Am. J. Hum. Genet. 55:305–313, 1994

Common Mutations in the Phosphofructokinase-M Gene in Ashkenazi Jewish Patients with Glycogenesis VII—and Their Population Frequency

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Summary

Phosphofructokinase (PFK) catalyzes the rate-limiting step of glycolysis. Deficiency of the muscle enzyme is manifested by exercise intolerance and a compensated hemolytic anemia. Case reports of this autosomal recessive disease suggest a predominance in Ashkenazi Jews in the United States. We have explored the genetic basis for this illness in nine affected families and surveyed the normal Ashkenazi population for the mutations we have found. Genomic DNA was amplified using PCR, and denaturing gradient-gel electrophoresis was used to localize exons with possible mutations. The polymorphic exons were sequenced or digested with restriction enzymes. A previously described splicing mutation, Δ5, accounted for 11 (61%) of 18 abnormal alleles in the nine families. A single base deletion leading to a frameshift mutation in exon 22 (ΔC-22) was found in six of seven alleles. A third mutation, resulting in a nonconservative amino acid substitution in exon 4, accounted for the remaining allele. Thus, three mutations could account for all illness in this group, and two mutations could account for 17 of 18 alleles. In screening 250 normal Ashkenazi individuals for all three mutations, we found only one Δ5 allele. Clinical data revealed no correlation between the particular mutations and symptoms, but male patients were more symptomatic than females, and only males had frank hemolysis and hyperuricemia. Because PFK deficiency in Ashkenazi Jews is caused by a limited number of mutations, screening genomic DNA from peripheral blood for the described mutations in this population should enable rapid diagnosis without muscle biopsy.

Received January 21, 1994; accepted for publication April 5, 1994.

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0002-9297/94/5502-0011$02.00

Introduction

Absence of activity of the enzyme phosphofructokinase (PFK; E.C.2.7.1.11), which converts fructose-6-phosphate to fructose-1,6-diphosphate, was recognized by Tarui in 1965 (Tarui et al. 1965; Layzer et al. 1967), as responsible for an exercise-related myopathy, glycogenosis type VII. The inheritance of the disease is autosomal recessive. Clinically, patients have an exertional myopathy, compensated hemolysis, and, often, hyperuricemia. Definitive diagnosis requires muscle biopsy.

PFK is a tetrameric allosteric enzyme of molecular weight of ~340 kD. There are three mammalian PFK isozymes, designated muscle (PFK-M), liver (PFK-L), and platelet (PFK-P), which are encoded by different genes (Vora et al. 1982, 1983*b*; Vora 1983; Van Keuren et al. 1986) and display differential tissue-specific regulation of glycolysis (Nakajima et al. 1990*a*). PFK-M is the predominant isozyme in skeletal muscle, heart, and brain. In these tissues, the active enzyme is a tetramer of four identical PFK-M subunits. The sequence of genomic DNA (Sharma et al. 1989; Yamasaki et al. 1991) located on chromosome 1 and of the full-length cDNA of PFK-M, which includes 22 coding exons (3–24, based on the terminology of Yamasaki [Yamasaki et al. 1991]), have been reported. Two noncoding exons in the 5′ UTR are alternatively spliced to generate three types of PFK-M mRNA (type A, type B, and type C) (Valdez et al. 1989; Nakajima et al. 1990*a*, 1990*c*). Type B mRNA, the dominant form in muscle, contains exon 2 and intron 2, which is spliced out in type A. Types A and B share a common promoter, located upstream from exon 2. Type C mRNA, which contains exon 1, is found in many tissues and is regulated by a different promoter. In normal tissue, including muscle, a splice variant with coding exon 9 deleted is found (Sharma et al. 1990).

While muscle is solely dependent on the PFK-M to utilize glucose as an energy source, in red blood cells the PFK-M and PFK-L subunits are both used. Defects in the structure of the PFK-M gene affect muscle and red-blood-cell metabolism, and the clinical symptoms reflect both deficits. From early childhood, myalgias and weakness occur after exercise and may lead to myoglobinuria and, in severe

cases, rhabdomyolysis. Red-cell survival is shortened, leading to reticulocytosis and macrocytosis, without anemia. In some patients, however, episodes of hemolysis accompanied by hyperbilirubinemia or even jaundice may occur.

A review of the literature shows a predominance of cases among Jews of eastern European descent (Ashkenazi) and Japanese (Rowland et al. 1986). Nakajima and his colleagues identified a g-to-t point mutation at the 5′-splice donor site of intron 15 for the muscle subunit (PFK-M) in a Japanese family (Nakajima et al. 1990*b*). This mutation results in recognition of a cryptic splice site within exon 15 and an in-frame 75-bp deletion in the mRNA. We have recently identified a second splicing defect in the PFK-M gene of two Ashkenazi sisters in whom a g-to-a substitution at the splice donor site of intron 5 leads to a precise deletion of exon 5 (Δ5) in the PFK-M mRNA (Raben et al. 1993).

To determine whether clinical heterogeneity reflects an underlying molecular heterogeneity and to develop a simple screening test based on the analysis of genomic DNA from circulating blood cells, we analyzed the molecular defect in eight additional Ashkenazi families. We have uncovered two previously unrecognized mutations in patients with this illness, and we have sought all three mutations in 250 people of Ashkenazi ancestry.

Subjects and Methods

Subjects

Eleven patients with PFK deficiency were identified with the help of physicians from the United States and Israel. The six male and five female patients came from nine unrelated Ashkenazi families (the family in whom the Δ5 was found [Raben et al. 1993] and eight additional families). Clinical data from eight of the nine families were collected using physician and patient questionnaires. The patients ranged from 22 to 71 years of age. In eight patients, clinical history and blood tests suggested the diagnosis, which was confirmed by muscle biopsy with measurements of PFK enzyme activity. One mildly symptomatic patient had his diagnosis confirmed by red-blood-cell PFK isoenzyme analysis (Vora et al. 1983*a*). The two remaining patients were siblings of documented cases and had clinical histories identical to their siblings, but no diagnostic evaluation had been performed. Three of the patients had been studied metabolically by Haller and his colleagues (Haller and Lewis 1991; Lewis et al. 1991).

The 11 patients and 4 first-degree relatives of patient 4 provided informed consent for genetic evaluation. This included survey questionnaires addressing the clinical, laboratory, and genetic history, completed by both subjects and physicians; collection of peripheral blood; and, in one case, a muscle biopsy.

Analysis of Genomic DNA

Genomic DNA samples for PCR were prepared from whole blood by using a rapid procedure (Higuchi 1989) and were amplified with 2.5 U of *Taq* DNA polymerase (Perkin Elmer Cetus) in a 100-μl PCR mixture containing 100 pmol each of the specific oligonucleotide primers located in the introns and flanking each of the 22 coding exons of the PFK-M gene (exons 3–24) (Yamasaki et al. 1991). Each of the upstream primers included a 40-bp GC-rich sequence (a GC-clamp) at the 5′ end, in addition to the specific sequence. After 5 min denaturation at 95°C, the reaction mixtures were subjected to 35 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 60 s. Amplification of exons 20–22 and 24 required increased concentration of $MgCl_2$, ranging from 2 to 4 mM. PCR products were analyzed using denaturing gradient-gel electrophoresis (DGGE), sequencing of the polymorphic exons, or digesting with appropriate restriction enzymes.

Denaturing gradient gels were prepared as described elsewhere (Myers et al. 1987). The gels were 6.5% acrylamide (acrylamide:bis-acrylamide = 37.5:1) in a TAE buffer (40 mM TRIS, 20 mM sodium acetate, and 1 mM EDTA; pH 7.4) with 25%–75% or 40%–70% linear gradient of denaturant (100% denaturant = 7 M urea and 40% formamide) parallel to the direction of electrophoresis. Aliquots of PCR products (200 ng of DNA) were electrophoresed at 60–80 V for 16–18 h in a Hoefer SE 600 vertical apparatus with the temperature maintained at 60°C. The gels were stained with ethidium bromide to visualize the bands.

Sequence Analysis and Restriction-Enzyme Digestion

Single-stranded DNA for direct sequencing was generated with nested primers in a two-stage PCR protocol as described elsewhere (Raben et al. 1991). It proved difficult to sequence exon 4 directly, so the PCR product was subcloned into the TA cloning vector PCR®-1000 (Invitrogen). DNA sequencing reactions were done with Sequenase 2.0, according to the manufacturer's directions (US Biochemical). Nucleotide sequences were confirmed by two independent PCR reactions. Eight clones containing exon 4 were sequenced. PCR products were purified with Millipore filters and digested with *Rma*I or *Ban*II at 37°C to screen for the mutations in exon 4 and exon 22, respectively. To screen for the Δ5 mutation, we designed an antisense primer with a mismatch located three nucleotides downstream from the mutation site (5′-GAGCAAATATGGGACAGGGAGTTCTCTGGAAGATA). This primer created an *Eco*RV restriction site in the PCR fragment from the mutated sequence but not in that from the normal sequence. After digestion, purified PCR products were size-fractionated on 1.5% agarose gels and stained with ethidium bromide.

Northern Analysis

Total RNA for northern analysis was extracted from frozen muscle-biopsy specimens by using the RNAzol method (Chomczynski and Sacchi 1987), and northern

blotting was performed as described elsewhere (Sambrook et al. 1989). The membranes were hybridized with ^{32}P-labeled control (a 1.1-kb cDNA from glyceraldehyde 3-phosphate dehydrogenase; Clontech) and PFK-specific (a 715-bp fragment [bp 848-1562] from PFK-M cDNA) probes and were washed for 20 min at 50°C in 0.1 × SSC with 0.1% SDS.

Population Study

To determine the frequency of Δ5, ΔC-22, and the base change in exon 4, DNA samples—provided under code, from 250 unrelated Ashkenazi individuals—were analyzed for these alleles by using PCR and DGGE as described above.

Results

We began our analysis by seeking the genetic defect that we had previously identified in two Ashkenazi sisters with PFK deficiency: a g-to-a substitution at the 5′ splice donor site of intron 5 (Δ5). We screened eight additional Ashkenazi patients with PFK deficiency by using a combination of PCR and DGGE. This technique had allowed us to detect heterozygous and homozygous individuals in the original family (Raben et al. 1993) (fig. 1*A*, *left*). The DGGE migration patterns (fig. 1*A*) suggest that six of the eight patients carry the Δ5 mutation. Three (1–3) are homozygous, three others (4, 5, and 8) are heterozygous, and two (6 and 7) have normal DGGE patterns.

To confirm the identity of the mutation, we used direct sequence analysis of the PCR products in two patients and restriction digestion in the remaining six. We sequenced the region of interest from patient 4, whose DGGE pattern suggested heterozygosity, and from patient 6, who was negative for the mutation by the DGGE analysis. As expected, both a g and an a are observed at the first nucleotide of intron 5 in patient 4; the exon 5/intron 5 junctional sequence is normal in patient 6 (fig. 1*B*). In both a normal subject and patient 7 (fig. 1*C*), *Eco*RV digestion (see Subjects and Methods) of the 173-bp PCR product resulted in a nondigested fragment. The 173-bp band was replaced by two digested products, of 148 bp and 25 bp (the smaller band is not visible), in homozygous patients 2 and 3 (patient 1 not shown). Both undigested and digested fragments were observed in heterozygous patients 5 and 8. Thus, the Δ5 mutation was found in 9 of 16 alleles, but it could account for PFK deficiency only in those patients (1–3) who are homozygous, because inheritance of the disease is autosomal recessive (Vora 1983). The remaining patients (three heterozygous for Δ5 and two with normal sequences) were expected to carry novel mutations.

Novel Mutations

We sought to detect other PFK mutations by amplifying each of the 22 translated exons (exons 3–24) from genomic DNA and by testing for heteroduplexes by DGGE. We

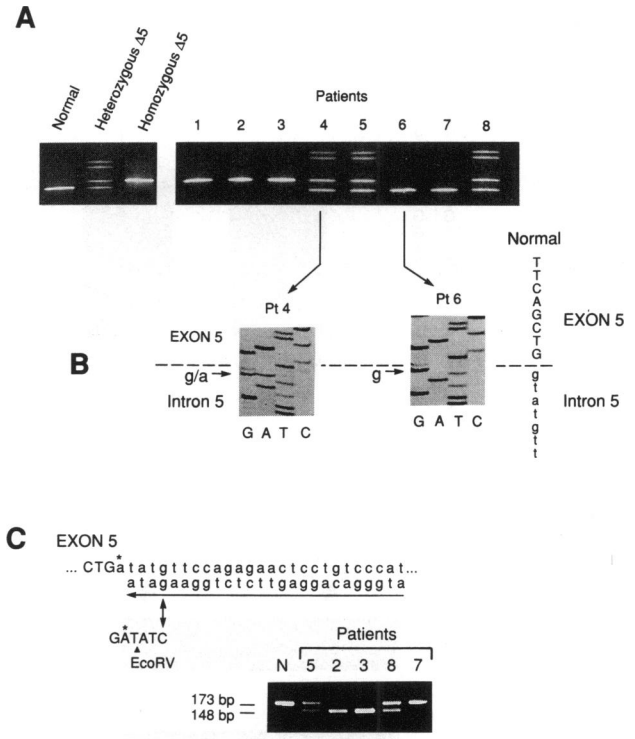

Figure 1 Screening for the Δ5 mutation of the PFK-M gene in eight Ashkenazi patients with PFK deficiency. *A*, DGGE patterns of the 173-bp PCR products spanning the 5′ splice site of intron 5. DGGE was carried out on a gel with a denaturant gradient of 25%–75%. Control DNA samples (*left*) are from a normal subject, a subject heterozygous for a g-to-a substitution at the first nucleotide of intron 5, and a patient with PFK deficiency homozygous for the mutation. Lanes 1–8 show DGGE patterns of DNA fragments from eight patients examined in this study. *B*, Direct sequencing of the exon 5/intron 5 region in patients 4 and 6. A g-to-a point mutation was found in one allele of patient 4. The sequence at the splice site was normal in patient 6. The position of the exon/intron boundary is indicated by a dotted line. *C*, *Eco*RV detection of the Δ5 mutation. Amplification was with primer 1, which has a single base mismatch to the native PFK-M gene sequence and creates an *Eco*RV restriction site in the product generated from a mutant template. The mismatched nucleotide is indicated by the arrow. The 173-bp PCR products were restricted with *Eco*RV, resulting in a nondigested fragment in a normal subject (N); only digested products of 148 bp and 25 bp (not visible, because of the small size) were observed in patients 2 and 3, indicating that the mutation is present in homozygous form; both undigested and digested fragments are seen in heterozygous patients 5 and 8.

initially studied patient 4 because the family was available for genetic analysis. A polymorphic exon 22 was identified (not shown), and direct sequencing revealed deletion of a C nucleotide at position 2079 in one allele of the gene (fig. 2*A*). This deletion introduces a frameshift and a premature stop codon. Thus, patient 4 harbors two different mutations: a junctional mutation in intron 5 (Δ5) and a frameshift mutation in exon 22 (ΔC-22). Each of the mutations would be predicted to generate a product with major structural abnormalities. To rule out the possibility that both mutations resided on the same allele and that another mutation on the second allele had been missed

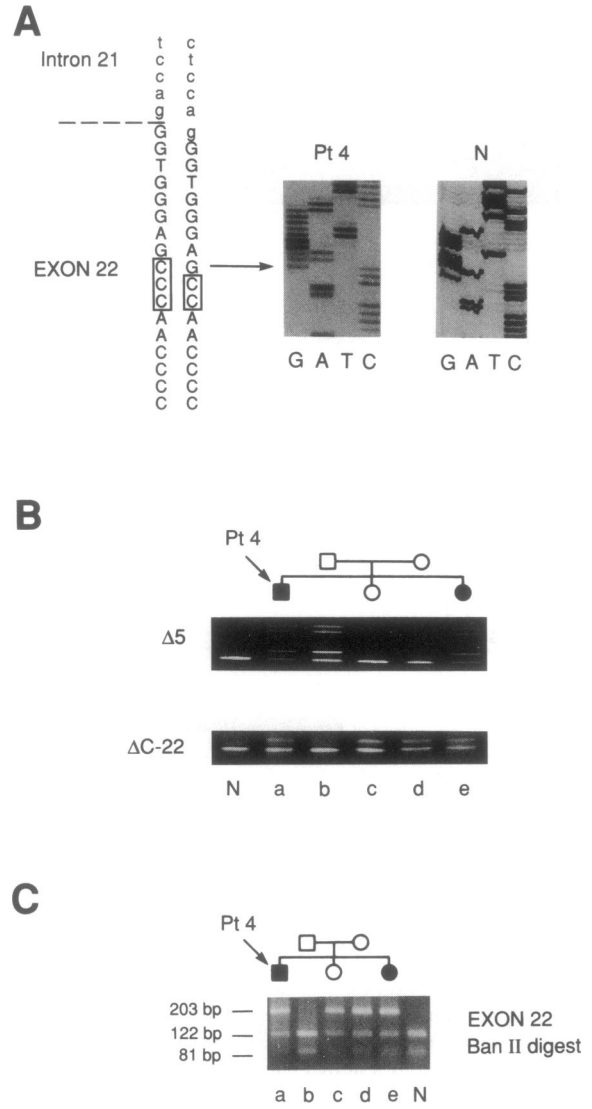

Figure 2 *A*, Partial sequence of exon 22 of the PFK-M gene. Shown are direct sequencing of the PCR-amplified DNA from a normal subject (*right*) and a compound heterozygous patient 4 (*left*). A C nucleotide deletion was detected on one allele in patient 4. *B*, Two-generation pedigree of family 4. Blackened symbols represent affected members of the family. Beneath the pedigree, genomic PCR amplifications of the exon 5/intron 5 junction and of exon 22 are resolved on DGGE with 25%–75% and 40%–70% gradients, respectively. The allele with Δ5 in the proband (lane a) and in the affected sister (lane e) was inherited from the father (lane b); the allele with ΔC-22 in all of the siblings (lanes a, c, and e) was inherited from the mother (lane d). N = DGGE pattern for a normal subject. *C*, Confirmation of ΔC-22 by restriction digest. *Ban*II cleaves the 203-bp PCR product into 136-bp and 87-bp fragments in a normal subject and in the father (lane b); the mutation destroys the restriction site, resulting in three bands in heterozygous individuals in the family (lanes a and c–e).

by the DGGE analysis, we screened the available family members (fig. 2*B*). The Δ5 mutation in the proband (lane a) and in his affected sister (lane e) was inherited from the father (lane b); the ΔC-22 mutation in the proband (lane a) and two sisters (lanes c and e) was inherited from the mother (lane d). The pedigree analysis establishes that the proband is a compound heterozygote for these two mutations in the PFK-M gene.

The deletion in exon 22 destroys a native *Ban*II restriction site in the wild-type sequence, resulting in an undigested 203-bp fragment in addition to two smaller digested fragments, of 122 bp and 81 bp, in the heterozygous mother (lane d) and three siblings (lanes a, c, and e) (fig. 2*C*). Only two digested fragments are observed in the father (lane b) and a normal control (lane N). Thus, restriction-enzyme digestions confirmed that the DGGE pattern was specific for the ΔC-22 mutation.

By using this screening test, ΔC-22 mutation was also identified in patients 5–7 (fig. 3*A*). Patients 6 and 7 appear to be homozygous for the ΔC-22 mutation, as indicated by the presence of only undigested fragments after cleavage with *Ban*II. Patient 5, who carries the splicing defect on one allele, also has the ΔC-22 mutation (three bands are detected after *Ban*II digestion). Therefore, two unrelated compound heterozygous patients, 4 and 5, share the same genetic defects of the PFK-M gene.

It is of interest that DGGE did not detect the ΔC-22 mutation in homozygous patients 6 and 7. DGGE, which relies on the melting behavior of DNA fragments in a gel with an increasing concentration of denaturant, allows nearly all *heterozygous* base-pair changes to be detected, but homozygosity can be missed more easily (Myers et al. 1985; Sheffield et al. 1989, 1992). To avoid possible PCR artifacts and sample cross-contamination, we directly sequenced independent PCR products corresponding to exon 22 in patients 6 and 7 and confirmed that both are homozygous for the one base deletion (fig. 3*B*, shown for patient 7). Fig. 3*C* illustrates the predicted effect of the mutation on the PFK product. Northern analysis (not shown) revealed a decreased amount of shortened mRNA from a muscle biopsy of patient 7, who was homozygous for the ΔC-22 mutation.

Restriction analysis of exon 22 with *Ban*II in patient 8, who was heterozygous for Δ5, demonstrated two normal digested bands (fig. 3*A*, patient 8), thereby excluding ΔC-22 deletion as his second mutation. Therefore, we again utilized DGGE analysis to seek another polymorphic exon. A polymorphic exon 4 was identified, cloned, and sequenced (fig. 4*A*). The patient was found to be heterozygous for yet another mutation, a G-to-T transversion that would predict the substitution of Leu for Arg at position 39. Because this mutation creates an *Rma*I site, we were able to verify its presence in DNA amplified by an independent PCR and *Rma*I cleavage. (fig. 4*B*). Thus, patient 8 appears to be a compound heterozygote with the Δ5 and a missense mutation in exon 4. In addition to the sites described above, we have identified two previously unrecognized silent mutations in the PFK-M gene. A C-to-T transition occurs at position 306 in exon 6, and a T-to-G transversion occurs at position 2334 in exon 24. Table 1

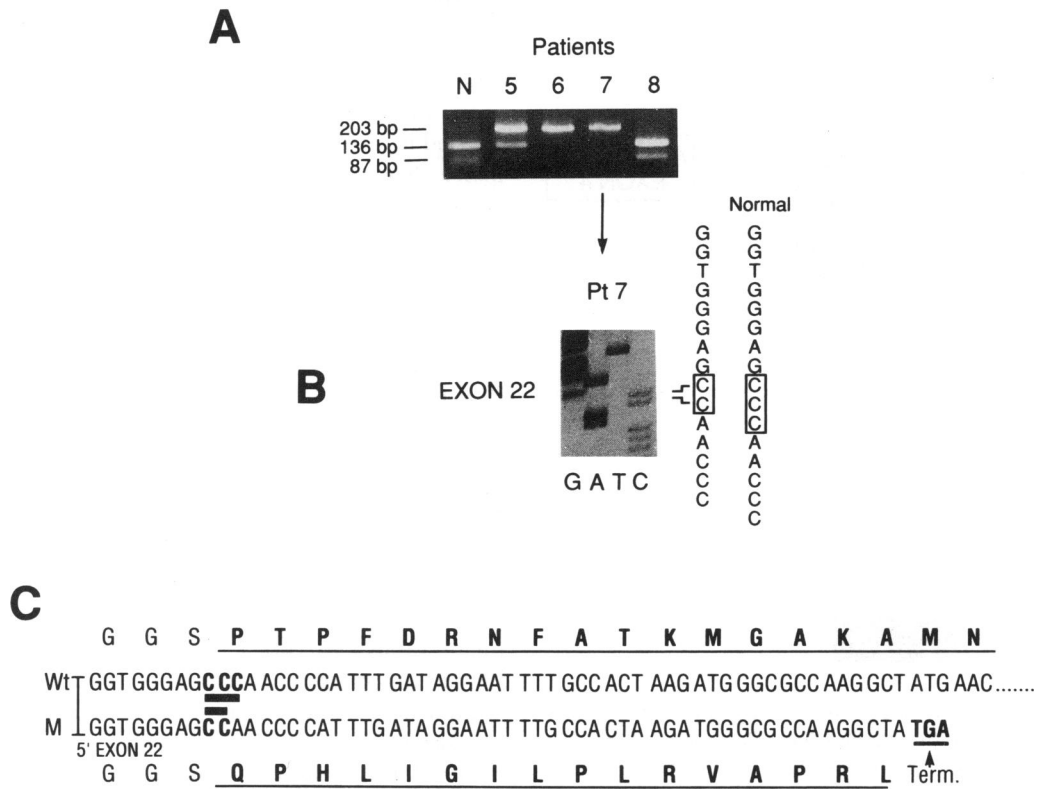

Figure 3 *A*, Screening for ΔC-22 by restriction digestion with *Ban*II endonuclease in patients 5–8. *Ban*II-digested 203-bp PCR products were size-fractionated on 2% agarose gel. The undigested 203-bp fragment, in addition to two digested fragments, is observed in heterozygous patient 5; only undigested products are present in patients 6 and 7, showing that they are homozygous for the mutation. The digestion pattern in patient 8 was similar to that in the control, indicating that he is negative for exon 22 mutation. *B*, Partial sequence of exon 22, showing a deletion of a C nucleotide in homozygous patient 7. *C*, Partial DNA and protein sequence of normal (Wt) and mutant (M) genes. The position of the deleted nucleotide is underlined. The premature translation-termination codon is indicated by an arrow.

summarizes the distribution of the mutant alleles and polymorphisms.

Clinical Correlations

All patients have symptoms characteristic of PFK deficiency (table 2), and most, in retrospect, have had them since childhood. Seven of 11 experience a second-wind effect while exercising. There was no correlation between any of the mutations and severity of the disease or extramuscular manifestations. Male patients had recorded creatine phosphokinase (CK) elevations ranging from 1,000 to 6,000, but these could be episodic, since three patients had normal values reported at different times. Only two of the women had documented CK elevations in the 1,000–2,000 range. Evidence of bouts of pronounced hemolysis, as demonstrated by episodes of jaundice, confirmed by elevations in serum bilirubin, and hyperuricemia were documented only in males. Myoglobinuria was not documented in the physician records, but, again, only three men recall episode of dark urine. Most of the men (four of five for whom data were available) pursued medical care and were diagnosed before age 40 years, whereas in the females the diagnosis was often incidental (three of the five) and made at an older age. Thus, Ashkenazi males appear to be more symptomatic than females. In fact, the oldest patient, a male, is the only one to have developed severe fixed weakness.

All of the families with the Δ5 and ΔC-22 mutations for whom data were available (eight of nine) were of eastern European—Russian or Polish—descent. Patients 6 and 7, the two who are homozygous for the ΔC-22, reported consanguinity in their families. Interestingly, patient 8, with the exon 4 mutation, was the sole individual with partial non–eastern European lineage.

Population Studies

Screening of 250 unrelated Ashkenazi individuals from the United States and Israel revealed no alleles with the exon 4 mutation, no alleles with the ΔC-22 mutation, and one allele containing the Δ5 mutation. The absence of these mutations in the normal population confirms that they are in fact mutations and not likely to be polymorphisms.

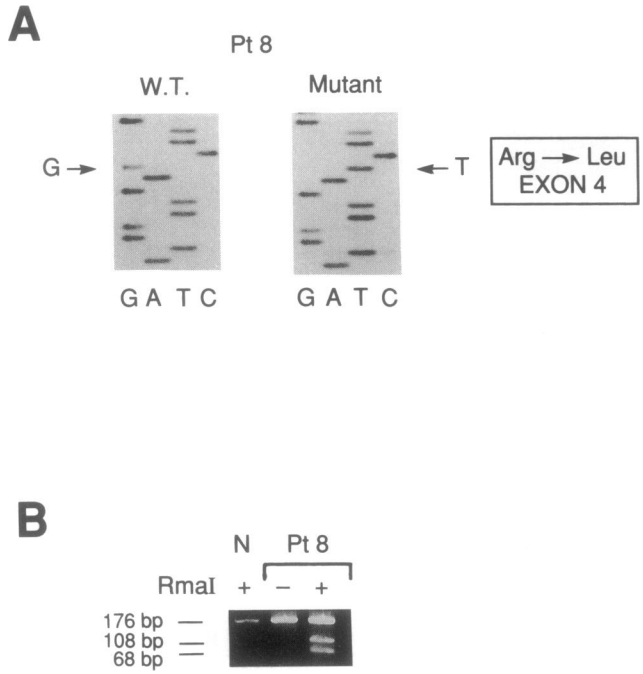

Figure 4 *A*, Sequence analysis of exon 4 in patient 8. A G-to-T transversion was found in six of eight clones. *B*, 176-bp PCR product digested with *Rma*I, resulting in undigested product in a normal subject. Undigested as well as two digested products, of 108 and 68 bp, are observed in the patient, consistent with the presence of G-to-T mutation in a heterozygous individual.

Discussion

In this study, we have sought to understand the molecular basis of the mutations in PFK deficiency in the Ashkenazi Jewish patients who constitute the majority of reported cases in the United States. We have described a g-to-a mutation at the normally highly conserved splice donor site (Mount 1982; Shapiro and Senapathy 1987), resulting in a neat exon-5 deletion in the mRNA (Δ5). We have now used PCR either in combination with DGGE or with restriction-enzyme digestion to screen for the junctional mutation and to localize two undescribed mutations in eight additional Ashkenazi families with PFK deficiency.

In the nine Ashkenazi families, Δ5 accounts for 11 (61%) of the 18 alleles. A newly recognized mutation, a base deletion in exon 22 (ΔC-22), which results in a frameshift and a premature stop codon, accounts for 6 (33%) of the 18 alleles. We have found a third mutation—a base-pair transversion from G to T in exon 4, which would result in a nonconservative amino acid substitution of leucine for arginine—as the mutation in the remaining allele.

The three mutations represent three different classes of genetic defects: (1) a splicing defect leading to an exon skip, (2) a single base deletion leading to a premature stop codon, and (3) a missense mutation. The first two would cause major structural alteration in a protein product. Transcription of DNA from patients bearing the Δ5 mutation results in a shortened message (Raben et al. 1993), as was true in the Japanese patient with a similar splicing mutation (Nakajima et al. 1990*b*). In the case of ΔC-22, a mutation located near the 3′ end of the transcript, a decreased level of mRNA was found, despite mounting evidence that premature stop codons result in severely decreased levels of mRNA only when a premature stop codon is located early in the transcript (Hamosh et al. 1991; Mashima et al. 1992; McIntosh et al. 1993).

The Arg at position 39 that is changed by the exon 4 mutation is conserved in evolution (Evans et al. 1981; Poorman et al. 1984) and is found in both the PFK-M and PFK-L isoforms (Vora et al. 1986; Nakajima et al. 1987; Hotta et al. 1991). Analysis of both bacterial and mammalian PFK suggests that the latter has arisen from a gene duplication event (Poorman et al. 1984). On the basis of the structure of bacterial PFK, the Arg in position 39 in human PFK-M is predicted to be part of the substrate binding site (Evans et al. 1981; Poorman et al. 1984; Hotta et al. 1991). Hence, a nonconservative substitution there might abolish enzyme activity. Analysis of red-blood-cell PFK in this patient (Vora et al. 1983*a*), however, demonstrated no evidence of PFK-M tetramer formation, suggesting that the mutation may inhibit subunit interaction or prevent the synthesis of stable monomers.

The particular mutations did not define clinical groups of PFK-M deficiency, but the clinical observations proved interesting, nevertheless. As reported in the literature, symptoms began in childhood, usually in early childhood, taking the form of exercise-induced weakness and myalgias, sometimes accompanied by nausea. However, the 60-

Table 1

Distribution of Mutant Alleles and Polymorphisms in Unrelated Ashkenazi Jewish Patients with PFK Deficiency

Subject	Mutation			Polymorphism(s)
	Δ5	ΔC-22	Exon 4	
Patients:				
1	+/+			
2	+/+			
3	+/+			
4	+	+		C to T, Thr to Thr in exon 7; T to G, Thr to Thr in exon 24
5	+	+		
6		+/+		
7		+/+		
8	+		+	C to T, Thr to Thr in exon 7
9[a]	+/+			C to T, Thr to Thr in exon 7
Normal control				C to T, Ala to Ala in exon 6

[a] Original family.

Table 2

Clinical Characteristics of 11 Patients with PFK Deficiency

Age at Diagnosis (years)	Onset of Symptoms	Mutation	Exercise Intolerance	Second Wind	Evidence of Hemolysis[a]	Hyperuricemia	Family No.	Origin
Women:								
31	Early Childhood	5/5	+	−	↑ retics	−	1	Germany/Russia
14	Early Childhood	5/22	+	+	−	−	4[b]	
50	Early Childhood	22/22	+	+	−	−	6	Poland
65	Early Childhood	5/5	+	+	↑ MCV and retics	−	9[c]	Russia
53	Early Childhood	5/5	+	−	↑ MCV and retics	−	9[c]	
Men:								
60	Childhood	5/5	+	+	↑ bili and retics, Jaundice	+	2	Russia (parents from same village)
13	12	5/5	+	+	↑ bili and retics, Jaundice	+	3	Russia/Germany
16	Childhood	5/22	+	+	↑ bili and retics	+	4[b]	Russia/Poland
NA	NA	5/22	NA	NA	NA	NA	5	NA
36	Childhood	22/22	+	−	↑ bili, MCV, and retics	+	7	Romania
20	Childhood	5/4	+	+	↑ bili, MCV, and retics	+	8	Poland/France

NOTE.—NA = not available.

[a] Retics = reticulocytes; MCV = mean corpuscular volume; and bili = bilirubin.

[b] Siblings.

[c] Siblings from original family (Raben et al. 1993).

year-old man (table 1), who is homozygous for the Δ5 mutation, had developed a progressive myopathy as an adult; at the present time we cannot predict the clinical course of the younger men who share his mutation.

Many of the patients described the second-wind phenomenon, an association that has not been well documented with PFK-M deficiency (Rowland et al. 1986) but is well known in McArdle disease. In contrast to McArdle disease, in which the defect is at the level of glycogen catabolism (Mommaerts et al. 1959; Schmid and Mahler 1959), the defect in PFK-M deficiency does not permit the utilization of glucose. In McArdle disease, glucose has been implicated as the alternative energy source sustaining muscle activity (Argov et al. 1987*a*, 1987*b*), whereas the presence of a second wind in PFK-deficient patients suggests that free fatty acids may be responsible for this phenomenon (Haller and Lewis 1991).

As would be predicted for an autosomal recessive disease, we did not find a gender predominance. However, episodes of marked hemolysis, as demonstrated by hyperbilirubinemia and jaundice, were documented only in men. Elevated uric acid was also a feature found only in men, but none of the patients in this series had gout (Mineo et al. 1987). It is unclear whether behavior or genetic factors predispose male patients with this disease to show stronger evidence of rapid red-cell turnover. The increased symptoms and the evidence of hemolysis may account for the apparent higher prevalence in men in reported series (Rowland et al. 1986).

The frequency of this disease may be underestimated because patients, even if symptomatic, may not pursue clinical evaluation. In a sample of the normal Ashkenazi population we found only one allele with the Δ5 mutation and no evidence of the ΔC-22 or the missense mutations.

The majority of the patients in this series traced their ancestry to Russia or Poland. The frequency (61%) of the Δ5 mutation in these families suggests the possibility of a founder effect, although the prevalence of the allele in the population is too low to confirm it. Both additional mapping of linked polymorphisms on chromosome 1 and fine mapping of the PFK gene would enable further clarification of this possibility. A second common mutation, ΔC-22, was found in 33% of the alleles in these Ashkenazi patients. The exon 4 mutation, identified in one heterozygous patient of French and Polish ancestry, may not have arisen in eastern Europe.

We have identified all the mutations in these nine families. Since two mutations account for 94% of the alleles, rapid genetic screening of Ashkenazi patients with suspected PFK deficiency is possible, perhaps eliminating the need for assay of the activity of this labile enzyme on a specimen of muscle.

Acknowledgments

We are grateful to Drs. Salvatore DiMauro, Ronald Haller, Alfred Slonim, James Sowa, and Alfred W. Wolfsohn, for referring patients and providing clinical data and samples; to George Poy for providing oligonucleotides; and to Drs. Daniel Kastner and Richard Proia, for reviewing the manuscript.

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