Muscle Phosphoglycerate Mutase Deficiency Revisited

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**Background:** Phosphoglycerate mutase (PGAM) deficiency (glycogen storage disease type X) has been reported in 12 patients of whom 9 were African American.

**Objective:** To describe 2 patients, 1 of Pakistani and 1 of Italian ethnic origin, with typical clinical and biochemical changes of glycogen storage disease type X and novel mutations in the gene encoding the muscle subunit of PGAM (PGAM2).

**Design:** Clinical, pathological, biochemical, and molecular analyses.

**Setting:** Tertiary care university hospitals and academic institutions.

**Patients:** A 37-year-old Danish man of Pakistani origin who had exercise-related cramps and myoglobinuria and a 65-year-old Italian man who had exercise intolerance and myalgia but no pigmenturia and had undergone long-term statin therapy.

**Main Outcome Measures:** Clinical course and biochemical and molecular features.

**Results:** Biochemical evidence showed severe isolated PGAM deficiency, and molecular studies revealed 2 novel homozygous mutations, a nonsense mutation and a single nucleotide deletion. Pathological studies of muscle showed mild glycogen accumulation but prominent tubular aggregates in both patients.

**Conclusions:** We found that glycogen storage disease type X is not confined to the African American population, is often associated with sarcoplasmic reticulum (SR) proliferation, and is genetically heterogeneous.

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**PHOSPHOGLYCERATE MUTASE** (PGAM; EC 5.4.2.1) is an enzyme of terminal glycolysis that catalyzes the reversible shift of the phosphate group between C-2 and C-3 of glycerate. There are 3 isoforms of this dimeric enzyme in mammalian tissue. The MM homodimer accounts for approximately 95% of the total PGAM activity in mature human muscle, whereas the BB homodimer and the MB heterodimer predominate in most other tissues. The first identified patient with muscle PGAM deficiency was an African American man with a lifelong history of myalgia, cramps, and pigmenturia after brief, intense bouts of exercise. He had chronically increased serum creatine kinase (CK) levels and gouty arthritis. A forearm ischemic exercise increased venous lactate levels only 1.3 and 1.5 times (reference range, 3- to 5-fold increase). All enzyme activities of glycogen metabolism and glycolysis were normal except for PGAM, whose 5% residual activity was completely accounted for by the BB isomorph. To date, PGAM deficiency (glycogen storage disease type X [GSDX], OMIM 261670) has been reported in 12 patients. Although 17 patients were described in a recent article by Oh et al., the enzyme defect was not satisfactorily documented in one family, and the pathogenic significance of the heterozygous mutation in a Japanese family is questionable. Of the 12 bona fide patients, 9 were African American, a fact that had initially suggested GSDX occurred due to a founder effect and was confined to the black population. However, this notion was dispelled by the report of an Italian family in 1994. After cloning the gene (PGAM2) encoding the muscle subunit, Tsujino and coworkers established the molecular basis of PGAM deficiency in all but the first patient, whose DNA was not available. We report the molecular defects in 2 more non–African American patients, 1 of Pakistani and 1 of Italian descent, and revisit some peculiar features of this rare glycogenosis.

**REPORT OF CASES**

**PATIENT 1**

Bicycle ergometer study results, muscle morphologic findings, and the effect of dantrolene treatment have been previ-
ously reported for this patient. The patient is a 37-year-old Danish man of Pakistani descent whose parents were first cousins. Since the age of 19 years, he has had symptoms of myalgia, cramps, and pigmenturia that have occurred after bouts of strenuous exercise. The results of physical and neurologic examinations and electromyography were normal, but his serum CK levels were occasionally increased. A muscle biopsy specimen showed no accumulation of glycogen but prominent tubular aggregates at the periphery of type 2b fibers. Enzymes of glycolysis and glycolysis were normal except for PGAM, which was 3% of the reference mean.

PATIENT 2

Although this 65-year-old man came from the same area of Southern Italy as the first Italian patients with PGAM deficiency, any family relationship was excluded. He had hypertension and hypercholesterolemia and had been undergoing statin therapy for the past 20 years. He had symptoms of exercise intolerance, myalgia, and premature fatigue but did not report discoloration of the urine. There was no family history of similar problems. Serum CK levels were elevated in the long term (recently as high as 1112 U/L; reference range, 200 IU/L; to convert to micromoles per liter, multiply by 0.0167). Electromyography showed a myogenic pattern, and a muscle biopsy specimen showed scattered atrophic fibers and prominent subsarcolemmal tubular aggregates. The activity of PGAM in muscle extracts was 5% of the reference mean, whereas other glycolytic enzyme levels were normal.

MOLECULAR ANALYSIS

Nucleic Acids Extraction

Muscle DNA was extracted from frozen tissue specimens using a commercially available kit (Gentra Systems, Minneapolis, Minnesota). Total RNA was extracted from frozen muscle using TRIzol isolation kit reagents and following the manufacturer’s recommended protocol (Invitrogen Corporation, Carlsbad, California).

PGAM2 Gene Sequence Analyses

The entire PGAM gene (ie, 5’UTR, 3 exons, 2 introns, and 3’UTR) was amplified and sequenced using BigDye reagent (Applied Biosystems, Foster City, California) and the cycle sequencing technique (primer sequences are available on request from the corresponding author).

Reverse Transcription–Polymerase Chain Reaction Analysis

Twenty-five milligrams of muscle tissue was homogenized in 1 mL of TRIzol solution, and RNA was extracted according to the manufacturer’s protocol. A total of 500 ng of RNAs was used for the 1-step reverse transcription–polymerase chain reaction amplification, using a SuperScriptIII One-Step reverse-transcription polymerase chain reaction kit (Invitrogen Corporation, Carlsbad) and following the manufacturer’s recommended protocol.

RESULTS

The first patient had the typical clinical presentation of PGAM deficiency because since adolescence he had experienced recurrent bouts of myalgia and cramps and often pigmenturia after brief strenuous exercise. He was otherwise healthy, except for occasionally increased resting serum CK levels and the presence of prominent tubular aggregates in his muscle biopsy specimen. This patient was found to have increased muscle Ca2+-adenine triphos-
Case Reports

In the first patient, who was in his 40s, PGM2A phosphatase and increased calcium concentrations in muscle, and his ischemic exercise-induced contractures subsided on treatment with the calcium release inhibitor dantrolene sodium.9

Sequence analysis of the PGAM2 gene in this patient detected a homozygous nonsense mutation, CGA (Arg) to TAG (stop), at codon 180 in exon 2 of the PGAM2 gene (Figure 1A). We also showed that the T→C transition at this position created a new splice site, which led to a 57–base pair (bp) truncation of the PGAM messenger RNA (mRNA), predicting a loss of 19 amino acids in the protein. Amplification and size determination of the complementary DNA prepared from the muscle mRNA of the patient revealed that, in fact, this novel mutation creates a new splice site, resulting in a 57-bp shorter mRNA (Figure 1B). Sequencing of the abnormal mRNA indicated that the deleted region encompasses 59 bp (17 codons) of exon 2, without affecting exon 3. Both parents were heterozygous for the same mutation.

The case of the second patient, who was in his 60s, was unusual in several respects. He presented with exercise intolerance and leg pain, but he had been undergoing statin treatment for 2 decades, which suggested a statin myopathy rather than a primary metabolic myopathy. The high serum levels of CK were also compatible with statin myopathy. However, finding tubular aggregates in the muscle biopsy specimen suggested the diagnosis of PGAM deficiency, which was confirmed biochemically. Although this patient lived in the area at the tip of the Italian peninsula, as did the first Italian family with PGAM deficiency, the molecular genetic defect, a single nucleotide deletion (Del532G) in exon 2, differed from that found in the first family (R90W), ruling out a founder effect (Figure 2A–C).

Figure 2. Genetic studies of the PGAM2 gene in patient 2. A, The sequence of PGAM2 shows a single nucleotide (G) deletion at position 532 of exon 2. B, The deletion causes a frameshift in exon 2 that results in a premature stop codon. C, Restriction analysis of a polymerase chain reaction–amplified fragment of the gene spanning the single nucleotide deletion. P indicates patient; C, control; U, undigested; and bp, base pairs. The deletion of nucleotide G at position 532 eliminates the HpaII recognition site.

Phosphoglycerate mutase deficiency (GSDX) is a rare and benign muscle glycogenosis that occurs due to a partial block of terminal glycolysis. The initial patient described in 19811 and 9 of the 13 patients described thus far (Table) have been African Americans. Although the first patient was never characterized at the molecular level, 7 of the 8 remaining patients were homozygous for a nonsense mutation in exon 1 (and 1 was compound heterozygous for the same mutation and a missense change), a finding that suggests that the prevalence of GSDX among African Americans is, in fact, due to a founder effect.
However, it soon became clear that PGAM deficiency is not an ethnic disease: in 1994 Vita and coworkers described a family from Southern Italy, in 1999 (and again in 2005) Vissing et al described a Danish patient of Pakistani descent, in 1999 Hadjigeorgiou et al identified a heterozygous mutation in a Japanese family, and we now describe a second Italian patient. Because the molecular defect in the Pakistani family had not been clarified, we revisited that patient and identified a novel and unusual mutation in exon 2. Together with the single nucleotide deletion identified in the new Italian patient, these are the first mutations to affect exon 2 of the PGAM2 gene.

From the clinical point of view, PGAM deficiency is a homogeneous entity. Patients are asymptomatic except when they engage in brief strenuous efforts, which trigger myalgia, cramps, and often muscle necrosis and myoglobinuria. An exception to this stereotypical clinical picture is the present Italian patient, who appeared to have a late-onset statin myopathy; this possibility cannot be excluded because there is evidence that exposure to statins may unmask clinically silent metabolic myopathies in homozygous or heterozygous patients.11-13

The benign clinical phenotype of typical patients is not surprising when one considers that PGAM is the most active enzyme in the glycolytic pathway; even when the activity is reduced to 5% of normal, this residual BB activity is only slightly less than the normal activity of the glycolytic rate-limiting enzyme phosphofructokinase (PFK). This finding is in agreement with cycle exercise data collected from our Pakistani patient and from 2 other typical patients with PGAM deficiency.6,8 The glycolytic responses to exercise in patients with PGAM deficiency are strikingly different from those obtained in patients with McArdle disease, in whom glycolysis is almost completely blocked.8 The residual PGAM activity was sufficient to allow near-normal maximal oxidative capacity, to allow near-normal production of lactate during dynamic exercise, and to prevent the occurrence of a “second wind.”8,14 Why, then, do these patients develop cramps and myoglobinuria? Because, at near-maximal work intensities, anaerobic glycolysis becomes a crucial source of substrate-level phosphorylation, and in its absence energy crises (reflected by cramps and myoglobinuria) ensue. In agreement with this hypothesis, phosphorus magnetic resonance spectroscopy shows accumulation of monophosphorylated carbohydrate intermediates, also known as phosphomonoesters, only at high work intensity but not during moderate exercise.8

Although PGAM deficiency is best explained as a prototypical defect of anaerobic glycolysis, there are a few unexplained facets to this disease. The first is the occurrence of manifesting heterozygotes, a finding that is unexpected in a disease in which even homozygotes are affected only in extreme circumstances. Yet, the brother of an African American patient, and a Japanese father and his son, had exercise intolerance, but no myoglobinuria, with intense exercise. Manifesting heterozygotes for the myophosphorylase gene (PYGM) have also been described,16,17 but careful physiologic studies revealed no signs of McArdle disease, a finding that questions the definition of manifesting carriers based solely on subjective symptoms.

The second unusual feature of PGAM deficiency is the frequent association with tubular aggregates: these have been seen in 5 of 15 patients (33%), whereas they have never been associated with other, more common muscle

### Table. Clinical, Ethnic, Muscle Biopsy, and Genetic Features of the 13 Patients With Phosphoglycerate Mutase Deficiency

<table>
<thead>
<tr>
<th>Sex/Age, y</th>
<th>Ethnicity</th>
<th>Clinical Features</th>
<th>Muscle Biopsy Findings</th>
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<td>Tubular aggregates</td>
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</table>

**Figure 3.** The muscle glycolytic pathway. The mean activity of each enzyme is depicted by a bar. The pale gray portion of the phosphoglycerate mutase (PGAM) bar shows the average residual activity in the muscle of patients with PGAM deficiency. ALD indicates aldolase; EN, enolase; GPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PGM, phosphoglucomutase; PHI, phosphohexose isomerase; and PK, pyruvate kinase.
glycogenoses, such as myophosphorylase deficiency (McArdle disease) or phosphofructokinase deficiency (Tarui disease), or with other defects of terminal glycolysis, such as phosphoglycerate kinase deficiency or lactic dehydrogenase deficiency. Because this association does not appear to be casual, there must be a specific trigger. The presence of phosphomonoesters in muscle is not an adequate explanation because (1) phosphomonoesters also accumulate in other defects of terminal glycolysis, where tubular aggregates are not seen, and (2) there is no obvious reason why phosphomonoesters should favor SR proliferation. More interesting was the observation in 2 patients with PGAM deficiency and tubular aggregates (including the Pakistani patient revisited in this article) that calcium concentration and Ca\(^{2+}\) adenine triphosphate activity were markedly increased in muscle. Although it is difficult to establish the relationship between cause and effect, it is tempting to speculate that increased calcium content may trigger proliferation of its main “sink,” the SR. However, a different scenario is suggested by the effect of dantrolene in preventing contractures in the Pakistani patient, namely, that excessive calcium is released from the overabundant SR during contraction. In this scenario, the trigger of SR proliferation would remain obscure.

Of course, tubular aggregates are a nonspecific pathological change seen in diverse conditions, including exposure to drugs, toxins, hypoxia, and muscle CK deficiency, and considered “an adaptive response of the SR to various insults to the muscle fiber.”\(^{19}\) The specific insult in PGAM deficiency remains a mystery.

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