

Mutation Frequencies for Glycogen Storage Disease Ia in the Ashkenazi Jewish Population

Josef Ekstein,^{1*} Berish Y. Rubin,² Sylvia L. Anderson,² David A. Weinstein,³ Gideon Bach,⁴ Dvorah Abeliovich,⁴ Michael Webb,⁵ and Neil Risch^{6,7}

¹Dor Yeshorim, The Committee for the Prevention of Jewish Genetic Diseases, Brooklyn, New York

²Department of Biological Sciences, Fordham University, Bronx, New York

³Department of Medicine, Division of Endocrinology, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts

⁴Department of Human Genetics, Hadassah Hebrew University Hospital, Jerusalem, Israel

⁵Tepnel Diagnostics Ltd., Abingdon Oxfordshire, England

⁶Department of Genetics, Stanford University School of Medicine, Stanford, California

⁷Division of Research, Kaiser Permanente, Oakland, California

Glycogen storage disease type Ia (GSDIa) is a severe autosomal recessive disorder caused by deficiency of the enzyme D-glucose-6-phosphatase (G6Pase). While numerous mutations have been found in cosmopolitan European populations, Ashkenazi Jewish (AJ) patients appear to primarily carry the R83C mutation, but possibly also the Q347X mutation found generally in Caucasians. To determine the frequency for both these mutations in the AJ population, we tested 20,719 AJ subjects for the R83C mutation and 4,290 subjects for the Q347X mutation. We also evaluated the mutation status of 30 AJ GSDIa affected subjects. From the carrier screening, we found 290 subjects with R83C, for a carrier frequency for this mutation of 1.4%. This carrier frequency translates into a predicted disease prevalence of 1 in 20,000, five times higher than for the general Caucasian population, confirming a founder effect and elevated frequency of GSDIa in the AJ population. We observed no carriers of the Q347X mutation. Among the 30 GSDIa affected AJ subjects, all were homozygous for R83C. These results indicate that R83C is the only prevalent mutation for GSDIa in the Ashkenazi population. © 2004 Wiley-Liss, Inc.

KEY WORDS: glycogen storage disease type Ia; Ashkenazi Jews; glucose-6-phosphatase

INTRODUCTION

Glycogen storage disease type Ia (von Gierke disease or GSDIa), is a recessive disease caused by deficiency of the enzyme D-glucose-6-phosphatase (G6Pase). This key enzyme catalyzes the synthesis of glucose from glucose-6-phosphate, and lack of activity results in severe hypoglycemia since both glycogenesis and gluconeogenesis are impaired [Wolfsdorf and

Weinstein, 2003]. The disease is rare in the general population (1 in 100,000), but is apparently increased in frequency in the Ashkenazi Jewish (AJ) population. The diagnosis of GSDIa was formerly dependent upon demonstration of impaired enzyme activity in liver biopsy specimens. With the identification of the gene for G6Pase [Lei et al., 1993], mutation analysis has become the preferred method for diagnosis. In addition, mutation analysis allows for carrier detection and prenatal diagnosis. To date, over 56 different mutations in the *G6Pase* gene from 300 unrelated patients have been reported [Rake et al., 2000]. Numerous studies document allelic heterogeneity in cosmopolitan European populations, with a few mutations occurring at an elevated frequency, namely R83C, 158delC, Q347X, R170X, and deltaF327 [Rake et al., 2000]. Such heterogeneity and the relatively high frequency of novel and/or unique mutations create an impediment to general population screening.

On the other hand, population isolates often have a more limited range of mutations. Such is the case in the AJ population, which has been shown repeatedly to have increased mutational homogeneity underlying Mendelian syndromes, such as the lysosomal storage diseases, familial dysautonomia, torsion dystonia, and Fanconi anemia. Indeed, such homogeneity has also been demonstrated in the AJ population for GSDIa. In a study from Israel, all eight AJ GSDIa patients were homozygous for the R83C mutation [Parvari et al., 1997]. In a prior study in the United States, six of eight AJ patients were reported to be R83C homozygotes, with the remaining two reported to be compound heterozygotes for R83C and Q347X [Lei et al., 1995]. Thus, of a total of 32 GSDIa mutations in AJ patients, 30 (94%) were identified as R83C and 2 (6%) as Q347X.

In this report, we describe our results of screening a large AJ population (from Dor Yeshorim, the Committee for the Prevention of Jewish Genetic Diseases) for the two mutations R83C and Q347X. We also report the results of mutation testing on 30 GSDIa patients with two AJ parents.

MATERIALS AND METHODS

The healthy subjects in this study were derived from the Dor Yeshorim Screening Program focusing on the orthodox AJ population. This study population has been described previously [Broide et al., 1993; Abeliovich et al., 1996; Bach et al., 2001; Risch et al., 2003], and has been previously screened for a variety of recessive diseases prevalent in the AJ population. For analysis of the GSDIa R83C mutation, a total of 20,719 subjects were studied; for analysis of the Q347X mutation,

*Correspondence to: Josef Ekstein, Dor Yeshorim, 429 Wythe Ave., Brooklyn, NY 11211. E-mail: jekstein@dor-yeshorim.com.

Received 3 November 2003; Accepted 2 April 2004

DOI 10.1002/ajmg.a.30232

4,290 subjects were examined. The subjects included in this study were anonymous and the researchers had no knowledge of any relationship among them. All were unselected as far as family history for GSDIa is concerned, with the only requirement being AJ ancestry. Therefore, the sample is a random representation of the AJ population with respect to GSDIa, and the carrier frequencies we estimated are unbiased. It is possible that some subjects were related, for example, as sibs or cousins, but given the restricted age range we expect the number to be small. In any event, such relatedness would not affect the estimated allele frequencies but rather the standard errors for these frequencies [Risch et al., 2003].

Genotyping of mutations was performed by three laboratories, Orchid BioSciences, Abingdon, England, Fordham University, Bronx, NY, and Hadassah Hospital, Jerusalem, Israel. Orchid uses allele specific amplification technology as implemented in the Amplification Refractory Mutation System (ARMSTM) [Newton et al., 1989]; the laboratory at Fordham uses SSCP analysis of PCR products; and the laboratory at Hadassah uses restriction analysis following PCR.

To estimate carrier frequencies, we divided the number of detected carriers by the number of tested subjects. To obtain allele frequencies, we simply divided the carrier frequency by two (a close approximation when the carrier frequency is small).

Thirty subjects affected with GSDIa with two AJ parents were genotyped at Children's Hospital, Boston. The mutations for none of these subjects have been reported in the literature previously. Complete sequencing of all five exons of the glucose-6-phosphatase gene was performed using the protocol described by Lei et al. [1995]. Mutations were confirmed in duplicate in both the forward and reverse directions.

These studies were approved by institutional review boards at the respective institutions, and informed consent was obtained for all adult subjects and parental consent for any minors where applicable.

RESULTS

Results from mutation screening for the two mutations R83C and Q347X are given in Table I. We found 290 carriers of the R83C mutation out of 20,719 subjects tested, for a carrier rate of 0.014. We found no carriers of the Q347X mutation out of 4,290 subjects tested, indicating a carrier rate for this mutation of less than $1/4,290 = 0.00023$ in the Ashkenazi population. Of the 30 AJ affected subjects tested, all were homozygous for the R83C mutation.

DISCUSSION

Prior studies of AJ subjects affected with GSDIa have shown that the R83C allele is the primary mutation in this population. However, one study found two of eight AJ subjects that were compound heterozygotes for R83C and Q347X, the latter being the most common mutation in Caucasians of Western European heritage. A primary concern in this study was to determine the frequency of the Q347X mutation along with the R83C mutation in the AJ population. The fact that we did not detect a single carrier of the Q347X mutation out of over 4,000 subjects tested suggests that the R83C mutation is the

only GSDIa mutation prevalent in this group. This conclusion is bolstered by the observation that the R83C mutation was the only one found among 30 affected AJ subjects.

When two prior studies are combined [Lei et al., 1995; Parvari et al., 1997], out of a total of 32 mutations observed in affected AJ subjects, 30 were found to be R83C and 2 were found to be Q347X, giving a frequency ratio of 15:1. In our study of over 20,000 AJ subjects, we observed a carrier frequency of the R83C mutation of 1.4%. On this basis, assuming a frequency ratio of 15:1, we would then predict a carrier frequency of 0.09% for the Q347X mutation in the AJ population. With this carrier frequency, out of 4,290 subjects screened for this mutation, we would have expected 4 carriers of the Q347X mutation, whereas we observed none. Assuming a Poisson distribution for the number of carriers, the probability of observing none out of 4,290 when four are expected is 0.018. Thus, it appears that the frequency of the Q347X allele in the AJ population is probably lower than what might have been predicted based on the data from the previously reported patients [Lei et al., 1995]. Supporting this conclusion is the fact that among 30 AJ patients screened in this study, all were homozygous for the R83C mutation, i.e., 60 out of 60 AJ alleles were R83C. One possible explanation for the discrepancy with a previous study [Lei et al., 1995] is that the two compound heterozygote patients observed in that study were of mixed ancestry (i.e., less than 100% AJ), allowing the introduction of another allele common in Caucasians into those individuals. In any event, our data provide a compelling argument that R83C is the only prevalent GSDIa mutation in the AJ population.

Also of interest is our estimated frequency for the R83C mutation in the AJ population, namely 0.7%. This frequency translates into an estimated disease prevalence of 1 in 20,000, which is 5 times higher than the rate suggested for the general Caucasian population. Thus, our data also support the presence of a founder effect for the R83C mutation that has given rise to an elevated frequency of GSDIa in the AJ population. We note that this mutation frequency is comparable or greater than those for a number of diseases currently undergoing population screening in the AJ population, including Niemann–Pick disease (0.5%) and Fanconi anemia (0.6%) [Risch et al., 2003].

Genetic prenatal screening has classically focused on early degenerative or fatal diseases. While treatments for GSDIa are improving and many patients are now surviving into adulthood, there is still significant morbidity associated with the disease. Severe hypoglycemia can occur within minutes, and constant care, particularly of infants and young children, is required. Frequent exposure to hypoglycemia can lead to severe mental retardation (IQ less than 65) in 3% of patients or borderline mental development (IQ between 65 and 85) in 18% of patients [Rake et al., 2002]. Seizures, hepatic adenomas, hepatocellular carcinoma, and renal failure are all common complications in young adults [Rake et al., 2002; Weinstein and Wolfsdorf, 2002]. Thus, despite clinical advances, screening for GSDIa in the AJ population, which is facilitated by homogeneity for the R83C mutation, may still be warranted and at least requires further discussion.

REFERENCES

- Abeliovich D, Quint A, Weinberg N, Verchezon G, Lerer I, Ekstein J, Rubinstein E. 1996. Cystic fibrosis heterozygote screening in the orthodox community of Ashkenazi Jews: The Dor Yeshorim approach and heterozygote frequency. *Eur J Hum Genet* 4:338–341.
- Bach G, Tomczak J, Risch N, Ekstein J. 2001. Tay-Sachs screening in the Jewish Ashkenazi population: DNA testing is the preferred procedure. *Am J Med Genet* 99:70–75.
- Broide E, Zeigler M, Eckstein J, Bach G. 1993. Screening for carriers of Tay-Sachs disease in the ultraorthodox Ashkenazi Jewish community in Israel. *Am J Med Genet* 47:213–215.

TABLE I. Results of Mutation Testing for Glycogen Storage Disease Type Ia (GSDIa) in the Ashkenazi Population

Mutation	Number tested	Number (%) carriers
R83C	20,719	290 (0.014)
Q347X	4,290	0 (0.000)

- 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 Ia. *Science* 262:580–583.
- Lei K-J, Chen Y-T, Chen H, Wong L-JC, Liu J-L, McConkle-Rosell A, Van Hover JLK, Ou HC-Y, Yeh NJ, Pan C-J, Chou JY. 1995. Genetic basis of glycogen storage disease type Ia: Prevalent mutations at the glucose-6-phosphatase locus. *Am J Hum Genet* 57:766–771.
- Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, Smith JC, Markham AF. 1989. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 17:2503–2516.
- Parvari R, Lei K-J, Bashan N, Hershkovitz E, Korman SH, Barash V, Lerman-Sagi T, Mandel H, Chou JY, Moses SW. 1997. Glycogen storage disease type Ia in Israel: Biochemical, clinical, and mutational studies. *Am J Med Genet* 72:286–290.
- Rake JP, ten Berge AM, Visser G, Verlind E, Niezen-Koning KE, Buys CH, Smit GP, Scheffer H. 2000. Glycogen storage disease type Ia: Recent experience with mutation analysis, a summary of mutations reported in the literature and a newly developed diagnostic flow chart. *Eur J Pediatr* 159:322–330.
- Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GPA. 2002. Glycogen storage disease type I: Diagnosis, management, clinical course, and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr* 161: S20–S34.
- Risch N, Tang H, Katzenstein H, Ekstein J. 2003. Geographic distribution of disease mutations in the Ashkenazi Jewish population supports genetic drift over selection. *Am J Hum Genet* 72:812–822.
- Weinstein DA, Wolfsdorf JI. 2002. Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type Ia glycogen storage disease. *Eur J Pediatr* 161:S35–S39.
- Wolfsdorf JI, Weinstein DA. 2003. Glycogen storage diseases. *Rev Endocr Metab Disord* 4:95–102.