

ORIGINAL ARTICLE

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Glucose-6-phosphatase gene mutations in Taiwan Chinese patients with glycogen storage disease type Ia

Received: January 4, 2000 / Accepted: February 28, 2000

Abstract Glycogen storage disease type Ia (GSD Ia) is caused by a deficiency of glucose-6-phosphatase (G6Pase) activity. Eighteen GSD Ia families were studied for *G6Pase* gene mutations. Thirty-two mutations were found in 36 GSD Ia chromosomes: 16 were 727 G→T (44.44%); 13 were R83H (327 G→T; 36.11%); 1 was 341delG; 1 was 933insAA; and 1 was 793 G→T. The 727 G→T and R83H mutations together accounted for 80.56% (29/36) of the GSD Ia chromosomes. These two mutations were easily examined by polymerase chain reaction-based methods, and the prenatal diagnosis of a non-affected fetus was successfully made. The 727 G→T mutation is the predominant mutation in Japanese GSD Ia patients, but is rarely seen in Western countries. The 727 G→T mutation is also the most prevalent mutation in Taiwan Chinese, although the incidence is not as high as in Japan.

Key words Glycogen storage disease type Ia · Glucose-6-phosphatase · Mutation · Chinese · Taiwan

Introduction

Glucose-6-phosphatase (G6Pase, E.C.3.1.3.9) is responsible for the final step of glycogenolysis — the conversion of glucose-6-phosphate to glucose (Chen and Burchell 1995; Foster et al. 1997). Deficiency of G6Pase induces glycogen storage disease type Ia (GSD Ia; von Gierke disease; MIM no. 232,200). Excessive glycogen storage is found in the

liver, kidney, and intestine of affected individuals. Human G6Pase gene catalytic unit (G6PC; GenBank U01120), located on chromosome 17q21, spans 12.5 kb and consists of five exons (Lei et al. 1993). Several common GSD Ia mutations have been identified in different ethnic groups, e.g., the R83C and Q347X mutations in Caucasians, the 130X in Hispanics, and the 727 G→T in Japanese (Foster et al. 1997; Lei et al. 1993; Lei et al. 1994; Lei et al. 1995; Chevalier-Porst et al. 1996). R83H (327 G→T) has been thought to be the most prevalent mutation in Chinese (Lei et al. 1995).

We previously analyzed several Taiwan Chinese GSD Ia patients, but found that the R83H mutation accounted for only 25% of the chromosomes (Hwu et al. 1995). Recently, the 727 G→T mutation was found to be prevalent in Hong Kong Chinese patients (Lam et al. 1998). In this article, we report on 18 GSD Ia families, including one fetus, who were studied for G6Pase gene mutations. We found that 727 G→T was the most prevalent mutation in Taiwan Chinese.

Subjects and methods

Eighteen families with GSD Ia were studied (see Table 1). The first eight patients listed in Table 1 and patient 13 have been described previously (Hwu et al. 1995; Hwu et al. 1999). All patients had typical clinical and laboratory findings, including short stature, hepatomegaly, hypoglycemia, lactic acidosis, hyperlipidemia, hyperuric acidemia, and flat glucose response to glucagon stimulation. Clinical information, including sex and age at diagnosis, is summarized in Table 1.

Genomic DNA was extracted from the peripheral blood of the patients and their parents. Chorionic villus sampling from the fetus of the mother of patient 13 was performed at 12 weeks of gestation after informed consent and permission of the ethics committee were obtained. DNA was extracted directly from villi without further culture. The five exons of the *G6Pase* gene were amplified from genomic DNA, as previously described (Lei et al. 1993). Polymerase chain reaction (PCR) products were purified with a Qiaex

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Table 1. Summary of mutations in GSD Ia patients

Patient no.	Sex	Age (years) at diagnosis	R83H mutation	727 G→T mutation	Other mutations
1	F	12	+	+	
2	F	4	-	+	
3	M	3	-	++	
4	M	2	-	+	793 G→T
5	M	3	-	+	
6	M	1	+	+	
7	M	3	+	+	
8	M	6	+	+	
9	F	1	+	-	
10	M	8/12	+	+	
11	F	1	-	++	
12	M	4/12	+	-	
13	M	5/12	+	-	341delG
14	M	7/12	-	++	
15	M	2	++	-	
16	F	2	+	+	
17	M	5/12	+	-	933insAA
18	F	2	+	+	

GSD, Glycogen storage disease; +, one positive allele; ++, two positive alleles; -, negative

Gel Extraction kit (Qiagen, Hilden, Germany) and sequenced either directly or after cloning by [$\alpha^{35}\text{S}$]-dATP incorporation with a CircumVent sequencing kit (BioLabs, Hestfordshire, England, UK).

The R83H and 727 G→T mutations were also checked by restriction fragment length polymorphism (RFLP). R83H creates a new *Fok* I restriction site, so that the exon 2 PCR product (191 bp) can be cut into 131-bp and 60-bp fragments (Lam et al. 1998). The 727 G→T mutation destroys an amplification-created *Bsr* GI site, and thus prevents the cleavage of an 89-bp PCR product into 59-bp and 30-bp fragments (Okubo et al. 1997). Both the *Fok* I and *Bsr* GI enzymes were purchased from BioLabs.

Results and discussion

Thirty-two mutations were found in 36 GSD Ia chromosomes: 16 were 727 G→T (44.44%); 13 were R83H (36.11%); 1 was 341delG; 1 was 933insAA; and 1 was 793 G→T (Table 1). The 933insAA is a novel frame-shift mutation, and the polypeptide stops 16 amino acids after lysine 285. The patient is heterozygous for this mutation (data not shown). The 793 G→T is a silent mutation which changes codon 238 from CTG to CTT (both CTG and CTT encoding leucine). The three other mutations have been described previously (Foster et al. 1997; Lam et al. 1998). The 727 G→T and R83H mutations together accounted for 80.56% of the GSD Ia chromosomes (29/36). In 66.67% of the patients (12/18), the mutations of both chromosomes could be identified by screening for these two mutations. These screening methods are, therefore, very helpful in the diagnosis and counseling of GSD Ia patients. Mutations in 5 of the 36 chromosomes were not found. They could be located in either the promoter or intronic regions.

Patient 13 is a compound heterozygote, in that he inherited the R83H mutation from his father, and the 341delG mutation from his mother. His mother had a second pregnancy, and analysis of DNA from the villi of the fetus, both by RFLP and by direct sequencing, showed no R83H mutation (data not shown). The presence of the 341delG mutation could not be excluded, but the signal for the mutant allele in direct sequencing is weak. This may be caused by maternal DNA contamination. Nevertheless, the fetus was not affected by GSD Ia.

Although 727 G→T is the most common mutation in both Taiwan and Hong Kong Chinese, R83H was found in seven of the ten mutant chromosomes in a Chinese population in the United States (Lei et al. 1995). Because most of the populations in Hong Kong and Taiwan come from the southeastern part of China, it is probable that the 727 G→T mutation is more prevalent in Southeast China.

In Japan, the 727 G→T mutation has been detected in 91% of GSD Ia patients (Okubo et al. 1997; Kajihara et al. 1995). The genotype homogeneity in Japan is probably the result of a founder effect. The elemental Japanese population may have originated in both Southeast and Northeast Asia (Hanihara 1992; Horai et al. 1996). From the similarity in the *G6Pase* gene mutation between Taiwan and Japan, we hypothesize that the 727 G→T mutation probably spread from Southeast Asia to Japan. This is analogous to findings with β -thalassemia, in that both the mutations and the associated haplotypes in the β -globin gene are identical in Chinese and Japanese (Naritomi et al. 1990). Future studies on the haplotypes of GSD Ia patients in these areas are required to prove this hypothesis.

Acknowledgments This work was supported in part by a Grant from the National Taiwan University (no. NTUH 85-163-A48).

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