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REVIEW

Familial Isolated Growth Hormone Deficiency: Genetics and Pathophysiology

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Introduction

HUMAN growth hormone (GH) is encoded by the *GH-I* gene which forms a gene cluster with *CSHP-I, CSH-I, GH-II* and *CSH-II* [1–3]. Human GH with a molecular mass of 22 kD is synthesized in the pituitary somatotrophs from which it is secreted into circulation. GH deficiency causes metabolic alterations and growth failure. Proportionate short stature, accompanied by decreased growth velocity and delayed bone maturation, in the absence of bone dysplasia, chronic disease or other pituitary hormone deficiencies, are important clinical findings for the diagnosis of isolated growth hormone deficiency (IGHD).

Familial IGHD is classified into three major types by inheritance. Type I IGHD is inherited in an autosomal recessive, type II in an autosomal dominant, and type III in an X-linked manner [4]. We have been investigating genetic abnormalities in Japanese patients with IGHD and have reported several novel mutations causing Type I [5–8] and Type II IGHD [9–12]. Also studied was the pathogenesis of Type II IGHD [13]. The aim of the present review is to summarize the genetics and pathophysiology of IGHD.

Type I IGHD

1) *GH-I gene defect*

Mutations in both alleles of the *GH-I* results in the absence of normal GH molecule in circulation, resulting in IGHD. Deletion encompassing all the exons of *GH-I* gene was first demonstrated by Southern blotting in 1981 and followed by a number of reports [4, 5, 14–18]. The upstream and downstream flanking regions of *GH-I* gene are homologous to each other, and crossover events of these regions generate 6.7-kb, 7.0-kb or 7.6-kb deletions as depicted in Fig. 1 [19]. Deletions with larger size have also been reported [20, 21]. The patients with homozygous *GH-I* gene deletion completely lack growth hormone molecule in circulation, and they usually produce anti-GH antibodies in response to exogenous GH, resulting in a limited response to replacement therapy.

A point mutation, and a single or two base deletion have also been identified in patients with type I IGHD ([6, 8, 17, 18, 22]; see Fig. 1). Patients are either homozygous for one of these mutations or compound heterozygous with a large deletion. Since the mutations in exon 2 result in mutant proteins which lack most of the GH molecule, the patients with these mutations develop anti-GH antibodies in response to exogenous GH, as in cases with patients with large *GH-I* gene deletion [17]. In contrast, patients with mutations in exon 3 or intron 4 do not develop the antibody and respond well to replacement therapy [6, 8, 18, 22], suggesting that mutant GHs generate immunological tolerance.

2) *GHRHR gene defect*

Identification of a missense mutation in GH releasing hormone receptor (GHRHR) in *little/little* mice revealed that GHRH action is required for synthesis and secretion of GH [23, 24]. A nonsense mutation

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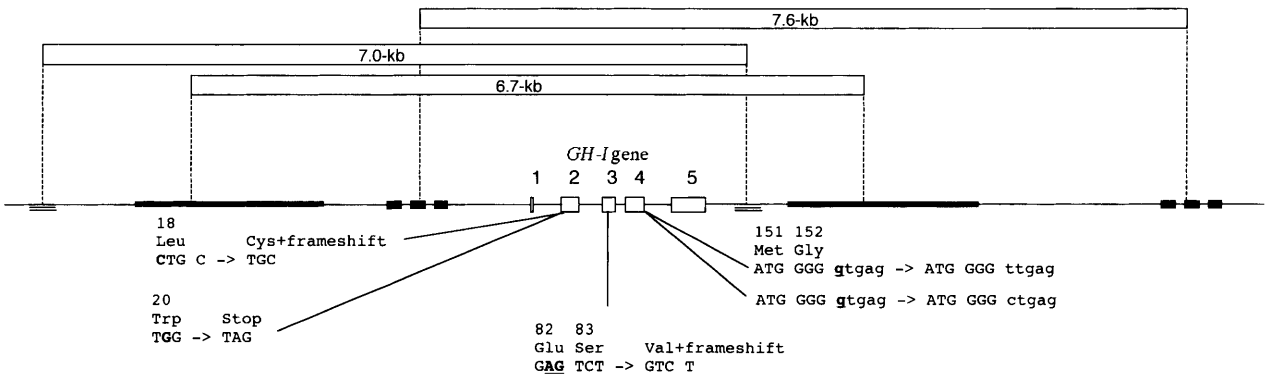

Fig. 1 Mutations in *GH-I* gene resulting in recessively inherited IGHD
GH-I gene and flanking region is schematically shown. Numbered boxes stand for exons. The three patterns of deletion are shown on the top of figure. The 6.7-kb, 7.6-kb and 7.0-kb deletions occur as crossovers of two homologous regions indicated as bold lines, dashed lines and double underlines, respectively. For exact sequence information, refer to GenBank HUMGHCSA and AC005803. A point mutation, a single or two base deletions identified as a compound heterozygote or a homozygote in patients with recessively inherited IGHD is also shown at the bottom of the figure. Nucleotides in exons and introns are shown in uppercase letter and lowercase letter, respectively. Amino Acid is shown in three-letter code, together with codon number. To obtain amino acid number in mature GH protein, subtract 26 (signal peptide) from the codon number.

in *GHRHR* (Glu72Stop), which terminates the GHRHR peptide in extracellular domain, was first identified in an Indian Moslem family [25]. Subsequently, the same mutation was identified in multiple families, all originating from the Indian subcontinent ([26, 27] and T. Kamijo, manuscript in preparation). This mutation is likely to have occurred in a single ancestor and spread in the Indian subcontinent by subsequently being transmitted to descendants.

Five other mutations in *GHRHR* gene have been so far reported in patients with recessively inherited IGHD, including a sporadic case. A G → A transition at the first nucleotide of intron 1 of *GHRHR* gene has been identified in a large Brazilian kindred [28]. A homozygous four-bp deletion in exon 12 of *GHRHR* gene has been reported in a Japanese patient [29]. Salvatori *et al.* very recently identified three kinds of *GHRHR* mutation (Leu144His, Phe242Cys, Ala222Glu) in three families with recessively inherited IGHD [30].

Type II IGHD

During the last decade, several different mutations were identified at the donor splice site of intron 3 of the *GH-I* gene in patients with IGHD inherited in an autosomal dominant manner ([9, 10, 13, 31–35]; see Fig. 2). Accordingly, the affected patients were heterozygous for the mutations. Since the patients have an intact copy of *GH-I* a very small, yet significant amount of GH is detectable after provocative tests. In contrast to the patients with homozygote *GH-I* gene deletions, these patients do not develop anti-GH antibody after replacement therapy [9, 11].

Among these mutations, a guanine (G) to adenine (A) transition of the first nucleotide of intron 3 (IVS3 + 1:G→A) is most frequently identified not only in familial IGHD but also in sporadic IGHD. We identified IVS3 + 1:G→A in 4 Japanese patients belonging to 3 families. It should be noted that patients with the *de novo* mutation were identified in all the families (Fig. 3). The mutation has also been reported in patients with IGHD of non-Japanese origin, and *de novo* cases were present in all the families [33, 34].

The high incidence of this mutation can be explained by the fact that the guanine base mutated is preceded by a cytosine base, resulting in a CpG dinucleotide which has been considered as a mutational hot spot. It is believed that CpG dinucleotide is frequently methylated in human genome and the methylcytosine residues can be converted to a thymidine base by deamination. Indeed, a C to T or a G to A transition in this dinucleotide is frequently identified in human genetic diseases or polymorphisms

[36, 37]. Thus, we believe that screening for this mutation, not only in familial but also in sporadic cases with IGHD, should detect more patients with this mutation. We as well as others have demonstrated that transcripts arising from the *GH-I* gene with IVS3 + 1:G→A lack exon 3 [13, 31]. The exon 3 of *GH-I* gene consists of 120-bp, thereby the protein encoded by the mutant mRNA skipping exon 3 lacks in-frame 40 amino acid residues. The deduced molecular mass of the protein translated from the mutant mRNA skipping or lacking exon 3 is 17.5-kD.

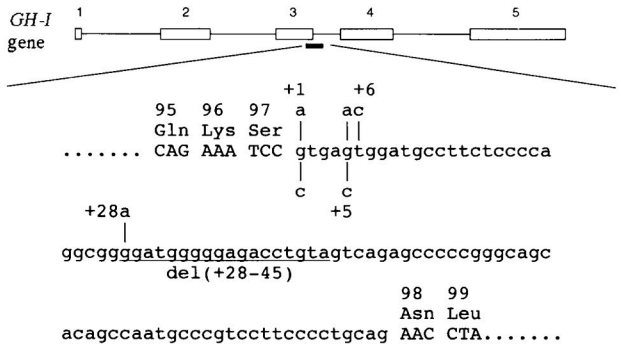

Fig. 2. Mutations in *GH-I* gene resulting in dominantly inherited IGHD
The structure of *GH-I* gene is presented on the top of the figure. Numbered boxes stand for exons and lines stand for introns. Bold line indicates the donor splice site of intron 3 and the sequence of the junction between exon 3 and intron 3 is indicated. Nucleotides in exons and introns are shown in uppercase letter and lowercase letter, respectively. Amino acid sequence is shown in three-letter code, together with codon number.

Metabolic labeling study using Cos-7 cell transfected with expression vectors revealed that the mutant 17.5-kD GH is retained in the cell, while the wild type 22-kD GH is readily secreted [13]. To further explore the pathogenesis of type II IGHD, we coexpressed 17.5-kD and 22-kD GH in various cell lines. While expression of 17.5-kD GH did not inhibit 22-kD GH secretion in Cos-7 cells derived from kidney or in HepG2 cells derived from hepatoblastoma, significant inhibition was observed in two pituitary-derived cells, MtT/S cells and AtT-20 [13]. These results suggested that a neuroendocrine cell-type specific feature, such as regulated secretion through secretory granules, is involved in the pathogenesis of type II IGHD [13]. Alternatively, accumulation of

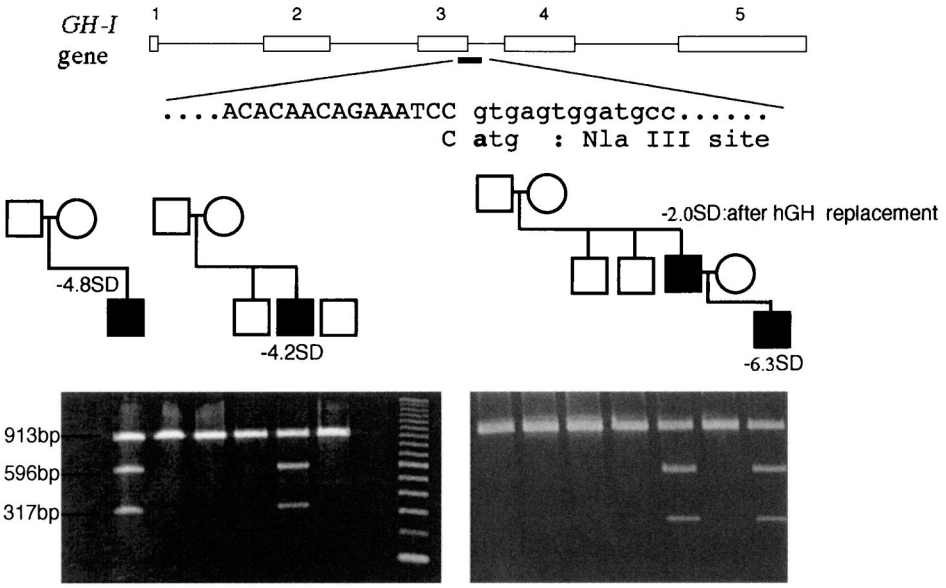

Fig. 3. *GH-I*:IVS3+1:G→A in 3 unrelated Japanese families with IGHD.
The structure of *GH-I* gene is presented on the top of the figure. Numbered boxes stand for exons and lines stand for introns. Bold line indicates the donor splice site of intron 3 and the sequence of the region as well as Nla III restriction site generated by *GH-I*:IVS3 + 1:G→A are shown. Pedigrees combined with the results of PCR-Nla III digestion analysis are shown in the bottom. In the presence of *GH-I*:IVS3 + 1:G→A, the 913-bp PCR fragments are digested by Nla III into two fragments (596-bp and 317-bp). Hundred-bases ladders are used as a molecular weight marker. Affected subjects are indicated as closed symbols and their height in SD are shown.

the mutant GH molecules in the secretory granules may result in somatotroph death. These results are schematically summarized in Fig. 4.

The other mutations depicted in Fig. 2 (IVS: + 1G →C, + 5G→A, + 5G→C, + 6T→C, + 28G→A and del (+ 28–45)) are much less frequent, compared to IVS + :1G→A. Indeed, each mutation is reported for one family [9, 11, 31, 32, 34, 35]. Among these mutations, four substitutions (IVS: + 1G→C, + 5G →A, + 5G→C and + 6T→C) result in complete skipping of exon 3 in the transcripts as in the case of IVS: + 1G→A [9, 13, 31, 32]. On the other hand, multiple transcripts can be generated from the other two mutations, IVS: + 28G→A and del (+ 28–45). mRNAs encoding 17.5-kD and normal 22-kD GH are transcribed from *GH-I* with IVS: + 28G→A. From *GH-I* gene with del (+ 28–45), mRNAs encoding 17.5-kD and 20-kD GH are transcribed [35]. The 20-kD GH is a splicing variant, which can be detected in significant amounts in healthy subjects [38]. The amount of 17.5-kD GH synthesized from the mutant allele should be less than 22-kD GH derived from the normal allele. Although clinical information on the IGHD subjects with *GH-I* (IVS: + 28G→A) or del (+ 28–45)) is not described in detail, this finding suggests that even when the amount of 17.5-kD GH is less than that of 22-kD GH, it is sufficient to inhibit GH secretion from somatotrophs in the affected subjects.

Type III IGHD

IGHD inherited in X-linked manner is very rare and associated with X-linked hypogammaglobulinemia (XLA). Only a few familial cases have been reported so far [39–43], but the genetic basis has yet to be elucidated. An intronic mutation in Bruton's tyrosine kinase (BTK) gene was recently identified in a sporadic patient with IGHD and XLA [44]. However, it is not clear whether the mutation in BTK gene is responsible for IGHD phenotype, because of a single case report without functional characterization of the mutant product. Furthermore, many mutations have been identified in BTK gene in patients with XLA, but without IGHD.

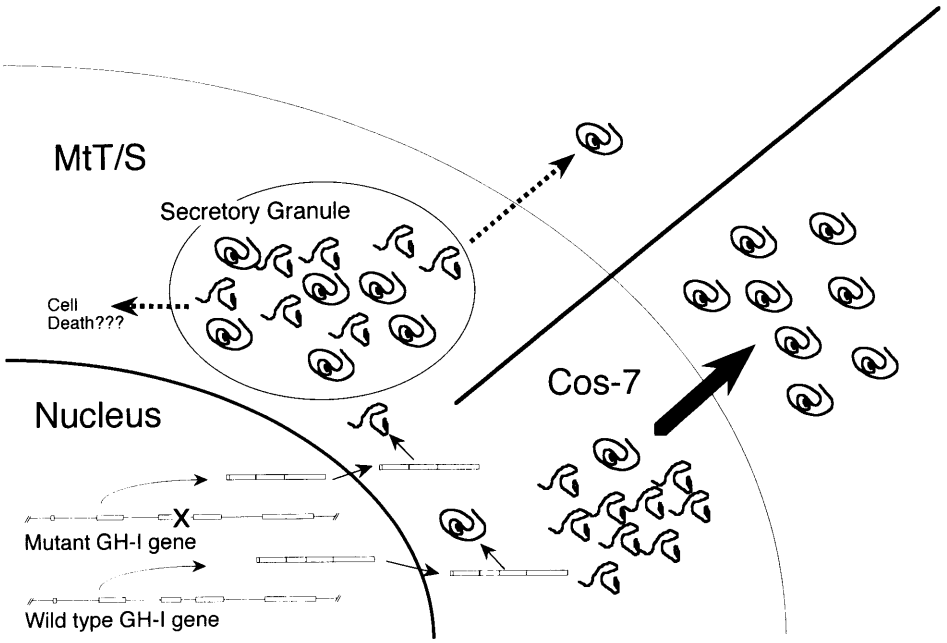

Fig. 4. Schematic summary for pathogenesis of type II IGHD.
In the nucleus, an mRNA skipping exon 3 is transcribed from a *GH-I* gene which has a point mutation at the donor splice site of intron 3. Malfolded 17.5 kD GH transcribed from the mRNA cannot be secreted and retained in the cell. When the wild-type 22-kD GH and the 17.5-kD GH is co-expressed, secretion of the wild-type GH is inhibited in pituitary derived MtT/S cells, but not in kidney derived Cos-7 cells.

Summary and prospective

Since this review focused on the pathogenesis of hereditary IGHD, we did not describe the mutations in *GH-I* gene producing bioinactive GH molecules which also causes short statures [45], Nor did we describe mutations in tissue specific transcription factors, which result in combined pituitary hormone deficiency [46–54].

Mutations in *GH-I* gene and *GHRHR* gene have been identified in familial as well as sporadic cases with IGHD. In addition to homozygous or compound heterozygous mutations identified in sporadic cases, *de novo* mutations in intron 3 of *GH-I* gene resulting in type II IGHD have been frequently identified. Thus, it is important to screen possible mutations in *GH-I* gene in patients with IGHD, even in sporadic cases. Although mutations in *GHRHR* gene result in IGHD, no mutation in growth hormone releasing hormone has been identified so far [55]. There appears to be a number of families with IGHD, in which mutations in *GH-I*, *GHRHR* and *GRF* genes and/or linkage to these loci were excluded ([55]; manuscript in preparation). We expect that whole genome linkage analysis in large families with IGHD and the progress of the human genome project [56, 57] should unveil genes involved in somatotroph development and GH synthesis.

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