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A Gene on Chromosome 11q23 Coding for a Putative Glucose-6-Phosphate Translocase Is Mutated in Glycogen-Storage Disease Types Ib and Ic

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Summary

Glycogen-storage diseases type I (GSD type I) are due to a deficiency in glucose-6-phosphatase, an enzymatic system present in the endoplasmic reticulum that plays a crucial role in blood glucose homeostasis. Unlike GSD type Ia, types Ib and Ic are not due to mutations in the phosphohydrolase gene and are clinically characterized by the presence of associated neutropenia and neutrophil dysfunction. Biochemical evidence indicates the presence of a defect in glucose-6-phosphate (GSD type Ib) or inorganic phosphate (Pi) (GSD type Ic) transport in the microsomes. We have recently cloned a cDNA encoding a putative glucose-6-phosphate translocase. We have now localized the corresponding gene on chromosome 11q23, the region where GSD types Ib and Ic have been mapped. Using SSCP analysis and sequencing, we have screened this gene, for mutations in genomic DNA, from patients from 22 different families who have GSD types Ib and Ic. Of 20 mutations found, 11 result in truncated proteins that are probably nonfunctional. Most other mutations result in substitutions of conserved or semiconserved residues. The two most common mutations (Gly339Cys and 1211–1212 delCT) together constitute ~40% of the disease alleles. The fact that the same mutations are found in GSD types Ib and Ic could indicate either that Pi and glucose-6-phosphate are transported in microsomes by the same transporter or that the biochemical assays used to differentiate Pi and glucose-6-phosphate transport defects are not reliable.

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Introduction

Glycogen-storage diseases type I (GSD type I [also known as "von Gierke disease") are recessive disorders due to a deficiency in glucose-6-phosphatase (reviewed in Chen and Burchell 1995), the enzymatic system that catalyzes the terminal step of gluconeogenesis and glycogenolysis in the endoplasmic reticulum (Hers et al. 1951). According to the substrate-transport model, this system comprises a hydrolase, whose catalytic site faces the lumen of the endoplasmic reticulum, as well as transporters for glucose-6-phosphate, Pi (Arion et al. 1975, 1980), and glucose (Waddell et al. 1991). The glucose-6-phosphate transporter is assumed to be specific for glucose-6-phosphate (as opposed to its 2-epimer mannose 6-phosphate), to account for the specificity of the enzyme in intact microsomes. The Pi transporter is apparently also able to transport inorganic pyrophosphate (PPi), at least at acidic pH, explaining the pyrophosphatase activity under these conditions (Arion et al. 1980). The glucose transporter had been identified as Glut 7 (Waddell et at. 1992), which has, however, been recently reported to be a cloning artifact (Burchell 1998). Alternative models, known as "conformational models," have been proposed, by other authors, both to account for the effect that detergents have on the kinetic properties of glucose-6-phosphatase (Schulze et al. 1986; Berteloot et al. 1991) and to explain the fact that glucose-6-phosphate transport and its hydrolysis appear to be tightly linked (St.-Denis et al. 1995).

The most frequent form of GSD type I, called "type Ia" (MIM 232200), is caused by mutations in the gene, present on chromosome 17, that encodes phosphohydrolase (Lei et al. 1993, 1995). This gene is not mutated in some patients with a more severe form of the disease, in which neutropenia and neutrophil dysfunction are

found (reviewed by Gitzelmann and Bosshard 1993) in addition to the clinical symptoms typical of GSD type I (i.e., hepatomegaly, hypoglycemia, and hyperlactatemia). In these patients, there is evidence of defects in either the glucose-6-phosphate translocase (type Ib; MIM 232220; Narisawa et al. 1978; Lange et al. 1980) or the Pi translocase (type Ic; MIM 232240; Nordlie et al. 1983). In both cases, the glucose-6-phosphatase activity is abnormally latent in intact microsomes. In type Ic, there is also a complete latency of inorganic pyrophosphatase (Nordlie et al. 1983). However, the two conditions are difficult to differentiate clinically (Chen and Burchell 1995), since both show neutropenia in addition to the classical symptoms of GSD type I.

We have recently cloned a cDNA encoding a putative glucose-6-phosphate translocase, which we found to be mutated in two patients with GSD type Ib (Gerin et al. 1997). Independently, GSD types Ib (Annabi et al. 1998) and Ic (Fenske et al. 1998) have been mapped to chromosomes 11q23 and 11q23-24.2, respectively. We now show that the gene encoding the putative translocase is in this region and is mutated in 26 patients from a total of 22 families, who have been diagnosed as GSD type Ib or type Ic.

Patients and Methods

Patients and Diagnosis of GSD Types Ib and Ic

All patients had hepatomegaly, hypoglycemia, lactic acidosis, and neutropenia. The diagnosis of GSD type Ib was based on both the finding of normal or elevated total glucose-6-phosphatase activity and the existence of a significant latency (latency is defined as the enzymic activity revealed by detergents, and it is often expressed in terms of percent of the total activity) of this enzyme (Narisawa et al. 1978; Lange et al. 1980). Assays were performed on sucrose homogenates prepared either from unfrozen liver biopsies or from liver samples that had been frozen immediately after being collected (Brown 1985).

One case with apparently normal glucose-6-phosphatase latency (patient 18c; table 1) was diagnosed as GSD type Ic, on the basis of complete latency of inorganic pyrophosphatase in intact microsomes (Nordlie et al. 1983); the two other affected members of the family were assumed to have the same form of disease; one of them indeed showed a normal glucose-6-phosphatase latency (Fenske et al. 1998). Patient 22 showed a subnormal glucose-6-phosphatase latency; the activity without detergent amounted to 54% of the activity with detergent; his condition was tentatively identified as GSD type Ic.

Mapping of the Gene to Chromosome 11

A 671-bp human-specific fragment of the glucose-6-phosphate translocase gene was amplified by a sense primer (5′-GGA GAG CAC CCA CAG CTA AGG GGA-3′) in exon 3 and by an antisense primer (5′-GGA CTC CGC AAC CTG GAC CCC ATG-3-′) in exon 4. This amplification reaction was used to screen the low-resolution GeneBridge 4 radiation-hybrid panel (Research Genetics), by means of the following PCR program: 95°C for 5 min; 35 cycles of 95°C for 30 s, 70°C for 30 s, and 72°C for 1 min; and 72°C for 5 min. Mapping was computed by the RH Mapper program, available on-line at the Whitehead Institute for Genome Research, Massachusetts Institute of Technology.

SSCP Analysis and Sequencing

Genomic DNA was isolated from leukocytes by use of the QIAmp Blood Kit (QIAGEN), according to the manufacturer's instructions. SSCP analysis (Orita et al. 1989) was used to screen all nine exons, as well as the flanking intronic sequences. PCR reactions were performed with primers in 10-μl volumes containing 10–20 ng of genomic DNA template, 1 × PCR buffer (Boehringer), 180 μM of each dNTP, 0.2 μM of each ^{32}P–end-labeled primers (table 2), and 0.5 U of *Taq* DNA polymerase (Boehringer). The PCR conditions were 95°C for 5 min; 30 cycles of 95°C for 30 s, 65°C for 1 min, and 72°C for 1 min; and 72°C for 5 min. Amplified samples were diluted 1:6.5 in formamide buffer (95% formamide, 10 mM NaOH, 0.025% bromophenol blue, and 0.025% xylene cyanol), denatured at 95°C for 5 min, and rapidly cooled on ice. For each sample, 5 μl was loaded onto a 0.5 × MDE, 0.6 × Tris-borate EDTA gel (FMC Bioproducts). The gels were run at 6 W (constant power) for 14–16 h at room temperature before being dried and subjected to autoradiography. Direct sequencing of PCR products showing a shift in SSCP analysis was performed on both strands by the dsDNA Cycle Sequencing System (GIBCO-BRL), according to the manufacturer's protocol. The PCR products were purified, before being sequenced, by means of a QIAquick PCR Purification Kit (QIAGEN).

The Gly339Cys mutation was detected by allele-specific PCR (Gerin et al. 1997), under the following conditions: 95°C for 5 min; 35 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min; and 72°C for 5 min. The sequences of the primers were as follows: TGG TAT TGG GAG CTG TAT TTG (5′ wild-type primer), TGG TAT TGG GAG CTG TAT TTT (5′ mutated primer), and GAG CGT GCA GGG GGA AGG CCA CCG (3′ common primer). The presence of this mutation was also confirmed by sequencing of exon 8 in the manner described above.

Table 1

Distribution of Mutations in Investigated Patients

Patient (Sex)	Mutation[a]		Origin/Ethnicity	Consanguinity	Diagnosis (GSD Type)
	Allele 1	Allele 2			
1 (M)	Gly20→Asp[b]	Gly20→Asp[c]	United States/Caucasian	No	Ib
2 (M)	Gly88→Asp	Gly88→Asp	Senegal/Black	Yes	Ib
3 (F)	Gly68→Arg[b]	Gly339→Cys[c]	Italy and United Kingdom/Caucasian	No	Ib
4 (F)	Gly339→Cys	Gly339→Cys	Belgium/Caucasian	No	Ib
5 (F)	Gly339→Cys	Gly339→Cys	Belgium/Caucasian	No	Ib
6a (M)	Gly339→Cys[b]	Gly339→Cys[c]	United States/Caucasian	No	Ib
6b (F)	Gly339→Cys[b]	Gly339→Cys[c]	United States/Caucasian	No	Ib
7 (F)	Gly339→Cys	Glu355→Stop	Belgium/Caucasian	No	Ib
8 (F)	Gly339→Cys	ΔRF Ala347[c]	United States/Caucasian	No	Ib
9 (F)	Gln218→Stop[b]	Ser55→Arg[c]	United States/Caucasian	No	Ib
10 (F)	Gln248→Stop	Gln248→Stop	Italy/Caucasian	No	Ib
11 (F)	ΔRF Ala347	Gly292→Pro; Leu293→Stop[c]	United States/Caucasian	No	Ib
12a (F)	ΔRF Pro153	ΔRF Ala347[c]	United Kingdom/Caucasian	No	Ib
12b (F)	ΔRF Pro153	ΔRF Ala347[c]	United Kingdom/Caucasian	No	Ib
13 (M)	ΔRF Thr312[b]	ΔRF Thr312[c]	Pakistan/Caucasian	Yes	Ib
	1393G→A (silent)	1393G→A (silent)			
14 (M)	ΔRF Pro345	ΔRF Ala347	Germany/Caucasian	No	Ib
15 (M)	ΔRF Ala347[b]	ΔRF Ala347[c]	Portugal/Caucasian	No	Ib
16 (M)	ΔRF Ala347[b]	ΔRF Ala347[c]	Tunisia/Caucasian	No	Ib
17 (F)	ΔRF Ala347[b]	Insertion of 4-amino-acid repeat after Met311[c]	United States/Caucasian	No	Ib
18a (M)	Σ Exon 8/intron 8[b]	Σ Exon 8/intron 8[c]	Pakistan/Caucasian	Yes	Ic
18b (F)	Σ Exon 8/intron 8[b]	Σ Exon 8/intron 8[c]	Pakistan/Caucasian	Yes	Ic
18c (M)	Σ Exon 8/intron 8[b]	Σ Exon 8/intron 8[c]	Pakistan/Caucasian	Yes	Ic
19 (M)	Σ Exon 8/intron 8[b]	Σ Exon 8/intron 8[c]	Pakistan/Caucasian	Yes	Ib
20 (M)	Σ Intron 8/exon 9	Σ Intron 8/exon 9	Sardinia/Caucasian	No	Ib
	Asn198→Ile (polymorphism?)	Asn198→Ile (polymorphism?)			
	1231C→T (silent)	1231C→T (silent)			
	1393G→A (silent)	1393G→A (silent)			
21 (M)	Arg28→Cys[b]	Gly150→Arg[c]	France/Caucasian	No	Ib
22 (F)	Trp96→Stop	Insertion of 4-amino-acid repeat after Met311	United Kingdom/Caucasian	No	Ic
	1393G→A (silent)				

[a] When available, the DNA from the parents was analyzed by SSCP and/or allele-specific PCR, to identify parental origin of the mutation. ΔRF = change in reading frame, and Σ = splice-site mutation.

[b] Paternal allele.

[c] Maternal allele.

Table 2

Primers Used for SSCP Analysis

Amplified Region	Primer[a]		Fragment Length (bp)
	Forward	Reverse	
Exon 1 (5′)	CAGTTTGGCGCTCAGTAATCTC[b]	ATGGCTGAGAAGATCACAGTGC[c]	300
Exon 1 (3′)	CAGGAACTGTGGTCAGAGGCTGT[c]	TGTCTGGCTGGTTCTGTGTCC	259
Exon 2	TCCTGTGTTTCTCCCCTGGTC	TTAGGCATCCTCTATGACAATCC	332
Exon 3	ATGGGCAGTAGGCTGGACACC	TGCCTGCTAAATGAGTGCCCC	334
Exon 4	GGTTCAGGTTGGGGAGAGCAG	TCCTGCTCCTTATGCCCACCC	262
Exon 5	CTTCCCTTCCCTCTTCCCACC	CGTGAAGACTGAAAGGGACCC	175
Exon 6	GGTGTTCTCTGCCCTGTTCTG	AGGGAGACAGAGTCAGTGGCC	238
Exon 7	CTCCTCCACTCTGGGCCTGG	CAAAGGTGAGACAGACCAGGAG	162
Exon 8	TCCGACTCTGAATGCCACTCC	TGCAAAGCACAGGTGGGGGTG	226
Exon 9	CCACTGGCTTAGGTTCTTCCC	ACCGTGTGATGGTGCTCCGG[c]	236

[a] Primers are intronic, unless noted otherwise.
[b] In the promoter region.
[c] Exonic primer.

Results

Chromosomal Localization of the Glucose-6-Phosphate Transporter

Using the GeneBridge 4 radiation-hybrid panel, we mapped the gene encoding the putative glucose-6-phosphate translocase (Gerin et al. 1997), between markers NIB 361 and WI-9009, to 11q23—that is, the region linked to GSD types Ib and Ic (Annabi et al. 1998; Fenske et al. 1998).

Mutation Detection in the Glucose-6-Phosphate Transporter

To enable us to search for mutations at the genomic level, we cloned the human glucose-6-phosphate translocase gene from a genomic DNA library, using as a probe the full-length cDNA (Gerin et al. 1997), and determined its intron-exon structure. Nine exons were identified, the first of which contained the initiating ATG codon and the last of which contained the stop codon and the 3′ UTR. Exon 7 is not present in liver cDNA (Gerin et al. 1997), but it is in expressed sequence tags derived from human brain libraries. It is 66 bp in length and introduces 22 residues between amino acids Lys337 and Leu338 (I. Gerin, M. Veiga-da-Cunha, and E. Van Schaftingen, unpublished data).

Intronic and, when necessary, exonic PCR primers were used to amplify genomic DNA from patients diagnosed as GSD types Ib or type Ic. Exon 1 was amplified in two parts, because of its size, and only the coding region of exon 9 was analyzed. The primers used and the sizes of the expected fragments are shown in table 2. As shown in figure 1*A*, SSCP analysis and sequencing allowed the identification of the mutations listed in table 3, except for the Gly339Cys mutation; this mutation, which had been previously identified in

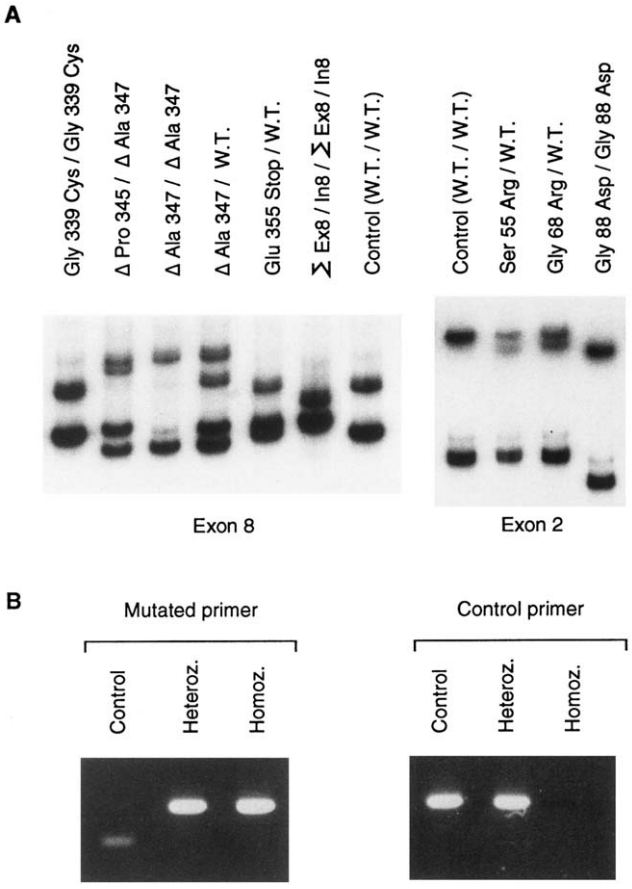

Figure 1 Representative SSCP patterns for mutations identified in exons 2 and 8 (*A*) and in allele-specific PCR analysis of the Gly339Cys mutation (*B*). In panel *B*, amplification products of genomic DNA from a control and from patients heterozygous (heteroz.) or homozygous (homoz.) for the Gly339Cys mutations are shown. Δ = change in reading frame, W.T. = wild type, and Σ = splice-site mutation.

Table 3

Mutations Identified in Patients with GSD Types Ib and Ic

Nucleotide Change[a]	Location	Effect on Protein[b]	Amino Acid(s) at Same Position in Bacterial Transporters	Patient(s)[c]
Substitutions:				
228G→A	Exon 1	Gly20→Asp	Gly	1
271C→T	Exon 1	Arg28→Cys	Arg	21
332A→C	Exon 2	Ser55→Arg	Leu, Gly, Cys	9
371G→A	Exon 2	Gly68→Arg	Gly, Ser	3
431G→A	Exon 2	Gly88→Asp	Gly, Ala	2
617G→A	Exon 3	Gly150→Arg	Gly, Ala	21
762A→T	Exon 3	Asn198→Ile (polymorphism? [see text])	Ala, Pro, Ser, Lys	20
1184G→T	Exon 8	Gly339→Cys	Gly	3–5, 6a, 6b, 7, 8
Nonsense mutations:				
456G→A	Exon 2	Trp96→Stop		22
821C→T	Exon 4	Gln218→Stop		9
911C→T	Exon 4	Gln248→Stop		10
1232G→T	Exon 8	Glu355→Stop		7
Deletions/insertions:				
629delA	Exon 3	ΔRF after Pro153, Stop at 211		12a, 12b
1103ins12 (see fig. 2*b*)	Exon 6	4-amino-acid repeat after Met311		17, 22
1105insA	Exon 6	ΔRF after Thr312, Stop at 325		13
1205insC	Exon 8	ΔRF after Pro345, Stop at 401		14
1211–1212 delCT	Exon 8	ΔRF after Ala347, Stop at 400		8, 11, 12, 14–17
Splice site mutations:				
1041ins9 (see fig. 2*a*)	Intron 5/exon 6	Gly292→Pro, Leu293→Stop		11
Deletion of 4 bp (see fig. 2*c*)	Exon 8/intron 8	Truncated protein?		18a, 18b, 18c, 19
Deletion of 2 bp (see fig. 2*d*)	Intron 8/exon 9	Truncated protein?		20

[a] The position refers to the cDNA sequence (EMBL/Genbank accession number Y15409).

[b] Residues found in the same position in the sequences of bacterial organophosphate transporters (Gerin et al. 1997) are shown. ΔRF = change in reading frame.

[c] Numbered as in table 1.

the cDNA from two patients, could be easily detected by amplification with allele-specific primers (fig. 1*B*). Eight substitutions were identified; six of them resulted in replacement of a residue that is either partially (Gly68, Gly88, and Gly150) or completely (Gly20, Arg28, and Gly339) conserved in the bacterial organophosphate transporters, which belong to the same protein family that contains the human glucose-6-phosphate translocase (Gerin et al. 1997). We also identified four nonsense mutations and four other nucleotide changes that resulted in a frameshift that was due to either insertion of 1 bp or deletion of 1 or 2 bp. Two splice-site mutations (exon 8/intron 8 and intron 8/exon 9), which are likely to cause exon skipping (fig. 2*c* and *d*), were also found. A 9-bp insertion at the intron 5/exon 6 boundary introduces a premature stop codon (fig. 2*b*), and a 12-bp insertion in exon 6 results in a 4-amino-acid insertion (fig. 2*a*) in a predicted transmembrane domain.

Distribution of Mutations

The distribution of mutations among the patients is shown in table 1. The two most frequent mutations are Gly339Cys, which is found in the homozygous state in patients from four unrelated families, and a 2-bp deletion resulting in a change in reading frame after Ala347, homozygous in two families. Most patients affected by one of these two mutations originate from western Europe or the United States. Other mutations were found in only one or two families. One patient was homozygous for both a substitution (Asn198Ile), which could be a polymorphism, and a splice-site mutation at the intron 8/exon 9 junction (fig. 2*d*), as well as for two silent nucleotide replacements. This multiple polymorphism could be related to the fact that this patient originates from Sardinia, which has a genetically isolated population.

Presence of Mutations in the Same Gene in GSD Type Ic

Interestingly, a splice-site mutation at the exon 8/intron 8 junction was found to be homozygous in two families of Pakistani origin, one diagnosed as GSD type Ic and the other diagnosed as GSD type Ib; the first family was used to map the *GSD Ic* locus to 11q23-24.2 (Fenske et al. 1998). A patient from another family, also diagnosed as GSD type Ic, was heterozygous for a mutation found in a patient with GSD type Ib (a 4-amino-acid insertion) and a missense mutation.

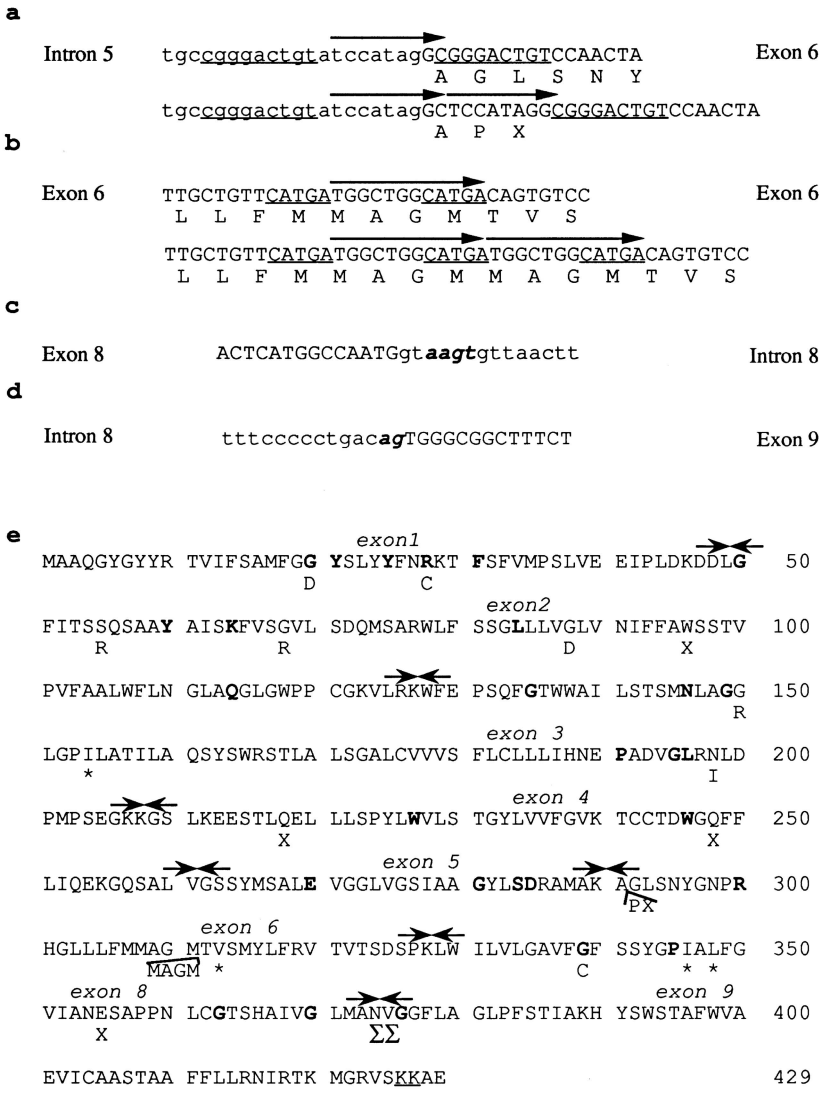

Figure 2 Complex mutations found in the translocase gene (*a–d*), and localization of exons and mutations in the amino acid sequence (*e*). In panels *a–d,* exonic sequences are represented as uppercase letters, intronic sequences as lowercase letters. In panels *a* and *b,* the repeated elements found in the wild-type gene are underlined, and the arrows denote the sequence that is repeated in the mutated allele. In panels *c* and *d,* the nucleotides that are missing in the mutated alleles are in boldface italics. In panel *e,* amino acid residues are represented in terms of the one-letter code, and exon limits are denoted by arrows; the retention signal for the endoplasmic reticulum is underlined. Mutations are shown below the sequence: letters indicate substitutions or insertions (X = stop codon), * = changes in reading frame, and Σ = splice-site mutation.

Discussion

Previous studies had indicated that GSD types Ib and Ic map to the same region of chromosome 11 (Annabi et al. 1998; Fenske et al. 1998). The results of the present study show that, in both these GSD types, the mutated gene is the one encoding the putative glucose-6-phosphate translocase, which we have now mapped to the same region. All patients have mutations that result either in (*a*) the substitution of highly conserved or semiconserved residues by amino acids with widely different size or charge or (*b*) the truncation of the translocase, with loss of the two lysine residues that are needed in positions −3 and −4 (or −5) from the C-terminus to retain proteins in the endoplasmic reticulum (Jackson et al. 1990). These considerations indicate that the putative glucose-6-phosphate translocase is most likely nonfunctional in all patients reported here.

In 12 families of the 22 that were investigated here, the patients were homozygous for some of the identified mutations. This homozygosity was expected in some cases, because of consanguinity (in the case of four families). It could be confirmed by analysis of the genomic DNA of the parents in five of the other eight homozy-

gous families. In 7 of the 10 combined-heterozygous families, the availability of parental DNA enabled us to show that the two mutations were on different chromosomes.

Our finding that, in GSD types Ib and Ic, the same mutations are present in the same gene could indicate that glucose-6-phosphate and Pi (and PPi) are transported by the same protein. Accordingly, the bacterial hexose 6-phosphate and glycerol 3-phosphate translocases, to which the human putative glucose-6-phosphate translocase is homologous (Gerin et al. 1997), act as inorganic phosphate/phosphate ester exchangers (Maloney et al. 1990). However, this argument, based on homology, is not conclusive (Maloney and Wilson 1996), since the same superfamily of transporters comprises uniports (transporting only one substrate at a time), symports (transporting two substrates in the same direction), and antiports (transporting two substrates in opposite directions; these are also known as "exchangers"). Furthermore, Arion et al. (1980) concluded, on the basis of kinetic studies performed on intact and detergent-treated microsomes, that the glucose-6-phosphate and the Pi transporters were different. This is also indicated by the discovery of a novel class of compounds, derived from chlorogenic acid, that behave as specific inhibitors of glucose-6-phosphate but not of PPi transport (Arion et al. 1998a, 1998b) as well as by the distinctive behavior that the two transport functions have in development (Goldsmith and Stetten 1979; Burchell et al. 1990). One might argue, though, that two different domains of the same protein could be involved in the transport of glucose-6-phosphate and Pi (or PPi).

If there are indeed two separate transporters for Pi and glucose-6-phosphate, then we have to admit that measurement of the latencies of glucose-6-phosphatase and pyrophosphatase does not allow us to differentiate correctly the Pi and glucose-6-phosphate transport defects. This would explain why patients with the same mutations have been diagnosed as GSD types Ib and Ic. The technical difficulties encountered in such assays should not be underestimated, since the diagnosis is best performed on homogenates prepared from fresh liver-biopsy material (Narisawa et al. 1978; Lange et al. 1980; Nordlie et al. 1983) and often has to be realized on small liver samples obtained by needle biopsy. Interestingly, Nordlie et al. (1983) concluded, in their original report on GSD type Ic, that their patient must also have a defect in the glucose-6-phosphate translocase. Their argument was that they did not observe the time-dependent inhibition of glucose-6-phosphatase that they would have expected to find if the decrease in glucose-6-phosphatase activity had been due only to accumulation of inorganic phosphate in the microsomes. Evidence for simultaneous defects in the transport of inorganic phosphate and glucose-6-phosphate was also reported for another case (Hawkins et al. 1995), though one patient recently studied by Marcolongo et al. (1998), in whom Pi and glucose-6-phosphate transport were measured by light scattering, was reported to have an isolated Pi-transport defect. Now that the gene encoding the putative glucose-6-phosphate translocase has been identified, it will be possible to determine whether all "non-a" forms of GSD type I are due to mutations in this gene. Moreover, functional studies should also enable to verify whether the protein encoded by the gene on chromosome 11q23 is a translocase acting independently of the hydrolase, which would argue against the conformational models.

All the patients described in table 1 had some degree of neutropenia, which is probably not due to functional glucose-6-phosphatase deficiency, since there is usually no neutropenia in GSD type Ia (Chen and Burchell 1995). Glucose-6-phosphate could be transported into the endoplasmic reticulum to supply substrate to enzyme(s) other than the glucose-6-phosphate hydrolase. One candidate is hexose-6-phosphate dehydrogenase, a glucose-6-phosphate dehydrogenase isozyme that is present in the microsomes in several tissues (Takahashi and Hori 1978). Both the existence of this isozyme in neutrophils and its role in their metabolism will need to be investigated. In conclusion, the present study opens the possibility of diagnosing, at the gene level, most if not all cases of GSD type I that are not due to mutations in the phosphohydrolase gene.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

EMBL, http://www.ebi.ac.uk
Genbank, http://www.ncbi.nlm.nih.gov/Entrez
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for GSD types Ia [MIM 232200], Ib [MIM 232220], and Ic [MIM 232240])
Whitehead Institute for Genome Research, http://genome.wi.mit.edu

References

Annabi B, Hiraiwa H, Mansfield BC, Lei K-J, Ubagai T, Polymeropoulos MH, Moses SW, et al (1998) The gene for gly-

cogen-storage disease type Ib maps to chromosome 11q23. Am J Hum Genet 62:400–405

Arion WJ, Canfield WK, Callaway ES, Burger H-J, Hemmerle H, Schubert G, Herling AW, et al (1998a) Direct evidence for the involvement of two glucose 6-phosphate binding sites in the glucose-6-phosphatase activity of intact liver microsomes. J Biol Chem 273:6223–6227

Arion WJ, Canfield WK, Ramos FC, Su ML, Burger H-J, Hemmerle H, Schubert G, et al (1998b) Chlorogenic acid analogue S3483: a potent competitive inhibitor of the hepatic and renal glucose-6-phopshatase systems. Arch Biochem Biophys 351:279–285

Arion WJ, Lange AJ, Walls HE, Ballas LM (1980) Evidence for the participation of independent translocases for phosphate and glucose 6-phosphate in the microsomal glucose-6-phosphatase system. J Biol Chem 255:10396–10406

Arion WJ, Wallin BK, Lange AJ, Ballas LM (1975) On the involvement of a glucose 6-phosphate transport system in the function of microsomal glucose 6-phosphatase. Mol Cell Biochem 6:75–83

Berteloot A, Vidal H, van de Werve G (1991) Rapid kinetics of liver microsomal glucose-6-phosphatase: evidence for tight coupling between glucose-6-phosphate transport and phosphohydrolase activity. J Biol Chem 266:5497–5507

Brown BI (1985) Diagnosis of glycogen storage disease. In: Wapnir PA (ed) Congenital metabolic myopathy. Marcel Dekker, New York, pp 227–250

Burchell A (1998) A re-evaluation of Glut 7. Biochem J 331: 973

Burchell A, Gibb L, Waddell ID, Giles M, Hume R (1990) Ontogeny of human hepatic microsomal glucose-6-phosphatase proteins. Clin Chem 36:1633–1637

Chen YT, Burchell A (1995) Glycogen storage diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 935–967

Fenske CD, Jeffery S, Weber JL, Houlston RS, Leonard JV, Lee P (1998) Localisation of the gene for glycogen storage disease type Ic by homozygosity mapping to 11q. J Med Genet 35:269–272

Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, Van Schaftingen E (1997) Sequence of a putative glucose 6-phosphate translocase mutated in glycogen storage disease type Ib. FEBS Lett 419:235–238

Gitzelmann R, Bosshard NU (1993) Defective neutrophil and monocyte functions in glycogen storage disease type Ib: a literature review. Eur J Pediatr 152 Suppl:S33–S38

Goldsmith PK, Stetten MR (1979) Different developmental changes in latency for two functions of a single membrane-bound enzyme: glucose-6-phosphatase activities as a function of age. Biochem Biophys Acta 523:133–147

Hawkins RA, Kamath KR, Scott HM, Burchell A (1995) Multiple transport protein defects in a patient with glycogen storage disease type I: GSD Ib/ICβ. J Inherit Metab Dis 18: 558–566

Hers HG, Berthet J, Berthet L, de Duve C (1951) Le système hexose-phosphatasique. III. - Localisation intra-cellulaire des ferments par centrifugation fractionnée. Bulletin de la Société de Chimie Biologique 33:21–41

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

Lange AJ, Arion WJ, Beaudet AL (1980) Type Ib glycogen storage disease is caused by a defect in the glucose-6-phosphate translocase of the microsomal glucose-6-phosphatase system. J Biol Chem 255:8381–8384

Lei KJ, Shelly LL, Lin B, Sidbury JB, Chen YT, Nordlie RC, Chou JY (1995) Mutations in the glucose-6-phosphatase gene are associated with glycogen storage disease types Ia and IaSP but not Ib and Ic. J Clin Invest 95:234–240

Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY (1993) Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type Ia. Science 262:580–583

Maloney PC, Ambudkar SV, Anantharan V, Sonna LA, Varadhachary A (1990). Anion-exchange mechanisms in bacteria. Microbiol Rev 54:1–17

Maloney PC, Wilson TH (1996) Ion-coupled transport and transporters. In: Neidhardt FC (ed) *Escherichia coli* and *Salmonella:* cellular and molecular biology. ASM Press, Washington, DC, pp 1130–1148

Marcolongo P, Bánhegyi G, Benedetti A, Hinds CJ, Burchell A (1998) Liver microsomal transport of glucose-6-phosphate, glucose, and phosphate in type I glycogen storage diseases. J Clin Endocrinol Metab 83:224–229

Narisawa K, Igarashi Y, Otomo H, Tada K (1978) A new variant of glycogen storage disease type I probably due to a defect in the glucose-6-phosphate transport system. Biochem Biophys Res Commun 83:1360–1364

Nordlie RC, Sukalski KA, Munoz JM, Baldwin JJ (1983) Type Ic, a novel glycogenosis: underlying mechanism. J Biol Chem 258:9739–9744

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human single-strand conformation polymorphisms. Proc Natl Acad Sci USA 86: 2766–2770

Schulze HU, Nolte B, Kannler R (1986) Evidence for changes in the conformational status of rat liver microsomal glucose-6-phosphate phosphohydrolase during detergent-dependent membrane modification. J Biol Chem 261:16571–16578

St-Denis J-F, Berteloot A, Vidal H, Annabi B, van de Werve G (1995) Glucose transport and glucose-6-phosphate hydrolysis in intact rat liver microsomes. J Biol Chem 270: 21092–21097

Takahashi T, Hori SH (1978) Intramembraneous localisation of rat liver microsomal hexose-6-phosphate dehydrogenase and membrane permeability to its substrates. Biochim Biophys Acta 524:262–276

Waddell ID, Scott H, Grant A, Burchell A (1991) Identification and characterization of a hepatic microsomal glucose transport protein: T3 of the glucose-6-phosphatase system? Biochem J 275:363–367

Waddell ID, Zomerschoe AG, Voice MW, Burchell A (1992) Cloning and expression of a hepatic microsomal glucose transporter: comparison with liver plasma-membrane glucose-transporter protein GLUT 2. Biochem J 286:173–177