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Exon Redefinition by a Point Mutation within Exon 5 of the Glucose-6-Phosphatase Gene Is the Major Cause of Glycogen Storage Disease Type 1a in Japan

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Summary

Glycogen storage disease (GSD) type 1a (von Gierke disease) is an autosomal recessive disorder caused by a deficiency in microsomal glucose-6-phosphatase (G6Pase). We have identified a novel mutation in the G6Pase gene of a individual with GSD type 1a. The cDNA from the patient's liver revealed a 91-nt deletion in exon 5. The genomic DNA from the patient's white blood cells revealed no deletion or mutation at the splicing junction of intron 4 and exon 5. The 3′ splicing occurred 91 bp from the 5′ site of exon 5 (at position 732 in the coding region), causing a substitution of a single nucleotide (G to T) at position 727 in the coding region. Further confirmation of the missplicing was obtained by transient expression of allelic minigene constructs into animal cells. Another eight unrelated families of nine Japanese patients were all found to have this mutation. This mutation is a new type of splicing mutation in the G6Pase gene, and 91% of patients and carriers suffering from GSD1a in Japan are detectable with this splicing mutation.

Introduction

Glucose-6-phosphatase (G6Pase) catalyzes the terminal step in gluconeogenesis and glycogenolysis (Nordlie et al. 1985; Sukalski et al. 1989). G6Pase is a single copy gene located on chromosome 17 (Lei et al. 1994). The human G6Pase transcript unit spans 12.5 kb and consists of 5 exons. The G6Pase catalytic unit consists of 357 amino acids with an apparent molecular mass of 35 kD (Lei et al. 1993). The deduced human G6Pase protein contains the ER protein retention signal, KK, located at residues 354 and 355 (Jackson et al. 1990; Lei et al. 1993).

Deficiency of G6Pase causes glycogen storage disease (GSD) type 1a (von Gierke disease), an autosomal recessive disorder with an incidence of 1 in 100,000–300,000. This metabolic disease typically manifests during the 1st year of life, with severe hypoglycemia and hepatomegaly caused by the accumulation of glycogen. Children with GSD1a exhibit growth retardation, delayed puberty, lactic acidemia, hyperlipidemia, and hyperuricemia, and adults have a high incidence of hepatic adenomas (Hers et al. 1989; Beaudet et al. 1991).

Recently, the gene that encodes G6Pase and the mutations that cause G6Pase deficiency (GSD1a) have been reported (Lei et al. 1993, 1994). The molecular genetic basis of GSD1a in the Japanese has not been explored. We report here a new type of splicing mutation not involving an authentic splicing site mutation, in 9 Japanese families of 11 patients with GSD1a.

Patients and Methods

Patients

The patient was a 26-year-old man and progeny of first-cousin parents. He had the history of hepatomegaly and hypoglycemia since childhood and was diagnosed as having GSD1a, on the basis of serum hypoglycemia, hypertriglycedemia, hyperuricemia, and liver biopsy findings. The residual G6Pase activity in his liver was determined using the methods described by Burchell et al. (1988) and was 18% of that of a normal control, including nonspecific phosphatase activity, in both fresh and previously frozen liver biopsy specimens. He was the third of four children, and his younger brother also was affected by this disease.

RNA Extraction and cDNA Analysis

Total RNAs of patient and control were isolated from a frozen liver-biopsy specimen by using methods de-

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Table 1

Primers Used in the Present Study

Number	Sense or Antisense?	Sequence	Position (nt)
1	Sense	5′-TCTGCTGACATCTTCCT-3′	Exon 1 (43–59)
2	Antisense	5′-GCCTTACAAAGACTTCTTGTG-3′	Exon 5 (1156–1136)
3	Sense	5′-GAGCCCCTCTGGCCATGCCA-3′	Exon 3 (421–440)
4	Antisense	5′-TCCAGAGTCCACAGGAGGTC-3′	Exon 5 (795–776)
5	Antisense	5′-TTGCTGTAGTAGTCAGTATC-3′	Exon 2 (366–347)
6	Sense	5′-GCTGTGCAGCTGAATGTCTG-3′	Exon 4 (560–579)
7	Sense	5′-TGCAAGAGTGCGGTAGTGCC-3′	Exon 5 (1161–1080)
8	Sense	5′-GCCAGGCTCCAACATTT-3′	Intron 3
9	Antisense	5′-GGAGAGAAACGGAATGG-3′	Intron 4
10	Sense	5′-CTTCCTATCTCTCACAG-3′	Intron 4
11	Sense	5′-GGGATTCTAGACTGTGCAGCTGAATGTCTG-3′	Exon 4 (550–579)
12	Antisense	5′-CTGGGCTTTCTCCAGGATCCACAGGAGGTC-3′	Exon 5 (805–776)

scribed elsewhere (Chomczynski and Sacchi 1987). The sources of the specimens for the normal control liver RNA and white blood cell (WBC) genomic DNA were individuals who had no signs or laboratory data consistent with GSD1a.

First-strand cDNA was generated from 5 μg of total RNA by using Molony murine leukemia virus reverse transcriptase (RT) (Bethesda Research) with random primers. PCR amplification of cDNA was performed for 40 cycles consisting of denaturation at 92°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 3 min with primers 1 and 2 (table 1) (Saiki et al. 1988). The amplified cDNA was subcloned into the TA vector (Promega), and the nucleotide sequence of the inserted cDNA was determined by primers 1–7, a dye terminator cycle sequencing kit (ABI), and a DNA sequencer (model 373A; ABI) (table 1).

Genomic DNA Analysis

Genomic DNA was isolated from WBCs, according to standard procedures (Kunkel et al. 1977). PCR fragments were amplified from genomic DNA of the patient by using primers 8 and 9, 4 and 10 (table 1) (Saiki et al. 1988). PCR fragments from exon 4 to exon 5 were amplified by the long PCR method for 40 cycles, consisting of denaturation at 98°C for 20 s, annealing and extension at 68°C for 7 min with primers 11 and 12 using a Takara LA PCR kit (Cheng et al. 1994). The PCR fragments were sequenced directly using the same primers. The 3′ site of intron 4 was sequenced with primer 4 (dye terminator cycle sequencing kit; ABI and DNA sequencer model 373A; ABI).

In Vitro Expression

PCR fragments from the patient and control genomic DNA were generated by the long PCR with primers 11 and 12. The fragments were digested with *Xba*I (within primer 11) and *Bam*HI (within primer 12), purified by centricon 100 (Amicon) and then ligated to the expression vector pSVL (Pharmacia LKB Biotechnology) to create minigene constructs that included the G6Pase sequence extending from within exon 4 to within exon 5. A plasmid carrying the same sequence as the mutant form (except that the mutation site was changed to normal) was constructed by substituting the *Sac*I (−286 bp from intron 4 3′ end)–*Bam*HI (within primer 12 of exon 5) fragment of the mutant sequence. The substitution of the mutation site was confirmed by direct sequencing. Transfection of COS cells was achieved by the calcium-phosphate method as described (Kingston et al. 1987). Total RNA was prepared ~48 h following transfection, was reverse-transcribed, and was PCR-amplified with primers 4 and 6. One-tenth of the reaction products were electrophoresed in 3% NuSieve agarose gels.

Dot Blotting

PCR of the intron 4–exon 5 junction of genomic DNA, which included the mutation site, was performed for 40 cycles by primers 4 and 10 and a thermal cycler (Perkin-Elmer PJ2000). A sample (5 μl) of each amplified DNA in 50 μl was denatured by 0.4 M NaOH and was blotted onto a Gene Screen Plus nylon membrane according to a method described elsewhere (Mullis et al. 1986). The filters then were hybridized in a solution of 5× SSPE, 0.5% SDS, with the following allele-specific oligonucleotide (ASO) probes labeled at the 5′ end: N727 (normal probe), 5′-AAGCTGAA<u>C</u>AGGAAGAA-3′; and M727 (mutant probe), 5′-AAGCTGAA<u>A</u>AG-GAAGAA-3′. Following hybridization for 8 h at 46°C, filters were washed twice in 3% SSC for 5 min and then in 6× SSC at 46°C for 5 min. The filters were autoradiographed for 8 h at −70°C by using an intensifying screen.

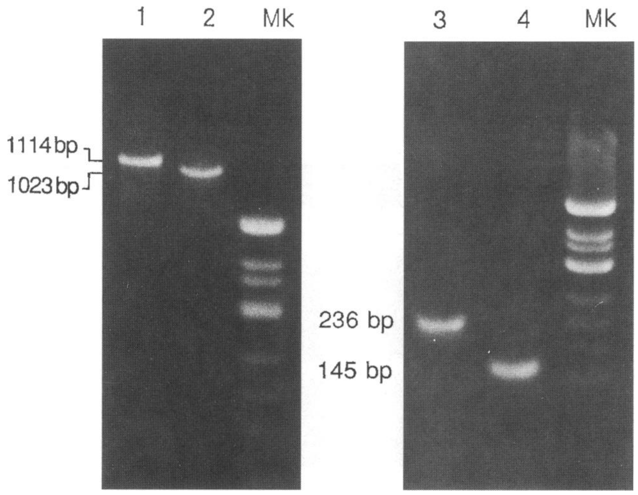

Figure 1 Agarose gel electrophoresis of RT/PCR fragments of RNAs from the patient and control livers. Primers 1 and 2 were used for amplification in lane 1 and 2. Primer 4 and 6 were used for amplification in lane 3 and 4. "Mk" refers to *Hinc*II-digested ψX174 phage DNA (Toyobo). Lane 1, control; lane 2, patient. Lane 3, control; lane 4, patient.

Results

Nucleotide Sequence of a Mutant G6Pase cDNA

We amplified and sequenced the entire coding region of the G6Pase cDNA from the patient. All the PCR products of the coding sequence of the patient were smaller in size than the control (fig. 1). There was a deletion of the front 91 nt (nt 642–732) of exon 5 in the patient's cDNA. The deletion altered the reading frame of the encoded protein such that a stop codon would be generated at codon 212 (nt 713–715). The predicted mutant G6Pase protein is a severely truncated protein of 211 amino acids. There were no other differences between the normal and mutant G6Pase cDNA.

Nucleotide Sequence of a Mutant G6Pase Gene

Analysis on a 3% NuSieve agarose gel of PCR products obtained from amplification of the patient's genomic DNA showed that product with primers 10 and 4 (intron 4, exon 5) was normal (fig. 2*A*, *B*). Sequence analysis of this fragment revealed no splice-junction mutation. There were an acceptor site at codon 218 (nt 732) and a PyNPyTPuAPy-like sequence (TTCTCAT), lariat site, which are necessary for splicing, located at codon 210–212 (nt 708–714) (fig. 2*C*) (Pikielny and Rosbash 1985; Ruskin et al. 1985). There was one nucleotide substitution (G to T) in the 5′ region of the new splice site in exon 5 (nt 727) (fig. 2*C*). Exon 4, including the 5′ and 3′ exon-intron junction, was amplified with primers 8 and 9 and directly sequenced; there were no exon-intron junction mutations (data not shown). The 3′ site of intron 4 was amplified with primers 11 and 12 and was directly sequenced with primer 4. There was no mutation in the PyNPyTPuAPy-like sequence (fig. 2*C*). These data demonstrate that although a splicing mutation had occurred, there were no mutations at either 5′ or 3′ intron 4 splicing sites, and only one nucleotide substitution was present in the 5′ region of the new splice site in exon 5 (nt 727) (fig. 2*C*). In this patient, although there was a normal splice site, no normal splice occurred.

A Single-Base Mutation Causes the Splicing Mutation

To examine whether the nucleotide substitution in exon 5 affected splicing, the amplified genomic DNA was subcloned into pSVL expression vector, and the pattern of splice-site selection was assessed by RT-PCR, following transfection into COS cells (Kingston 1987). A schematic diagram of the expression vector construct

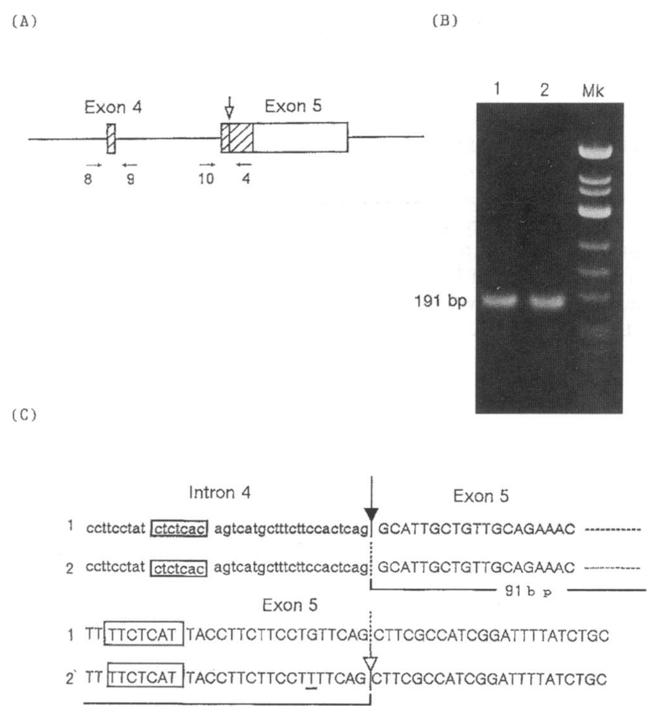

Figure 2 *A*, Partial schematic representation of the G6Pase gene. The boxes represent the exons. The boxes with diagonal lines indicate the coding region of G6Pase. The unlined boxes indicate the noncoding region. The positions of the primers used for amplification of genomic DNA are indicated by arrows. The larger arrow indicates the new exon-intron junction in the patient. *B*, Agarose gel electrophoresis of PCR fragments of genomic DNAs from the patient's and control WBCs. The primers 4 and 10 were used for amplification. "Mk" refers to *Hinc*II-digested ψX174 phage DNA (Toyobo). Lane 1, control; lane 2, patient. *C*, DNA sequence of the analyzed exon-intron transitions of the G6Pase gene. Exon sequences are shown in uppercase, intron sequences in lowercase. The point mutation in the patient is underlined. The blackened arrow indicates the exon-intron junction. The unblackened arrow indicates the new splice site. Boxes indicate the PyNPyTPuAPy-like sequence. 1, control; 2, patient.

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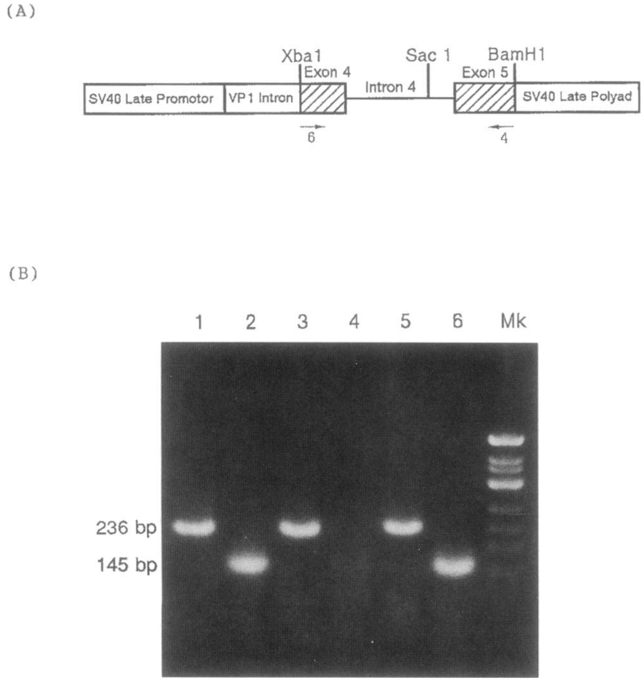

Figure 3 Expression of splicing phenotype in COS cells. *A,* A schematic of the pSVL expression vector construct with the position of the primers (*arrows*) employed to amplify cDNAs derived from transfected COS cell RNAs. *B,* RT-PCR amplification products from COS cell RNAs, prepared following transfection with the normal, mutant, reconstructed, and vector-alone allelic constructs, are shown in lanes 1, 2, 3, and 4, respectively. RT-PCR products from the normal and the patient's liver RNAs are shown in lanes 5 and 6, respectively. Mk refers to DNA size marker *Hinc* II-digested ψX174 phage DNA (Toyobo).

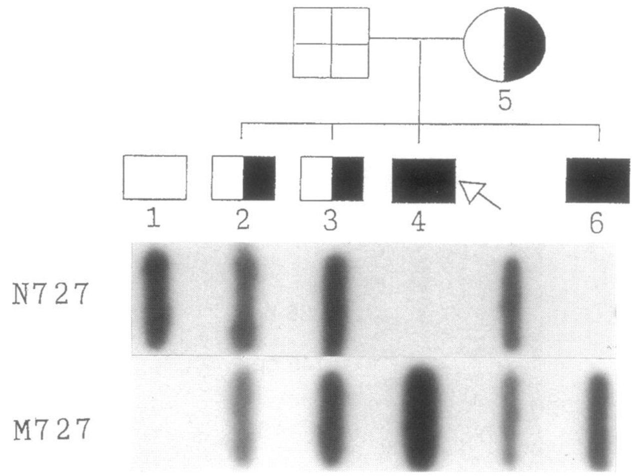

Figure 4 Genotype analysis of an affected pedigree. Exon 5 DNA of the individual was specially amplified by PCR, with primers 4 and 10, and was hybridized with the following probes: N727-normal probe; M727-mutant probe. The parents are cousins. Unblackened and blackened symbols represent the normal haplotype and the mutant haplotype, respectively. 1, normal unrelated individual; 2, unaffected brother; 3, unaffected brother; 4, propositus (*arrow*); 5, mother; 6, affected brother

and the amplification strategy is shown in figure 3*A*. The RT-PCR products were identified by size (normal processed mRNA, 236 bp; mutant, 145 bp). The results indicated that the COS cells carrying the normal control made the normal spliced product, whereas no normal spliced product was detected in the mutant or in the cells carrying vector alone, and only the mutant product was detected in the mutant form. The COS cells carrying the reconstructed form made a normal spliced product (fig. 3*B*).

Mutational Analysis of the Affected Pedigree

To determine whether the patient was homozygous for the mutation at nt 727 (GSD1aG727T), the sequence of exon 5 was amplified by PCR and was examined by dot blot hybridization with an ASO probe, N727 (normal) or M727 (mutant). His family members also were examined. The N727 probe hybridized with the genomic DNAs of his mother, his two elder brothers, and normal control, while the M727 probe hybridized with the genomic DNAs of the proband, his affected younger brother and his mother (fig. 4). These findings indicated that the patient and his affected younger brother were homozygous for this mutation, and that the unaffected brothers and the unaffected mother were heterozygous for this mutation.

Family members of eight unrelated Japanese patients with GSD1a were analyzed by dot blot hybridization with PCR and ASO probes. Of these, seven patients were homozygous for the GSD1aG727T mutation, and, in the eighth family, two patients were heterozygous for the mutation (fig. 5). Eleven normal controls had no GSD1aG727T mutation (data not shown).

Discussion

This study characterized a new type of splicing mutation in the gene encoding the G6Pase. The cDNA prepared from the patient's liver had a deletion of 91 nt, and there was no normal-sized cDNA. This mutation

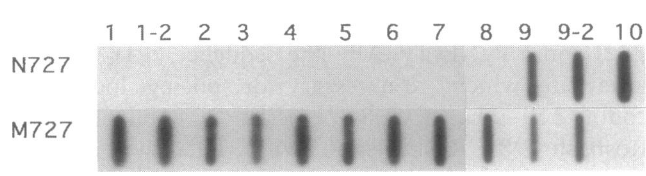

Figure 5 Genotype analysis of GSD1a patients in Japan. Exon 5 DNA of the patients was specially amplified by PCR, with primers 4 and 10, and was hybridized with the following probes: N727-normal probe; M727-mutant probe. 1–9, unrelated patients affected with GSD1a; 1 and 1-2, 9 and 9-2, affected siblings; 10, normal unrelated individual.

Table 2

Phenotypic Characteristics of GSD1a Patients with Homozygous GSD1aG727T Mutation

	Patient Number 1	2	3	4	5	6	7	8	
Initials	YF	SS	YS	FN	TT	HN	ME	MK	
Age (years)	31	37	32	27	31	18	27	39	
Sex	M	F	F	M	M	M	M	F	
Characteristic									Total[a]
Age at clinical onset:									
<10 years	+	−	+	+	+	+	+	−	6/8
>10 years	−	+	−	−	−	−	−	+	2/8
Growth retardation	+	+	+	+	−	+	−	+	6/8
Hypoglycemia	−	+	+	+	+	−	−	−	4/8
Enlarged liver	+	+	+	+	+	+	+	+	8/8
Liver dysfunction	+	−	−	+	+	+	+	+	6/8
Hepatic adenoma	+	+	+	+	+	+	+	−	7/8
Hyperlactiacidemia	+	+	+	+	+	+	+	+	8/8
Hypertriglycedemia	+	+	+	+	+	+	+	+	8/8
Hypercholesterolemia	+	+	−	+	+	−	−	−	4/8
Hyperuricemia	+	+	+	+	+	+	+	−	7/8
Renal dysfunction	+	−	+	+	−	−	−	+	4/8
Death	−	−	+	−	−	−	−	−	1/8

[a] No. of patients with phenotypic characteristic/total no. of patients.

results in a G6Pase polypeptide that is 146 amino acids shorter (211 amino acids) than the normal gene product (357 residues) located at the carboxy-terminal end. It is reported that an 11-amino-acid deletion at the carboxy-terminal (Q347SP mutation) results in G6Pase inactivation (Lei et al. 1994). This truncated G6Pase product is thought to have no G6Pase activity. The residual activity of G6Pase in the patient's liver was 18% of normal, including nonspecific phosphatase activity. This might reflect the nonspecific phosphatase activity other than G6Pase, because normal G6Pase cDNA was not detected by the PCR amplification with wild-type specific primers that were deleted in the mutant G6Pase cDNA (data not shown). The activity of the G6Pase were analyzed in several unrelated homozygous GSD1a patients with this mutation, and, in all of them, the activity was low or not detectable (data not shown).

Analysis of the mutant G6Pase genomic DNA verified a single nucleotide substitution (G to T) at nt 727 (fig. 2C). Although the patient's splice site in intron 4 and exon 5 had a normal consensus sequence, a normal splice did not occur. The mutation at nt 727 is thought to be the cause of the splicing mutation, and it was confirmed by in vitro splicing experiments.

Many types of splicing mutations have been reported in human diseases, causing exon skipping or activation of cryptic splice sites (Fukumaki et al. 1982; Grandchamp et al. 1989; Lee et al. 1991; Horst et al. 1993; Hoshide et al. 1993; Katrin et al 1994; Tsujino et al 1994). In the case of this G6Pase splicing mutation, a 1-nt substitution, located far from the splice junction, altered the splicing site. According to the database containing aberrant splicing mutations of mammalian genes, new splice-site creation is relatively infrequent (11% of aberrant splice mutations). In new splice-site creation, there is no alteration in the authentic splice-site sequences, and creation of the new 5′ and 3′ sites is only observed in the upstream region of the authentic 5′ and 3′ splice sites (Nakai and Sakamoto 1994). To our knowledge, this G6Pase mutation (GSD1aG727T) is the first case of a human inherited disease in which the new 3′ splice occurs in the downstream region of a normal splice site without any normal splice-site mutations.

A scoring system described by Schapiro and Senapathy (1987) assigns a numerical value to any proposed 5′ or 3′ consensus sequence. The score of the proper splice-acceptor site at intron 4 of the G6Pase gene is 97%; the scores of the newly appearing splice acceptor sites at exon 5 in the patient and the control are 93% and 91%, respectively. The score of restricted pyrimidine-rich site (polypyrimidine tract) is 93.3% in the proper splice site; the scores of newly appearing splice site in the patient and the control are 96.8% and 92.6%, respectively. By restricting the scoring system of splice-acceptor-site recognition to the polypyrimidine tract, the newly appearing splice-acceptor site of mutant G6Pase gene may be more easily spliced out than would the normal splice acceptor site of intron 4. Polypyrimidine tract affects the first step of splicing system, 5′ splice-site cleavage and lariat formation (Ruskin et al. 1985). By this point mutation, the polypyrimidine tract of the new splice site may become to be more efficient for 5′ splice-site cleavage and lariat formation.

By genotype analysis of the affected pedigree, the patient and his affected brother were found to be homozygous for this mutation, while his unaffected two brothers and mother were heterozygous. The genotype of this mutation was compatible with the phenotype of GSD1a, (autosomal recessive), in the pedigree.

GSD1a is the predominant type of GSD. The incidence is 1 in 100,000–300,000. Approximately 400 cases of GSD1a have been described in Japan. GSD1a has been analyzed at the nucleotide level, and several GSD1a patients have been detected by DNA analysis of their blood (Lei et al. 1993, 1994). In the United States, the mutation sites are heterogenous. Six mutations occur in 12 patients (Chou et al. 1994). It appears that the mutation described here is another new type.

The genotype analysis of eight families of another nine unrelated patients, with the ASO probes described here, revealed that seven families of seven patients were homozygous for this mutation and that one family of two patients who were thought to be compound heterozygote was heterozygous for this mutation. By this analysis, this G6Pase mutation (GSD1aG727T) is thought to be the major cause of GSD1a in Japan. The phenotypic characteristics of patients with homozygous GSD1aG727T mutation revealed that most patients have almost all manifestations: growth retardation, hepatomegaly, hypoglycemia, hyperlipidemia, hyperuricemia, and hepatic adenoma (table 2). The reason for this may be that, because G6Pase with the GSD1aG727T mutation is thought to have no activity, the phenotype may become severe.

According to our genotype analysis, 91% of GSD1a patients and carriers in Japan are detectable with GSD1aG727T mutation analysis. The reason for genotype homogeneity in Japan may be the homogeneity of Japanese. Although we analyzed 9 families of 11 patients with GSD1a, the ASO probes described here could also be used to help diagnosis and heterozygote detection in Japan. In addition, the new type of splice mutation in this patient will be of help in the analysis of the splicing mechanism.

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References

Beaudet AL (1991) The glycogen storage diseases. In: Wilson JD, Braunwald E, Isselbacher KJ, Petersdorf RG, Martin JB, Fauci AS, Root RK (eds) Harrison's principles of internal medicine, 12th ed. McGraw-Hill, New York, pp 1854–1860

Burchell A, Hume R, Burchell B (1988) A new microtechnique for the analysis of the human hepatic microsomal glucose-6-phosphatase system. Clin Chem Acta 173:183–192

Cheng S, Fockler C, Barnes WM, Higuchi R (1994) Effective amplification of long targets from cloned inserts and human genomic DNA. Proc Natl Acad Sci USA 91:5695–5699

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Chou JY, Lei K-J, Shelly LL (1994) Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. Am J Hum Genet Suppl 55:A5

Fukumaki Y, Ghosh PK, Benz EJ, Reddy VB, Lebowitz P, Forget BG, Weissman SM (1982) Abnormally spliced messenger RNA in erythroid cells from patients with β^+ thalassemia and monkey cells expressing a cloned β^+-thalassemic gene. Cell 28:585–592

Grandchamp B, Picat C, Rooij F, Beaumont C, Wilson P, Deybach JC, Nordmann Y (1989) A point mutation G→A in exon 12 of the porphobilinogen deaminase gene results in exon skipping and is responsible for acute intermittent porphyria. Nucleic Acids Res 17:6637–6649

Green MR (1986) Pre-mRNA splicing. Annu Rev Genet 20:671–708

Hers H-G, Van Hoof F, de Barsy T (1989) Glycogen storage disease. In: Scriver CR, Beaudet AL, Charles R, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 425–452

Horst K, Kurt U, Charalampos A, Petra F, Karl JL, Gerd S (1993) A splice junction mutation causes deletion of a 72-base exon from the mRNA for lysosomal acid lipase in a patient with cholesteryl ester storage disease. J Clin Invest 92:2713–2718

Hoshide R, Matsuura T, Haraguchi Y, Endo F, Yoshinaga M, Matsuda I (1993) Carbamyl phosphate synthetase I deficiency. J Clin Invest 92:1884–1887

Jackson MR, Nilsson T, Paterson PA (1990) Identification of a consensus motif for retention of transmembrane protein in the endoplasmic reticulum. EMBO J 9:3153–3162

Katrin W, Thilo D, Manfred S, Thomas M, Rosemarie B-H, Burkhard T, Jorg S (1994) A novel exon in the cystic fibrosis transmembrane conductance regulator gene activated by the nonsense mutation E92X in airway epithelial cells of patients with cystic fibrosis. J Clin Invest 93:1852–1859

Kingston RE (1987) Transfection of DNA into eukaryotic cells. In: Ausubel FM, Brent R, Kingston RE, Moor DD, Sceidman JG, Smith JA, Struhl K (eds) Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, pp 9.1.1–9.1.4

Kunkel LM, Smith DK, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Berg RW, et al (1977) Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. Proc Natl Acad Sci USA 74:1245–1249

Lee B, Vitale E, Superti-FA, Steinmann B, Ramirez F (1991) G to T transversion at position +5 of a splice donor site causes skipping of the preceding exon in the type III procollagen transcripts of a patient with Ehlers-Danlos syndrome type IV. J Biol Chem 266:5256–5259

Lei K-J, Pan CJ, Shelly LL, Liu J-L, Chou JY (1994) Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. J Clin Invest 93:1994–1999

Lei K-J, Shelly LL, Pan C-J, Sidbury JB, Chou JY (1993) Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease tpe 1a. Science 262:580–583

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp Quant Biol 51:263–274

Nakai K, Sakamoto H (1994) Construction of a novel database containing aberrant splicing mutations of mammalian genes. Gene 141:171–177

Nordlie RC, Sukalski KA (1985) Multifunctional glucose-6-phosphatase: a critical review. In: Martonosi AN (ed) The enzymes of biological membranes, 2d ed. Plenum, New York, pp 349–398

Padgett RA, Grabowski PJ, Konarska MM, Seiler S, Sharp PA (1986) Splicing of messenger RNA precursors. Annu Rev Biochem 55: 1119–1150

Pikielny CW, Rosbash M (1985) mRNA splicing efficiency in yeast and the contribution of nonconserved sequences. Cell 41:119–126

Ruskin B, Greene JM, Green MR (1985) Cryptic branch point activation allows accurate in vitro splicing of human β-globin intron mutants. Cell 41:833–844

Saiki RK, Gelfand DH, Stofeel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:489–491

Schapiro MB, Senapathy P (1987) RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acid Res 15:7155–7174

Sukalski KA, Nordlie RC (1989) Glucose-6-phosphatase: two concepts of membrane-function relationship. Adv Enzymol Relat Areas Mol Biol 62:93–117

Tsujino S, Servidei S, Tonin P, Shanske S, Azan G, Di-Mauro S (1994) Identification of three novel mutations in non-Ashkenazi Italian patients with muscle phosphofructokinase deficiency. Am J Hum Genet 5411:812–819