Am. J. Hum. Genet. 57:766–771, 1995

# Genetic Basis of Glycogen Storage Disease Type 1a: Prevalent Mutations at the Glucose-6-Phosphatase Locus

Ke-Jian Lei,[1] Yuan-Tsong Chen,[2] Hungwen Chen,[1] Lee-Jun C. Wong,[3] Ji-Lan Liu,[1] Allyn McConkie-Rosell,[2] Johan L. K. Van Hove,[2] Henry C.-Y. Ou,[1] Nan Jung Yeh,[1] Lorraine Y. Pan,[1] and Janice Yang Chou[1]

[1]Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda; [2]Department of Pediatrics, Duke University Medical Center, Durham, NC; and [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston

## Summary

Diagnosis of glycogen storage disease (GSD) type 1a currently is established by demonstrating the lack of glucose-6-phosphatase (G6Pase) activity in the patient's biopsied liver specimen. Recent cloning of the G6Pase gene and identification of mutations within the gene that causes GSD type 1a allow for the development of a DNA-based diagnostic method. Using SSCP analysis and DNA sequencing, we characterized the G6Pase gene of 70 unrelated patients with enzymatically confirmed diagnosis of GSD type 1a and detected mutations in all except 17 alleles (88%). Sixteen mutations were uncovered that were shown by expression to abolish or greatly reduce G6Pase activity and that therefore are responsible for the GSD type 1a disorder. R83C and Q347X are the most prevalent mutations found in Caucasians, 130X and R83C are most prevalent in Hispanics, and R83H is most prevalent in Chinese. The Q347X mutation has thus far been identified only in Caucasian patients, and the 130X mutation has been identified only in Hispanic patients. Our results demonstrate that the DNA-based analysis can accurately, rapidly, and noninvasively detect the majority of mutations in GSD type 1a. This DNA-based diagnosis now permits prenatal diagnosis among at-risk patients and serves as a database in screening and counseling patients clinically suspected of having this disease.

## Introduction

The absence of the key enzyme glucose-6-phosphatase (G6Pase) in the homeostatic regulation of blood glucose concentrations causes glycogen storage disease (GSD) type 1a (von Gierke disease), an inborn error of metabolism (Nordlie and Sukalski 1985; Beaudet 1991; Chen and Burchell 1995). The enzyme is normally expressed in the liver, kidney, and intestinal mucosa, and the absence of G6Pase activity is associated with excessive accumulation of glycogen in these organs. GSD type 1a has an overall frequency of ~1/100,000 live births and has been reported in Caucasians, Hispanics, and Orientals but not, thus far, in African Americans. The disease manifests itself during the first year of life, with severe hypoglycemia, growth retardation, hepatomegaly, bleeding diathesis, lactic acidemia, hyperlipidemia, and hyperuricemia. Long-term complications include gout, hepatic adenomas, osteoporosis, and renal disease (Chen et al. 1988; Beaudet 1991; Chen and Burchell 1995).

Currently, the diagnosis of type 1a GSD is established by demonstrating the lack of G6Pase activity on a liver biopsy specimen. Reliable carrier testing is not available, and prenatal diagnosis requires a fetal liver biopsy, an invasive high-risk procedure. Whereas enzyme-based diagnosis can only be performed in the organ normally expressing the enzyme, DNA-based diagnosis can be performed on readily available tissues, such as blood, skin, or, in the case of prenatal testing, chorionic villi or amniocytes. Our recent cloning of the G6Pase cDNA, characterization of the gene for human G6Pase, and identification of mutations in the G6Pase gene that cause GSD type 1a (Lei et al. 1993, 1994, 1995) allow us to develop a noninvasive DNA-based diagnosis. The labor-intensive linkage analysis requiring multiple polymorphic DNA markers is frequently used as a DNA-based diagnostic method (Ott 1986). The more direct and unequivocal mutational analysis of the target gene, eliminating the need for extensive data on affected siblings and family members, is employed in this study. To establish a DNA-based diagnosis based on mutation analysis, it is desirable to determine the prevalent mutations and to develop a rapid screening method for detecting the majority of these mutations. In order for diagnosis to be widely applicable for all GSD type 1a patients, it is also necessary to establish prevalent mutations in different ethnic/racial groups. In this report, we have characterized mutations in the G6Pase gene of 70 unrelated GSD type 1a patients of different ethnic backgrounds, including 12 patients whose mutations had been characterized in earlier studies (Lei et al. 1993, 1994, 1995).

Received May 19, 1995; accepted for publication June 26, 1995.

Address for correspondence and reprints: Dr. Janice Chou, Human Genetics Branch, Building 10, Room 9S242, National Institutes of Health, Bethesda, MD 20892.

© 1995 by The American Society of Human Genetics. All rights reserved.

0002-9297/95/5704-0004$02.00

## Patients and Methods

### *Patients*

We have analyzed the G6Pase gene of 70 GSD type 1a patients, including 12 patients whose mutations had been previously identified (Lei et al. 1993, 1994, 1995). These patients presented with clinical manifestations of hypoglycemia, hepatomegaly, and growth retardation and were positively diagnosed as GSD type 1a by the lack—or greatly reduced level—of G6Pase activity in liver biopsy samples. Genomic DNA preparations were extracted from blood samples by use of a Nucleon II kit obtained from Scotlab. Peripheral blood samples were obtained with the informed consent of the patients and/or parents.

### *Analysis of the G6Pase Gene by SSCP and DNA Sequencing*

The G6Pase gene of GSD type 1a patients and available family members was characterized by amplifying the coding and intron/exon junction regions by PCR. Reaction mixtures (20 μl) contained 25 mM TAPS (N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid), pH 9.3, 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.2 mM each of dATP/dCTP/dGTP/dTTP, 2.5 μCi $^{32}$P-dCTP (6,000 Ci/mmol), 1.25 μM of both primers, and 1 unit *Taq* polymerase. PCR was carried out for a total of 30 cycles. Oligonucleotide primers containing intronic, 5′ untranslated sequences, or 3′ untranslated sequences of the human G6Pase gene were employed, and exons I–IV were amplified as described elsewhere (Lei et al. 1993). Exon V was amplified into two overlapping fragments, termed "V-5′" and "V-3′," by the following primers: V-5′, 5′-CTTCCTATCTCTCACAG-3′ (sense) (Lei et al. 1993) and 5′-TGGAGTTGAGAGC-CAGC-3′ (antisense; nucleotides [nt] 895–911); and V-3′, 5′-CTCAAGAACCTGGGCACGCTC-3′ (sense; nt 863–883) and 5′-TCACTTGCTCCAAATACC-3′ (antisense) (Lei et al. 1993).

The PCR amplified fragments–I (306 bp), II (192 bp), III (209 bp), IV (259 bp), V-5′ (292 bp), and V-3′ (389 bp)—were analyzed by SSCP (Orita et al. 1989) by electrophoresing wild-type and mutant target DNAs side-by-side through MDE nondenaturing gels (AT Biochem), according to the protocols provided by the manufacturer. To increase sensitivity of SSCP analysis, we also analyzed the amplified fragments on MDE gels containing 5% glycerol. Mutations in the target DNA were visualized by the differential migration of one or both of the mutant strands (Orita et al. 1989). Six previously identified G6Pase gene mutations in the same study population—R83C, Q347X, 130X, ΔF327, R295C, and G222R (Lei et al. 1993, 1994, 1995)—were included as controls.

The mutation-containing fragments identified by SSCP were subcloned by a TA cloning kit obtained from Invitrogen and were characterized by DNA sequencing. The new mutations were introduced into the G6Pase cDNA by site-directed mutagenesis as described elsewhere (Lei et al. 1993), by use of the phG6Pase-1 cDNA containing nt 77–1156 of the entire coding region of the human G6Pase cDNA as a template.

### *Allele-Specific Oligonucleotide (ASO) Hybridization Analysis*

For ASO hybridization analysis, the coding and intron/exon junction regions of the G6Pase gene were amplified by PCR as described elsewhere (Lei et al. 1993). Two microliters of the PCR products were spotted onto Hybond-$N^+$ nylon membranes (Amersham) and were denatured and neutralized (DeMarchi et al. 1994). Hybridization and washing conditions were essentially as described elsewhere (DeMarchi et al. 1994). The wild-type and mutant oligonucleotide probes are R83 (5′-AGTATGGACGCTGTCCAAA-3′; antisense) / R83C (5′-AGTATGGACACTGTCCAAA-3′; antisense), 130 (5′-AGGTGTATACTACGTGATG-3′; sense)/130X (5′-GGTGTATATACTACGTGAT-3′; sense), Q347 (5′-CCAGGACCTGGGCGAGGCA-3′; antisense)/Q347X (5′-CCAGGACCTAGGCGAGGCA-3′; antisense), F327 (5′-GTCTTGTCCTTCTGCAAGA-3′; sense)/ΔF327 (5′-CGTCTTGTCCTGCAAGAGT-3′; sense), R295 (5′-AGC-TGAGGCGGAATGGGAG-3′; antisense)/R295C (5′-AGCTGAGGCAGAATGGGAG-3′; antisense), and G222 (5′-TTCGCCATCGGATTTTATC-3′; sense)/G222R (5′-TTCGCCATCCGATTTTATC-3′; sense).

### *Expression in COS-1 Cells and Phosphohydrolase Assay*

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 wild-type or mutant G6Pase cDNA in a pSVL vector was transfected into COS-1 cells by the DEAE-dextran/chloroquine method (Ausubel el al. 1992). Mock transfections of COS-1 cells with the pSVL vector alone were used as controls. After incubation at 37°C for 3 d, the transfected cultures were harvested for G6Pase assays.

Phosphohydrolase activity was determined essentially according to the method described by Burchell et al. (1988). Reaction mixtures (100 μl) contained 50 mM cacodylate buffer, pH 6.5, 10 mM glucose-6-phosphate, 2 mM EDTA, and appropriate amounts of cell homogenates and were incubated at 30°C for 10 min. Sample absorbance was determined at 820 nm and is related to the amount of phosphate released, according to a standard curve determined with a stock of inorganic phosphate solution.

## Results

### *Identification of Mutations in the G6Pase Gene of GSD Type 1a Patients*

In earlier studies, we characterized the G6Pase gene of 12 GSD type 1a patients and uncovered a total of six

Am. J. Hum. Genet. 57:766–771, 1995

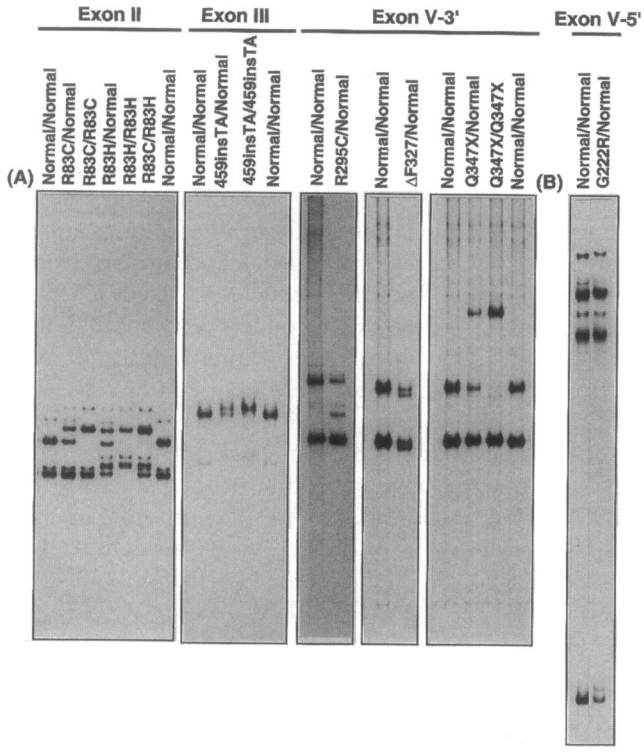


**Figure 1** SSCP analysis of the 6 known mutations, R83C, Q347X, 130X, ΔF327, R295C, G222R, and a new mutation, R83H, in the G6Pase gene of GSD type 1a patients. The exon-containing fragments were amplified by PCR and were analyzed by electrophoresis on (*A*) MDE gels or (*B*) MDE gels containing 5% glycerol.

independent mutations (Lei et al. 1993, 1994, 1995). This was accomplished by amplifying the coding and intron/exon junction regions by PCR, subcloning the amplified fragments, and sequencing five or more subclones of each fragment. Although DNA sequencing is the best approach to pinpoint the exact nature of the mutation, it is time-consuming and impractical for mutation analysis in a large number of patients. In the present study, we were able to use SSCP analysis (Orita et al. 1989) to detect mutations in the G6Pase gene, in ≤2 d. We also expanded our study to a total of 70 unrelated GSD type 1a patients. The human G6Pase gene consists of five exons (Lei et al. 1993). Each exon and the associated intron/exon junctions were amplified and analyzed as one fragment, except for exon V, which was amplified into two overlapping fragments—292 bp (V-5′) and 389 bp (V-3′)—because of its large size.

SSCP analysis on MDE gels in the absence (fig. 1*A*) or presence (fig. 1*B*) of glycerol could detect the six previously identified (known) mutations—R83C (exon II), 130X (exon III), R295C (exon V-3′), ΔF327 (exon V-3′), Q347X (exon V-3′), and G222R (V-5′). Moreover, a given mutation existing in one or both alleles could be accurately distinguished by SSCP, as demonstrated for R83C, Q347X, and 130X (fig. 1*A*).

We also employed ASO hybridization assays (DeMarchi et al. 1994) designed to detect the six known mutations in the G6Pase gene of GSD type 1a patients and the available family members (data not shown). Identical results were obtained by SSCP and ASO assays.

In addition to the 6 known mutations, SSCP analysis of the 140 G6Pase alleles revealed the existence of 10 additional mutations (158delC/35X, G268A/W63X, G327A/R83H, C611T/P178S, G641A/G188S, T785A/W236R, C803T/Q242X, 813insG-822delC/CEQP245-248WRAA, G888T/G270V, and T1113G-C1114A/L345R), which were further characterized by DNA sequencing (table 1). The 10 mutations include 2 in exon I (35X and W63X [fig. 2*A*]), 1 in exon II (G327A/R83H [fig. 1*A*]), 2 in exon IV (C611T/P178S [fig. 2*B*] and G641A/G188S [fig. 2*A*]), 3 in exon V-5′ (T785A/W236R, C803T/Q242X, and 813insG-822delC/CEQP245-248WRAA [fig. 2*B*]), and 2 in exon V-3′ (G888T/G270V [fig. 2*B*] and T1113G-C1114A/L345R [fig. 2*A*]). The Q347X (fig. 2*A*) and G222R (fig. 2*B*) mutations were included to demonstrate that SSCP analysis could accurately identify several mutations in the same amplified fragment. To date, we have detected no normal sequence polymorphism in the screening of nearly 180 G6Pase alleles.

The liver biopsy specimens of all patients analyzed had very low or undetectable phosphohydrolase activity, suggesting that mutations identified in the G6Pase gene of these patients inactivated the G6Pase enzyme. Therefore, we constructed G6Pase mutants and analyzed phosphohydrolase activities after transient expression of wild-type or mutant G6Pase cDNA into COS-1 cells (table 2). The R83H, P178S, G188S, CEPQ245-248WRAA, G270V, and L345R mutations abolished G6Pase activity, whereas the W236R mutation greatly reduced G6Pase activity.

### *Mutation Frequency and Relationship of G6Pase Mutations and Ethnic Groups*

The mutations and their respective frequencies in the 70 patients with GSD type 1a are summarized in table 3. A total of 16 mutations were identified. Of the 70 patients analyzed, 3 contained no identifiable mutant alleles, 11 contained only one mutant allele, and 56 contained two mutant alleles. Of these 56 patients, 26 are homozygotes and 30 are compound heterozygotes.

The most prevalent mutations are R83C (37%) and Q347X (22%), representing 59% of the genetic lesions seen in GSD type 1a. These were followed by 130X and R83H, each representing ~6% of the disease-causing alleles. Together, the 16 mutations account for 88% of GSD type 1a alleles. R83C (41%) and Q347X (28%) are the most prevalent mutations found in Caucasians, R83C (28%) and 130X (50%) are most prevalent in Hispanics, and R83H (70%) is most prevalent in Chinese (table 4). The Caucasians studied were all North

**Table 1**

**Ten New Mutations in the G6Pase Gene of GSD Type 1a Patients**

| Nucleotide(s)/Amino Acid(s) | Comments |
|---|---|
| 158delC/35X | Deletion of C at nt 158; generation of a stop at position 35 |
| G268A/W63X | G→A transition at nt 268; Trp-63→stop codon |
| G327A/R83H | G→A transition at nt 327; Arg-83→His |
| C611T/P178S | C→T transition at nt 611; Pro-178→Ser |
| G641A/G188S | G→A transition at nt 641; Gly-188→Ser |
| T785A/W236R | T→A transversion at nt 785; Trp-236→Arg |
| C803T/Q242X | C→T transition at nt 803; Gln-242→stop codon |
| 813insG-822delC/CEQP245-248WRAA | Insertion of G at nt 813 and deletion of C at nt 822; Cys, Glu, Gln, and Pro at codons 245–248→Trp, Arg, Ala, and Ala, respectively |
| G888T/G270V | G→T transversion at nt 888; Gly-270→Val |
| T1113G–C1114A/L345R | TC→GA transversion at nt 1113–1114; Leu-345→Arg |

Americans. Although they represent a population of mixed heritage, 66% of those responding to questions regarding ancestry claimed heritage from Eastern Europe, including eight Jewish families. Six of the Jewish patients were homozygous for R83C, and two were heterozygous for R83C and Q347X. The Q347X mutation has thus far been identified only in Caucasian patients. We have analyzed 18 G6Pase alleles of Hispanic origin; seven patients (14 alleles) have two Hispanic parents, and four patients have one Hispanic parent. It appears that the 130X mutation was inherited exclusively from the patient's Hispanic parent. Of the five Chinese patients analyzed, two were homozygous for the R83H mutation and three contained one R83H allele (7 of the 10 alleles). On the other hand, the R83H mutation was detected in only 1 of the 112 G6Pase alleles of Caucasian patients.

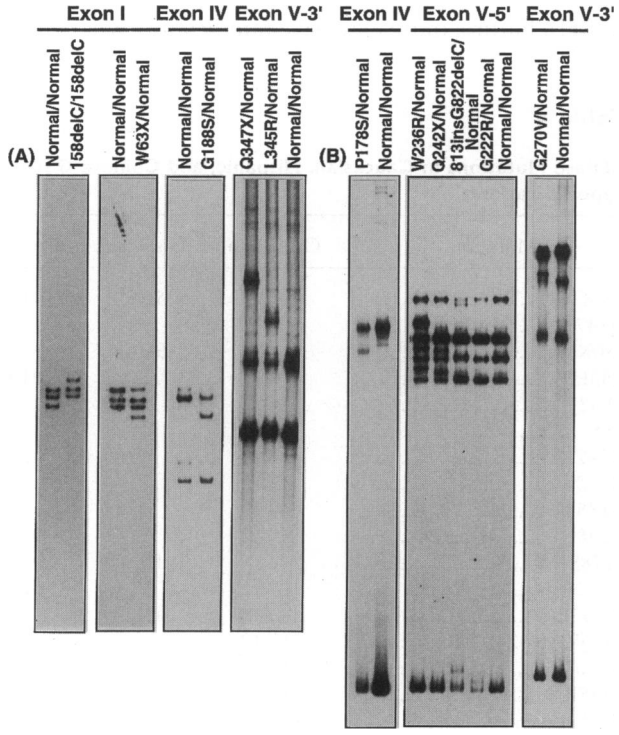


**Figure 2** SSCP analysis of new mutations in the G6Pase gene of GSD type 1a patients. The exon-containing fragments were amplified by PCR and were analyzed by electrophoresis on (*A*) MDE gels or (*B*) MDE gels containing 5% glycerol.

## Discussion

We have employed an SSCP assay to screen for mutations that are present in the coding region and intron/exon junctions of the G6Pase gene in 70 unrelated GSD type 1a patients diagnosed by enzymatic assays. A total of 16 mutations were uncovered, which were further

**Table 2**

**G6Pase Activity Encoded by Wild-Type and Mutant G6Pase cDNAs**

| Construct | Mean ± SEM[a] Phosphohydrolase Activity[b] (nmol/min/mg protein) |
|---|---|
| Mock | 14.4 ± 1.6 |
| Wild type | 139.4 ± 7.8 |
| R83H | 14.1 ± 1.3 |
| P178S | 16.4 ± 1.6 |
| G188S | 13.6 ± 1.0 |
| W236R | 19.6 ± 1.2 |
| CEPQ245–2458WRAA | 13.0 ± .6 |
| G270V | 13.1 ± .4 |
| L345R | 15.2 ± 1.0 |

[a] Standard error of the mean.

[b] In whole homogenates, assayed in reactions containing 10 mM glucose-6-phosphate, with two independent isolates of each construct.

**Table 3**

**Mutations in the G6Pase Alleles of GSD Type Ia Patients**

| Mutations | No. of Alleles |
|---|---|
| R83C | 52 (37.1%) |
| Q347X | 31 (22.1%) |
| 130X | 9 (6.4%) |
| R83H | 8 (5.7%) |
| ΔF327 | 4 (2.9%) |
| 35X | 4 (2.9%) |
| Q242X | 3 (2.1%) |
| W63X | 2 (1.4%) |
| G188S | 2 (1.4%) |
| R295C | 2 (1.4%) |
| P178S | 1 (.7%) |
| G222R | 1 (.7%) |
| W236R | 1 (.7%) |
| CEQP245–248WRAA | 1 (.7%) |
| G270V | 1 (.7%) |
| L345R | 1 (.7%) |
| Unidentified[a] | 17 (12.1%) |
| Total | 140 |

NOTE.—SSCP analysis has identified 16 independent mutations in the G6Pase gene of 70 unrelated GSD type 1a patients, including 12 patients whose mutations had been previously characterized (Lei et al. 1993, 1994, 1995). The presence of six previously identified mutations—R83C, Q347X, 130X, ΔF327, R295C, and G222R—was also confirmed by ASO hybridization assays.

[a] G6Pase alleles that include 3 patients with no identifiable mutations and 11 patients with only one identifiable mutation.

characterized by DNA sequencing. Transient-expression assays using mutant G6Pase cDNAs were employed to demonstrate that all 16 mutations inactivate or greatly reduce G6Pase activity. The 16 different mutations include 6 previously identified mutations—R83C, 130X, G222R, R295C, ΔF327, and Q347X (Lei et al. 1993, 1994, 1995)—and 10 newly identified mutations—R83H, 35X, Q242X, W63X, G188S, P178S, W236R, CEQP245-248WRAA, G270V, and L345R. We also used ASO hybridization assays to confirm the presence of the six previously known mutations in these patients. Together these 16 mutations account for 88% of mutant G6Pase alleles.

SSCP analysis, however, failed to identify G6Pase lesions in 12% of the alleles of the GSD type 1a patients. Generally, analysis of mutations by SSCP detects only 70%–95% of mutations in PCR products (Michaud et al. 1992; Sheffield et al. 1993). Furthermore, the primers that we are using at present allow us to detect only mutations in the coding region and intron/exon junctions of the gene. Therefore, mutations located in the 5′ UTR or 3′ UTR of the human G6Pase gene, which may affect the stability of the G6Pase mRNA (Cleveland and Yen 1989; Peltz et al. 1991), will elude detection. Also, the present assay cannot identify mutations in the control regions of the G6Pase gene, including the 5′ and 3′ flanking regions. These mutations can affect the rate of gene transcription (Lai and Darnell 1991; Roeder 1991), resulting in an altered G6Pase mRNA expression and synthesis. We are now designing oligonucleotide primers to look for mutations in the 5′ control region as well as the 3′ UTR of the G6Pase gene.

Sixty G6Pase alleles (43%) in GSD type 1a patients contain a mutation that alters codon 83—R83C or R83H. The two mutations occur at a CpG dinucleotide that involves either a C→T transition at nt 326 (R83C) or a G→A transition at nt 327 (R83H). Studies have shown that a large number of human genetic diseases are caused by cytosine methylation at CpG dinucleotides (Cooper and Youssoufian 1988; Holliday and Grigg 1993; Tasheva and Roufa 1993). Most of the mutations at CpG dinucleotides result from a 5-methylcytosine→T transition, although G→A transitions also occur (Magewu and Jones 1994). The CpG dinucleotide at codon 83 of the G6Pase gene appears to be a hotspot for mutations in GSD type 1a.

The 70 GSD type 1a patients consist of 54 Caucasians, 7 Hispanics, 4 Caucasian/Hispanic, and 5 Chinese. Thus, we were able to identify the prevalent mutations—R83C and Q347X—in Caucasian patients (112 G6Pase alleles). Although the numbers of Hispanic and Chinese patients analyzed are statistically low, there does seem to be a bias in the prevalent mutation present in Hispanic GSD type 1a patients (mutations 130X and R83C) and in Chinese GSD type 1a patients (mutation R83H). Future, more extensive family study will demon-

**Table 4**

**G6Pase Mutations in Caucasian, Hispanic, and Chinese GSD Type Ia Patients**

| Mutation | Caucasian | Hispanic | Chinese |
|---|---|---|---|
| R83C | 46/112 | 5/18 | 1/10 |
| Q347X | 31/112 | ... | ... |
| 130X | ... | 9/18 | ... |
| R83H | 1/112 | ... | 7/10 |
| ΔF327 | 4/112 | ... | ... |
| 35X | 4/112 | ... | ... |
| Q242X | 3/112 | ... | ... |
| W63X | 2/112 | ... | ... |
| G188S | 2/112 | ... | ... |
| R295C | 2/112 | ... | ... |
| P178S | ... | 1/18 | ... |
| G222R | 1/112 | ... | ... |
| W236R | ... | 1/18 | ... |
| CEQP245–248WRAA | 1/112 | ... | ... |
| G270V | 1/112 | ... | ... |
| L345R | 1/112 | ... | ... |
| Unidentified | 13/112 | 2/18 | 2/10 |

NOTE.—Data are nos. of mutant alleles/total no. of G6Pase alleles analyzed. For patients with both Caucasian and Hispanic parents, mutation analysis by SSCP was also performed on the G6Pase gene of both parents.

strate whether this bias stands. Thus far, the Q347X mutation has been identified only in Caucasian patients, and the 130X mutation has been identified only in Hispanic patients.

The prevalence of different mutations in various ethnic/racial groups has practical clinical implications. Our data suggest that screening of one or two ethnic-specific mutations is sufficient to detect >65% of mutations in each ethnic/racial group. More important, screening for 16 mutations by SSCP followed by either DNA sequencing or ASO hybridization assay will positively identify 88% of all mutant alleles carrying GSD type 1a, regardless of ethnic/racial background. A robotics-assisted procedure can be used for large-scale mutation analysis (DeMarchi et al. 1994). The small size of the G6Pase gene is amenable to automated DNA sequencing, which should further improve the mutation-detection rate. In summary, a DNA-based diagnosis can be used as an initial screening for patients clinically suspected of having GSD type 1a, thereby avoiding liver biopsy. The DNA-based diagnostic method can also be used for carrier testing and prenatal diagnosis of GSD type 1a.

## Acknowledgments

We thank Kerri Lamance, Dr. Arthur L Beaudet, and the other metabolic physicians at Baylor College of Medicine for providing patient samples; the GSD families and the Association for Glycogen Storage diseases for supports; Ms. C.-J. Pan for technical assistance; and Drs. Margaret Chamberlin and Ida Owens for critical reading of the manuscript. This study is supported in part by National Center for Research Sources (General Clinical Research Centers Program) grant MO1-RR30.

## References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1992) Current protocols in molecular biology. Greene and Wiley-Interscience, New York, pp 9.2.1–9.2.6

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. Clim Chim Acta 173:183–192

Chen Y-T, Burchell A (1995) Glycogen storage diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited diseases, 7th ed. McGraw-Hill, New York, pp 925–965

Chen Y-T, Coleman RA, Scheinman JI, Kolbeck PC, Sidbury JB (1988) Renal disease in type I glycogen storage disease. N Engl J Med 318:7–11

Cleveland DW, Yen TJ (1989) Multiple determinants of eukaryotic mRNA stability. N Biol 1:121–126

Cooper DW, Youssoufian H (1988) The CpG dinucleotide and human genetic disease. Hum Genet 78:151–155

DeMarchi JM, Richards CS, Fenwick RG, Pace R, Beaudet AL (1994) A robotics-assisted procedure for large scale cystic fibrosis mutation analysis. Hum Mutat 4:281–290

Holliday R, Grigg GW (1993) DNA methylation and mutation. Mutat Res 285:61–67

Lai E, Darnell JE Jr (1991) Transcriptional control in hepatocytes: a window on development. Trends Biosci 16:427–430

Lei K-J, Pan C-J, 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, Lin B, Sidbury JB, Chen Y-T, Nordlie RC, Chou JY (1995) Mutations in the glucose-6-phosphatase gene are associated with glycogen storage disease type 1a and 1aSP but not 1b and 1c. J Clin Invest 95:234–240

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 type 1a. Science 262:580–583

Magewu AN, Jones PA (1994) Ubiquitous and tenacious methylation of the CpG site in codon 248 of the p53 gene may explain its frequent appearance as mutational hotspot in human cancer. Mol Cell Biol 14:4225–4232

Michaud J, Brody LC, Steel G, Fontaine G, Martin LS, Valle D, Michell G (1992) Strand-separating conformational polymorphism analysis: efficacy of detection of point mutations in the human ornithine delta-aminotransferase gene. Genomics 13:389–394

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

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

Ott JA (1986) A short guide to linkage analysis. In: Davies KE (ed) Human genetic diseases: a practical approach. IRL Press, Oxford, pp 19–32

Peltz SW, Brewer G, Bernstein P, Hart PA, Ross J (1991) Regulation of mRNA turnover in eukaryotic cells. Crit Rev Eukaryot Gene Expr 1(2): 99–126

Roeder RG (1991) The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. Trends Biosci 16:402–408

Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM (1993) The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16:325–332

Tasheva ES, Roufa DJ (1993) Deoxycytidine methylation and the origin of spontaneous transition mutations in mammalian cells. Somat Cell Mol Genet 19:275–283