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Research Article

Glycogen storage disease (GSD) type 1a is an autosomal recessive inborn error of metabolism caused by a deficiency in microsomal glucose-6-phosphatase (G6Pase), the key enzyme in glucose homeostasis. Southern blot hybridization analysis using a panel of human-hamster hybrids showed that human G6Pase is a single-copy gene located on chromosome 17. To correlate specific defects with clinical manifestations of this disorder, we identified mutations in the G6Pase gene of GSD type 1a patients. In the G6Pase gene of a compound heterozygous patient (LLP), two mutations in exon 2 of one allele and exon 5 of the other allele were identified. The exon 2 mutation converts an arginine at codon 83 to a cysteine (R83C). This mutation, previously identified by us in another GSD type 1a patient, was shown to have no detectable phosphohydrolase activity. The exon 5 mutation in the G6Pase gene of LLP converts a glutamine codon at 347 to a stop (Q347SP). This Q347SP mutation was also detected in all exon 5 subclones (five for each patient) of two homozygous patients, KB and CB, siblings of the same parents. The predicted Q347SP mutant G6Pase is a truncated protein of 346 amino acids, 11 amino acids shorter than the wild type G6Pase of 357 residues. Site-directed mutagenesis and transient expression assays demonstrated that G6Pase-Q347SP was devoid of G6Pase activity. G6Pase […]

Identification of Mutations in the Gene for Glucose-6-Phosphatase, the Enzyme Deficient in Glycogen Storage Disease Type 1A

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Abstract

Glycogen storage disease (GSD) type 1a is an autosomal recessive inborn error of metabolism caused by a deficiency in microsomal glucose-6-phosphatase (G6Pase), the key enzyme in glucose homeostasis. Southern blot hybridization analysis using a panel of human–hamster hybrids showed that human G6Pase is a single-copy gene located on chromosome 17. To correlate specific defects with clinical manifestations of this disorder, we identified mutations in the G6Pase gene of GSD type 1a patients. In the G6Pase gene of a compound heterozygous patient (LLP), two mutations in exon 2 of one allele and exon 5 of the other allele were identified. The exon 2 mutation converts an arginine at codon 83 to a cysteine (R83C). This mutation, previously identified by us in another GSD type 1a patient, was shown to have no detectable phosphohydrolase activity. The exon 5 mutation in the G6Pase gene of LLP converts a glutamine codon at 347 to a stop (Q347SP). This Q347SP mutation was also detected in all exon 5 subclones (five for each patient) of two homozygous patients, KB and CB, siblings of the same parents. The predicted Q347SP mutant G6Pase is a truncated protein of 346 amino acids, 11 amino acids shorter than the wild type G6Pase of 357 residues. Site-directed mutagenesis and transient expression assays demonstrated that G6Pase-Q347SP was devoid of G6Pase activity. G6Pase is an endoplasmic reticulum (ER) membrane–associated protein containing an ER retention signal, two lysines (KK), located at residues 354 and 355. We showed that the G6Pase-K355SP mutant containing a lysine-355 to stop codon mutation is enzymatically active. Our data demonstrate that the ER protein retention signal in human G6Pase is not essential for activity. However, residues 347–354 may be required for optimal G6Pase catalysis. (*J. Clin. Invest.* 1994. 93:1994–1999.) Key words: inborn error of metabolism • genetic mutation • endoplasmic reticulum and/or retention signal

Introduction

Glucose-6-phosphatase (E.C. 3.1.3.9) (G6Pase)[1] catalyzes the terminal step in gluconeogenesis and glycogenolysis and is therefore the pivotal enzyme in the homeostatic regulation of glucose, the major metabolic fuel for all mammalian cells (1, 2). Deficiency of microsomal G6Pase causes glycogen storage disease (GSD) type 1a (von Gierke disease), an autosomal recessive disorder. 1 in 100,000–300,000 people are affected with this genetic disease (3, 4), which manifests during the first year of life with severe hypoglycemia and hepatomegaly. Individuals with GSD type 1a, first described in 1952 by Cori and Cori (5), also exhibit a wide range of clinical symptoms and biochemical abnormalities, including short stature, lactic acidemia, hyperlipidemia, hyperuricemia, tendency to bleed, neutropenia, hepatic adenomas, and renal dysfunction (3, 4). The present treatments for GSD type 1a focus on relieving symptomatic hypoglycemia by frequent ingestion of glucose by mouth or continuous nighttime feeding by a nasogastric tube. The oral administration of uncooked cornstarch for management of GSD type 1 patients has also been shown to be an effective alternative to nocturnal nasogastric glucose feeding (6).

Our laboratory has recently reported the isolation of the cDNA and gene encoding human G6Pase (7), an enzyme associated with the endoplasmic reticulum (ER) and nuclear membranes (1–4). The human G6Pase transcription unit spans 12.5 kb and consists of five exons. We identified mutations in the G6Pase gene of two patients diagnosed with GSD type 1a by PCR amplification of the coding region of each of the five exons and all exon/intron junctions (7). Site-directed mutagenesis followed by transient expression assays confirmed that the mutations abolished G6Pase activity, establishing the molecular basis of GSD type 1a.

Little is known about the structure-function relationship of the G6Pase protein. Isolation of the G6Pase cDNA and characterization of the in vitro expressed protein has demonstrated that the G6Pase catalytic unit consists of 357 amino acids with an apparent molecular mass of 35 kD (7). The deduced human G6Pase is an extremely hydrophobic protein containing six putative membrane-spanning segments (7). Studies have shown that proteins destined for the ER contain signals for their retention (8–10). One signal that confers ER residence for transmembrane proteins is two lysines (KK) located at three and four or five residues from the carboxy terminus (9, 10). The deduced human G6Pase protein contains the ER protein retention signal, KK, located at residues 354 and 355 (7).

In the present study, we describe mutations in the G6Pase gene of two additional patients diagnosed with GSD type 1a and their family members to expand our knowledge of the structure-function of G6Pase and to correlate specific defects with clinical manifestations of the disorder. We further investigate the role of the KK motif on G6Pase catalysis.

Methods

Genomic DNA isolation and Southern blot hybridization analysis. Genomic DNA preparations from the patient KB (GM11215), KB's affected sister, CB (GM11416) (submitted by Dr. Y.-T. Chen, Duke Uni-

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1. *Abbreviations used in this paper:* ER, endoplasmic reticulum; GSD, glycogen storage disease; G6Pase, glucose-6-phosphatase; WT, wild type.

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versity, Durham, NC), patient LLP (GM11468), and the father (GM11471) and mother (GM11470) of LLP (submitted by Dr. A. S. Knisely, Children's Hospital of Pittsburgh) were isolated from lymphoblasts obtained from National Institute of General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository (Camden, NJ). Genomic DNA preparations from the parents of KB and CB were extracted from blood samples kindly provided by Dr. Y. -T. Chen. KB and CB, siblings of unrelated Caucasian parents, experience hypoglycemia and hepatomegaly. LLP suffers occasional episodes of severe hypoglycemia with lactic acidosis, hepatomegaly, and slow growth. Liver biopsy specimens of KB, CB, and LLP had no detectable G6Pase activity. Genomic DNA was isolated using a Nucleon II kit obtained from Scotlab (Stratclyde, Scotland).

For Southern blot analysis (11), human genomic DNA was digested with BamHI or EcoRI, separated on a 0.8% agarose gel, transferred to a Nytran membrane (Schleicher & Schuell, Inc., Keene, NH), and hybridized with a phG6Pase-1 (7) probe prepared by random priming. Filters containing the human hamster hybrids were obtained from BIOS Laboratories (New Haven, CT).

Analysis of the G6Pase gene by PCR and DNA sequencing. Five pairs of oligonucleotide primers containing intronic sequences of the G6Pase gene (7) were used to amplify by PCR the coding regions of each of the five exons and the corresponding intron-exon junctions in the G6Pase genes of GSD type 1a patients and available family members. The primers for amplifying exon 1 are: 5'-TCTGCTGACATCTTCCT-3' (sense) and 5'-GCCTCTTTTCTTGCTGA-3' (antisense); exon 2, 5'-GCATTCATTCAGTAACCC-3' (sense) and 5'-TCCACTCAGCTTCTGTCT-3' (antisense); exon 3, 5'-CACCTTTACTCCATTCTC-3' (sense) and 5'-GTGGTGTGTCAGCTACA-3' (antisense); exon 4, 5'-GCCAGGCTCCAACATTT-3' (sense) and 5'-GGAGAGAAACGGAATGG-3' (antisense); and exon 5, 5'-CTTCCTATCTCTCACAG-3' (sense) and 5'-TCACTTGCTCCAAATACC-3' (antisense). The amplified fragments, I (306), II (191), III (209) IV (259), and V (646), were subcloned using the TA cloning kit obtained from Invitrogen (San Diego, CA), and five or more subclones of each exon were sequenced.

Construction of G6Pase mutants. The phG6Pase-1 cDNA (G6Pase-WT) containing nucleotides 77–1156 of the entire coding region of the human G6Pase cDNA) (7) was used as a template for mutant construction by PCR. The 5' primer for the G6Pase-Q347SP and G6Pase-K355SP mutants is 5'-AGGATGGAGGAAGGAATGAA-3' (nucleotides 77–96 of the phG6Pase cDNA). The 3' primer for G6Pase-Q347SP is 5'-TTACAACGACTTCTTGTGCGGCTGGCCCAGGACCTaGG-3' (nucleotides 1153–1116); for G6Pase-K355SP, 5'-TTACAACGACTaCTTGTGCGG-3' (nucleotides 1153–1136). The 5' and 3' primers contain an additional XhoI or XbaI linker, respectively. The amplified fragments were digested with XhoI and XbaI and ligated either into a pSVL (Pharmacia Fine Chemicals, Piscataway, NJ) or a pGEM7Z(+) (Promega Biotec, Madison, WI) vector. All constructs were confirmed by DNA sequencing (12).

In vitro transcription and translation. In vitro transcription-translation of wild type (WT) and mutant G6Pase cDNAs was performed using the TnT-coupled reticulocyte lysate system obtained from Promega Biotec. The G6Pase cDNAs were analyzed in both sense and antisense orientations and the in vitro synthesized proteins were analyzed by 10% SDS-PAGE and fluorography.

Expression in COS-1 cells and Northern blot hybridization analysis. COS-1 cells were grown at 37°C in Hepes-buffered Dulbecco modified minimal essential medium supplemented with streptomycin, penicillin, and 4% fetal bovine serum. The WT or mutant G6Pase cDNA in pSVL vectors were transfected into COS-1 cells by the DEAE-dextran/chloroquine method (13). After incubation at 37°C for 3 d, the transfected cultures were either harvested for microsomal preparation, G6Pase assays, or lysed for RNA isolation. Microsomal membranes were isolated by the method of Burchell et al. (14) from freshly prepared homogenates of transfected COS-1 cells. Disrupted microsomal membranes were prepared by incubating intact membranes in 0.2% deoxycholate for 20 min at 0°C. The latency or intactness of microsomal preparations was assessed by assaying mannose-6-phosphohydrolysis in intact versus detergent-disrupted microsomes (15). Mock transfections of COS-1 cultures by the pSVL vector were used as controls.

RNA was isolated by the guanidinium thiocyanate/CsCl method (16), separated by electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde (17), and transferred to Nytran membranes. The filters were hybridized at 42°C in a buffer containing 150 mM NaCl/15 mM Na-citrate (1× SSC), 50% formamide, 50 mM Na-phosphate, pH 6.5, 8× Denhardt, 1% SDS, 200 μg/ml sonicated salmon sperm DNA, and phG6Pase-1 probe. RNA blots were washed two times in 2× SSC containing 0.5% SDS for 30 min at room temperature, then four times in 0.1× SSC containing 0.1% SDS for 60 min at 65°C.

Phosphohydrolase assay. Phosphohydrolase activity was determined essentially as described by Burchell *et al.* (14). Reaction mixtures (100 μl) contained 50 mM cacodylate buffer, pH 6.5, 10 mM glucose-6-P, 2 mM EDTA, and appropriate amounts of cell homogenates or microsomal proteins. After incubation at 30°C for 10 min, reactions were stopped by the addition of 4 vol of a solution containing 2.1 mM ammonium molybdate, 0.33 M sulfuric acid, 3.3% SDS, and 0.07 M ascorbic acid. Sample absorbance was determined at 820 nm and is related to the amount of phosphate released using a standard curve constructed by a stock of inorganic phosphate solution.

Results

Chromosomal localization of the human G6Pase gene. In an earlier study (7), we showed that the human G6Pase gene spans ~ 12.5 kb and consists of five exons: I (309 bp), II (110 bp), III (106 bp), IV (116 bp), and V (2,451 bp including a coding region of 509 bp) (Fig. 1). Southern blot hybridization analysis was performed on human genomic DNAs digested with BamHI or EcoRI and hybridized with the phG6Pase-1 cDNA

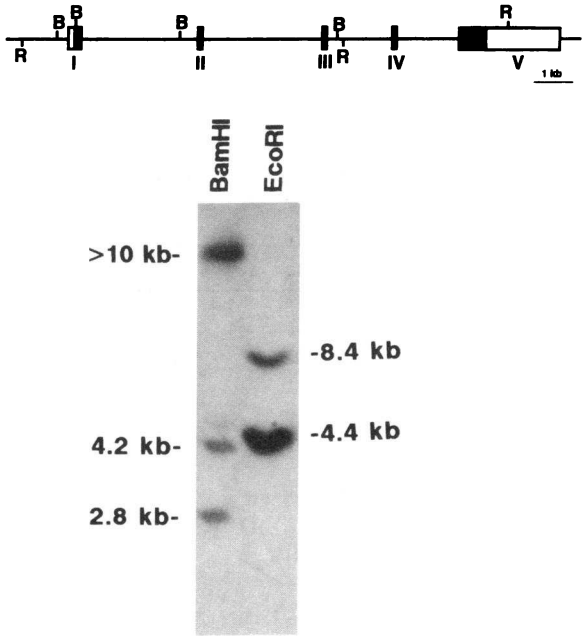

Figure 1. Southern blot hybridization of human genomic DNA to the phG6Pase-1 cDNA. Human lymphocyte DNA was digested with BamHI or EcoRI, separated on a 0.8% agarose gel, transferred to a Nytran membrane, and hybridized with a phG6Pase-1 cDNA (7) probe prepared by random priming. λDNA digested with HindIII was used as markers. The structural organization of the human G6Pase transcription unit is illustrated on the top of the figure.

probe containing the entire coding region of human G6Pase. Exon sequences were contained in three BamHI (2.8, 4.2, and > 10 kb) or two EcoRI (4.4 and 8.4) fragments as predicted from the G6Pase gene (Fig. 1). The 0.6-kb BamHI fragment containing mainly the 5'-untranslated region in exon I was not detected by this probe. These data suggest that human liver G6Pase is a single copy gene.

A panel of human–hamster hybrids were used to identify the chromosomal localization of the human G6Pase gene. Southern blots from cell hybrids were hybridized to the phG6Pase-1 cDNA probe, which cross-hybridized only slightly with the hamster genomic DNA, but detected two specific human EcoRI fragments of 4.4 and 8.4 kb (data not shown). The fragments segregated together and their presence could be correlated with that of chromosome 17 (Table I). We concluded that the human G6Pase gene is located on chromosome 17.

Identification of mutations in the G6Pase gene of a compound heterozygous GSD type 1a patient. To identify mutations in the G6Pase gene of GSD type 1a patients, we PCR amplified the coding regions of each of the five exons and all intron-exon junction regions of this gene using five pairs of oligonucleotide primers containing intronic sequences of the human G6Pase gene (7). The amplified fragments were cloned and at least five subclones of each exon were sequenced. Sequencing data of the defective gene were compared with that of a normal G6Pase gene to identify mutations.

Analysis of the G6Pase gene in a GSD type 1a patient, LLP, showed that exons 1, 3, and 4 were normal (data not shown). In exon 2 subclones of LLP, a C to T mutation at nucleotide 326 converting an arginine codon to a cysteine (codon 83, Fig. 2) was found in three of the five subclones. A second mutation was observed in two of the five independent exon 5 subclones of patient LLP. This mutation changed a C to T at nucleotide 1118, converting a glutamine at codon 347 to a stop codon (Fig. 2). Human WT G6Pase is a polypeptide of 357 residues (7). The predicted mutant G6Pase (G6Pase-Q347SP) is a truncated protein of 346 amino acids, lacking eleven residues at the carboxy terminus. Our results suggest that patient LLP is a compound heterozygote with different mutations in the two G6Pase alleles. This was confirmed by sequencing exon subclones obtained from the G6Pase gene of both parents. The father had a normal exon 2 and the mother had a normal exon 5. Four of the six exon 5 subclones from the gene of the father contained a C to T mutation at nucleotide 1118 converting a glutamine to a stop at codon 347. Two of the six exon 2 subclones from the gene of the mother contained a C to T mutation at nucleotide 326 converting an arginine to a cysteine at codon 83 (data not shown).

Identification of mutations in the G6Pase genes of two homozygous GSD type 1a patients of the same family. Sequence analysis of the G6Pase genes of two siblings, KB and CB, diagnosed with GSD type 1a showed that exons 1, 2, 3, and 4 were normal (data not shown). However, exon 5 of both G6Pase genes had a C to T mutation at nucleotide 1118 that converts a glutamine at codon 347 to a stop codon (Fig. 3). This mutation was identified in each of the five exon 5 subclones of each patient, indicating that KB and CB are homozygous for this mutation and predicting that both parents are heterozygous for the C to T substitution at nucleotide 1118. As expected, a C to T mutation at nucleotide 1118 was demonstrated in two of six exon 5 subclones of the gene from the mother and three of the five exon 5 subclones of the gene from the father (data not shown).

The truncated G6Pase mutant of 346 residues is enzymatically inactive. The liver biopsy specimens of patients LLP, KB, and CB had no detectable phosphohydrolase activity, suggesting that a mutation that converts an arginine-83 to a cysteine (G6Pase-R83C) or a glutamine-347 to a stop codon (G6Pase-Q347SP) inactivates G6Pase activity. In earlier studies, we showed that the G6Pase-R83C mutant had no detectable phosphohydrolase activity (7). To demonstrate that the G6Pase of 346 residues is devoid of G6Pase catalytic activity, we constructed a G6Pase-Q347SP mutant that changes the glutamine-

Table I. Segregation of the G6Pase Locus in Human–Hamster Hybrids

	Human chromosomes*																						
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Positive hybrids																								
811								+									65%	65%						
937	+				+									+	+		+				+			
Negative hybrids																								
507			+		+							+		65%						40%		25%		+
683					+							+		+					+		+	+		
734					+				+									+						
756					D	65%	+					+	65%	45%					+	+	+			+
803				+	+			+			15%											+	+	
852		+																						
860			30%		+	+				15%									35%		+			
867	60%				+								+	+				+	+					
909					D	+		+						+									+	
967					+			+								+								
1006				55%	+		+	+					+		+				+		+			
1049					+						+													

* Percentage numbers are the percent of the cell population containing the noted chromosome; "+" indicates >75%; *D*, deleted at 5p15.1–5p15.2.

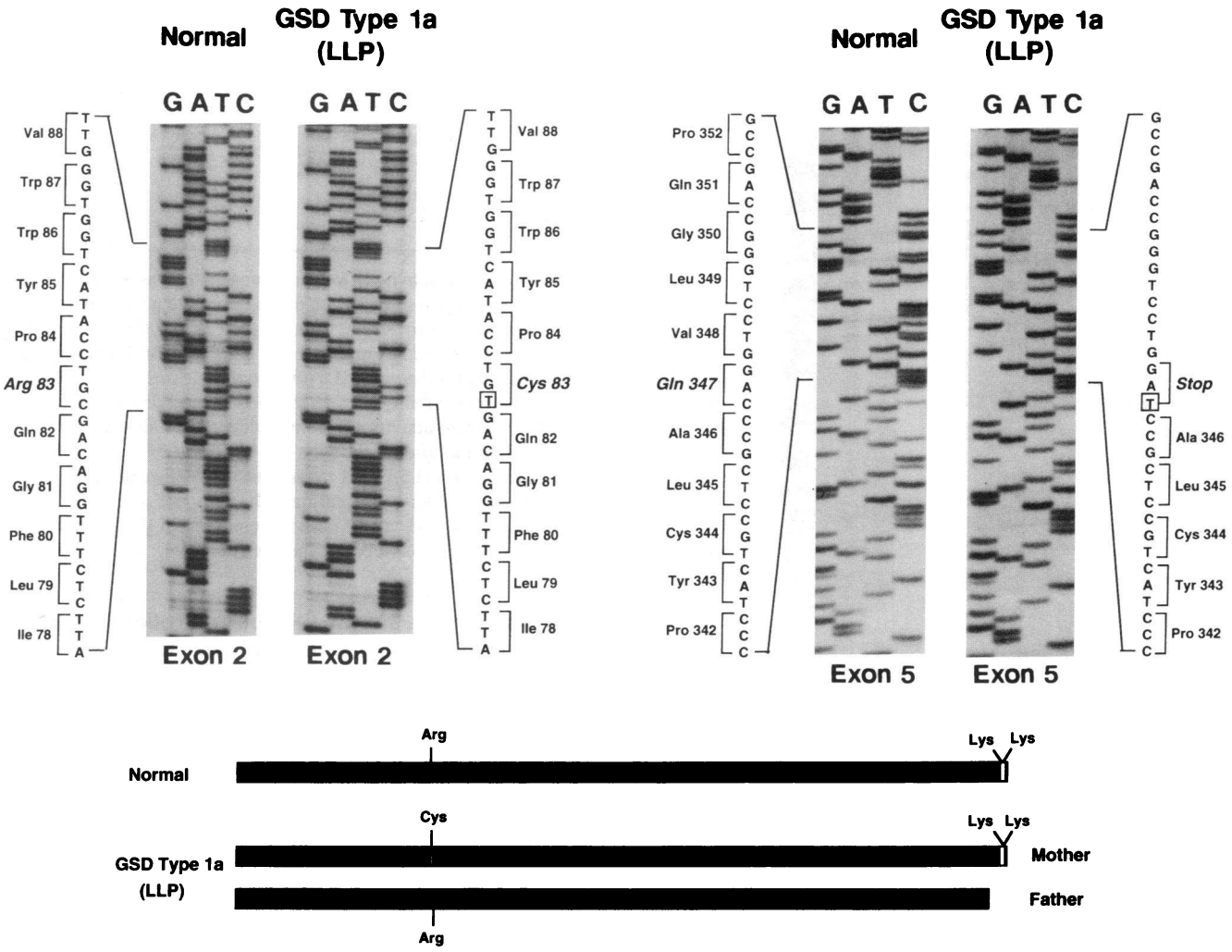

Figure 2. Autoradiograms of Sanger nucleotide sequencing reactions of the G6Pase gene from normal and GSD type 1a patient LLP. The G6Pase gene of patient LLP contains two C to T mutations (*boxed*) at nucleotides 326 (exon 2) and 1118 (exon 5), respectively. The predicted mutant G6Pase of patient LLP contains an arginine-83 to cysteine mutation in one G6Pase allele and a glutamine-347 to a stop codon mutation in the second allele. LLP inherited the exon 5 mutation from the father and the exon 2 mutation from the mother, shown diagrammatically at the bottom. The genomic DNAs of patient LLP and both parents were isolated from lymphoblasts (GM11468, GM11471, and GM11470) obtained from NIGMS Human Genetic Mutant Cell Repository. The liver biopsy specimen of patient LLP had no detectable G6Pase activity.

347 to a stop codon. Phosphohydrolase activities were analyzed after transient expression of WT or mutant G6Pase in COS-1 cells (Table II). As predicted, a truncation of the carboxy terminal 11 residues in human G6Pase abolished G6Pase activity. Northern hybridization analysis of G6Pase transcripts from transfected cells showed that WT and Q347SP mutant G6Pase mRNAs were expressed at similar levels (Fig. 4 *A*). The G6Pase-WT mRNA supported the synthesis of a polypeptide of 35 kD (Fig. 4 *B*). The G6Pase-Q347SP mRNA directed the synthesis of a polypeptide that migrated slightly faster than the WT enzyme, consistent with a deletion of 11 residues.

The ER retention signal KK is not essential for G6Pase activity. The G6Pase-Q347SP mutant lacking the 11 carboxy-terminal amino acids has no detectable phosphohydrolase activity, indicating that these residues are essential for G6Pase catalysis. The carboxy terminus contains the ER transmembrane protein retention signal, KK, at residues 354 and 355 in the G6Pase protein (7). To determine if the loss of the ER motif is responsible for the lack of enzymatic activity of the G6Pase-Q347SP mutant, we created a mutant G6Pase, G6Pase-K355SP, that converts the lysine residue at position 355 to a stop codon. Phosphohydrolase activities were examined in whole homogenates after transient expression of WT or mutant G6Pase in COS-1 cells (Table II). In contrast to the enzymatically inactive G6Pase-Q347SP mutant, the G6Pase-K355SP mutant is active, retaining at least 75% of WT G6Pase activity. The G6Pase-K355SP mRNA also supported the synthesis of a 35-kD polypeptide like the WT transcript (Fig. 4 *B*). Our data show that neither the ER protein retention signal nor the last three residues (355–357) in human G6Pase is essential for G6Pase catalysis.

As the KK motif has been shown to signal transmembrane proteins to reside in the ER (9, 10), it is possible that the G6Pase-K355SP mutant lacking an ER retention motif remained in the soluble rather than microsomal fractions of the transfected cells. Therefore, we examined phosphohydrolase activities in microsomal and soluble fractions of COS-1 cells transfected with either the WT or G6Pase-K355SP mutant (Ta-

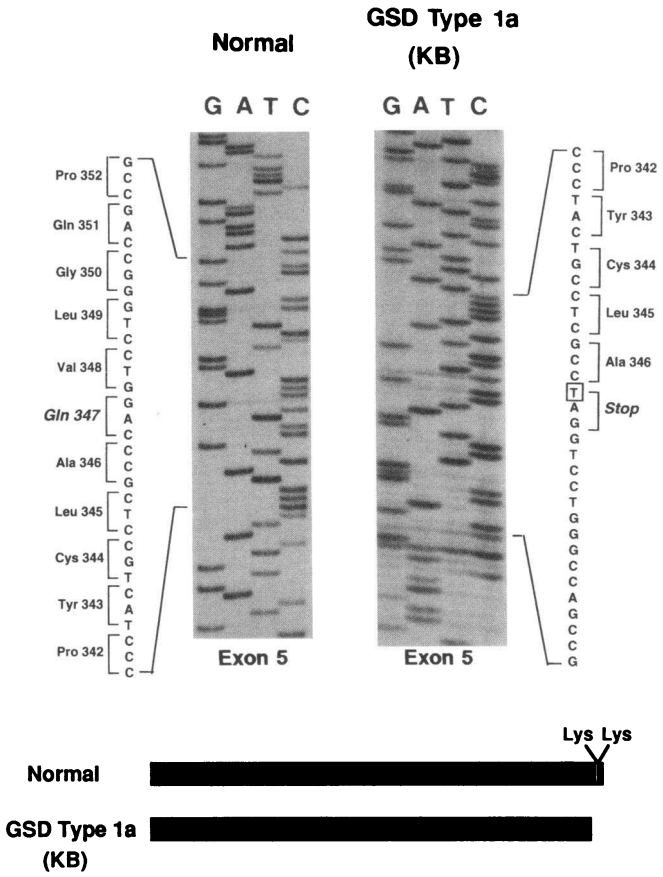

Figure 3. Autoradiograms of Sanger nucleotide sequencing reactions of the G6Pase gene from normal and GSD type 1a patient KB. Patient KB contains a C to T mutation (*boxed*) at nucleotide 1118 (exon 5) of the G6Pase gene. The predicted mutant G6Pase of patient KB contains a glutamine-347 to a stop codon mutation. A similar mutation was found in patient CB, the sibling of KB. Both parents were heterozygous for this mutation. The genomic DNAs of patients KB and CB were isolated from lymphoblasts (GM11215 and GM11416) obtained from NIGMS Human Genetic Mutant Cell Repository. The genomic DNAs of both parents were isolated from blood samples. The liver biopsy specimens of patients KB and CB exhibited no detectable G6Pase activity.

ble II). G6Pase activities were associated primarily with microsomal preparations; low or undetectable levels of enzyme activity were found in the soluble fractions of WT or K355SP mutant–transfected COS-1 cells. Moreover, microsomal G6Pase in both cells exhibited similar latencies and heat sensitivities (Table II), suggesting that ER residence of G6Pase is not affected by the removal of the retention signal.

Discussion

GSD type 1a is an autosomal recessive disorder that affects ~ 1 in 100,000–300,000 people (3, 4). We have previously reported the isolation of the human G6Pase gene and established a correlation between mutations in the gene and the presence of GSD type 1a (7). In the present study, we show that human G6Pase is a single copy gene located on chromosome 17. In addition, we have expanded our analysis of patients with GSD type 1a and identified two genetic lesions in the G6Pase gene.

Table II. Analysis of G6Pase Phosphohydrolase Activity of WT and Mutant G6Pase cDNAs in COS-1 Cells

Constructs	Phosphohydrolase activity	Latency	Thermal stability
Mock	30±1.5	–	–
WT	346.4±12	28.2%	6.0%
Q347SP	29.2±3.2	–	–
K355SP	259.5±9.5	28.0%	6.8%

Phosphohydrolase activity in whole homogenates was assayed in reactions containing 10 mM glucose-6-phosphate using two independent isolates of each construct. Data are presented as the mean±SEM. Latencies were assessed by mannose-6-P phosphohydrolysis in intact (I) versus detergent-disrupted (D) microsomes, defined as (1 − I/D) × 100. Thermal stability was determined by assaying glucose-6-P phosphohydrolase activity in deoxycholate (0.2%)-disrupted microsomes before and after incubation for 10 min at 37°C in 50 mM cacodylate buffer, pH 5.0, and refers to the percentage of enzyme activity remaining after heat treatment. In both WT and K355SP-transfected cells, <3% of the activity remained in the supernatant fractions (soluble) after pelleting the microsomes.

We also show that a putative ER protein retention signal at the carboxy terminus of G6Pase is not required for the subcellular localization of this enzyme.

Both genetic lesions were found in patient LLP, who was shown to be a compound heterozygote. One G6Pase allele of LLP contains a C to T transition in exon 2, converting an arginine to a cysteine at position 83 (R83C). The other allele has a C to T transition in exon 5, converting a glutamine codon to a stop (Q347SP). LLP inherited the defective exon 2 (R83C) from the mother and the defective exon 5 (Q347SP) allele from the father. Both parents also have normal G6Pase alleles and are therefore phenotypically unaffected. We have previously reported that the R83C mutation results in an inactive G6Pase (7); in this report we also show that the Q347SP mutation inactivates the enzyme.

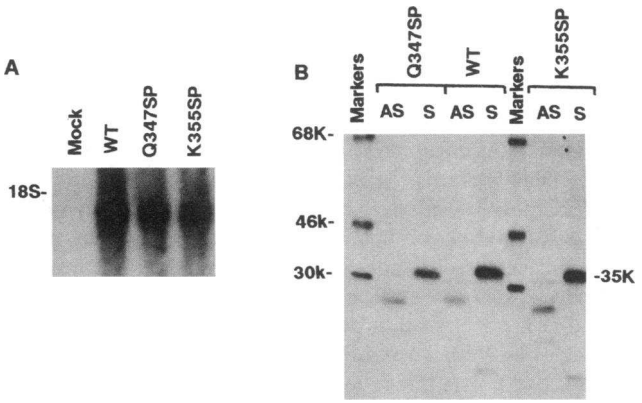

Figure 4. (*A*) Analysis of mRNA expression after transient expression of WT and mutant G6Pase cDNAs in COS-1 cells. Total RNA was separated by formaldehyde-agarose gel electrophoresis, blotted onto a Nytran membrane, and hybridized to the phG6Pase-1 probe labeled by random priming. (*B*) SDS-PAGE electrophoresis analysis of G6Pase polypeptides. In vitro transcription-translation of WT or mutant G6Pase cDNA in a pGEM-7Zf(+) vector was performed using the TnT-coupled reticulocyte lysate system and analyzed in both sense (*S*) and antisense (*AS*) orientations.

The Q347SP mutation was also found in two affected siblings, KB and CB, who are homozygous for this mutation. Both parents of the patients are heterozygotes with one normal allele and one allele with the Q347SP mutation. It is interesting to note that even though a very small sample of patients have been examined thus far, the same kind of mutations arise in apparently unrelated patients, suggesting that these mutations may represent mutational hot spots. The C to T mutation in exon 2 (CGT to TGT) may result from deamination of 5-methylcytosine to thymine (18). Mutations within CpG doublets have been reported for a variety of congenital human diseases (19, 20).

The Q347SP mutation results in a polypeptide that is 11 amino acids shorter than the normal gene product (357 residues) at the carboxy-terminal end. The lack of catalytic activity of the Q347SP mutant suggests that amino acids downstream of 346 are important for human G6Pase catalysis. The deleted region contains two lysines at amino acids 354 and 355, a motif (KK) associated with the retention of transmembrane proteins in the ER (9, 10). To determine if deletion of the KK retention signal was responsible for the lack of enzymatic activity observed in mutant Q347SP, we constructed a mutant that converts one of the lysines to a stop codon (G6Pase-K355SP). This mutant did not lose enzymatic activity, and furthermore it remained associated with microsomal membranes demonstrating that ER residence of G6Pase is not dictated by the KK signal. Several transmembrane ER proteins, such as the human ribophorins I and II, are retained in the ER in the absence of this motif (10). These proteins may not require a discrete structural motif for ER retention. Our data also suggest that amino acids 347–354 play a role in G6Pase catalysis. The role of the carboxy-terminal amino acids in G6Pase catalytic activity is currently under investigation.

It has been demonstrated that GSD type 1a patients do not have G6Pase activity in their livers. The identification of mutations in the G6Pase gene has established the molecular basis for this lack of enzymatic activity and provides us with molecular reagents for prenatal diagnosis and screening of potential carriers. In addition, knowledge of the mutations may be applied to design therapeutic approaches, including gene therapy, for this disorder.

Acknowledgments

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