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Glycogen Storage Disease Type 1a in Israel: Biochemical, Clinical, and Mutational Studies

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Glycogen storage disease type 1a (von Gierke disease, GSD 1a) is caused by the deficiency of microsomal glucose-6-phosphatase (G6Pase) activity which catalyzes the final common step of glycogenolysis and gluconeogenesis. The recent cloning of the G6Pase cDNA and characterization of the human G6Pase gene enabled the characterization of the mutations causing GSD 1a. This, in turn, allows the introduction of a noninvasive DNA-based diagnosis that provides reliable carrier testing and prenatal diagnosis. In this study, we report the biochemical and clinical characteristics as well as mutational analyses of 12 Israeli GSD 1a patients of different families, who represent most GSD 1a patients in Israel. The mutations, G6Pase activity, and glycogen content of 7 of these patients were reported previously. The biochemical data and clinical findings of all patients were similar and compatible with those described in other reports. All 9 Jewish patients, as well as one Muslim Arab patient, presented the R83C mutation. Two Muslim Arab patients had the V166G mutation which was not found in other patients' populations. The V166G mutation, which was introduced into the G6Pase cDNA by site-directed mutagenesis following transient expression in COS-1 cells, was shown to cause complete inactivation of the G6Pase.

The characterization of all GSD 1a mutations in the Israeli population lends itself to carrier testing in these families as well as to prenatal diagnosis, which was carried out in 2 families. Since all Ashkenazi Jewish patients harbor the same mutation, our study suggests that DNA-based diagnosis may be used as an initial diagnostic step in Ashkenazi Jews suspected of having GSD 1a, thereby avoiding liver biopsy. Am. J. Med. Genet. 72:286–290, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: **glycogen storage disease type 1a; Israeli mutations; Ashkenazi Jews; Muslim Arabs**

INTRODUCTION

Glycogen storage disease type 1a (von Gierke disease, GSD 1a), one of the most common types of glycogen storage diseases, is caused by the deficiency of microsomal glucose-6-phosphate (G6Pase) activity which catalyzes the final step of glycogenolysis. The enzyme is normally expressed in liver, kidney, and intestinal mucosa and its absence is associated with excessive accumulation of glycogen in these organs. GSD 1a is an autosomal recessive trait with an estimated overall frequency of 1:100,000. The disease manifests itself with severe hypoglycemia, hepatomegaly, growth retardation, and a bleeding diathesis. Biochemically, in addition to rapidly decreasing fasting blood glucose values, patients have lactic acidemia, hyperlipidemia, and hyperuricemia. Long-term complications include gout,

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hepatic adenomas, osteoporosis, nephrolithiasis, and progressive renal disease [Chen et al., Beaudet, 1991; Chen and Burchell, 1995]. Currently, the diagnosis of GSD 1a is established by demonstrating the lack of G6Pase activity on a liver biopsy specimen. Carrier identification and prenatal diagnosis by enzymatic activity are not feasible. The recent cloning of the G6Pase cDNA and characterization of the human G6Pase gene by Lei et al., [1993] enabled the identification of the mutations causing GSD 1a [Lei et al., 1993, 1994, 1995a,b; Parvari et al., 1995]. This, in turn, permits performance of DNA-based diagnosis for carrier testing and prenatal diagnosis. The present study provides biochemical, clinical, and mutational analyses on 12 Israeli GSD 1a patients from different families, including 7 patients who were analyzed for mutations and reported previously [Parvari et al., 1995], but their clinical and biochemical data were not yet presented.

METHODS

In all 12 patients, the biochemical diagnosis of GSD 1a was based on liver glycogen concentration which was determined as described by Johnson et al. [1993]. G6Pase activity was assayed on fresh liver homogenate before and after disruption of microsomes as described previously [Narisawa et al., 1983].

Extraction of DNA, polymerase chain reaction, (PCR) amplification, single-stranded conformational polymorphism (SSCP), and sequence analysis were performed as described elsewhere [Parvari et al., 1995]. The V166G mutation was introduced into the G6Pase cDNA by site-directed mutagenesis and both the mutated and the wild-type constructs were tested for enzymatic activity after transient expression in COS-1 cells as described by Lei et al. [1995b].

RESULTS

Clinical and Biochemical Aspects

The biochemical parameters of the Israeli cases are presented in Table I next to their corresponding mutations. The mutations and G6Pase enzymatic activities, but not the biochemical data, of the patients marked with a dagger appeared in our previous communication [Parvari et al., 1995]. G6Pase activity was low in intact and in disrupted liver membranes in all patients. This procedure differentiates between GSD 1a and 1b patients: GSD 1a patients show no activity in intact as well as in disrupted microsomes while GSD 1b patients show a lack of activity in intact membrane preparations only. Glycogen concentrations were elevated in most cases. Elevated blood lactic acid levels were, as expected, found in all cases. Lactic acid levels, which usually reflect metabolic control, were moderately elevated in all cases. The exception was patient F.O. who presented with evidence of severe metabolic derangement, as indicated by marked elevation of lactic and uric acid levels. Uric acid levels were abnormal in 3 more patients, one of whom (F.A.) had clinical manifestations and imaging evidence of nephrolithiasis. In addition, he had computerized tomographic evidence of hepatic adenomata which did not change markedly over the last 7 years. Hyperlipidemia, another hallmark of this disease, is evident by the strikingly elevated serum triglyceride levels in all patients. Hypercholesterolemia was present in most cases, though the elevation of cholesterol levels was not as excessive as the triglyceride levels. The 2 types of mutations do not appear to differ in their effect on biochemical parameters or in their clinical presentation.

Mutation Analyses

Mutations were identified in each patient (the right column of Table I). R83C was the only mutation detected in all 9 unrelated Jewish patients studied, 8 of whom were of Ashkenazi origin and one of whom was of mixed Egyptian Greek and Syrian origin. Figure 1 presents a characteristic SSCP analysis of exon 2 from an affected Ashkenazi family. The migration pattern of the patient's DNA and the heterozygous parents and sister is distinct from that of the normal control. All other exons did not show any different migration pat-

TABLE I. Biochemical, Enzymatic, and Molecular Genetic Studies in 12 Israeli GSD 1a Patients*

			Liver			Serum				
				G6Pase activity (nmol/h/mg protein)						
Initials	Sex	Ethnic origin	Glycogen (g/100 g liver)	Int	Disrupt	Lactate (mmol)	Uric acid (mg%)	Triglycerides (mg%)	Cholesterol (mg%)	Molecular genetic diagnosis
T.Y.†	M	Ashk	7.1	0.10	0.10	2.5	6.7	567	253	R83C
L.G.	M	Ashk	2.1	0.00	0.10	2.4	6.0	166	142	R83C
U.S.†	M	Ashk	7.4	0.20	0.30	4.6	6.4	898	269	R83C
G.Y.†	M	Ashk	3.8	0.00	0.00	3.3	9.0	988	287	R83C
K.O.†	F	Ashk	5.9	0.42	0.39	3.9	5.8	1133	212	R83C
V.A.†	M	Ashk	10.0	0.41	0.45	2.0	4.6	415	179	R83C
F.O.	F	Ashk	4.2	0.0	0.12	10.6	15.9	—	223	R83C
B.M.	F	Ashk	5.5	0.00	0.00	7.0	7.5	941	224	R83C
F.A.†	M	Seph	4.3	0.30	0.20	5.0	9.1	1820	334	R83C
A.A.	M	Musl	x[a]	x[a]	x[a]	6.76	3.0	440	152.6	R83C
G.H.†	M	Musl	2.0	0.10	0.05	3.1	7.7	1119	335	V166G
D.S.	M	Musl	11.7	0.10	0.16	4.1	10.2	853	193	V166G
Controls			<4.0	3.4 ± 0.5	3.8 ± 0.6	0.8–1.5	2.0–7.0	35–138	124–200	

*Ashk, Ashkenazi Jew; Seph, Sephardic Jew; Musl, Muslim Arab; Int, intact liver tissue; Disrupt, disrupted liver membranes.
†Liver glycogen levels, G6Pase activity, and mutation previously reported.
[a]Data were abnormal but could not be traced.

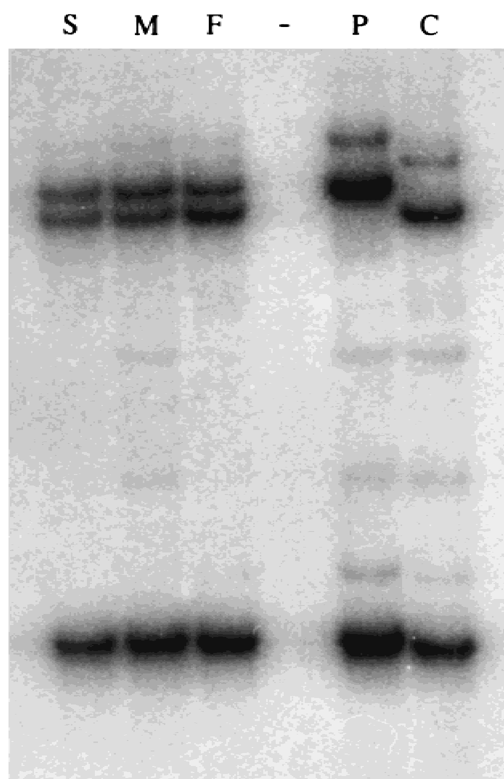

Fig. 1. SSCP analysis of exon 2 in a Jewish GSD 1a patient (P), homozygote for the R83C mutation, as compared to control (C), his parents (M,F), and sister (S).

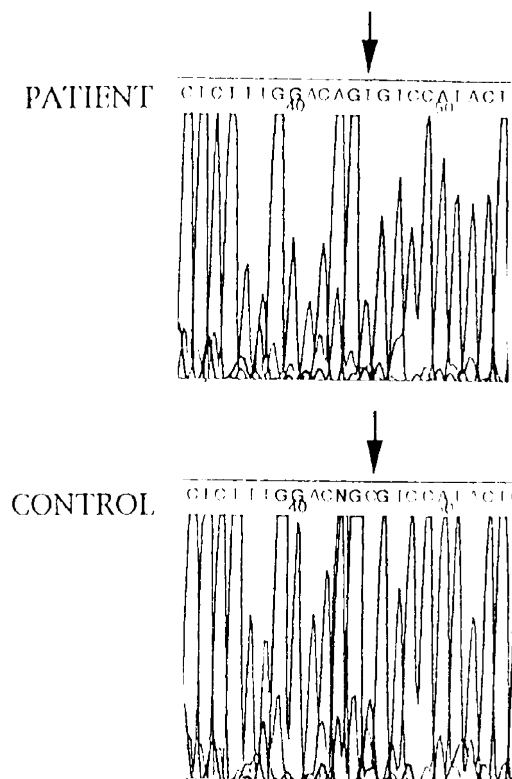

Fig. 2. Sequence analysis of exon 2 of a Jewish GSD 1a patient compared to control. The arrows point to the change of the C $\rightarrow$ T at nucleotide 326. The sequence analysis presented was performed with the forward primer.

tern. Sequencing, with both forward and reverse primers of exon 2 of the G6Pase gene from the Jewish patient as compared to control, demonstrated a change from C to T at nucleotide 326 (Fig. 2), causing the above-mentioned mutation of R83C.

Of the 3 Muslim Arab unrelated patients studied, one had the R83C mutation while the other 2 showed a different SSCP migration in exon 4, distinct both in the homozygote patient and in the heterozygote mother (Fig. 3). All their other exons presented the normal migration pattern. Sequencing of exon 4 showed a T to G mutation at nucleotide 576 (Fig. 4), resulting in the change of valine to glycine at position 166. This mutation had not yet been detected in any other population. In order to prove that the V166G is a missense mutation, this mutation was introduced into a G6Pase construct by site-directed mutagenesis and the phosphohydrolase activity after transient expression in COS-1 examined in comparison to the phosphohydrolase activity of the wild-type construct. The replacement of valine by glycine rendered the enzyme completely inactive (Table II), thus proving that this is the mutation causing the disease in the GSD 1a Arab patients.

DISCUSSION

Glycogen storage disease type 1 is a severe metabolic disease involving carbohydrate, purine, and lipid metabolism and manifesting hepatomegaly (which is partially dependent on metabolic control and can be especially marked in the young, as a rule showing a relative decrease with age) and fasting hypoglycemia. At a young age, the postprandial capacity to maintain normal blood glucose levels is often very short and improves with age. The characteristic "doll face" found in most patients represents an abnormal distribution of fat tissue which is frequently also manifested in the form of a "stubby" body build. Growth retardation is commonly present, becomes more prominent during school age, leading to markedly short stature. Delayed onset of puberty is found in most cases. Long-term complications, which occur after the first or second decade of life and are apparently partly related to poor metabolic control, include renal complications in the form of hypertension, glomerular hyperfiltration, and microalbuminuria, leading to overt proteinuria and progressive renal failure. Histologically, focal segmental sclerosis and interstitial fibrosis can be found. None of our patients, most of whom are still young, had evidence of advanced kidney disease. Another late complication of GSD 1a is the appearance of hepatic adenomata as demonstrated in our 20-year-old patient F.A. These adenomata have a low-grade change of malignant transformation.

In terms of phenotype-genotype correlation, patients with R83C and with V166G mutations show clinical and biochemical manifestations that are similar to the known pattern of the disease. This is expected in view of the finding that R83C and V166G mutations cause

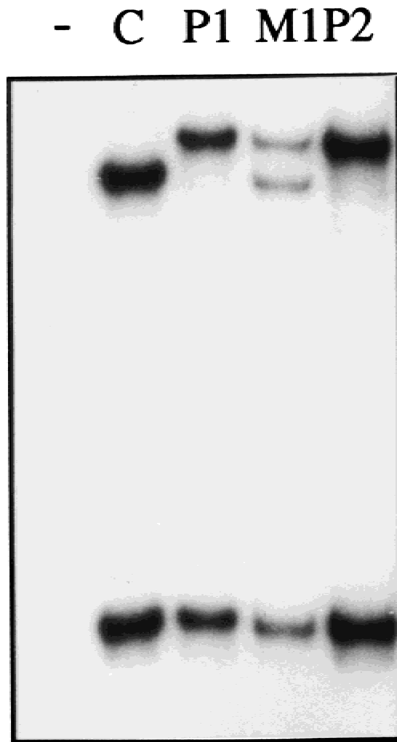

Fig. 3. SSCP analysis of exon 4 from 2 Muslim Arab GSD 1a patients (P1, P2), homozygous for the V166G mutation, and the mother of patient 1 (M1) as compared to control (C).

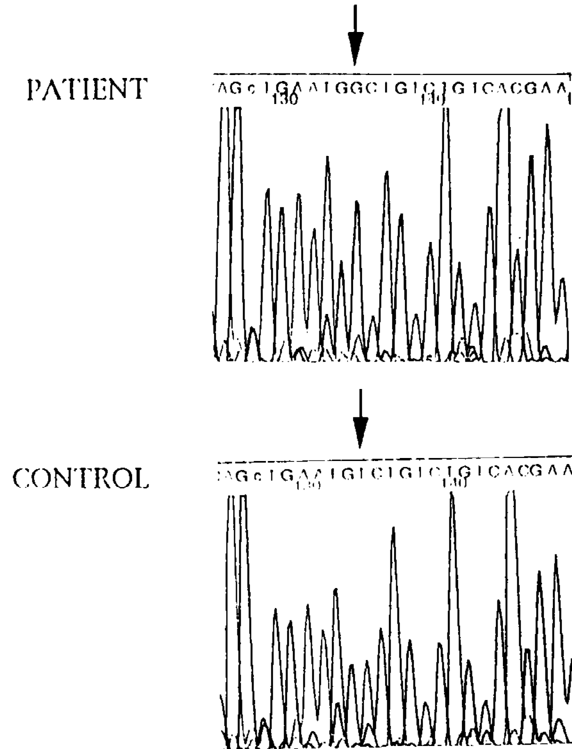

Fig. 4. Sequence analysis of exon 4 of a Muslim Arab GSD 1a patient compared to control. The arrows point to the change of T → G at nucleotide 576. The sequence analysis presented was performed with the forward primer.

complete inactivation of G6Pase enzymatic activity [Lei et al., 1993; this study].

The complete inactivation of G6Pase by replacement of the nonpolar valine with the uncharged polar glycine at position 166 in the third putative transmembrane domain [Lei et al., 1995a] demonstrates the hydrophobic requirement at this position of this membrane-spanning segment. Two other mutations in transmembrane domains 4 and 6, G222R and ΔF327, were shown to cause inactivation of the G6Pase [Lei et al., 1993, 1994, respectively]. This demonstrates the importance of the structure of the transmembrane domains to the enzymatic activity of the hydrophobic G6Pase.

Our findings of R83C as the only mutation in all 8 unrelated Ashkenazi patients is consistent with the findings of Lei et al. [1995a]. They reported that 6 of 8 Eastern European Jewish patients examined were homozygous for R83C and 2 were compound heterozygotes for the R83C and Q347X mutations. This phenomenon of a predominant mutation causing GSD 1a in the Ashkenazim has been amply demonstrated in other frequent inborn error of metabolism in the Jewish Ashkenazi population such as Tay Sachs [Paw et al., 1990], Gaucher disease [Beutler et al., 1991], and Canavan disease [Kaul et al., 1994]. The appearance of the R83C in the Sephardic Jew and in one Muslim Arab patient is in accordance with the finding of Lei et al. [1995a,b], who showed that this mutation was the most common one in the Caucasian patient population, with a prevalence of 41% (46/112 alleles examined). The V166G mutation appears to be unique to Muslim Arabs in Israel, as it has not yet been reported elsewhere, including the United States [Lei et al., 1995a]. In their study, among 70 GSD 1a patients examined, 16 new mutations of the G6Pase gene were found.

The identification of the mutations causing GSD 1a enables the families to benefit from DNA-based analysis for the detection of heterozygotes and for prenatal diagnosis. The distinct SSCP patterns of both R83C and V166G mutations enable easy detection of affected heterozygotes. Indeed, prenatal diagnosis was successfully employed in 2 of the GSD 1a families presented here [T.J. and G.H.; Parvari et al., 1996]. The identification of 2 mutations causing GSD 1a in all Ashkenazi Jewish patients, together with the high prevalence of the R83C mutation found in this population (100% in the 9 patients analyzed in Israel and 87.5% in the 8 Jewish patients in the United States), suggests the use

Table II. Analysis of Phosphohydrolase Activity of Wild Type and V166G Mutant Constructs in COS 1 Cells*

Constructs	Phosphohydrolase activity
Mock	8.6 ± 0.5
G-6-Pase WT	117.4 ± 9
V166G	8.5 ± 1.2

*Phosphohydrolase activity in whole homogenate was assayed in reactions containing 10 mM Glucose-6-P, using two different isolates of each construct in two separate transfections. The activity is expressed as nmol/min/mg prot. and data are expressed as mean ± DS. The Mock constructs represent cells transfected with the expression vector. G-6-Pase WT are the wild type cDNA sequence and V166G represents the wild type construct into which the T → G at nucleotide 576 was introduced.

of DNA-based diagnosis as an initial diagnostic test of Ashkenazi Jewish patients clinically suspected of suffering from GSD 1a, thereby avoiding liver biopsy.

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