INVITED REVIEW

Genetic control of growth

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Abstract

The application of the powerful tool molecular biology has made it possible to ask questions not only about hormone production and action but also to characterize many of the receptor molecules that initiate responses to the hormones. We are beginning to understand how cells may regulate the expression of genes and how hormones intervene in regulatory processes to adjust the expression of individual genes. In addition, great strides have been made in understanding how individual cells talk to each other through locally released factors to coordinate growth, differentiation, secretion, and other responses within a tissue. In this review I (1) focus on developmental aspects of the pituitary gland, (2) focus on the different components of the growth hormone axis and (3) examine the different altered genes and their related growth factors and/or regulatory systems that play an important physiological and pathophysiological role in growth. Further, as we have already entered the ‘post-genomic’ area, in which not only a defect at the molecular level becomes important but also its functional impact at the cellular level, I concentrate in the last part on some of the most important aspects of cell biology and secretion.

Introduction

Growth is an inherent property of life. Normal somatic growth requires the integrated function of many of the hormonal, metabolic, and other growth factors involved in the hypothalamo-pituitary growth axis. The application of the powerful tool molecular biology has made it possible to ask questions not only about hormone production and action but also to characterize many of the receptor molecules that initiate responses to the hormones. Therefore, significant progress has been made in unravelling the events that lead to the final cellular expression of hormonal stimulation. As more details of intracellular signalling emerge the complexities of parallel and intersecting pathways of transduction have become more evident. We are beginning to understand how cells may regulate the expression of genes and how hormones intervene in regulatory processes to adjust the expression of individual genes. In addition, great strides have been made in understanding how individual cells talk to each other through locally released factors to coordinate growth, differentiation, secretion, and other responses within a tissue. In this review I (1) focus on developmental aspects of the pituitary gland, (2) focus on the different components of the growth hormone axis and (3) examine the different altered genes and their related growth factors and/or regulatory systems that play an important physiological and pathophysiological role in growth. Further, as we have already entered the ‘post-genomic’ area, in which not only a defect at the molecular level becomes important but also its functional impact at the cellular level, I concentrate in the last part on some of the most important aspects of cell biology and secretion.

Development of the pituitary gland and its impact on hormonal deficiencies

Overview

Discovery of transcription factors responsible for pituitary cell differentiation and organogenesis has had an immediate impact on the understanding and diagnosis of pituitary hormone deficiencies. Importantly, combined pituitary hormone deficiencies have been associated with mutations in genes coding for transcription factors that control organogenesis or multiple cell lineages, whereas isolated hormone deficiencies are often caused by transcription factors controlling late cell differentiation. However, as there may be a strong phenotypic variability in familial combined pituitary hormone deficiency caused by different transcription factors, e.g. PROP1 (prophet of Pit1), it is of high clinical importance to have some knowledge about the various steps in pituitary gland development (Fig. 1) (1–12).

As summarized in Fig. 2 the formation of the pituitary gland involves many factors that control various
processes during development; these include factors for early patterning (Fig. 2; Rathke’s pouch: dorsal, Pax-6; ventral, Isl-1 and Brn-4; early pituitary gland: within anterior lobe, dorsal, Pax-6, Prop-1; ventral, Isl-1, Brn-4, Lhx-4 and GATA-2; within intermediate lobe, Six-3 and Pax-6) and organogenesis, for control of cell proliferation and, finally, for differentiation of individual lineages. Some of these transcription factors contribute to more than one process at different times. For example the Pitx-1 and Pitx-2 factors contribute to very early organogenesis, but they are also involved in late functions such as expansion of the gonadotroph and thyrothroph lineages in Pitx (13) and the control of transcription of hormone-coding genes (Figs 1 and 2).

**Lineage commitment and differentiation**

All lineages of the anterior and intermediate pituitary gland derive from the epithelial cells of the Rathke’s pouch which projects as a diverticulum from the roof of the stomadeum – in humans – in the middle of the fourth week (Figs 1 and 2). Molecular markers indicate that the pouch cells are not equivalent along the dorso-ventral axis, and this may be taken as an indication that the commitment to different pituitary lineages may be determined at an early developmental stage. In mice, at stage e9.5-11.5 (embryonic days 9.5–11.5), transcription factors such as Prop1 and Pax6 are preferentially expressed in the dorsal pouch, whereas factors Isl-1, Brn-4, Lhx-4 and GATA-2 are primarily expressed on the ventral side. Only one of these factors, Prop1, may be a commitment factor. Prop1 is initially expressed in the dorsal pouch and developing anterior pituitary where the somato-lactotrophs and definitive thyrothrophs will eventually appear (14, 15). Further Prop1 is required for expression of Pit1 (pituitary-specific transcription factor 1), which itself is necessary for differentiation of the same lineages (16). Therefore, if data suggest that Prop1 may commit the dorsal pituitary to give rise to the somato-lactotroph
and thyrotop lineages, there is no evidence of a counterpart of Prop1 that may commit the ventral pituitary to give rise to gonadotroph or corticotroph lineages. Isl1 and GATA-2 may be candidate factors for this function (12, 17, 18). Based on the description of gradients of signalling molecules and transcription factors in and around the development of the pituitary gland, a combinatorial model was proposed in which these regulatory molecules define territories within the developing gland. Only the unique combination of signals and/or factors would be responsible for differentiation towards one rather than the other lineages (19). This model, however, reflects the fact that precise relations among all the lineages are not clear at all (20). As it has been recently shown that the corticotroph and gonadotroph lineages, which both arise ventrally, may have a common precursor (17, 18, 21) a simple binary model to account for all pituitary differentiation events starting from a common precursor has been proposed (Fig. 1d). This model would be consistent with the clear commitment of the dorsal pituitary by Prop1 to form the pre-somato-lacto-thyrothroph precursors (PSLT), from which thyroid-stimulating hormone (TSH), growth hormone (GH) and prolactin (PRL) lineages will later arise through the action of Pit1, GATA-2 and other factors. Further, pre-corticol-gonadotrophs are committed at a similar time, and these will later be driven to differentiate into corticotrophs via pre-cortico-melanotrophs under the influence of Tpit or NeuroD1, or even later into gonadotrophs under the influence of SF1 and GATA-2. However, for the time being it is not clear which factors may commit the ventral pituitary to the pre-corticol-gonadotroph fate. Signalling gradients are also likely to be involved and affect differentiation in this model, but these gradients may rather act in a stochastic fashion on a cell-by-cell basis rather than by defining specific territories within the developing gland.

**Pituitary hormones and maintenance of normal cell function**

In addition, it is not only important to get a certain function, but also to maintain it. Therefore, it has to be highlighted that a specific cell function (production of any hormone) might be lost over time because of a lack of cellular crosstalk, as has to be suggested in patients suffering from PROP1 gene defects in terms of adrenocorticotropic hormone (ACTH) production (14). Further, not only the different transcription factors but also the distinct and well-tuned hormonal feedback loops (e.g. GH-releasing hormone (GHRH), GHRH receptor, GH, GH receptor (GRH) and insulin-like growth factor I (IGF-I)) may play a major role at the level of maintenance of each hormonal cell activity. As an example, GHRH receptor mutant mice (little mice) present with a hypoplastic anterior gland and phenotypically with dwarfism lacking GH secretion.
**Transcription factors of clinical importance**

**HESX1** The paired-like homeobox gene HESX1, a transcriptional repressor, has been implicated in patients suffering from septo-optic dysplasia (SOD), often referred to as 'de Morsier syndrome' (OMIM 182230). It is characterized by the classical triad of optic nerve hypoplasia and midline defects mainly combined with neuro-radiological abnormalities, such as agenesis of the corpus callosum and pituitary hypoplasia with consequent panhypopituitarism. Dattani et al. (22) described the first HESX1 gene defect (R160C) in two siblings. Importantly, the phenotype is highly variable, and may include any two of the three classical features even in the same family, suggesting incomplete penetrance (Table 1) (22–28). In addition to the two homozygous missense mutations (R160C, I26T), three heterozygous mutations (S170L, T181A and Q6H) have been described in association with milder phenotypes characterized by isolated GH deficiency (IGHD) with or without an ectopic posterior pituitary gland. Recently, a de novo heterozygous mutation (insertion, 306/307 ins AG) within exon 2 was reported in a family from Japan (26). This patient, however, presented with severe combined pituitary hormone deficiency (CPHD) including ACTH deficiency (26).

**PROP1** Wu et al. (29) described four families in which CPHD was associated with homozygosity or compound heterozygosity for inactivating mutations of the PROP1 gene. PROP1 (prophet of Pit1) is a paired-like homeodomain transcription factor and originally a mutation in this gene (S83P) was found to cause the Ames dwarf (dj) mouse phenotype (30). In mice, Prop1 gene mutation primarily causes GH, PRL and TSH deficiency, and in humans PROP1 gene defects also appear to be a major cause of CPHD. In agreement with the model of Prop1 playing a role in commitment of dorsal lineages (GH, PRL and TSH) Prop1 mutant mice exhibit a dorsal expansion of gonadotrophs that normally arise on the ventral side. To date, many different missense, frameshift and splicesite mutations, deletions and insertions have been reported and it has been realized that the clinical phenotype varies not only between the different gene mutations but also among the affected siblings with the same mutation (1, 2). In addition, although the occurrence of the hormonal deficiency varies from patient to patient (1), the affected patients as adults were not only GH-, PRL- and TSH-deficient, but also gonadotropin-deficient (Table 1). The three tandem repeats of the dinucleotide GA at location 296-302 in the PROP1 gene represents a hot-spot for CPHD (1–3). Low levels of cortisol have also been described in some patients with PROP1 gene mutations (31, 32). In addition, pituitary enlargement with subsequent involution has been reported in patients with PROP1 mutations (31). The mechanism, however, underlying this phenomenon remains still unknown.

**POU1F1 (PIT1)** The pituitary transcription factor PIT1 is a member of the POU family of homeoproteins, which regulates important differentiating steps during embryological development of the pituitary gland and regulates target gene function within the postnatal life (33–37). Further, it is 291 amino acids in length, contains a transactivation domain as well as two conserved DNA-binding domains, the POU homeodomain and the POU-specific domain (33–37). As PIT1 is confined to the nuclei of somatotropes, lactotrops and thyrotrops in the anterior pituitary gland, the target genes of PIT1 include GH, PRL and TSH subunits, and the POU1F1 gene itself (38). Therefore, the defects in the human POU1F1 gene known so far have all resulted in a total deficiency of GH and PRL, whereas a variable hypothyroidism due to an insufficient TSH secretion, at least during childhood, has been described (39–41) (Table 1). Although it is important to stress that the clinical variability is due to other factors than the exact location of the mutation reported, the type of inheritance, however, seems to correlate well with the genotype (34, 36, 37, 41–47). Beside one exception, which is a C-terminal-located mutation in the POU1F1 gene (V272ter), the following rule was deduced: mutations lying within the DNA-binding domains, either the POU-specific domain or the POU homeodomain, cause autosomal recessively inherited CPHD, whereas CPHD caused by mutations outside these two specific regions may follow the autosomal-dominant pattern of inheritance (48, 49). Further, the dominant-negative effect of the R271W POU1F1 form has been challenged recently by Kishimoto et al.

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**Table 1** Comparison of phenotypes caused by a defect of various transcription factors of the pituitary gland. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

<table>
<thead>
<tr>
<th>Factor</th>
<th>POU1F1 (PIT1)</th>
<th>PROP1</th>
<th>LHX3</th>
<th>HESX1</th>
<th>LHX4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior pituitary gland</td>
<td>Normal to hypo</td>
<td>Hypo to hyper</td>
<td>Normal</td>
<td>Hypo</td>
<td>Hypo</td>
</tr>
<tr>
<td>Posterior pituitary gland</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Ectopic eyes, brain</td>
<td>Ectopic skull defects</td>
</tr>
<tr>
<td>Other manifestations</td>
<td>None</td>
<td>None</td>
<td>Neck rotation: normal, 160–180°; patients, 75–85°</td>
<td>septo-optic dysplasia</td>
<td>Sella turcica</td>
</tr>
</tbody>
</table>

www.eje-online.org
LHX3 LHX3 encodes a LIM-type homeodomain protein that contains two N-terminal tandemly repeated LIM motifs and a C-terminal homeodomain with DNA-binding activity (51, 52). In Lhx3−/− mice, the Rathke’s pouch formed but failed to differentiate, except for the corticotroph lineage. Further, it is selectively expressed in both anterior and intermediate pituitary in the mature mice and is also transiently expressed in the developing ventral neural cord brainstem (52). The Lhx3 protein acts synergistically with the Pit1 protein to transcriptionally activate genes that control pituitary differentiation. Two mutations in LHX3, a missense mutation changing a tyrosine to a cysteine (Y116C) and an intragenic deletion that results in a truncated protein lacking the DNA-binding homeodomain, have been identified in humans. These mutations were identified in patients with retarded growth and combined pituitary hormone deficiency, except for ACTH, and also abnormal neck (rigid cervical spine leading to limited head rotation) and cervical spine development were described (Table 1) (53). Further, in some of the affected subjects severe pituitary hypoplasia was diagnosed, whereas one patient presented at the age of 19 years an enlarged anterior pituitary gland that had not been documented 10 years earlier (53).

LHX4 Machinis et al. (54) reported a family with an LHX4 germline splice-site mutation that resulted in a disease phenotype characterized by short stature due to GHD (as well as deficits of other anterior pituitary hormones associated with hypoplastic anterior hypophysis) and by pituitary and hindbrain defects (Chiari malformation type I; OMIM 118420) in combination with abnormalities of the sella turcica of the central skull base (Table 1). Most importantly, the affected subjects presented with a heterozygous intronic point mutation (G to C transversion) involving the invariant dinucleotide (AG) of the acceptor splice site preceding exon 5 (54).

SOX3 Sox3 is a member of the Sox family (approximately 20 genes) that encode a group of proteins carrying a 79-amin acid DNA-binding domain (HMG box) (55). Recently, in both mice and humans, Sox3/ SOX3, which in humans is a single exon gene on the X chromosome (Xq26-17), has been implicated in X-linked hypopituitarism (55–57). In humans, in a single pedigree with X-linked GHD and mental retardation, a polyalanine expansion (26 alanine residues instead of 15) was identified in SOX3 C-terminal to the HMGbox domain (55). The phenotype consists of variable mental retardation, facial anomalies and isolated GHD (IGHD). The final height of the untreated subjects ranged from 135 to 159 cm. Additionally, a deletion of nine alanine residues within the same polyalanine tract was found in two boys with mental retardation, short stature, microcephaly and abnormal faces (58). This may be a gene of high clinical as well as scientific importance as, interestingly, another group of patients with X-linked hypopituitarism harbour duplications of the region of the X chromosome that includes SOX3. The phenotype in these subjects is most likely due to the increased dosage of the gene (59).

Different components of the GH axis

The GHRH-GH axis is shown in Fig. 3. GH is regulated by two hypothalamic peptides, GHRH, which is stimulatory, and GH-inhibiting factor (GHIF), which is inhibitory. There are membrane receptors for both GHRH and GHIF (somatostatin) on anterior pituitary cells. These two peptides are in turn influenced by an array of neurotransmitters. Pituitary GH encoded by the GH-1 gene is secreted in pulses and binds to GHR in the liver and other target organs. Receptor occupancy increases production and release of IGF-I. This mediator of GH action binds to IGF-I receptors (IGF-IRs) in target tissues such as growth plates at the end of the long bones. There is a tight feedback control of GH release, involving GH and IGF-I in regulation of GHIF and probably GHRH. Additional genes are of importance to the GHRH-GH axis including pituitary transcription factors (e.g. PIT1 and POU1F1). Further, classification of genetic defects in the development of the GH axis illustrates that basically the site of these defects, both reported and hypothetical, may be located at any level from the hypothalamus to the target receptors of skeletal tissues (Fig. 3, Table 2).

Classification of isolated GHD

Structure and function of GH and CS genes

The GH gene cluster consists of five very similar genes in the 5’-to-3’ order GH-1, CSHP (chorionic somatomammotropin pseudogene), CSH-1 (chorionic somatomammotropin gene), GH-2 and CSH-2, encompassing a distance of about 65 kb on the long arm of chromosome 17 at bands q22-24 (Fig. 4) (73). The GH-2 gene encodes a protein (GH-V) that is expressed in the placenta rather than in the pituitary gland and differs from the primary sequence of GH-N (the product of the GH-1 gene) by 13 amino acids. This hormone replaces pituitary GH in the maternal circulation during the second half of pregnancy (74). The CSH-1 and CSH-2 genes encode proteins of identical
Regulation of GH secretion/GHRH-GH axis. GH secretion is regulated by various factors. The sites of derangements responsible for various familial disorders of the GH axis are indicated on the right.

Table 2

<table>
<thead>
<tr>
<th>Hypothalamus</th>
<th>Pituitary gland</th>
<th>Liver</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factors</td>
<td>Transcription factors</td>
<td>TPIT</td>
<td>IGF-1 Receptor</td>
</tr>
<tr>
<td>GHRH gene</td>
<td>GHRH receptor</td>
<td>SOX3</td>
<td>GH receptor (primary: extracellular, transmembrane, intracellular)</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>GH gene cluster</td>
<td>HESX1</td>
<td>GH receptor (primary: extracellular, transmembrane, intracellular)</td>
</tr>
<tr>
<td>GH-deficiency/bio-inactivity</td>
<td>GH insensitivity</td>
<td>LHX3</td>
<td>GH insensitivity (secondary)</td>
</tr>
<tr>
<td>GH target organs</td>
<td>Signalling (JAK2/STAT5b/ERK)</td>
<td>LHX4</td>
<td>Malnutrition (e.g. anorexia)</td>
</tr>
<tr>
<td>GH receptor (primary: extracellular, transmembrane, intracellular)</td>
<td>GH insensitivity (secondary)</td>
<td>PROP1</td>
<td>Liver disease (e.g. Byler's disease)</td>
</tr>
<tr>
<td>GHRH gene</td>
<td>GH target organs</td>
<td>POU1F1</td>
<td>Chronic illness</td>
</tr>
<tr>
<td>GH-deficiency/bio-inactivity</td>
<td>GH target organs</td>
<td>GHRH receptor</td>
<td>Anti-GH antibodies</td>
</tr>
<tr>
<td>IGF-I defects</td>
<td>GH target organs</td>
<td>GH gene cluster</td>
<td>IGF-I defects</td>
</tr>
<tr>
<td>IGF-I transport/metabolism/clearance</td>
<td>GH target organs</td>
<td>GH gene cluster</td>
<td>IGF-I transport/metabolism/clearance</td>
</tr>
<tr>
<td>IGF-1 receptor defect (type I)</td>
<td>GH target organs</td>
<td>GH gene cluster</td>
<td>IGF-1 receptor defect (type I)</td>
</tr>
<tr>
<td>IGF-1 signalling (post-receptor defect)</td>
<td>GH target organs</td>
<td>GH gene cluster</td>
<td>IGF-1 signalling (post-receptor defect)</td>
</tr>
</tbody>
</table>

Figure 3 Regulation of GH secretion/GHRH-GH axis. GH secretion is regulated by various factors. The sites of derangements responsible for various familial disorders of the GH axis are indicated on the right.

Figure 4 Deletions of various loci in the GH gene cluster. The locations and sizes of the deletions within the GH gene cluster are indicated. Further, a schematic representation of the GH gene cluster and its localization on the long arm of chromosome 17 is shown. Exons, introns and untranslated sequences are depicted by solid, open and shaded rectangles, respectively. The sizes are indicated in kb.
sequences, whereas CSHP encodes a protein that differs by 13 amino acids and contains a mutation (donor splice site of its second intron) that should alter its pattern of mRNA splicing and, therefore, the primary sequence of the resulting protein (73). The extensive homology (92–98%) between the immediate flanking, intervening and coding sequences of these five genes suggests that this multigene family arose through a series of duplicational events (75). With the exception of CSHP, each gene encodes a 217-amino-acid pre-hormone that is cleaved to yield a mature hormone with 191 amino acids and a molecular mass of 22 kDa. The expression of GH-1 gene is controlled by cis- and trans-acting elements and factors. In Fig. 5, the 5’ untranslated and promoter regions of the human GH-1 gene are depicted. In addition in this figure, well-known and putative or inferred binding sites of transcription factors are indicated.

**Familial isolated GHD**

Short stature associated with GHD has been estimated to occur in about 1/4000–1/10 000 in various studies (76–79). Whereas most cases are sporadic and believed to result from environmental cerebral insults or developmental anomalies, 3–30% of cases have an affected

**Figure 5** Structure of the 5’ untranslated and promoter region of the human GH-1 gene. Transcription is regulated by proteins (trans-acting factors) that bind to regulatory (cis-acting) elements. The first nucleotide of the start site is designated +1, by convention, and the 5’ nucleotides are counted backwards from −1. Known, putative and inferred binding sites for transcription factors are indicated by bold and underlining. In addition, the TATA box, a Chi-like element and the ATG translation initiation sites are underlined. CRE, cAMP-responsive element; GRE, glucocorticoid-responsive element; TRE: thyroid hormone responsive element; POU1F1: Pit1 (pituitary-specific transcription factor); USF, upstream stimulatory factor; NF1, nuclear factor 1; SP1, specificity protein 1; ΔG, deletion of a G. Further, the polymorphic sites are indicated.
first-degree relative suggesting a genetic aetiology (77, 78). Since magnetic-resonance examinations detect only about 12–20% of anomalies of either the hypothalamus or pituitary gland in patients suffering from IGHD one might assume that many genetic defects may not be diagnosed and that a significantly higher proportion of sporadic cases may indeed have a genetic cause (80). Familial IGHD is associated with at least four Mendelian disorders (Table 3). These include two forms that have autosomal-recessive inheritance (IGHD types IA and IB) as well as autosomal-dominant (IGHD type II) and X-linked (IGHD type III) forms (81).

**IGHD type IA** IGHD type IA was first described by Illig (82) in three Swiss children with unusually severe growth impairment and apparent deficiency of GH. Affected individuals occasionally have short length at birth and hypoglycaemia in infancy but uniformly develop severe growth retardation by the age of 6 months. Their initial good response to exogenous GH was hampered by the development of anti-GH antibodies leading to dramatic slowing of growth (82, 83).

**GH-1 gene deletions** In 1981, Phillips et al. (84) examined genomic DNA from the Swiss children reported on by Illig and discovered using Southern blotting that the GH-1 gene was missing (84). Subsequently, additional cases of GH-1 gene deletions have been described as responding well to GH treatment, making the presence of anti-GH antibodies an inconsistent finding in IGHD type IA patients with identical molecular findings (homozygosity for GH-1 gene deletions) (85). The frequency of GH-1 gene deletions as a cause of GHD varies among different populations and the criteria and definition of short stature chosen. Analysing patients with severe IGHD (≤ −4 to −4.5 SDS) the prevalence reported was 9.4% in Northern Europe (n = 32), 13.6% in the Mediterranean region (n = 22), 16.6% in Turkey (n = 24), 38% in Oriental Jews (n = 13), 12% in Chinese (n = 26) and 0% in Japanese (n = 10) (86–89). The sizes of the deletions are heterogeneous, with the most frequent (70–80%) being 6.7 kb (86). The remaining deletions described include of 7.6, 7.0 and 45 kb, and a double deletion within the GH gene cluster (Fig. 4). At a molecular level these deletions involve unequal recombination and crossing over within the GH gene cluster at meiosis (90, 91). Interestingly, crossing over is reported to occur in 99% homologous regions (594 bp) flanking the GH-1 gene, rather than in Alu repeat sequences (91). Although Alu repeats, which are frequent sites of recombination, are adjacent to the GH-1 gene, they were not involved in any of the recombinational events studied. These highly homologous regions flanking the GH-1 gene were used in a PCR amplification method to screen for gene deletions (88). Inasmuch as the fusion fragments associated with the 6.7 kb deletions differ in the size of fragments produced by certain restriction enzymes (Smal), homozygosity and heterozygosity for these deletions can easily be detected by enzyme digestion following PCR amplification (92). The PCR approach is rapid, requires very small quantities of DNA and can even be done on filter-paper spots of capillary blood samples.

**GH-1 gene frameshift and nonsense mutations** The frameshift and nonsense mutations diagnosed so far causing the different types of GHD are summarized in Table 4. It is worthwhile stressing that single base-pair deletions and nonsense mutations of the signal peptide result in an absent production of mature GH and are bound to produce anti-GH antibodies on exogenous replacement therapy.

**IGHD type IB** Patients with IGHD type IB are characterized by low but detectable levels of GH (<7 mU/l; <2.5 ng/ml), short stature (<−2 SDS for age and sex), significantly delayed bone age, an autosomal-recessive inheritance (two parents of normal height; two siblings affected), no demonstrable direct and/or endocrine cause for IGHD, and a positive response and immunological tolerance to treatment with exogenous GH. This subgroup of IGHD has been broadened and reclassified on the basis of the nature of their GH gene defects and now includes splice-site mutations of the GH gene; even an apparent lack of GH has been found by RIA. The phenotype of IGHD type IB, therefore, is more variable than type IA. In one family, the children may resemble IGHD type IA, whereas in other families, growth during infancy

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**Table 3** Types of IGHD.

<table>
<thead>
<tr>
<th>Category</th>
<th>Inheritance</th>
<th>GH RIA</th>
<th>Candidate gene</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGHD type IA</td>
<td>Recessive</td>
<td>Absent</td>
<td>Human GH-1</td>
<td>Deletions/mutations, frameshift</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human GH-1</td>
<td>Splice-site mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GHRH</td>
<td>Unlikely</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GHRH receptor</td>
<td>Mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trans-acting factors</td>
<td>Mutations/deletions</td>
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<td></td>
<td></td>
<td></td>
<td>Cis-acting elements</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Human GH-1</td>
<td></td>
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<tr>
<td>IGHD type II</td>
<td>Dominant</td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHD type III</td>
<td>X-linked</td>
<td>Low</td>
<td>Unknown</td>
<td></td>
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</table>

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is relatively normal and growth failure is not noted until mid-childhood. Similarly, GH may be nearly lacking or simply low following stimulation testing. This heterogeneous phenotype suggests that there is more than one candidate gene causing the disorder. Possible candidate genes involved are noted in Table 3.

Splice-site and nonsense mutations and frameshifts within the GH-1 gene The GH-1 gene has often been amplified and screened for small deletions and mutations that have been found and described (Table 4). However, generally speaking functional studies are necessary to prove the importance of all these alterations found in any gene. Therefore, for instance, all the mutations causing a suggested mRNA splicing error need transfection of the mutant gene into a cultured cell system allowing reverse transcription followed by cDNA sequencing. Thereafter, the impact of the changes of the amino acids encoded by the mutant genes is studied. Studies with bovine GH mutants have shown that not only the stability and biological activity of the mutants may be altered but also the intracellular targeting of GH protein products to the secretory granules important for secretion may be deranged (110, 111).

<table>
<thead>
<tr>
<th>Table 4 Mutational spectrum of GHD.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microdeletions</strong></td>
</tr>
<tr>
<td><strong>Deficiency</strong> <strong>type</strong></td>
</tr>
<tr>
<td>IA</td>
</tr>
<tr>
<td>IA</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>IA</td>
</tr>
<tr>
<td><strong>Single-base-pair substitutions in the GH-1 gene coding region</strong></td>
</tr>
<tr>
<td><strong>Mutation</strong></td>
</tr>
<tr>
<td>IA</td>
</tr>
<tr>
<td>IA</td>
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<tr>
<td>II</td>
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<td>II</td>
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IGHD, mutational changes in genes specific to the GHRH-GH axis are of importance and there is a need to focus on them.

**GHRH gene** Ours and several other laboratories have tried to define GHRH gene alterations and have failed so far (112, 113). Therefore, if GHRH mutations do cause IGHD in humans, they must be very rare.

**GHRH receptor gene** In 1992, Mayo (114) cloned and sequenced the rat and human GHRH receptor gene which provided the opportunity to examine the role of the GHRH receptor in growth abnormalities that involve the GH axis. Sequencing of the GHRH receptor gene in the little mouse (lit/lit) showed a single-nucleotide substitution in codon 60 that changed aspartic acid to glycine (D60G), eliminating the binding of GHRH to its own receptor (115). As the phenotype of IGHD type IB in humans has much in common with the phenotype of homozygous lit/lit mice, including autosomal-recessive inheritance, features of growth retardation, diminished secretion of GH and IGF-I, proportionate reduction in weight and skeletal size, and delay in sexual maturation, the GHRH receptor gene was searched for alteration in these patients suffering from IGHD type IB (116, 117). In our laboratory, 65 children with IGHD type IB were studied, of whom 12 did not respond to exogenous GHRH. None of the analyses revealed any structural abnormalities in these patients (116). However, it has to be mentioned that at that time this study was limited due to its ability to analyze only the sequence of the extracellular domain of the GHRH receptor gene. The GHRH receptor is a member of a large family of heptahelical transmembrane receptors that couple to G proteins upon receptor activation. Binding of GHRH to GHRH receptors expressed on the surface of somatotroph cells activates G_s and leads to a consequent increase in cAMP synthesis that induces cellular proliferation and GH secretion. Wajnrajch et al. (117) reported a nonsense mutation similar to the little mouse in an Indian Muslim kindred. Furthermore, in two villages in the Sindh area of Pakistan, Baumann & Maheshwari (118) reported another form of severe short stature caused by a point mutation in the GHRH receptor gene resulting in a truncation of the extracellular domain of this receptor. Individuals who are homozygous for this mutation are very short (~7.4 SDS) but normally proportioned. They appear of normal intelligence, and at least some are fertile. Biochemical testing revealed that they have normal levels of GHRH and GH-binding protein (GHBP), but undetectable levels of GH and extremely low levels of IGF-I. Later, families from Sri Lanka, Brazil, the United States, Spain and Pakistan were reported (119–122). Mutations in the GHRH receptor gene have been described as the basis for a syndrome characterized by autosomal-recessive IGHD and anterior pituitary hypoplasia, defined as pituitary height more than 2 SDS below age-adjusted normal, which is likely due to depletion of the somatotroph cells (OMIM 139190). In a most recent report, however, certain variability in anterior pituitary size, even in siblings with the same mutation, was described (123).

**Specific trans-acting factor to the GH gene** Any alteration to the specific transcriptional regulation of the GH-1 gene may produce IGHD type IB (Fig. 5). Mullis et al. (124) have reported a heterozygous 211 bp deletion within the retinoic acid receptor a gene, causing the IGHD type IB phenotype.

**IGHD type II** The autosomal-dominant form of IGHD type II (IGHD II) is mainly caused by mutations within the first 6 bp of intervening sequences 3 (5’IVS-3) (125), which result in a missplicing at the mRNA level and the subsequent loss of exon 3, producing a 17.5 kDa human GH isoform (106). This GH product lacks amino acids 32-71 (del32-71GH), which is the entire loop that connects helix 1 and helix 2 in the tertiary structure of human GH (126, 127). Skipping of exon 3 caused by GH-1 gene alterations other than those at the donor splice site in 5’IVS-3 has also been reported in other patients with IGHD II. These include mutations in the exon splice enhancer (ESE1 in exon 3 (E3): E3 + 1G → T, ESE1m1; E3 + 5A → G, ESE1m2) and within the suggested intron splice enhancer (ISE: IVS-3 + 28G → A, ISEm1; IVS-3del + 28-45, ISEm2) sequences (94, 105, 107, 108, 125, 128, 129). Such mutations lie within purine-rich sequences and cause increased levels of exon 3-skipped transcripts (94, 105, 108, 125, 128, 129), suggesting that the usage of the normal splicing elements (ESE1 at the 5’ end of exon 3 as well as ISE in intron 3) may be disrupted (94, 128, 129). The first seven nucleotides in exon 3 (ESE1) are crucial for the splicing of GH mRNA (130) such that some nonsense mutations might cause skipping of one or even more exons during mRNA splicing in the nucleus. This phenomenon is called nonsense-mediated altered splicing; its underlying mechanisms are still unknown (131). In addition to the above-described splice-site mutations that result in the production of del32-71GH, three other mutations within the GH-1 gene (missense mutations) are reported to be responsible for IGHD II, namely the substitution of leucine for proline, histidine for arginine and phenylalanine for valine at amino acids 89 (P89L), 183 (R183H) and 110 (V110F), respectively (98–100).

At the functional level, the 17.5 kDa isoform exhibits a dominant-negative effect on the secretion of the 22 kDa isoforms in both tissue culture and transgenic animals (132–134). The 17.5 kDa isoform is initially retained in the endoplasmic reticulum, disrupts the Golgi apparatus, impairs both GH and other hormonal trafficking (135), and partially reduces the stability of
Circulation). A variety of studies have indicated that
abnormally low IGF-I in the face of normal to high GH in
circulation (146, 147). In its purest and most dramatic forms, primary IGFD has been identified with three classes of molecular defect: (1) GH insensitivity syndrome (GHIS) resulting from mutations within the GHR gene, primarily called Laron’s syndrome (148, 149), (2) genetic defects affecting the GH signalling pathway; mainly the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 5b (STAT5b) (150, 151) and (3) deletions or mutations of the IGF-I gene itself (152). Further, the concept of dysfunctional GH variants and/or bio-inactive GH molecules has been proposed for years (153) and opens an interesting platform to study the elements between GHD and IGFD, as some of these patients excellently respond to the exogenous GH treatment. In addition, there are reports on abnormal GHR signalling in children with ISS in the absence of any GHR or GH gene alteration (154, 155).

Syndrome of bio-inactive GH

The diagnosis ‘syndrome of bio-inactive GH’ has often been discussed and suggested in short children with a phenotype resembling IGHD but who had normal or even slightly elevated basal GH levels in combination with low IGF-I concentrations that increased after treatment with exogenous GH, excluding the diagnosis of Laron syndrome. Takahashi et al. described two cases heterozygous for point mutations in the GHI-I gene (R77C and D112G) (101, 156, 157). The R77C GH mutant bound with unusually high affinity to the GHBp and abnormally to the GHR. Further, it was able to inhibit tyrosine phosphorylation in the GH signalling pathway, presumably acting in a dominant-negative fashion as a GH antagonist, as IGF-I levels were not measurable following exogenous recombinant human GH (rhGH) treatment. However, as the patient’s father, who was also heterozygous for the same mutations, was phenotypically normal and of normal stature (101) many questions remain unanswered. The D112G mutant involved a single A → G substitution in exon 4 in a girl with short stature (157). The locus of the mutation was found within site 2 of the GH molecule in binding to the GHR/GHBp, which purportedly prevented dimerization of the GHR (157). The patient presented with high levels of GH and low levels of IGF-I, but responded well to the rhGH, and not only IGF-I increased but also the height velocity, leading to improved somatic growth (11 cm/year compared to 4.5 cm/year before therapy). Therefore, the authors claimed to report in this girl the first patient affected with a “real” bio-inactive GH. In addition, in a most recent report Millar et al. (137) described several dysfunctional GH variants associated with a significantly reduced ability to activate GHR-mediated JAK/STAT signal transduction.

IGF-I deficiency/GH insensitivity

Because IGF-I plays a pivotal role in growth, where it mediates most, if not all, of the effects of GH, GHD could also be considered somehow as a IGF-I deficiency (IGFD) (Table 2). Although IGFD can develop at any level of the GHRH-GH-IGF axis I would like to differentiate; however, between GHD (absent or low GH in circulation) and IGFD (normal to high GH in circulation). A variety of studies have indicated that approximately 25% of children evaluated for idiopathic short stature (ISS) have primary IGFD presenting with
**GH insensitivity and defects in the GHR gene**

Our understanding of the mechanism of action of GH has increased significantly since the characterization of the GHR and demonstration of a partial gene deletion in two patients with Laron-type dwarfism by Godowski et al. (158). GHR is a transmembrane receptor that is a member of the cytokine receptor superfamily. It has a soluble, circulating counterpart (GHBP) that consists of the extracellular domain and displays GH-binding activity in humans (159). The GHR gene was characterized by Godowski et al. (158) in 1989 who demonstrated that the coding and 3' untranslated regions of the receptor are encoded by nine exons, numbered 2-10. Exon 2 corresponds to the secretion signal peptide, exons 3-7 encode the extracellular domain, exon 8 encodes the transmembrane domain and exons 9 and 10 contain the cytoplasmic domain and the 3' untranslated region. Physiologically, the biological actions of GH are mediated through the activation of the GHR. As each GH molecule has two highly specific binding sites, ensuring the binding of GH to the GHR, a homodimer structure of the receptor is formed (160). Dimerization of the GHR following GH binding is the first step and a key event in the activation of target cells. Subsequently, tyrosine phosphorylation of JAK2 and STAT5 proteins plays a crucial role in the activation process, which ultimately results in gene transcription.

The GHR has been implicated in GHIS, a rare autosomal-recessive GH-insensitive form of short stature, first described in a group of Oriental Jewish children and reviewed by Laron, which is characterized by low serum concentrations of IGF-I and high levels of circulating GH (148, 161, 162). In addition, GHIS is confirmed by the failure of exogenously administered GH to elevate levels of IGF-I or IGF-binding protein 3 (IGF-BP3) significantly. In contrast, GHBP was initially found to be absent, but more recent reports have suggested that some patients with GHIS may also have normal levels of GHBP (158, 159). Genetic and mutation analyses have verified the high molecular heterogeneity of this syndrome: to date, more than 50 different mutations in nearly 300 cases worldwide have been identified (162–165). All classes of alteration have been reported: deletion, frameshift, nonsense, missense and splicing defects (166). Of these, missense mutations are of particular interest as they have the potential to provide critical information on the structure/function relationship of the GHR and related molecules. Patients with atypical forms of GHIS have detectable plasma GH-binding activity, associated with complete or partial GHIS (146, 167). However, molecular analyses of the phenotype with complete GHIS have revealed the existence of a missense mutation in the exoplasmic domain mainly located in exons 2-7 that impairs first receptor action by affecting GH binding and second, therefore, abolishes receptor homodimerization, thereby providing in vivo evidence for the critical role of the dimerization process in the growth-promoting action of GH (168). Similarly, missense mutations in the cytoplasmic region, which would not be expected to affect GH-binding activity, should contribute to the identification of other important domains involved in signal transduction. The intracellular tyrosine kinase JAK2 is associated with the cytoplasmic tail of GHR. After GH binding, two JAK2 molecules are brought into close proximity resulting in cross-phosphorylation of both each other and tyrosine residues on the cytoplasmic tail on GHR. These phosphotyrosines act as docking points for cell signalling intermediates such as STAT5 (166). STAT5 binding to phosphorylated receptor tail then brings it into close proximity to JAK2, resulting in its own phosphorylation by JAK2. Phospho-STAT5 dimerizes and translocates to the nucleus in which it transactivates GH-responsive genes leading to the observed biological effects of GH (166).

Interestingly, one patient with GHIS was reported to have mutations located in the intracellular region (169). Surprisingly, this patient, having very low serum GH-binding activity, presented two mutations on a single GHR allele (C422F and P561T), whereas no other abnormality was detected on the remaining allele. The P561T mutation has already been excluded to be of any importance in causing the disorder by simply studying a sufficiently large control group (170). Therefore, most importantly, in vitro studies are required to test the functional consequences of all these identified missense mutations.

In theory, partial GHIS could encompass a wide range of distinct phenotypes with variable degrees of GH resistance (146, 171). Heterogeneity could result from a missense GHR mutation or from a quantitative GHR mRNA defect due to a mutation in the promoter, or to abnormal RNA maturation; this latter hypothesis was indeed recently confirmed (164, 172–174). As previously mentioned, because GHBP is basically cleaved from the extracellular portion of the GHR, it is common knowledge that serum GHBP concentration is generally decreased in GHIS caused by any GHR gene defect in exons 2-7. There are, however, several cases of GHR defects reported associated with normal or raised GHBP levels in patients with mutations involving extracellular, transmembrane or intracellular domains. Duquesnoy et al. (168) reported a D152H mutation in exon 6 causing positive GH-binding activity but abolished GHR homodimerization. In contrast, in two subjects with severe GHIS caused by a 5' splice donor site mutation within IVS-8, serum GHBP was massively increased, because the mutation resulted in a truncated GHR molecule (175). In fact, complete exon 8 was skipped, producing a mutant GHR protein lacking transmembrane and intracellular domains. The authors predicted that this mutant protein would not be anchored in the cell membrane and would be
measurable in the circulation as GHBP, hence explaining the phenotype of severe GH resistance combined with elevated circulating GHBP (175). Interestingly, a similar defect was reported by Silbergeld et al. (176). Analysis of the GHR gene performed in the proband revealed a G → T substitution at nucleotide 785, one nucleotide before exon 8 (the 3' acceptor site). This mutation, which destroys the invariant dinucleotide of the splice acceptor site, is expected to alter GHR mRNA splicing and to be responsible for skipping exon 8 (176). Furthermore, two defects have been described which were associated with autosomal dominant GHRIS (177, 178). First, a single G → C transversion in the 3'-splice acceptor site of intron 8 causing skipping of exon 9 (177); second, a G → A transversion at the +1 position of the 5'-donor splice site of intron 9 causing skipping of exon 9 and a premature stop codon in exon 10 (178). At the functional level it has been shown that GH-induced tyrosine phosphorylation of STAT5 is inhibited and caused autosomal-dominant GHRIS (179).

Evidence is accumulating that abnormalities in the intracellular signalling of GHR distal to the intracellular domain but proximal to IGF-I synthesis can also cause GHRIS (180). In this report, Clayton et al. (180) described two families with a defective signalling pathway. In the first (D152H mutation of the GHR gene) neither STAT nor the mitogen-activated protein kinase (MAPK) pathways were activated, whereas in the second GH activated STAT but failed to activate MAPK (180).

Until recently, although several patients with a phenotype of GHRIS and a normal GHR gene have been described and no specific molecular downstream defect of the GHR identified (154), there is only one patient reported so far with the clinical and biochemical characteristics of GHRIS presenting a homozygous missense mutation in the gene for STAT5b (151). As child suffering from a IGF-I gene defect experienced respiratory difficulties with increased oxygen requirements (151, 152).

Furthermore, mutations of the GHR gene were reported recently in a study on a highly selected group of patient with ISS (181). In this analysis, four out of 14 children presented mutations in the region of the GHR gene, which codes for the extracellular domain of the receptor. One of the four children with mutations was a compound heterozygote, with one mutation that reduced the affinity of the receptor for GH and a second mutation that may affect function other than ligand binding (181). The remaining three patients had a heterozygous mutation in the GHR gene. However, it is of importance to stress that in one patient the mother presented the same heterozygous mutations but was of normal stature. Follow-up studies on the possible impact of heterozygous GHR gene mutations highlight the impact on short stature, especially in ISS (182). Indeed, whereas many obligate carriers of GHRIS have obtained normal height, others have not (149, 183). However, it may be a challenging concept that GHR gene mutations are responsible for about 5% of all ISS patients and it underscores the fact that these mutations should be considered when other causes of short stature have been eliminated. In addition, abnormal GHR signalling may also underlie ISS even in the absence of GHR gene mutations (154, 184). Although until recently it had been assumed that GH signalling following GHR homodimerization was mediated primarily by the JAK/STAT pathway and that the extracellular signal regulated kinase (ERK) pathway does not induce hepatic IGF-I production (185), a novel dysfunctional GH variant (I179M) exhibiting a decreased ability to activate the ERK pathway, resulting in short stature, has been described (186).

It could also be anticipated that, in some instances, the GHR gene is not involved in the GH-resistant phenotype, a hypothesis which can be tested by means of genetic linkage using the described intragenic GHR polymorphisms. This could help to identify other genes that control GHR expression or are required at different steps of the signal transduction pathway (187). In this regard, the availability of a possible animal model (e.g. sex-linked dwarf chicken strains) for Laron syndrome could open new ways in the identification of GH-inducible genes (188).

**Primary defects in IGF-I**

**IGF-I synthesis** Ours and many laboratories have made intense searches to find gene alterations of the IGF-I gene causing GH resistance (189, 190). In 1996, Woods et al. (152) reported a patient, a 15 year old boy, who had severe intrauterine and postnatal growth retardation, sensorineural deafness, mental retardation and hyperactivity, due to a homozygous deletion of exons 4 and 5 of the IGF-I gene (152). Importantly, the parents were heterozygous for the same defect and possibly slightly affected as they were rather small and presented with slightly low IGF-I levels. This report remains up to now the only confirmed case of an IGF-I gene defect (191). This patient is of particular interest in that he presents a unique opportunity to unravel the direct effects of GH from its indirect effects via IGF-I. Treatment for 1 year with IGF-I improved the patient’s height velocity from 3.8 to 7.8 cm/year, normalized his GH levels and improved his insulin sensitivity (192). In addition, focusing on the metabolic effects it has been shown that recombinant human IGF-I improved body composition and normalized the insulin sensitivity (193).

Furthermore, there is increasing evidence that IGF-I might be a major determinant of fetal growth. IGF-I knockout mice are born at 60% of their expected weight. This raises the possibility that defects of the IGF-I gene may contribute significantly to impaired
fetal growth, which has been recently studied. However, Johnston et al. (184, 194) concluded that IGF-I gene defects are likely only to be a very rare cause of impaired intrauterine growth.

**IGF-I resistance** IGF-IR−/− mice were shown to be severely affected (birth weight was 45% of normal weight) with the affected neonates dying from respiratory depression (195, 196). More than 50 mutations of the human insulin receptor gene have been reported until now (197). In contrast, there is only one recent report on two patients with IGF-IR gene alterations (198). Patient 1 presented with a compound heterozygosity for missense mutations (R108Q and K115N) within the highly conserved ligand-binding domain of the IGF-IR gene resulting in high GH and IGF-I serum concentrations reflecting severe IGF-I resistance (198). Patient 2 was heterozygous for the point mutation C → A leading to a stop codon (TAG; R59X) in exon 2 (198). This fact may lead to most interesting studies in future. Patients who are haploinsufficient for the IGF-IR gene because of an aneuploidy of chromosome 15 typically are dysmorphic and mentally as well growth retarded (199). However, although the impact of the loss of contiguous genes on chromosome 15 is unclear, a clear gene-dosage effect on somatic growth was suggested in patient 2. In addition, the parents of patient 1 had marginal growth retardation at birth and an adult height substantially below the population mean. Further, many unexpected findings in these patients are awaiting an answer: (1) bone age being less delayed than is typical in GHD, (2) doubling of the height velocity on exogenous GH, (3) normal mental development and (4) highly variable phenotype (198).

**IGF-I transport/clearance** Two possible clinical syndromes involving primary defects of IGF-I transport could theoretically present with growth failure (12). The first would be an excess of IGF-BPs, which might compete with the IGF-IR for binding and, therefore, inhibit IGF-I action. Further, in order to remain functionally in circulation the normal formation of the ternary complex (acid-labile subunit, IGF-BP and IGF) is necessary. Any defect at that level might have an impact on clearance as IGF peptides are not effectively bound and specific half-lives are changed. Along that line, Barreca et al. (200) reported a boy of short stature associated with high IGF-BP1 and high IGF-II levels responsive to exogenous rhGH treatment. They speculated that the increased IGF-BP1 levels may inhibit (1) the biological activity of IGF-I (IGF-I resistance), (2) the formation of the 150 kDa ternary complex (increased clearance) and (3) the feedback action on GH (increased GH levels) resulting in reduced stature.

**Cell biology/post-genomic defects**

As we have already entered the ‘postgenomic’ area it is most important to broaden our views and to focus, having defined the possible disorders at the DNA/RNA level, on function and to re-analyse the specific defects at the cellular level. An example is autosomal-dominant isolated GHD (IGHD II). Heterozygous GH-I gene mutations yielding an unfolded or misfolded GH protein do not have to cause GHD ultimately. Some are dominant and others are recessive. This fact is of importance and suggests possible mechanisms in the secretory pathway. Furthermore, IGHD II caused by various gene defects may produce the same clinical phenotype. However at the cellular level the disorder does have distinctive causes. Normally, secretory proteins are synthesized on polysomes attached to the endoplasmic reticulum and transported through its membrane into its lumen, where the proteins fold (201). Vesicular or tubular structures transport folded proteins to the cis-region of the Golgi complex, and the proteins process through the stacks of the Golgi complex to the trans-side, after which the vesicles deliver secretory proteins to the cell surface (202, 203). The disorder might be caused at any (or all) of these different stages of the secretory process (204–206). Proteins which are not properly folded are often retained in the endoplasmic reticulum and thereby targeted for degradation by the ubiquitin/20 S proteasome pathway (207–209). Furthermore, the defect may be within the Golgi complex, as well as in the regulated secretory pathway, thus having an effect on protein secretion (210–213). All these mechanisms are far from being confirmed and are still a major challenge to the whole scientific community focusing on the pathway of secretory proteins. Particularly interesting is the fact that identical phenotypes might be caused by different genotypes causing completely different defects at the cellular and therefore functional levels.

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