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Prevalence of Human Growth Hormone-1 Gene Deletions among Patients with Isolated Growth Hormone Deficiency from Different Populations

P. E. MULLIS, A. AKINCI, CH. KANAKA, A. EBLÉ, AND C. G. D. BROOK

Department of Paediatrics, Inselspital, Bern, Switzerland [P.E.M., Ch.K., A.E.]; Department of Paediatric Endocrinology, Dr. Sami Ulus Childrens Hospital, Ankara, Turkey [A.A.]; and Endocrine Unit, The Middlesex Hospital, London W1N 8AA, United Kingdom [C.G.D.B.]

ABSTRACT. Familial isolated growth hormone deficiency type IA results from homozygosity for either a 6.7-kb or a 7.6-kb hGH-1 gene deletion. Genomic DNA was extracted from circulating lymphocytes of 78 subjects with severe isolated growth hormone deficiency (height < −4.5 SD score) and studied by polymerase chain amplification and by restriction endonuclease analysis looking for gene deletions within the hGH-gene cluster. The individuals analyzed were broadly grouped into three different populations (North-European, *n* = 32; Mediterranean, *n* = 22; and Turkish, *n* = 24). Ten out of 78 patients studied presented with an hGH-1 gene deletion; eight out of these 10 showed a 6.7-kb gene deletion, the remaining two a 7.6-kb hGH-1 gene deletion. Five of the 10 subjects developed anti-hGH antibodies to hGH replacement followed by a stunted growth response. Family studies of the affected patients were performed, revealing consanguinity in all the families, and the corresponding heterozygosity for the deletion was present in each of the parents. The results of our study revealed a prevalence for an hGH-1 gene deletion in three out of 32 North-European, three out of 22 Mediterranean, and four out of 24 Turkish patients with growth hormone deficiency (height < 4.5 SD score). These data are important for prenatal diagnosis of at-risk pregnancies and for families at risk for recurrence and underline clearly the fact that the hGH-I gene deletion represents a common cause for growth hormone deficiency associated with severe growth retardation (height < −4.5 SD score). (*Pediatr Res* 31: 532–534, 1992)

Abbreviations

IGHD, isolated growth hormone deficiency
PCR, polymerase chain reaction
GH, growth hormone
hCS, human chorionic somatomammotropin gene

Short stature associated with abnormalities of GH secretion results from a variety of genetic and acquired interruptions in the hypothalamo-pituitary-peripheral axis. In the United Kingdom, the prevalence of GH deficiency is reported to be between 1 in 4000 and 1 in 10 000 (1). Five to 30% of GH-deficient patients have a GH-deficient relative; therefore, the disorder is suggested to be familial (2). Four distinct familial types of IGHD are well-differentiated on the basis of inheritance and other hormone deficiencies (3). One form is IGHD type IA, resulting from a GH-1 gene deletion (4, 5). Subjects with IGHD type IA may have short body length at birth and present occasionally with hypoglycemia, but severe growth retardation by 6 mo of age is a constant finding. Treatment with hGH is frequently complicated by the development of anti-hGH antibodies in a titer sufficient to cause arrest of response to the hGH replacement (6). However, an increasing number of patients have been reported in whom immune intolerance after GH treatment has not developed (7, 8). This has led to the idea that IGHD type IA cannot be identified on the basis of anti-hGH antibodies causing growth arrest. A second autosomal recessive type of IGHD (type IB) is characterized by the production of insufficient amounts of hGH after provocative stimuli; in contrast to IGHD type IA, no detectable hGH gene deletions are described (3). A third form (IGHD type II) has an autosomal dominant mode of inheritance, whereas a fourth one (IGHD type III) is X-linked inherited (3). The same criteria used to diagnose IGHD type IB apply to IGHD type II and type III.

Using Southern blot analysis, we found neither homozygous nor heterozygous patterns of hybridization for hGH-I gene deletions among 53 British children with milder forms of IGHD and concluded that structural abnormalities of the hGH gene cluster detectable by endonuclease analysis are an uncommon cause of GH deficiency (9). Recently, a rapid method using PCR amplification has been developed to facilitate detection of hGH-1 gene deletions (10). To determine the prevalence of hGH-1 gene deletions in different populations (North-European, Mediterranean, and Turkish) and to challenge the generally held assumption that the hGH-1 gene deletion is uncommon in IGHD, we analyzed patients with severe forms of IGHD (height < −4.5 SD score) using the PCR amplification method and Southern blotting.

MATERIALS AND METHODS

Patients. The individuals studied were broadly grouped into three populations, North-European, Mediterranean, and Turkish. The North-European population included individuals from the northern part of the Alps, and the Mediterranean group comprised Italians, Spaniards, and Greeks who were either living in Switzerland or who were referred to our outpatient clinic from abroad because of short stature. The Turkish population living in the area of Ankara composed the third group. Seventy-eight subjects with severe growth retardation due to IGHD were studied. Diagnostic criteria included a pretreatment height of < −4.5 SD score, [except in one baby aged 3 mo (−3 SD score)], decreased growth rate, retarded bone age, normal karyotype, normal thyroid function, and peak GH levels below 4 μg/L after

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Correspondence and reprint requests: Dr. Primus E. Mullis, M.D., Department of Paediatrics, Inselspital, CH-3010 Bern, Switzerland
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stimulation test (insulin-induced hypoglycemia). Details of the patients are shown in Table 1.

The families of the patients with either a 6.7- or a 7.6-kb hGH-1 gene deletion were also studied to analyze the parental heterozygosity for the disorder.

Methods. DNA isolation. Genomic DNA was isolated from peripheral leukocytes of subjects and relatives as previously described (9, 11). The concentration of each sample was determined by measuring the OD of the purified DNA at 260 and 280 nm.

Restriction endonuclease digestion and Southern blot analysis. Samples of DNA (5 μg) were digested to completion with the restriction enzymes *Bam*HI and *Hin*dIII. After electrophoresis in 0.5–1.2% (wt/vol) agarose gels, the DNA fragments were transferred onto nylon membranes (Hybond-N; Amersham International, Little Chalfont, Buckinghamshire, UK) and hybridized to the hGH probe as previously described (9, 11). The filters were then washed at 65°C and autoradiographed at −70°C using intensifying screens (9, 11).

DNA amplification. The hGH-1 gene encodes the hGH peptide (12). Deletions of this locus can be detected by Southern blot analysis after *Hin*dIII or *Bam*HI digestion and hybridization to the hGH-1 cDNA probe. Eighty percent of the hGH-1 gene deletions characterized are 6.7 kb in length, and the remaining cases mainly involve 7.6-kb deletions (3, 5). Because there is extensive homology between regions in the 5′ and 3′ flanking region of the hGH-1 gene (5, 12), unequal recombination between these two highly homologous regions is the main cause for 6.7-kb deletions of genomic DNA encompassing the hGH-1 gene (5). DNA amplification of these highly homologous regions was performed using PCR, as described by Vnencak-Jones *et al.* (10). The PCR analysis has been done with oligonucleotide primers that anneal to the 5′ and 3′ ends of these homologous regions (10). After amplification, 25 μL of each reaction mixture were digested with the restriction endonucleases *Sma*I, *Bgl*I, and *Hae*II. Because differences in restriction endonuclease sites exist, the analysis of the fragment pattern after enzyme digestion allows the definition of the hGH-1 gene deletion. The digested PCR products were visualized after electrophoresis on a 0.8% agarose gel.

RESULTS

PCR amplification analysis. PCR amplification of the homologous regions flanking the hGH-1 gene produced two DNA fragments (1900 and 1921 bp) from genomic control DNA. After digestion of these PCR amplification products with *Sma*I, the 1900-bp fragment, deriving from the amplification of the first highly homologous block 5′ to the hGH-1 gene, was not cleaved, whereas the 1921-bp amplification product from the homologous block 3′ to the hGH-1 gene yielded fragments of 761, 712, and 448 bp. The pattern of DNA fragments obtained by *Sma*I digestion of PCR amplification products in the control patient is shown in Figure 1.

Unequal recombinational events between these highly homologous regions 5′ and 3′ to the hGH-1 gene produce 6.7-kb deletions of genomic DNA containing the hGH-1 gene and fusion fragments of 1918 bp composed of portions from both homologous regions. After digestion with *Sma*I, the 1918-bp fusion fragments derived from homozygotes for the 6.7-kb gene deletion yielded fragments of 1470 and 448 bp. Therefore, the pattern of DNA fragments after *Sma*I digestion of the PCR amplification products in patients homozygous for 6.7-kb deletions involving the hGH-1 gene are 1470 and 448 bp in length (Fig. 1*a*).

Unequal recombination between two 29-bp perfect repeats is believed to give rise to the 7.6-kb gene deletions (5), and, after PCR amplification, the 1900-bp PCR product is retained whereas the 1921-bp product is lost as a result of the 7.6-kb deletion of the genomic DNA. In these cases, because the 1900-bp fragment has no *Sma*I restriction site, the highly homologous block 5′ to the hGH-1 gene is retained after *Sma*I digestion, yielding a 1900-bp fragment (Fig. 1*b*).

In 12.8% of the patients analyzed, an hGH-1 gene deletion could be found. Eight individuals were homozygous for a 6.7-kb gene deletion, whereas only two patients, previously described by Braga *et al.* (8), were homozygous for a 7.6-kb deletion of genomic DNA (Table 2). There was a pair of affected brothers with the 6.7-kb gene deletion among the North-European population, a pair of sisters with the 7.6-kb gene deletion among the Mediterranean population (8), and two pairs of affected brothers with the 6.7-kb gene deletion among the Turkish population (Table 2).

Southern blot analysis. The size of the deletion was analyzed by performing Southern blotting.

In 10 out of the 78 patients, the 3.8-kb *Bam*HI band was missing, supporting the existence of an hGH-1 gene deletion. Inasmuch as both hGH-1 and hCS-L reside in a 25-kb *Hin*dIII-derived fragment, the size of the majority of the deletions (6.7 and 7.6 kb) can be determined after *Hin*dIII digestion of the DNA (4). The data obtained by Southern blotting were identical to the PCR data.

In three out of the 10 patients (all 6.7-kb deletions), the hGH replacement was stopped after 6–12 mo because the anti-hGH antibodies produced caused growth arrest, whereas in patients (two) with 7.6-kb deletions the anti-hGH antibody production did not blunt the growth effect of hGH treatment.

Family studies. The 78 individuals studied belonged to 15 North-European, eight Mediterranean, and eight Turkish families. The families were unrelated, and each had one or more individuals with IGHD. The family members of the affected children were analyzed for heterozygosities of the reported hGH-1 gene deletion using Southern blotting and the PCR amplification method. In each parent of normal stature, the corresponding heterozygous pattern for the hGH-1 gene deletion could be found. Most interestingly, there was a history of consanguinity in all of the families with an affected child, whereas a history of consanguinity was found in none, one, and two of the remaining North-European, Mediterranean, and Turkish families without hGH-1 gene deletions, respectively. The different degree of consanguinity in the three subpopulations selected may be the cause of the higher prevalence of hGH-1 gene deletions in the Turkish and Mediterranean subpopulations compared with the North-European subpopulation with severe IGHD.

Table 1. *Clinical details**

	North-European	Mediterranean	Turkish
n	32	22	24
Sex (F/M)	13/19	14/8	10/14
Age (y)	5.8 (2.0–10)	6.2 (0.25–8.3)	12 (4–24)
Bone age (y)	2.9 (0.9–5.4)	2.5 (0.2–3.5)	7.0 (2.0–12.5)
Height SD score for chronologic age	−7.5 (−5.3 to −9.6)	−6.2 (−3 to −9.3)	−6.4 (−4.5 to −9.2)
Height velocity (cm/y)	2.5 (1.2–3.3)	2.1 (1.5–3.1)	1.0 (0.5–2.3)
hGH peak on ITT (ng/mL)†	<0.3–1.5	<0.3–2.0	<0.3–3.6

* Values given are the median (range).

† ITT, insulin tolerance test.

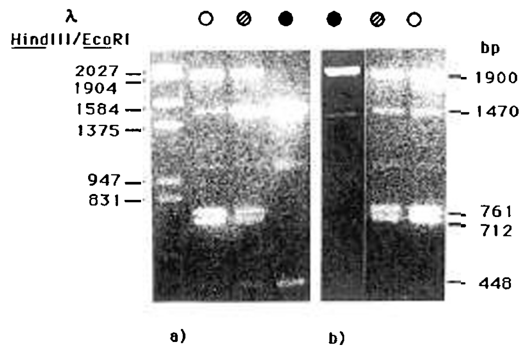

Fig. 1. DNA fragments obtained by PCR amplification after *Sma*I digestion and electrophoresis on a 0.8% agarose gel. *Open circles* represent subjects without a deletion; *hatched circles* represent subjects heterozygous for hGH-1 gene deletions, and *filled circles* represent subjects homozygous for hGH-1 gene deletions. *a*, The 6.7-kb hGH-1 gene deletion; *b*, the 7.6-kb hGH-1 gene deletion.

Table 2. *Prevalence of hGH-1 gene deletions in different populations*

	North-European	Mediterranean	Turkish
n	3/32 (9.4%)	3/22 (13.6%)	4/24 (16.6%)
Sex (F/M)	1/2*	2*/1	0/4†
6.7-kb hGH-1 gene deletion (F/M)	1/2	0/1	0/4
7.6-kb hGH-1 gene deletion (F/M)	0/0	2/0	0/0

* Siblings.
† Two unrelated siblings.

DISCUSSION

The hGH gene cluster consists of five very similar genes in the order 5′ (hGH-1, hCS-L, hCS-A, hGH-2, hCS-B) 3′ spanning approximately 66.5 kbp and is located on the long arm of chromosome 17 at bands q22–24 (12). The hGH-1 gene encodes the hGH, a polypeptide, that is synthesized by the somatotropic cells of the anterior pituitary.

Parks *et al.* (13) reported that 38% (five out of 13) of Oriental Jewish patients with severe (height < −4 SD score) IGHD had an hGH-1 gene deletion. None of these children produced a titer of anti-hGH antibodies sufficient to cause growth arrest (13). Studies among Chinese and Japanese subjects found a prevalence of hGH-1 gene deletions of 12% (three out of 26) and 0% (zero out of 10), respectively (10, 14).

The results of our study analyzing a total of 78 patients revealed a prevalence for hGH-1 gene deletion of 9.4, 13.6, and 16.6% in the subpopulation of children with severe IGHD (height < −4.5 SD score) of North-European, Mediterranean, and Turkish descent, respectively.

In 80% (eight out of 10), the hGH-1 gene deletion found was spanning 6.7 kb, which is in accordance with the figure reported by Phillips *et al.* (4). In addition, the affected subjects could not be distinguished from the children without gene deletion based on clinical findings. Notably, children without an hGH-1 gene deletion and those with a deletion of genomic DNA encompassing the hGH-1 gene can have equally severe growth retardation.

Compared with the PCR technique, Southern blotting is slow, time consuming, and laborious. Therefore, the PCR amplification method provides a real alternative to Southern blotting for identification and screening for homozygotes for hGH-1 gene deletions.

It is of interest that the two patients with a 7.6-kb deletion did not show any growth arrest, even though they developed a level of anti-hGH antibodies (68% maximum binding of ^{125}I-hGH) similar to that of two patients with a 6.7-kb deletion (64% and 66% maximum binding of ^{125}I-hGH) in whom the hGH treatment became blunted.

This study revealed, in contrast to our previous data, a high prevalence of hGH-I gene deletions in the subpopulation of children with severe IGHD (height < −4.5 SD score), selected from three apparently highly inbred populations of North-European, Mediterranean, and Turkish descent. In addition, the fact that all the families with hGH-1 gene deletions were consanguineous is of interest for the prenatal diagnosis of at-risk pregnancies and for families at risk for recurrence.

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