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THE GROWTH HORMONE RECEPTOR GENE MUTATION OF A JAPANESE PATIENT WITH LARON SYNDROME

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Summary Deletions and point mutations of the growth hormone (GH) receptor gene (*GHR*) have been identified in patients with Laron syndrome. We report the first detection of the *GHR* mutation among Japanese patients with Laron syndrome. Using the Japanese female patient's genomic DNA as a template, all exons and flanking portions of introns of *GHR* were amplified by polymerase chain reaction (PCR). Sequencing of the PCR products showed that the patient was homozygous for a G to A substitution at the first position of intron 4. This substitution was same as that detected in a Spanish patient and a north European patient. The base change occurred at the 5′ splice consensus sequence of intron 4, resulting in the abolition of a *Ban*I restriction site. Since this substitution was not detected by a *Ban*I restriction analysis in 85 control individuals, it is more likely a disease-related splice mutation than a polymorphism. The mutation in our patient was predicted to destroy the original 5′ splice site of intron 4 of *GHR* and to produce a new cryptic splice site, leading to abnormal mRNA processing and a lack of GH binding activity of GH-binding protein (GHBP).
Key Words *GHR*, Laron syndrome, splice mutation, the first Japanese case, sequencing

INTRODUCTION

Laron syndrome (Laron *et al.*, 1966), a rare autosomal recessive disorder, is characterized by a severely short stature resistant to growth hormone (GH). The cause of the syndrome, a partial deletion and a mutation of the growth hormone receptor (GHR) gene (*GHR*), were identified in 1989 (Godowski *et al.*, 1989;

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Amselem *et al.*, 1989). Although the short stature in Laron syndrome patients is clinically indistinguishable from that in isolated GH-deficient patients (Laron *et al.*, 1966), other manifestations such as a low level of insulin-like growth factor-I (IGF-I) despite a high GH level, a lack of GH binding in the liver microsomal membrane (Eshet *et al.*, 1984), a low level or absence of serum GH-binding protein (GHBP) (Daughaday and Trivedi, 1987; Baumann *et al.*, 1987; Laron *et al.*, 1989), and no response to exogenous GH (Laron *et al.*, 1989) characterize this syndrome.

GHR is the first member of a new class of transmembrane receptors and its extracellular domain including GH-binding sites is shown to be the serum GHBP. *GHR* located to 5p13-p11 is quite large, spanning more than 87 kb, and consists of 1 non-coding spliced exon and 9 exons that encode 620 amino acids: exon 1 is the non-coding spliced exon; exon 2 encodes the signal peptide; exons 3–7 the extracellular domain found as a circulating GHBP in serum; exon 8 the transmembrane domain; and exons 9 and 10 the intracellular domain (Godowski *et al.*, 1989; Leung *et al.*, 1987). In many tissues, *GHR* generates two different mRNAs by an alternative splicing of exon 3, one for a full length receptor and the other for an isoform that lacks exon 3 (Urbanek *et al.*, 1992; Sobrier *et al.*, 1993; Mercado *et al.*, 1994). Although previous studies suggested that exon 3 is not necessary for GH binding, the functional significance of the splice variant without exon 3 remains unknown (Mercado *et al.*, 1994; Bass *et al.*, 1991). Several different *GHR* mutations have been reported among Laron syndrome patients of different ethnic origins. In Japan, 4 cases of Laron syndrome have been reported. However the *GHR* mutation among Japanese is hitherto undescribed. We report here a mutation of *GHR* in a Japanese patient with the syndrome.

MATERIALS AND METHODS

Subjects. The patient, a Japanese female with the clinical features of Laron syndrome, was born to unrelated parents. Her parents and three siblings are of normal height. At age 36 years, her height was 125 cm (−6.6SD) and weight 56 kg. Her basal GH level was 9.2 ng/ml and peak GH levels after the insulin and arginine loading tests were high, being 60.8 ng/ml and 104.8 ng/ml, respectively, while her IGF-I level was low (42 ng/ml). Serum GHBP was below the detection limit of the assays, and IGF binding protein (IGFBP) 3 was low (0.51 μg/ml). Genomic DNA was isolated by the standard method from the peripheral blood leukocytes of the patient, 67 unrelated, phenotypically normal Japanese individuals and 18 growth-deficient children who did not have any clinical or biological features of Laron syndrome.

Sequencing of the GHR gene. All exons and flanking splice donor and acceptor sites of introns of *GHR* were amplified by polymerase chain reaction (PCR) with intronic primers shown in Table 1. One microgram of genomic DNA was added to a 100 μl reaction mixture containing 10 mM Tris-HCl (pH 8.3)/50

mM KCl/2.5 mM $MgCl_2$/0.1% Triton X-100/200 mM of each dNTP/100 pmol of each primer/0.8 U *Taq* polymerase. After initial denaturation of DNA at 94°C for 5 min, PCR was performed for 30 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, followed by a 5-min extension at 72°C. The PCR products were cloned into pGEM-T vectors (Promega Corp., WI). After these clones were purified by an automatic plasmid isolation system (PI-100, KURABO, Japan), they were labeled with ^{35}S-dATP and sequenced by the dideoxy method.

Restriction analysis. Exon 4 with the 5′ portion of intron 4 of *GHR* was PCR-amplified from the genomic DNA of the patient and 85 control individuals using GHR4F (AGGATCACATATGACTCACCT)/GHR4R (AGTGTACTTT-AGTAGGTACATC) as a primer pair. The condition for the PCR was the same as above. The PCR products were digested with *Ban*I at 30°C for 3 hr. The digests were then electrophoresed on 4% polyacrylamide gel and stained with ethidium bromide for visualization.

RESULTS

All of the *GHR* exons with exon/intron boundaries were amplified from the patient. Each exon had normal size, and there is no deletion of exons. Then All exons and exon/intron boundaries were sequenced. By sequencing of the plasmid clones, a G to A transition at the first position of intron 4 of *GHR* was identified

Table 1. Primers used for PCR amplification of *GHR*.

Exons	Primers*	Sequences (5′-3′)
2	GHR2F	GTCTGCTTTTAATTGCTGGGC
	GHR2R	ACACTGAGGGTGGAAATGGA
3	GHR3F	CCTCTTTCTGTTTCAGCCAC
	GHR3R	GGATAGTAGCTTAATTACACTA
4	GHR4F	AGGATCACATATGACTCACCT
	GHR4R	AGTGTACTTTAGTAGGTACATC
5	GHR5F	TAAGCTACAACATGATTTTTGG
	GHR5R	TTAGTCTAAAACTATGTCAAATG
6	GHR6F	GTGTCTGTCTGTGTACTAATG
	GHR6R	AGAAAAGTCAAAGTGTAAGGTG
7	GHR7F	TAGTGTTCATTGGCATTGAG
	GHR7R	ACAAAAGCCAGGTTAGCTAC
8	GHR8F	AAACTGTGCTTCAACTAGTCG
	GHR8R	GGTCTAACACAACTGGTACA
9	GHR9F	GAATATGTAGCTTTTAAGATGTC
	GHR9R	CATATGACAGGAGTCTTCAGGTG
10	GHR10F	GAGTTTCTTTTCATAGATCTTC
	GHR10R	GGTTTAAACATTGTTTTGGC

* F for sense strand; R for antisense strand.

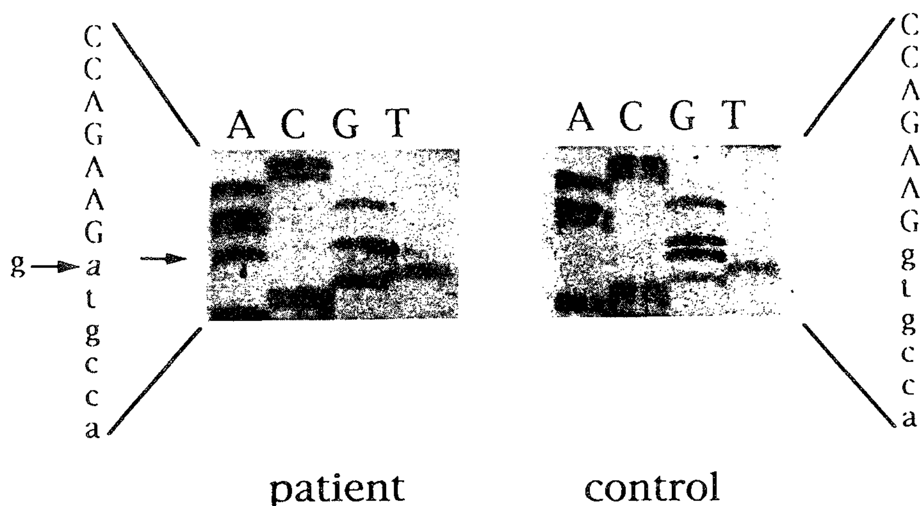

Fig. 1. DNA sequence at the exon 4/intron 4 boundary of *GHR* of the patient and the normal control. Arrow shows a G to A substitution at the first position of intron 4.

as a possible mutation in the patient (Fig. 1). All exons and other exon/intron boundaries showed normal sequences of nucleotides. The substitution was expected to cause the abolition of a *Ban*I site at the 5′ splice consensus sequence of intron 4. If a person has the site and another does not have it, *Ban*I digestion may give two fragments of 186 bp and 30 bp in the former and a 216 bp fragment in the latter. Restriction analysis revealed that the *Ban*I-digested PCR product from the patient gave only a longer 216 bp fragment compared with an expected 186 bp and 30 bp fragments, indicating that the patient did not have the *Ban*I site and was homozygous for the G-A transition. Then, in order to learn whether this substitution would affect the *GHR* expression, we analyzed this *Ban*I site among 85 control individuals. The results showed that all of them had only 186 bp and 30 bp fragments and retained the site (Fig. 2).

DISCUSSION

The first *GHR* mutation in a Japanese patient with Laron syndrome was demonstrated in this study. Though 4 cases of Laron syndrome have been reported in Japan, none of them was analyzed on the GHR gene. Our patient is the 5th case of Laron syndrome in Japan. We identified a G to A substitution at the 5′ splice consensus sequence of intron 4 of *GHR* in the patient. This substitution occurs within an extracellular domain and was not observed in any of the 85 control individuals. In addition, the substitution in our patient was same in position as

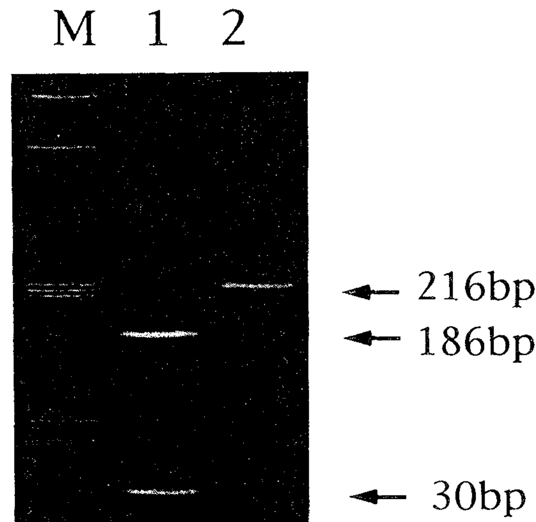

Fig. 2. *Ban*I digested exon 4 of *GHR*. All of 85 control individuals had only 186 bp and 30 bp fragments (lane 1). The patient had only a 216 bp fragment (lane 2). M: MW marker (pUC19/*Alu*I).

that detected in a Spanish patient (Berg *et al.*, 1993) and a north European patient (Amselem *et al.*, 1993). Thus, the detected G-A substitution is more likely a disease-related splice mutation than a polymorphism. Previous studies demonstrated that nine positions making up the 5′ splice consensus sequence play an important role in fixing a splice site and two nucleotides, GT, at the 5′ site act as the beginning position of splicing (Iida, 1990; Ohshima and Gotoh, 1987). A substitution at this position causes inactivation of the 5′ splice site resulting in an abnormal splicing. Thus, the substituted nucleotide observed in our patient is predicted to be deleterious and to destroy the original 5′ splice site in intron 4 of *GHR*, and it may produce a new cryptic splice site. As a result, the mutation may lead to abnormal mRNA processing and a lack of GH binding activity of GHBP. This hypothesis is coincident with the lack of serum GHBP in our patient. It remains to be seen whether or not the cellular expression of the *GHR*-cDNA is reduced in the patient.

GHR mutations observed so far among Laron syndrome patients have included deletions and nonsense, missense and frame-shift mutations, in addition to splice mutations. The reported nonsense mutations in *GHR* due to CG→TG or CG→CA changes all occurred at CpG dinucleotides, known as a 'mutation hot-spot', though the splice mutation that we identified is unrelated these base pairs. All mutations observed among Laron syndrome patients, including our patient, occur within an extracellular domain and may have caused deleted extracellular, transmembrane, and/or intracellular exoplasmic domains of GHR,

resulting in the lack of detectable GHBP in patients with the syndrome. This prediction was supported by the finding that cells transfected with a mutant cDNA having a missense mutation (F96S) lacked the GH binding activity (Duquesnoy *et al.*, 1991; Edery *et al.*, 1993).

Since a majority of patients with the syndrome is sporadic, most of the previously reported mutations seem to be confined to specific families. However a few identical mutations were found in patients from different geographic areas. The splice mutation in our patient is the first presentation of *GHR* mutation among Japanese patients and was identical to those detected in two different ethnic origins. Unfortunately both of her parents were already dead, and her siblings do not want to co-operate with us in this study. Thus we could not analyze genes of her family members. Recently, *GHR* mutations were identified among children with idiopathic short stature (Goddard *et al.*, 1995). We suppose that the *GHR* mutation may not be race- or family-specific and it would be detected in Japanese patients with Laron syndrome or other type of growth deficiency.

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