated in GHD and GHRD; in GHRD it is the most abundant IGFBP and is strongly inversely related to insulinemia. IGFBP-2 is present at a mean 300% of control concentrations in children with GHRD and 175% of control in affected adults *(83)*. This is a significant difference, and the IGFBP-3 levels in adults with GHRD are significantly greater than in affected children, as well.

Comparison with GHRH-R deficiency

In contrast to the experience with GHRD, no differences were found in the very low levels of IGF-I or IGFBP-3 between the seven sexually mature adults and seven affected children with GHRH-R who were tested *(70)*. The adults had levels comparable to those of children with GHRD. This finding is inconsistent with the hypothesis that sex steroids independently stimulate IGF-I/IGFBP-3 production.

Treatment

Soon after the cloning of the human IGF-I cDNA, human IGF-I was synthesized by recombinant DNA techniques (rhIGF-I) *(84)* Subcutaneous preparations of rhIGF-I became available in 1990. Results have been reported for 69 patients with GHI treated with rhIGF-I for 12 mo or longer. Seven of these patients had GH gene deletion with aquired GHI due to GH inhibiting antibodies. The rest had primary GHI, including Pakistani and Arab patients thought to have post-receptor defects.

Dose Response (Table 3)

In the European multicenter study *(80)*, twice-daily dosages varied according to response, but average dosage was similar to that used in the North Carolina *(85,86)* and Ecuadorian *(81,82)* study populations. As predicted from short-term studies, IGFBP-3 levels did not increase during long-term treatment with rhIGF-I. In the only direct comparison of dosages, there was no difference in growth response between 80 mcg/kg body weight and 120 mcg/kg twice daily; apparently defining a plateau effect *(82)*. Improvement in mean height SDS over 2 yr was 1.2 in the European study, 1.5 for the higher dose and 1.3 for the lower dose in Ecuador, and 1.3 in the North Carolina study. The European multicenter study and Ecuadorian study patients achieved two-thirds of their improvement in the initial year. In the Israeli patients treated with single daily injections of rhIGF-I (120 mcg/kg), there was an improvement of only 0.4 SDS during the first year of treatment with no further improvement for the six patients completing 2 yr of therapy *(87)*. This supports the rationale for twice-daily administration, which was based on kinetic studies in normal controls.

Comparison of growth response of 22 rhIGF-I-treated GHRD patients and 11 GH-treated GHD patients in the same setting demonstrated growth velocity increments in those with GHRD to be 63% of those achieved with GH treatment of GHD in the first year and less than 50% in the second and third years *(82)*. This lesser response in GHRD could be attributable to failure to increase IGFBP-3 levels, a direct GH effect, but three children who had defective IGF-I synthesis attributed to a post-receptor defect did not grow better while receiving IGF-I than did subjects with GHRD, despite their normal IGFBP-3 levels *(49,86)*. Furthermore, acromegaloid facial changes in some patients indicate that the amount of IGF-I reaching tissues is supraphysiologic *(88,89)*.

The more likely explanation for the relatively modest growth response is the absence of the direct GH effects at

Table 3
IGF-I Treatment of Children with Primary GHI for One Year or Longer

Number	Age (yrs)	IGF-I dose (μg/k)	Height SDS[a] Pretreatment	1 yr	2 yr
European Study Group *(80)*					
26	3.7–19.6	40–120 bid	–6.8(1.6)	–6.1(1.5)	X
18[b]	3.7–16.7	40–120 bid	–6.4(1.7)	–5.6(1.6)	–5.2(1.9)
Ecuador *(82)*					
15	4.7–17.1	120 bid	–8.5(1.3)	–7.5(1.1)	–7.0(1.2)
7	3.1–15.2	80 bid	–8.0(1.8)	–7.2(1.8)	–6.7(1.8)
Israel *(86)*					
9[c]	0.5–14.6	150–200/d	–5.6(1.5)	–5.2(1.7)	X
6[d]	0.5–14.6	150–200/d	–6.2(1.5)	–6.0(1.6)	–5.8(1.2)
North Carolina Study (85)					
8	2.3–11	80–120 bid	–5.6 (1.1)	NA	–4.5(1.3)

[a]SD in parentheses. NA = not available
[b]Same cohort as all 26
[c]Patient age 0.5 excluded because of rapid GV 1st 6 mo of life before Rx (18 cm/yr)
[d]Same cohort as all 9

the growth plate. These effects include epiphyseal prechondrocyte differentiation, increased responsiveness to IGF-I, and enhancement of local production of IGF-I that stimulates clonal expansion of the differentiating chondrocytes *(90)*. Comparable ratios of change in height age to change in bone age over two years of treatment in children with GHI treated with rhIGF-I and GHD children treated with GH, however, suggest that the absence of a direct effect of GH may have a temporal rather than an absolute effect on long-term growth response of GHRD patients to rhIGF-I *(82)*. Longer-term studies of treatment to maximal height are needed to resolve this issue. Also of interest would be studies of the administration of recombinant IGFBP-3 with rhIGF-I. The pursuit of further treatment studies with rhIGF-I, however, is limited by the decision of manufacturers to no longer produce IGF-I.

Adverse Events

Hypoglycemia is frequent in children with GHRD, and was a concern with rhIGF-I treatment because of the very low IGFBP-3 levels resulting in greater amounts of free IGF-I. Severe hypoglycemic episodes have been reported in the European treatment study *(78)*. During a 6-mo placebo controlled trial of rhIGF-I treatment in children with GHRD, however, there was no difference in the frequency of hypoglycemia between placebo and treatment groups *(81)*. Headache is a frequent complaint among treated patients *(80)*, but also did not vary in frequency between placebo-treated and rhIGF-I-treated patients *(81)*. Pain at the injection site is common and injection into lumps may result in cessation of response. Tachycardia, reflecting the inotropic effect of IGF-I *(91)*, is uniformly present early in treatment, but clears after several months *(92)*. Less frequent side effects include parotid swelling, facial nerve palsy, lymphoid hyperplasia which may require tonsillectomy or adenoidectomy, papilledema, and pseudotumor cerebri *(80,81,85)*. Coarsening of the facial features with mandibular hyperplasia and excessive weight gain are also seen in some patients *(88,89)*. Hyperandrogenism manifested by oligomenorrhea or amenorrhea, acne, and elevated serum androgens has been described in prepubertal and young adult patients given single daily injections of rhIGF-I *(93)*.

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