Isolated Growth Hormone Deficiency Type 1A without Apparent Growth Hormone 1 Gene Deletion

Shinya Tamai, Nobutake Matsuo, Seiji Sato, Jun Kudoh and Nobuyoshi Shimizu

Department of Pediatrics (ST, NM, SS) and Molecular Biology (JK, NS), School of Medicine, Keio University, Tokyo, Japan

Abstract We document a Japanese girl who, without apparent growth hormone (GH)-1 gene deletion, had a clinical phenotype identical to that of isolated growth hormone deficiency type 1A (IGHD-1A). The restriction fragment length polymorphism (RFLP) linkage analysis of the patient and her family members indicates that her GH-1 gene is structurally intact, though the molecular basis of her GH deficiency remains to be determined. We suspect that several congenital disorders other than GH-1 gene mutations may be responsible for IGHD-1A phenotype.

Key words: isolated growth hormone deficiency type 1A, growth hormone, growth hormone-1 gene, growth hormone-releasing factor gene

Introduction

IGHD-1A is characterized by: 1) extreme short stature, 2) typical facial appearance, 3) absent human growth hormone (hGH) secretion on multiple hGH stimulation tests, and 4) formation of high anti-hGH antibody titer to exogenous GH (1,2). The molecular analysis to date has disclosed that the great majority of the affected subjects suffer from complete deletion of the GH-1 gene (3). We describe a 5-year-old Japanese girl who, without apparent GH-1 gene deletion or mutation, had a clinical phenotype identical to that of IGHD-1A.

Case Report

This Japanese female patient was the product of a full term normal pregnancy and delivery. She measured 45 cm (mean -2.4 SD) and weighed 2.62 kg (mean -1.5 SD) at birth, and remained extremely short thereafter. Her unrelated parents and three siblings are normal in height. At 1 $\frac{1}{12}$ years of age, she was evaluated for short stature in another hospital, and was diagnosed as having isolated GH deficiency. The basal and stimulated serum GH levels with insulin (0.05 U/kg intravenously), arginine-HCl (0.5 g/kg intravenously), and glucagon-propranolol (0.5 mg/kg of propranolol orally 2 hours before intramuscular injection of 0.03 mg/kg of glucagon) were undetectable (<2 μ g/L). Her luteinizing hormone (LH) and

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Correspondence: Dr. Shinya Tamai, Department of Pediatrics, School of Medicine, Keio University, 35 Shinano-machi, Shinjuku-ku, Tokyo 160 Japan

follicle stimulating hormone (FSH) responses to luteinizing hormone-releasing hormone (LHRH; 100 $\mu\text{g}/\text{m}^2$ intravenously) and thyrotropin (TSH) response to TSH-releasing hormone (TRH; 300 $\mu\text{g}/\text{m}^2$ intravenously) were normal (peak serum levels of LH, FSH, and TSH were 61.3 IU/L, 44.0 IU/L, and 16.4 mU/L, respectively). At 2·4/12 years of age, she began to receive hGH therapy (0.6 U/kg/wk) to

which she responded very poorly, although she had a brief, initial growth spurt (3.9 cm/3 months) (Fig. 1).

At 3·8/12 years of age, she was referred to our service for work-up. Physical examination revealed her to be an extremely short girl with a prominent forehead and hypoplastic nose. Height was 71.0 cm (mean -6.9 SD), weight was 6.97 kg (mean -4.9 SD), and head circumference was 45.5 cm (3rd percentile). She had no pubertal sign (Tanner stage 1). There were no other associated dysmorphic features or asymmetry. Her intelligence was normal. Pertinent laboratory findings included: 1) bone age of 2 years (Greulich and Pyle's criteria (4)). 2) a normal anterior pituitary on magnetic

Fig. 1 Growth curve of the patient prior to and following treatment with hGH, plotted on the 1980 national growth standard for Japanese children (24), and the serum anti-hGH antibody titers following treatment.

Fig. 2 Sagittal T-1 weighed spin-echo image of the sella. A soft tissue intensity, representing an anterior pituitary, is present within the sella (arrowhead). The height of the anterior pituitary is about 3 mm and its upper border shows upward concavity. The pituitary stalk is clearly visible in the suprasellar cistern (open arrow). The posterior pituitary lies within the sella, easily identified by its characteristic bright signal intensity (black arrow).

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resonance imaging (MRI) (Fig. 2). 3) a karyotype, 46, XX. 4) the basal and stimulated levels of adrenocorticotropin (ACTH) and prolactin (PRL) were 8 and 23 pmol/L (34 and 102 pg/mL) and 19 and 53 μ g/L, respectively. 5) urine osmolality reached 971 mmol/kg after overnight thirst. 6) the serum 3,5,3'-triiodothyronine (T_3) and thyroxine (T_4) levels were 2.6 nmol/L (168 ng/dL) and 80 nmol/L (6.2 μ g/dL), respectively. 7) plasma insulin-like growth factor-I (IGF-I) concentrations ranged between 0.34 and 0.97 U/mL (n=4, Nichols Institute). 8) the serum anti-hGH antibody titer was 1:10⁴.

At 5.2/12 years of age, she received recombinant human IGF-I (hIGF-I) therapy (0.05-0.2 mg/kg/dose subcutaneously, on alternate days for two weeks) with definite biological

responses: 1) her blood glucose level fell from 4.1 to 2.4 mmol/L (73 to 44 mg/dL) 3 hours after 0.2 mg/kg/dose of hIGF-I, though breakfast was offered simultaneously. 2) nitrogen balance increased in stepwise fashion from 0.6 to 2.6 g/day during the two weeks (5).

Methods

Southern blot analysis of the GH-1 gene and the growth hormone-releasing factor (GRF) gene was performed in the patient and her family members.

Genomic deoxyribonucleic acid (DNA) was prepared from leucocytes of the patient and her family members according to standard methods (6). A 0.8-kb nearly full-length hGH

Fig. 3 Autoradiogram patterns of DNA from the patient, her family members, and a control subject (C) after digestion with *Bam*HI (Fig. 3a) or *Hind*III (Fig. 3b), and hybridization to the GH-1 cDNA probe.

complementary DNA (cDNA) (kindly donated by Dr. Peter H. Seeburg (7,8)) and a 0.6-kb nearly full-length human GRF (hGRF)-8 cDNA (kindly donated by Dr. Ueli Gubler (9)) were radiolabeled with [α - 32 P] deoxycytidine triphosphate (dCTP) by multiple DNA labeling system (10), and were used as Southern blot probes. Aliquots (6 μ g) of the nuclear DNA prepared from the subjects were digested to completion, using various restriction endonucleases (*Bam*HI, *Hind*III, *Hinc*II, *Bgl*II, *Msp*I for the GH-1 gene and *Bam*HI, *Hind*III, *Bgl*II for the GRF gene). the resulting fragments were separated using agarose gel electrophoresis, and were transferred to nitrocellulose filters prior to hybridization to the probes (11). Following hybridization the filters were washed and autoradiographed.

Results

Southern blot analysis of the GH gene

*Bam*HI and *Hind*III endonuclease analysis

The 3.8-kb *Bam*HI and 26-kb *Hind*III frag-

ments were found in all family members including the patient (Fig. 3). These fragments are known to include the GH-1 gene.

*Hinc*II, *Bgl*II, and *Msp*I endonuclease analysis

The results are shown in Table 1. For *Hinc*II analysis, the proband and her three siblings had 4.5-and 6.7-kb fragments, and her father was homozygous for 6.7-kb fragment and mother was homozygous for 4.5-kb fragment. For *Bgl*II a,b and *Msp*I a,b analysis, the proband and her three siblings had identical allelotype.

Southern blot analysis of the GRF gene

Each subject and normal control had identical *Bam*HI, *Hind*III, and *Bgl*II fragments. These fragments consisted of 6.6-, 3.5-kb for *Bam*HI, 6.8-, 6.6-kb for *Hind*III and 23-, 5.2-, 3.0-kb for *Bgl*II, respectively (Table 2).

Discussion

The phenotype of our patient fits the criteria for IGHD-1A described by Illig *et al.* (1,2).

Table 1 Restriction endonuclease analysis (GH gene)

	<i>Bam</i> HI	<i>Hind</i> III	<i>Hinc</i> II	<i>Bgl</i> IIa	<i>Bgl</i> IIb	<i>Msp</i> Ia	<i>Msp</i> Ib
proband	3.8	26	6.7/4.5	13/10.5	8.1/3.0	4.3/3.6	3.9/3.3
father	3.8	26	6.7	13	8.1/3.0	3.6	3.3
mother	3.8	26	4.5	10.5	8.1	4.3/3.6	3.9
sister(I)	3.8	26	6.7/4.5	13/10.5	8.1/3.0	4.3/3.6	3.9/3.3
brother	3.8	26	6.7/4.5	13/10.5	8.1/3.0	4.3/3.6	3.9/3.3
sister(II)	3.8	26	6.7/4.5	13/10.5	8.1/3.0	4.3/3.6	3.9/3.3

(unit: kb)

Table 2 Restriction endonuclease analysis (GRF gene)

	<i>Bam</i> HI	<i>Hind</i> III	<i>Bgl</i> II
proband	6.6 , 3.5	6.8 , 6.6	23, 5.2, 3.0
father	6.6 , 3.5	6.8 , 6.6	23, 5.2, 3.0
mother	6.6 , 3.5	6.8 , 6.6	23, 5.2, 3.0
3 siblings	6.6 , 3.5	6.8 , 6.6	23, 5.2, 3.0
control	6.6 , 3.5	6.8 , 6.6	23, 5.2, 3.0

(unit: kb)

IGHD-1A without GH-1 Gene Deletion

Since the present data indicate that our patient has GH-1 genes, her molecular defect may represent a hitherto undescribed cause of the IGHD-1A phenotype. In contrast with our patient, the great majority of IGHD-1A patients studied so far were found to have deletions that include the GH-1 genes (3,12). Most of such patients reported had 6.7-kb deletion, some had 7.0- or 7.6-kb deletion (13,14). A few patients had a single-base deletion or substitution in the GH-1 gene (15,16). As the common underlying mechanism for various GH-1 gene deletions, Vnencak-Jones *et al.* proposed unequal cross-over between sequences just upstream to the GH-1 and CSHP-1 genes, as studied in 9 patients with IGHD-1A (17). They suspect that the unequal cross-over is probably facilitated by highly homologous sequences that are repeated within the GH gene cluster (18).

Other than a complete GH-1 gene deletion, various other abnormalities such as frameshift and nonsense mutations have recently been found in the GH-1 genes of subjects with IGHD-1A, leading to complete absence of GH (19-21). We first suspected that a similar single base substitution or a subtle rearrangement within or near the GH-1 gene, rather than a gross deletion, was the most likely molecular defect of our patient. In order to detect such a minor abnormality of the GH-1 gene, linkage analysis using the restriction fragment length polymorphisms (RFLPs) at the GH-1 gene may be a useful tool. However, we failed to disclose the tight linkage between *HincII*, *BglII*, and *MspI* RFLPs and conceptual gene mutation resulting in GH deficiency. Supposing that this disorder is transmitted by an autosomal recessive trait, it follows that each sibling was a healthy carrier and received

a mutant GH-1 gene from father by *BglII* b RFLP segregation analysis. However, *MspI* a RFLP analysis indicated the opposite in that each received a mutant GH-1 gene from mother. Both RFLP patterns were thus mutually inconsistent, refuting mutation within the GH-1 locus.

It is also conceivable that complete deletion of the GRF gene may result in the phenotype of IGHD-1A, but there is very limited information on the mutant GRF gene. Rogol *et al.* (22) and Mullis *et al.* (23) reported that GRF gene is not polymorphic for *BamHI*, *BglI*, *BglII*, *EcoRI*, *HincII*, *HindIII*, *MspI*, *PstI*, and *PvuII* fragments, precluding the RFLP linkage analysis. In our family, we also failed to disclose gross deletion or informative RFLPs at the GRF gene. It is apparent, however, that further detailed studies of GRF gene is needed to exclude possible subtle alteration in the GRF gene in our patient.

In summary, the molecular basis of a complete absence of GH in our patient remains to be determined, but we suspect that her GH-1 gene may be structurally intact and that several congenital disorders other than GH-1 gene mutations may be responsible for IGHD-1A phenotype.

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