Genetic defects in GH synthesis and secretion

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Abstract

Growth hormone (GH) is a multifunctional hormone produced in the anterior pituitary that promotes postnatal growth of skeletal and soft tissues. GH secretion and release are complex phenomena depending on several intrinsic and extrinsic factors modulating the release of two hypothalamic hormones, GH releasing hormone and somatostatin. Any genetic or acquired disorder that impairs GH secretion or action causes a pathological phenotype characterized by harmonic short stature with isolated GH deficiency (IGHD) or combined pituitary hormone deficiency (CPHD). In this article we report current knowledge about the genetic basis of IGHD and CPHD.

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Introduction

Growth hormone (GH) is a multifunctional hormone produced in the anterior pituitary that promotes postnatal growth of skeletal and soft tissues. Over the last twenty years several genes implicated in growth regulation have been identified including genes encoding the growth hormone itself, the GH releasing hormone (GHRH) and its receptor (GHRHR), and pituitary transcriptional factors (Pit-1, Prop-1, Hesx-1, Lhx-3, Lhx-4).

Isolated GH deficiency (IGHD)

Short stature associated with IGHD has been estimated to occur in 1 in 4000–10 000 new-borns (1, 2). Most cases are sporadic, but 3–30% have an affected relative, suggesting a genetic etiology of the disease. Sporadic IGHD cases, which represent the majority, are believed to result mainly from environmental cerebral insults or developmental anomalies. However, *de novo* mutations in the GH encoding gene (GH1) have been detected in patients with sporadic IGHD (3, 4).

GH1 gene

GH1 is the most extensively studied gene in IGHD. It includes 5 exons and 4 introns for a total of 1800 bp and it is located on chromosome 17q23 within a 66 Kb cluster including five highly homologous (92–98%) genes of which GH1 is expressed in the pituitary, CSH-1, CSH-2 and GH-V in the placental syncytiotrophoblast cells, and CSH-P is a pseudogene.

Mutations in the GH1 gene have been detected in about 12.5% of familial and 10% of sporadic IGHD and include deletions of the entire gene and nonsense mutations in the most severe forms, and splicing mutations in the milder forms (5). These mutations

cause different types of IGHD classified as IA, IB, II and III on the basis of the severity and the mode of inheritance (Table 1). The most severe form of IGHD (IGHD IA), characterized by the total absence of GH, has an autosomic recessive mode of inheritance and the patients carry gross deletions removing the entire GH1 or, in a few cases, nonsense mutations leading to a premature stop codon. The milder forms (characterized by a very low but detectable amount of GH and responsiveness to exogenous GH therapy) include IGHD IB, autosomic recessive, (that is the most common form) and IGHD II, autosomic dominant. In these forms patients carry nucleotide substitutions affecting mRNA splicing. IGHD III is inherited as an X-linked recessive trait, but its molecular basis is, to date, unknown (6, 7).

The GH1 coding sequence is highly conserved. In contrast, the GH1 promoter exhibits an unusually high level of nucleotide variations (8). There are some indications that promoter polymorphisms influence GH secretion and height (9) but none of them has, to date, been clearly associated with IGHD. In a study including 21 Italian IGHD patients, all the promoter nucleotide substitutions were detected with comparable frequencies in the patients and in normal controls with the possible exception of –1T that was increased in the patients with a borderline significance (10).

In most IGHD patients no GH1 mutation is found. Since several factors take part in GH secretion, it is likely that genetic forms of GH deficiency can result from mutations in other genes such as GHRH, GHRHR, PIT-1, PROP-1, HESX1, LHX3, and LHX4.

GHRHR gene

The GHRHR gene consists of 13 exons spanning a 15 Kb DNA segment on chromosome 7p15 (11).

Table 1 Genetically determined IGHD.

Type	Mode of inheritance	Features	Etiology
IGHD-IA	Autosomal recessive	Total deficiency Development of anti-GH antibodies	GH-1 deletions Nonsense mutations
IGHD-IB	Autosomal recessive	Partial deficiency	Splicing mutations
IGHD-II	Autosomal dominant	Partial deficiency	Missense and splicing mutations
IGHD-III	X-linked	Partial deficiency ± agammaglobulinemia	Unknown

Eleven different mutations have been detected to date in the human GHRHR gene in homozygous or combined heterozygous patients with IGHD IB (Fig. 1) (12–14). These mutations make the GHRHR functionally inactive and cause a characteristic phenotype called ‘Dwarfism of Sindh’, inherited as an autosomic recessive trait and characterized by very low GH levels and severe short stature. Patients show good responsiveness and immunological tolerance to exogenous GH therapy. Preliminary results indicate that mutations in the GHRHR gene constitute 10% of IGHD IB. Mutations in the GHRHR are not limited to the coding sequence. Promoter mutations that impair Pit-1 binding might as well reduce expression of the GHRHR gene. A recent report described a patient with isolated GH deficiency type IB who was heterozygous for a missense mutation (K329E) and an A → C transversion (position –124) in one of the two sites of the promoter region binding the pituitary-specific transcription factor Pit-1 required for GHRHR expression. Functional studies showed that cells transfected with the mutant promoter yielded significantly less luciferase activity than with the wild-type promoter. DNA binding studies confirmed that the A → C base change markedly reduces DNA binding of the Pit-1 protein (15).

Combined deficiency

Combined pituitary hormone deficiency (CPHD) denotes impaired production of GH and one or more of the other five anterior pituitary-derived hormones (thyrotropin (TSH), prolactin (PRL), adrenocorticotropin (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH)).

A great deal has been learned about the genetic causes of CPHD with the discovery of transcriptional activation factors that direct the embryonic development of the anterior pituitary (16, 17). Formation and subsequent Rathke’s pouch differentiation into the anterior pituitary gland are regulated by the combined action of specific transcription activating factors including Hesx-1, Ptx-1, Ptx-2, Lhx-3, Lhx-4, Prop-1 and Pit-1.

PIT-1 gene

Pit-1 is a pituitary-specific factor essential for development of somatotrope, lactotrope, and thyrotrope cells in the anterior pituitary and it transactivates expression of the genes encoding GH, PRL, and TSH-β. The human PIT-1 gene, designed as POUF1, is located on chromosome 3p11 (18).

Figure 1 GHRHR structure and known mutations.

Mutations in the human PIT-1 are responsible for a CPHD with deficiency of GH, PRL or TSH, while the production of ACTH, LH and FSH are preserved. There is phenotypic variability in the degree of CPHD and in the pituitary size of the patient. GH deficiency is generally severe with severe growth retardation and failure to thrive usually diagnosed before the age of two years. There is more variability in the degree of hypothyroidism. Some patients are born with features of cretinism; others are euthyroid at diagnosis and develop mild hypothyroidism during GH treatment. Although LH and FSH are normal, some patients with PIT-1 abnormalities may experience delayed puberty because of delay in thyroxine or GH treatment. Magnetic resonance imaging shows a moderately hypoplastic pituitary in some patients and a gland of normal shape and size in other subjects (16).

To date, 1 gross deletion and 18 point mutations have been detected in humans (Fig. 2) (16, 19–21). These are mainly located within exons 4 and 6. Most of the mutations alter residues important for DNA binding or the predicted α helical nature of POU-domains; sometimes mutations cause the complete or partial loss of the transcriptional activating function.

PROP-1 gene

Prophet of Pit-1 gene (PROP-1) maps to chromosome 5q and encodes a transcription factor involved in the early embryonic pituitary development. It is necessary for expression of Pit-1, for the differentiation of Pit-1-dependent cell lineage and for gonadotrope differentiation (22). PROP-1 mutations are the most common causes of genetically determined CPHD (16).

So far, 13 different mutations have been identified within PROP-1 (Table 2) (23). All the mutations control a recessive trait and affect the domain involved in the binding of the transcriptional factor to its cognate

Figure 2 Point mutations of human PIT-1. The underlined mutations are found in the heterozygous state. The others are found in the homozygous or combined heterozygous state.

Table 2 PROP-1 mutations.

Type of mutation	Location	Nucleotide change	Aminoacid change	Frequency (# reported alleles)	Reference
Frame shift	Exon 2	112-124 del 13bp	S480 X	2	Agarwal <i>et al.</i> (25)
Frame shift	Exon 2	150 del A	V164X	1	Riepe <i>et al.</i> (27)
Missense	Exon 2	149–150 del GA	S109X	3	Fofanova <i>et al.</i> (43), Deladoey <i>et al.</i> (44)
Missense	Exon 2	C211T	R71C	1	Paracchini <i>et al.</i> (23)
Missense	Exon 2	G212A	R71H	1	Paracchini <i>et al.</i> (23)
Missense	Exon 2	C217T	R73C	7	Duquesnoy <i>et al.</i> (22), Vallette-Kasic <i>et al.</i> (45)
Missense	Exon 2	G218A	R73H	2	Vallette-Kasic <i>et al.</i> (45)
Missense	Exon 2	T263C	F88S	2	Osorio <i>et al.</i> (46)
Missense	Exon 2	C295T	R99X	1	Vallette-Kasic <i>et al.</i> (45)
Missense	Exon 2	G296A	R99Q	1	Vieira <i>et al.</i> (47)
Frame shift	Exon 2	301–302 del AG	S109X	58	Wu <i>et al.</i> (48), Fofanova <i>et al.</i> (43), Rosembloom <i>et al.</i> (49), Duquesnoy <i>et al.</i> (22), Mendonca <i>et al.</i> (50), Astier <i>et al.</i> (26), Pernasetti <i>et al.</i> (51), Crone <i>et al.</i> (52)
Splice site	Intron 2	A → T nt 343-2	—	1	Duquesnoy <i>et al.</i> (22), Deladoey <i>et al.</i> (44)
Missense	Exon 3	T349A	F117I	4	Wu <i>et al.</i> (48), Deladoey <i>et al.</i> (44), Crone <i>et al.</i> (52)
Missense	Exon 3	C358T	R120C	10	Wu <i>et al.</i> (48), Fluck <i>et al.</i> (24), Deladoey <i>et al.</i> (44), Arroyo <i>et al.</i> (53)

DNA sites. Mutations in PROP-1 have been reported as a cause of CPHD involving GH, PRL, TSH as well as gonadotropins and, occasionally, ACTH. The clinical phenotype associated with PROP-1 mutations varies considerably, especially with respect to the occurrence and the severity of the different pituitary hormone deficiencies. There is no correlation between genotype and phenotype (16, 24).

The most consistent presenting feature is growth retardation mainly caused by GH deficiency – rarely is TSH the first symptom. Most patients fail to enter puberty and show consistently low LH and FSH responses to LH releasing hormone (LHRH) stimulation. In rare cases, patients may reach spontaneous pubertal development with normal LH and FSH responses to an LHRH stimulation test, but, in any case, they are destined later to develop hypogonadism (16). A progressive late onset ACTH deficiency that can induce an acute cortisone insufficiency, with risk of shock with advancing age, has been reported in only a few cases. Therefore, a molecular analysis of all subjects affected by CPHD becomes necessary in order to identify patients carrying PROP-1 mutations as they will require a life-long evaluation of the ACTH–adrenal axis (25, 26). Phenotypic variability also applies to pituitary size that may be normal, hypoplastic or even enlarged. Most patients have a hypoplastic anterior pituitary; in contrast, there are few reports of patients with PROP-1 mutations and pituitary enlargement. Longitudinal follow-up of these patients showed that the pituitary enlargement was followed by a progressive involution during the second decade of life resulting in pituitary hypoplasia. The pituitary enlargement during early childhood must not be mistaken for craniopharyngioma, pituitary adenoma, dysgerminoma or Rathke's pouch cyst, since pituitary surgery is not indicated in patients with PROP-1 mutations (27).

HESX1 gene

HESX1 is a member of a paired-like class of homeobox genes that maps at locus 3p21.1-21.2 and codes for a transcriptional factor. Its expression is essential for the development of the optic nerve and regulates

some of the earliest stages in pituitary development. Mice homozygous for a mutation in *Hesx1* display multiple defects in placodally derived anterior structures including the eye, olfactory epithelium, forebrain and pituitary. This phenotype is similar to the abnormalities observed in the human disorder called septo-optic dysplasia (SOD), a syndromic form of congenital hypopituitarism which is characterized by the triad of pituitary hypoplasia and/or optic nerve hypoplasia and/or agenesis of midline brain structures, including the corpus callosum and septum pellucidum (28). To date, 5 missense mutations in the human HESX1 have been described in individuals with phenotypes ranging from severe SOD to relatively mild CPHD or isolated GH deficiency occasionally associated with undescended/ectopic posterior pituitary (Table 3) (28–30). The milder phenotype is present in heterozygous patients who have inherited the mutation from a healthy parent. Since GH deficiency is the most common manifestation of reduced HESX1 among heterozygous individuals, it will be important to assess the relative contribution of heterozygous HESX1 mutations in patients with sporadic and familial pituitary hypoplasia, in particular with isolated GH deficiency (29). More recently, a *de novo* heterozygous mutation (306/307 ins AG) within exon 2 was associated with panhypopituitarism and anterior pituitary hypoplasia, an ectopic posterior pituitary, and left optic nerve hypoplasia in a Japanese patient (31).

LHX3 gene

LHX3, located at 9q34, encodes a member of the LIM homeodomain family of transcription factors that is critical for both early structural development of the pituitary gland and for the specification and proliferation of the gonadotrope, lactotrope, somatotrope, and thyrotrope pituitary cell lineages. *Lhx-3* knockout mice do not develop a mature pituitary gland and only form a few corticotrope cells. Similarly, a recent report has described two mutations (1 deletion and 1 missense mutation) in the human LHX3 gene that are associated with CPHD characterized by the loss of all but one (adrenocorticotropin) of the five hormones

Table 3 HESX1: Genotype–phenotype correlations.

No.	HESX1 mutation	Endocrine disorder	MRI
1-1 ^a	R160X (homoz.)	CPHD	ACC, ONH
1-2 ^a	R160X (homoz.)	CPHD	ACC, ONH
2-1 ^b	S170L (heteroz.)	IGHD	ONH bilateral
2-2 ^b	S170L (heteroz.)	IGHD	Normal
3	T181A (heteroz.)	IGHD	Anterior pituitary hypoplasia
4	Q6H (heteroz.)	GH, TSH, LH/FSH deficiency	Anterior pituitary hypoplasia, ectopic posterior pituitary
5	S170L (heteroz.)	IGHD	Anterior pituitary hypoplasia, ectopic posterior pituitary
6	306/307 ins AG (heteroz.)	CPHD	ONH monolateral, anterior pituitary hypoplasia, ectopic posterior pituitary
7	I26T (homoz.)	CPHD	Anterior pituitary hypoplasia, ectopic posterior pituitary

^{a,b}Familial cases: ^afamily 1; ^bfamily 2; ACC, agenesis of corpus callosum; ONH, optic nerve hypoplasia; homoz., homozygous; heteroz., heterozygous.

produced in the anterior pituitary resulting in severe growth retardation. Patients also displayed a rigid cervical spine leading to limited head rotation. Two of these patients had a severe pituitary hypoplasia, whereas one patient presented secondarily with an enlarged anterior pituitary (32).

Because LHX3 appears to be important for early formative processes of the developing pituitary gland, it was hypothesized that its mutations would cause ectopic posterior pituitary disease associated with CPHD. However, molecular analysis of LHX3 in patients with posterior pituitary ectopia did not confirm this hypothesis (33).

LHX4 gene

LHX4 is a LIM-homeobox gene, located at 1q25, which has been shown to regulate, together with the closely related LHX3, proliferation and differentiation of pituitary lineages. In mice loss of both *Lhx3* and *Lhx4* causes earlier developmental arrest and only rudimentary pouch formation is detected; *Lhx4*-deficient pituitaries exhibit correct specification of all five hormone-producing cell types, but the expansion of each specialized cell is reduced dramatically. *Lhx4* mutants have a less severe phenotype than *Lhx3* mutants; all five anterior cell types are present, albeit severely reduced in number (34).

So far, one mutation is described in human LHX4 responsible of a disease phenotype characterized by short stature and by pituitary and hindbrain (i.e. cerebellar) defects in combination with abnormalities of the sella turcica of the central skull base. Some patients present with GH, TSH and ACTH deficiencies, which is consistent with the hormonal profile of *Lhx4*^{-/-} mice. Nevertheless there is variability in the LHX4 mutant phenotype and one human pedigree includes individuals with CPHD and with IGHD. In addition, some but not all patients display an ectopic pituitary gland posterior hypophysis. Finally, the disease phenotype includes the pointed cerebellar tonsils observed in patients with Arnold Chiari malformation (35).

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