Mutation analysis in 24 French patients with glycogen storage disease type 1a

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Abstract

Both alleles of 24 French glycogen storage disease type 1a patients were sequenced: 14 different mutations allowed the identification of complete genotypes for all the patients. Nine new gene alterations are reported. Five mutations, Q347X, R83C, D38V, G188R, and 158delC, account for 75% of the mutated alleles. These data show that the molecular pathology of the glycose-6-phosphatase gene is heterogeneous in this population. Complete genotyping of the index case by systematic sequencing is necessary to allow prenatal diagnosis in chorionic villi for at risk couples.

Key words: glucose-6-phosphatase; mutations; French.

Glycogen storage disease (GSD) type 1a is an autosomal recessive inborn error of metabolism resulting from deficiency of microsomal glucose-6-phosphatase (G6Pase), the enzyme catalysing the terminal step in gluconeogenesis and glycogenolysis. The gene involved in this disease has recently been isolated and sequenced. The human G6Pase gene is located on chromosome 17; its transcription unit spans 12-3 kb and is composed of five exons. The protein encoded by this gene contains 357 amino acids and is an endoplasmic reticulum (ER) membrane associated protein containing the ER retention signal (KK), and possesses six putative membrane spanning segments. The identification of the G6Pase gene allowed us to study a panel of 24 unrelated French patients with GSD type 1a. We report nine new mutations among the 14, allowing the determination of the complete genotype of each patient.

Materials and methods

SAMPLE COMPOSITION

Mutation analysis was performed on 24 unrelated GSD type 1a patients and their families: 20 were French, one Portuguese, one Italian, and two from North Africa.

METHODS

The diagnosis of GSD type 1a was established for the 24 unrelated patients by showing G6Pase deficiency in liver biopsy. Glycogen content and glucose-6-phosphatase activity were determined in frozen liver biopsies as described.

Genomic DNA was extracted from leucocytes of each patient and both parents except for the patient with the genotype R83C/L211P (no sample from the parents was available).

The five exons of the G6Pase gene and their flanking intron-exon junctions were amplified according to Lei et al from genomic DNA. Direct sequencing of purified PCR products was performed with sense and antisense primers using the “ds DNA Cycle Sequencing System” (GIBCO BRL). The five exons were sequenced for each patient. For the patient heterozygous for the 813 ins G mutation, an aliquot of the PCR reaction was subcloned directly into pGEM-T Vector (Promega) and eight clones were sequenced with sense and antisense primers, with the DNA Sequencing Kit Version 2.0 (Amersham).

Table 1  Mutations identified in 24 unrelated GSD 1a patients

<table>
<thead>
<tr>
<th>Name</th>
<th>Amino acid change</th>
<th>Nucleotide change</th>
<th>Exon</th>
<th>Alleles bearing mutation (% of total mutant alleles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>158 del C</td>
<td>Frame shift</td>
<td>1 bp deletion</td>
<td>1</td>
<td>3 (6)</td>
</tr>
<tr>
<td>D38V</td>
<td>Asp 38→Val</td>
<td>A192→T</td>
<td>1</td>
<td>4 (8)</td>
</tr>
<tr>
<td>W77R</td>
<td>Trp 77→Arg</td>
<td>T308→C</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>309 + 4 A→G</td>
<td>Splice mutation?</td>
<td>A309 + 4→G</td>
<td>Intron 1</td>
<td>1</td>
</tr>
<tr>
<td>R83C</td>
<td>Arg 83→Cys</td>
<td>C326→T</td>
<td>1</td>
<td>2 (9)</td>
</tr>
<tr>
<td>E110K</td>
<td>Glu 110→Lys</td>
<td>G407→A</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A124T</td>
<td>Ala 124→Thr</td>
<td>G449→A</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>G184E</td>
<td>Gly 184→Glu</td>
<td>G630→A</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>G188R</td>
<td>Gly 188→Arg</td>
<td>G641→C</td>
<td>4</td>
<td>3 (6)</td>
</tr>
<tr>
<td>L211H</td>
<td>Leu 211→Pro</td>
<td>T711→C</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>813 ins G</td>
<td>Frameshift</td>
<td>1 bp insertion</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>G270V</td>
<td>Gly 270→Val</td>
<td>G685→T</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>ΔF327</td>
<td>Deletion of Phe 327</td>
<td>3 bp deletion</td>
<td>5</td>
<td>17 (35)</td>
</tr>
<tr>
<td>Q347X</td>
<td>Glu 347→Stop</td>
<td>C1118→T</td>
<td>5</td>
<td>17 (35)</td>
</tr>
</tbody>
</table>

The new mutations identified in this study are in bold letters.

Results

The mutations identified in the 24 French unrelated GSD type 1a patients are listed in Table 1.

The most frequent mutation was the nonsense mutation Q347X (17/48 mutant alleles) identified in seven homozygous patients (five French, one Portuguese, one North African) and three French compound heterozygotes. R83C was the second most frequent mutation in our study (9/48 mutant alleles): one North African and one Italian patient were homozygous, and five French patients were compound heterozygotes for this mutation. The 158 C deletion was identified in a French homozygous patient born to related parents and in another French compound heterozygote patient. The G270V mutation was identified in a French homozygous patient. The deletion ΔF327 was identified in a French homozygous patient born to related parents. These five mutations have already been reported by Lei et al; all the others are previously undescribed.

D38V

The mutation D38V changes an acidic amino acid (aspartic acid) to a non-polar hydrophobic one (valine) in the middle of the first predicted transmembrane spanning domain. This missense mutation was found in four unrelated French compound heterozygotes.

W77R

The mutation W77R changes a non-polar hydrophobic amino acid (tryptophan) to a basic one (arginine). This missense mutation was found in a French compound heterozygote.

309 + 4 A→G

An A to G substitution at position +4 of the 5' donor splice site of intron 1 was identified in a French patient heterozygous for G188R.

E110K

The missense mutation E110K changes an acidic amino acid (glutamic acid) to a basic one (lysine). This mutation was identified in a French compound heterozygote diagnosed at the age of 2 months.

A124T

The mutation A124T changes a non-polar amino acid (alanine) to a polar one (threonine). This mutation, inherited from the mother, is associated with the mutation Q347X in two affected sibs of French origin.

G184E

G184E changes a non-polar hydrophobic amino acid (glycine) to an acidic one (glutamic acid). This mutation was found in a homozygous French patient from a family with no known consanguinity.

G188R

This missense mutation changes a non-polar hydrophobic amino acid (glycine) to a basic one (arginine). G188R was present in three unrelated French compound heterozygotes. G184E and G188R are both located in the putative cytoplasmic domain of the G6Pase.8

L211P

L211P changes a leucine to a proline. This leucine is the first amino acid of the fourth putative transmembrane domain. This mutation was identified in a French patient heterozygous for R83C: the segregation in the family could not be studied as no sample was available from the parents.

813 INS G

The mutation 813 ins G shifts the reading frame and introduces a stop codon nine codons downstream. The predicted mutant G6Pase is a truncated polypeptide of 253 amino acids. This insertion was identified in a French girl who inherited 813 ins G from her father and R83C from her mother.

One hundred normal subjects were tested for the missense mutations D38V, W77R, E110K, A124T, G184E, G188R, L211P, and G270V, and for the 309 + 4 A→G substitution. None of these substitutions was found among these 200 normal alleles tested.
Discussion

This study shows that the five most common mutations Q347X, R83C, D38V, G188R, and 158delC account for 75% of the mutant alleles in our 24 French patients. The other mutations are "private".

Substitution 309 + 4 A → G was the only alteration found in the coding region of a mutated allele. This alteration was not found in the 200 normal alleles studied. Although the effect of this substitution was not studied in the patient's RNA, changes at the +4 position of introns have been previously described and have been shown to be involved in the splicing mechanism.7

The insertion 813 ins G is not associated in our patient with the deletion 822 del C, as described by Lei et al.2 The absence of 822 del C was confirmed by ASO hybridisation.

The seven other new mutations identified in this study are missense mutations. These substitutions were not found in the 200 normal alleles studied and were the only alteration found in the coding region and splice junctions of the positive alleles. Furthermore, the segregation of the different mutations through the families (studied in all families except one) was as expected. All of these missense mutations concern amino acids conserved in rat and mouse liver G6Pase. The homology between G6Pase amino acid sequence in humans and these mammals is more than 90%.10 These missense mutations, except A124T, are non-conservative changes and could affect G6Pase activity or stability. Furthermore, G188R caused by the substitution of G641 → C may affect the correct splicing of intron 4, as G641 is the last nucleotide of exon 4. Two different changes have been described at this nucleotide position: G641 → C (our data) and G641 → A.7

From our data (table 2) and others,11 it is not possible to correlate the genotype with the biochemical data, such as G6Pase activity and glycogen content. Studies using site directed mutagenesis and transient expression assays in cell lines are necessary to show how these missense mutations affect the mutated proteins.12

Besides the five most frequent mutations (Q347X, R83C, D38V, G188R, and 158delC), the others are "private" and systematic sequencing of the entire coding region is necessary to identify the complete genotype of each patient. The knowledge of the complete genotype will allow reliable prenatal diagnosis in chorionic villi for at risk couples.

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