Case Report

Novel heterozygous mutations in the *PGAM2* gene with negative exercise testing

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A B S T R A C T

Pathogenic variants in the *PGAM2* gene are associated with glycogen storage disease type X (GSDX) and is characterized by exercise induced muscle cramping, weakness, myoglobinuria, and often tubular aggregates in skeletal muscle. We report here a patient diagnosed with GSDX at 52 years of age with a normal increase in post-exercise lactate with both anaerobic and aerobic exercise. Genetic testing found two novel *PGAM2* variants (c.426C > A, p.Tyr142Ter and c.533delG, p.Gly178Alafs*31).

1. Introduction

The phosphoglycerate mutase (PGAM) protein is encoded by the *PGAM2* gene on chromosome 7p13 [1]. It reversibly transfers the phosphate group from the 3rd carbon to the 2nd carbon of the glycate molecule in the 8th reaction of the glycolysis pathway [2]. There are three isoforms of PGAM, with the muscle (MM) form, accounting for 95% of PGAM activity. The other two forms, brain (BB), and mixed (MB) account for $\sim$ 5% residual activity in muscle, and are preferentially expressed in other tissues [3–5]. PGAM enzyme is so highly active, that even in a deficient state, it's activity is only slightly lower than that of phosphofructokinase (PFK), the rate limiting step in glycolysis [1]. PGAM deficiency is associated with glycogen storage disease type X (GSDX) [2], and was first reported in 1981 in an African-American man with muscle cramping, weakness, and pigmenturia following intense exercise [6]. He had elevated creatine kinase levels, an attenuated increase in lactate on forearm ischemic exercise testing, and had glycogen accumulation in skeletal muscle. Testing for the more common glycogenolytic (phosphorylase) and glycolytic (phosphofructokinase) defects was negative, but glycolytic enzyme testing showed a severe reduction in phosphoglycerate mutase (PGAM) activity at 3.6% of controls [6].

GSDX is characterized by exercise induced muscle cramping, weakness, contractures, and myoglobinuria, often with tubular aggregates in skeletal muscle [7–12]. As is the case with nearly all cases of muscle glycogenosis, most patients will show an attenuation of the normal lactate rise in response to forearm exercise/ischemic exercise testing. There have been 14 cases of GSDX identified thus far in the

literature [1,2,4,6,9,11,13–18] (Table 1). This is likely an under-reported condition since the symptoms are usually less severe than the two canonical muscle glycogenosis (myophosphorylase and PFK deficiency), and patients can remain asymptomatic for decades [15]. Diagnosis can also be difficult because some patients have been reported to perform at near normal maximal exercise with a normal peak lactate response [15]. We report here a Caucasian patient who presented with over 20 years of exercise induced cramps and pigmenturia, normal post-exercise lactate rise after a forearm ischemic test, maximal cycle ergometry testing lactate responses. These tests initially led away from a diagnosis of a glycolytic/glycogenolytic defect and the diagnosis was made after a next generation myopathy panel identified compound heterozygous nonsense mutations in *PGAM2*. Subsequent testing confirmed a partial reduction of PGAM enzyme activity in skeletal muscle.

2. Case report

A Caucasian male patient presented to the neuromuscular and neurometabolic clinic at 43 years of age for evaluation of exercise induced muscle cramps, pain, and weakness present since the age of 17 years. He reported eight definite episodes of pigmenturia following exercise with several hospital admissions for rhabdomyolysis with serum creatine kinase (CK) levels > 20,000 U/L [*N* < 225 U/L]. All bouts of rhabdomyolysis were associated with rapid onset high-intensity activities such as running up a flight of stairs or running to catch a bus. He did not experience any episodes with lower intensity activities, especially with a slow warm-up. He did not report a "second wind phenomenon" nor were his episodes superimposed with fasting or flu-

Table 1
Review of the reports of *PGAM2* mutations and the phenotype.

Patient age (y)	Sex	Enzyme activity (% of normal)	Response to forearm ischemic testing (FIT)		Skeletal muscle histology and ultrastructure		Ethnicity	Reference
			Lactate rise	Ammonia rise	Tubular aggregates	Increased glycogen		
53	M	21%	3.6×	16×	No	Yes	Italian	Current Case [22]
23	M	8.1% (mean)	2×	–	No	Yes	Italian	[18]
25	M	3%	2×	7×	Yes	–	African-American	[9]
20	M	3%	2×	6×	Yes	No	African-American	[9]
52	F	8%	–	–	Yes	–	African-American	[2]
44	M	3%	1×	6×	–	No	Italian	[11]
52	M	3.6%	Low	–	–	Yes	African-American	[6]
65	M	5%	–	–	Yes	–	Italian	[1]
17	F	6%	2×	Normal	–	Yes	Unknown	[13]
24	M	5.3%	1.9×	–	Yes	No	African-American	[14]
17	F	2.1%	–	–	No	No	African-American	[5]
30	M	2.6%	–	–	No	No	African-American	[5]
31	F	–	2×	–	–	–	Italian	[16]
25	M	2.4%	2×	7×	–	–	Pakistan	[17]
43	M	22%	Normal	Normal	Yes	Yes	African-American	[15]

– Not done/No info.

like illness and there was no relationship to the fed or fasted state. His medical history was significant for hypertension, dyslipidemia, and hypothyroidism but did not note a change in symptoms in relationship to the onset of these conditions, nor with treatment with rosuvastatin (10 mg/d). His CK level normalized between episodes. His family history was non-contributory. His neurological examination was normal between episodes.

The initial forearm ischemic test (FIT) at age 43 years showed a 3.6 fold increase in lactate (1.4 mmol/L [reference 0.5–2.2 mmol/L] to 5.1 mmol/L (1 min post)) and a 16 fold increase in ammonia (23 μmol/L [reference 9–33 μmol/L] to 367 μmol/L (1 min post)) [19]; however, he developed a contracture in his forearm during the test. He then completed an aerobic cycle ergometry test to maximal fatigue with a normal 6.5 fold increase in lactate (1.9 mmol/L [reference 0.5–2.2 mmol/L] to 12.9 mmol/L (1 min post)) and a 4 fold increase in ammonia (38 μmol/L [reference 9–33 μmol/L] to 165 μmol/L (1 min post)) [20]. A muscle biopsy of the *Vastus lateralis* showed non-specific myopathic changes and non-membrane bound glycogen accumulation in the sub-sarcolemmal regions [21]. Given the symptoms and the increased glycogen in skeletal muscle, muscle enzyme testing was sent and normal for myophosphorylase, phosphofructokinase, and phosphorylase b kinase activities (Robert Guthrie Biochemical & Molecular Genetics Laboratory, Kaleida Health System, Buffalo, NY).

He was reassessed at 53 years of age for unrelated issues (paraesthesias) but also requested further testing into his initial complaints of exercise intolerance and rhabdomyolysis. Given the many possibilities and the lack of pathological direction from the initial investigations, a 183 gene next generation sequencing myopathy panel was sent (MNG Laboratories, Atlanta, GA). The only variants of potential pathogenicity and significance were two novel heterozygous variants in exon 2 of the *PGAM2* gene (c.426C > A, p.Tyr142Ter; c.533delG, p.Gly178Alafs*31). This patient's genetic results were previously reported (Patient M53 [22]). Phosphoglycerate mutase enzyme activity was 21% of normal activity (49.9 μmol/min/g tissue [reference mean = 236.2 +/− 51.9 μmol/min/g tissue]), Robert Guthrie Biochemical & Molecular Genetics Laboratory, Kaleida Health System, Buffalo, NY).

3. Discussion

The symptoms of high intensity exercise induced rhabdomyolysis and cramps are consistent with disorders of glycolysis and glycogenolysis and are canonical features of GSDX [1,7–12]. Our patient reported many of the previously reported clinical symptoms of GSDX such as exercise intolerance, myalgias, cramping, weakness, as well as contractures. This patient's symptoms were lessened when he engaged in a warm-up routine prior to more intense exercise. A challenge in establishing the diagnosis in this patient was the fact that he showed a normal increase in post-exercise lactate with both anaerobic and aerobic exercise. This led away from the diagnosis because a failure of lactate to rise with a normal to exaggerated rise in post-exertional ammonia following a FIT test is considered to be very sensitive for most of the muscle glycogenosis [23], and most cases of GSDX show some attenuation of lactate rise (Table1). A failure of lactate to rise with FIT testing has a sensitivity of 1.00 for myophosphorylase deficiency (no false negatives) [19]; however, false negative testing has been reported in phosphorylase b kinase deficiency [20,24], phosphoglycerate kinase deficiency [25], and in one previous case of GSDX [15]. Consequently, although a negative forearm exercise test has high sensitivity for many of the muscle glycogen storage diseases, a normal test does not rule out the three aforementioned types. Clues to the impairment in anaerobic energy transduction during high intensity exercise in our patient, and possibly in other GSDs with a normal lactate response, is the fact that the muscle had a contracture during the FIT test and that the ammonia response was very high (16×).

An exaggerated ammonia rise in response to FIT testing is a classical response in myophosphorylase and PFK deficiency [26] and is also seen in phosphorylase b kinase deficiency [24], as it was in our patient. The increase of ammonia in response to a glycogenolytic or glycolytic defect is due to the obligatory flux through adenylate kinase and myoadenylate deaminase that generates ammonia (and ultimately uric acid), called "myogenic hyperuricemia" [26]. A partial reason for the increase in lactate in our patient is likely due to the fact that, as in the other GSDX patient reported to have a normal increase in lactate [15], the residual enzyme activity was in the 21–22% range, which, when combined with the inherently high flux through the PGAM enzyme [1], was likely sufficient to allow for some lactate generation. This explanation

however, does not explain why there would be then a need for the high adenlyate kinase/myoadenylate deaminase flux reflected in the high ammonia rise, nor the contractures. It is possible that there are regional foci of energy insufficiency in response to intense activity, particularly in the triadic gap (12 nm) where glycolytic enzymes are known to be compartmentalized [27]. It is also known that there is a coupling between glycolytic enzymes (including PGAM) and the calcium-ATPase (SERCA2) on the sarcoplasmic reticulum [28]. Collectively, a regional attenuation of glycolytic flux at or near the triadic gap could impair inositol triphosphate re-synthesis and inhibit ryanodine receptor function [29] and/or SERCA2 channel impairment could further lead to an exaggerated increase in intra-cellular calcium and induce contractures. This latter postulation is supported by the observation of contracture resolution in a patient with PGAM deficiency with dantrolene [17]. Both *PGAM2* mutations (c.426C > A, p.Tyr142Ter and c.533delG, p.Gly178Alafs*31) are expected to cause a stop codon within the first 90% of the transcript, thus increasing the risk of nonsense mediated decay; however, the residual activity at 21% of normal indicates that the decay of both transcripts was not complete.

We suggest that PGAM deficiency/GSDX should be considered as part of the differential diagnosis of recurrent myoglobinuria in response to high intensity exercise and that a normal FIT or aerobic lactate response does not rule it out. The presence of a contracture during FIT testing and an exaggerated ammonia response are important investigational clues to the diagnosis. Another factor to consider is the ratio of the rise in ammonia to the rise in lactate in response to the FIT. In healthy controls this ratio is 2.6:3.4 = 0.76 [19]; however, in the current study the ratio was dramatically higher at 4.4. Given that a high ammonia:lactate ratio is characteristic of all glycogenolytic and glycolytic defects, it is likely best to proceed with next generation sequencing panels of either pathway specific genes or a comprehensive myopathy panel that includes both metabolic (eg. *PGAM2* and other enzymes in glycolysis) and pseudo-metabolic genes (i.e., dystrophinopathies, sarcoglycanopathies, malignant hyperthermia, etc.) [22,30].

Acknowledgements

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