COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Whole-exome sequencing detects *PYGM* variants in two adults with McArdle disease

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Abstract McArdle disease is a debilitating glycogen storage disease with typical onset in childhood. Here, we describe a former competitive athlete with early adult-onset McArdle disease and a septuagenarian with a history of exercise intolerance since adolescence who was evaluated for proximal muscle weakness. Exome sequencing identified biallelic variants in the *PYGM* gene for both cases. The former athlete has the common, well-known pathogenic variant p.(Arg50Ter) in *trans* with a novel missense variant, p.(Asp694Glu). The second individual has a previously described homozygous missense variant, p.(Arg771Gln). Here, we describe the clinical course, enzyme-testing results using muscle tissue, and molecular findings for the individuals and add to the knowledge of the genotypic spectrum of this disorder.

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CASE PRESENTATION

Individual 1 is a 28-yr-old woman of Hispanic origin, who presented at the age of 25 years with episodic rhabdomyolysis, postexercise myalgia, and myoglobinuria. Medical history is significant for hypothyroidism, migraine headaches, anxiety, and attention deficit hyperactivity disorder. Family history is negative for similarly affected individuals. She was a competitive cheerleader, gymnast, and snowboarder from age 13 to age 24 years, but had muscle pain with exertion. At the age of 25 years, during an evening of drinking alcohol and dancing, she developed acute painful leg cramps and swelling as well as pigmenturia. She was admitted to a local hospital with markedly elevated creatine kinase (CK) of 107,000 U/L, which gradually declined over 10 days. Subsequently, she had more than 10 acute episodes of exertion-induced hyper-CKemia (>10,000 U/L). Because of a combination of anxiety about recurrent exercise-induced myalgias and hyper-CKemia as well as inadequately treated hypothyroidism in her late 20s, she became deconditioned, fatigued, and felt subjectively weak with a normal neurological examination. With increased levothyroxine and a mild exercise program, her symptoms improved; however, she reports marked exercise intolerance with activities such as moving the headboard of her bed, which provoked myalgias and CK elevation to 14,000. She also has daily swelling and pain in her lower extremities after minimal exertion, followed by pain and swelling in her upper extremities on subsequent days.

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Individual 2 is a 73-yr-old man with history of muscle weakness, exercise-induced hyper-CKemia, and myoglobinuria. In addition, he had gout, hyperlipidemia, ischemic heart disease, and type 2 diabetes mellitus. The onset of the myopathic symptoms began at the age of 15 years, when he developed acute back pain when attempting to lift a heavy object. He subsequently noted leg pain when he ran more than 300 meters and reported chronic weakness. At age 61, after a traumatic fall, he had another episode of hyper-CKemia, and he developed transient renal insufficiency requiring dialysis. At age 72 years, he noted leg weakness, with difficulty rising from chairs, climbing stairs, and walking more than 15 steps, because of leg "heaviness" without pain or cramps. At age 73 years, neurological examination revealed proximal limb weakness (deltoids 4+/5, biceps 4/5, and hip flexors 4/5). Family history includes a younger sister who has similar symptoms of periodic hemoglobinuria and exercise intolerance. The family is of Eastern European Ashkenazi-Jewish ancestry.

Table 1 compares the clinical phenotypes seen in the two individuals (1 and 2) using the Human Phenotype Ontology (HPO) terms typically associated with McArdle disease.

McArdle disease (Glycogen Storage Disease, type V; OMIM # 232600) caused by biallelic pathogenic variants in myophosphorylase (*PYGM* gene, OMIM # 608455) results from the inability to utilize glycogen to form glucose-1-phosphate during physical activity. It is characterized by exercise-induced pain, cramps, rhabdomyolysis, markedly elevated creatine kinase, and a "second wind" phenomenon in which affected individuals are able to return to physical activity after a brief period of rest (Martín et al. 2006). Most individuals with McArdle disease become symptomatic in childhood or early teenage years, although milder late-onset disease has been described (Pourmand et al. 1983; Petrou et al. 2015).

TECHNICAL ANALYSIS

Trio whole-exome sequencing for Individual 1 and her parents (in 2016) and singleton proband only whole-exome sequencing for Individual 2 (in 2018) was performed at the Laboratory of Personalized Genomic Medicine at Columbia University Medical Center on DNA extracted from peripheral blood mononuclear cells. Written consent was obtained and exome sequencing libraries were prepared from genomic DNA from individuals using Agilent SureSelectXT (Human All Exon v.5 + UTRs) capture kit according to the manufacturers' protocol. Paired-end sequencing was performed on the Illumina HiSeq 2500 platform. The sequence data were aligned to hg19 and annotated using NextGENe (version 2.3; SoftGenetics) software. Variant filtering and annotation were performed using an in-house developed pipeline and reviewed as part of the clinical workflow for constitutional clinical exome sequencing in the laboratory of Personalized Genomic Medicine at Columbia University Medical Center (Wang et al. 2016).

INTERPRETATION OF RESULTS

Previous laboratory results for Individual 1 were negative for elevated levels of lactate and pyruvate and positive for significantly elevated creatine kinase (resting CK; 5547.0 U/L, normal 40.0–308.0 U/L). CK was especially elevated during episodes of myoglobinuria (107,000 U/L) as described in the case presentation. Urine organic acids and plasma acylcarnitine profiles were unremarkable.

Glycolytic enzyme activity assay in the biopsied muscle revealed undetectable activity of myophosphorylase measured spectrophotometrically by nicotinamide-adenine dinucleotide phosphate reduction in the supernatant of muscle homogenate (mean ± SD activity of 118 control samples: 24 ± 7.4 μmol glucose-l-phosphate liberated per minute per gram of

Table 1. Comparison of clinical features seen in the two individuals with Human Phenotype Ontology (HPO) terms typically associated with McArdle disease

HPO#	Clinical feature	Individual 1	Individual 2
HP:0003201	Rhabdomyolysis	Y	Y
HP:0002875	Exertional dyspnea	NR	NR
HP:0001919	Acute kidney injury	NR	Y
HP:0003546	Exercise intolerance	Y	Y
HP:0012378	Fatigue	Y	Y
HP:0008305	Exercise-induced myoglobinuria	Y	Y
HP:0002015	Dysphagia	NR	NR
HP:0003738	Exercise-induced myalgia	Y	Y
HP:0009045	Exercise-induced rhabdomyolysis	Y	Y
HP:0030234	Highly elevated creatine kinase	Y	Y
HP:0008967	Exercise-induced muscle stiffness	Y	Y
HP:0005216	Impaired mastication	NR	NR
HP:0003652	Recurrent myoglobinuria	Y	Y
HP:0040319	Dark urine	Y	Y
HP:0001649	Tachycardia	NR	NR
HP:0009073	Progressive proximal muscle weakness	NR	Y
HP:0003710	Exercise-induced muscle cramps	Y	NR
HP:0012622	Chronic kidney disease	NR	NR
HP:0030973	Postexertional malaise	Y	Y
HP:0003202	Skeletal muscle atrophy	NR	NR
HP:0009051	Increased muscle glycogen content	NR	unk
HP:0001639	Hypertrophic cardiomyopathy	NR	NR
Additional clinical features			
HP:0000821	Hypothyroidism[a]	Y	NR
HP:0001997	Gout[a]	NR	Y
HP:0003077	Hyperlipidemia	NR	Y
HP:0001677	Coronary artery disease	NR	Y
HP:0005110	Atrial fibrillation	NR	Y
HP:0005978	Type 2 diabetes mellitus	NR	Y

(Y) Yes, (NR) not reported, (unk) unknown.
[a]Recent association with McArdle disease reported in Pizzamiglio et al. (2021).

fresh tissue). Activities of other enzymes in muscle were normal including phosphofructokinase, phosphoglycerate kinase, carnitine palmitoyltransferase, lactate dehydrogenase, phosphoglycerate mutase, and phosphorylase kinase (DiMauro et al. 1982).

Trio whole-exome sequencing revealed biallelic variants in *PYGM* NM_005609.3: c.[148C>T];[2082C>A], NP_005600.1:p.[(Arg50Ter)];[(Asp694Glu)] (Table 2; ClinVar accession numbers SCV001980710.1 and SCV001443154.1). These variants were confirmed by Sanger sequencing. The c.148C>T, p.(Arg50Ter) variant identified in this individual is one of the most common pathogenic variants described in *PYGM* (Martín et al. 2006) and has multiple independent pathogenic curations in ClinVar (VarID:2298). cDNA studies have suggested that the c.148C>T, p.(Arg50Ter) variant is subject to nonsense-mediated decay, as mature cDNA transcripts were not detected from this allele in individuals harboring the

Table 2. Biallelic *PYGM* variants identified in individuals in this study, with relevant population frequencies, computational predictions, and classification

Genomic coordinates (hg19)	Ref allele	Alt allele	HGVS cDNA	HGVS protein (inheritance)	Variant classification	gnomAD (v2.1.1) allele frequency	Computational predictions			
							Provean	SIFT	CADD (v1.6)	REVEL
Chr 11: 64517943 (Individual 1)	G	T	c.2082C > A	p.Asp694Glu (paternal)	Likely pathogenic	Not found	Deleterious (score −3.86)	Damaging (score 0.000)	14.29	0.707
Chr 11: 64527223 (Individual 1)	G	A	c.148C > T	p.Arg50Ter (maternal)	Pathogenic	1.4×10^{-3}, no homozygotes	n/a	n/a	33	n/a
Chr 11: 64514696 (Individual 2)	C	T	c.2312G > A	p.Arg771Gln	Likely pathogenic	7.1×10^{-6}, no homozygotes	Deleterious (score −3.38)	Tolerated (score 0.165)	35	0.913

The Refseq transcript used for annotation is NM_005609.3. Chr 11:64517943-G-T, VAF: 0.42, 134/321 total reads; Chr 11:64527223-G-A, VAF: 0.49, 127/259 total reads; Chr 11:64514696-C-T, VAF: 1, 43/43 total reads.
(VAF) Variant allele fraction.

variant (Nogales-Gadea et al. 2008). The c.2082C>A, p.(Asp694Glu) is a rare missense variant in the carboxy-terminal phosphorylase domain, downstream from the binding site for cofactor pyridoxal phosphate (Withers et al. 1981). In silico programs predict a damaging effect of this variant on protein function (Table 2). It has not been previously reported in any affected individuals and is classified as likely pathogenic as per American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015; PM2 + PM3 + PP3 + PP4 [biochemical evidence]).

Previous laboratory testing for Individual 2 was significant for abnormal resting serum creatine kinase: 900–3000 U/L. Proband's singleton whole-exome sequencing revealed an apparently homozygous, rare, missense variant in *PYGM*, NM_005609.3:c.[2312G>A];[2312G>A], NP_005600.1:p.[(Arg771Gln)];[(Arg771Gln)], confirmed by Sanger sequencing (Table 2; ClinVar accession number SCV001977607.1). In silico predictions for this missense variant are conflicting (Table 2). The variant alters the last nucleotide of the coding exon 18 and is also predicted to affect splicing (TraP score: 0.989; dbscSNV: 0.9999). It has been previously reported in at least two affected individuals, once in *trans* with the pathogenic p.(Arg50Ter) *PYGM* variant (compound heterozygous; Nadaj-Pakleza et al. 2009) and in another individual as a homozygous variant (Viéitez et al. 2011), and is classified as likely pathogenic as per ACMG guidelines (Richards et al. 2015; PM3_strong + PM2_supporting + PP2 + PP3 [consistent splicing predictions]). Individual 2's sister was not available for genetic testing.

SUMMARY

For McArdle disease, nonsense variants in *PYGM* account for 30%–35% of disease-causing variants, and the most common pathogenic variant seen in affected individuals is a nonsense variant p.(Arg50Ter). An additional 50% of the pathogenic variants described in *PYGM* are missense variants, and they are distributed throughout the protein with no specific hotspot or domain associated with pathogenic variation (Nogales-Gadea et al. 2015). Furthermore, with few exceptions, most previously described pathogenic variants result in undetectable enzyme function and/or reduced transcript levels, presumably through RNA-mediated decay or mRNA degradation (Nogales-Gadea et al. 2008; García-Consuegra et al. 2009). Although there is no observed genotype–phenotype correlation associated with *PYGM*

variants, identification of novel variants and associated clinical phenotypes is critical to expanding the body of knowledge of the genotypic and phenotypic spectrum of this disorder.

Here we report two cases of McArdle disease with varying ages at onset and severity, and wherein exome sequencing identified biallelic variants in *PYGM* gene. The first case is of a former competitive athlete who presented in young adulthood with recurrent episodes of exertional hyper-CKemia, myalgia, and myoglobinuria. This individual's exceptional exercise capacity in youth with abrupt onset of debilitating exertional myalgias and hyper-CKemia at age 25 years highlights the uniqueness of this case. Enzyme studies showed undetectable myophosphorylase enzyme activity, confirming a diagnosis of McArdle disease. One of the missense variants identified in this individual (c.2082C>T, (p.Asp694Glu)) has not been previously reported and thus expands the genotype spectrum of *PYGM* variants. The second individual had adolescent-onset recurrent exercise-induced hyper-CKemia and subjective weakness, with late-adult onset fixed proximal muscle weakness, and carried an apparently homozygous c.2312G>A, (p.Arg771Gln) *PYGM* variant. Clinical phenotype descriptions in previously reported individuals with this variant are limited, with normal muscle strength reported in one individual with McArdle disease in Nadaj-Pakleza et al. (2009). The early onset of weakness seen in Individual 2 is atypical for McArdle disease. In addition, Individual 1 also had hypothyroidism, whereas Individual 2 had gout; these additional clinical features have been recently associated with McArdle disease in a large cohort study (Pizzamiglio et al. 2021).

ADDITIONAL INFORMATION

Data Deposition and Access

The variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV001443154.1, SCV001977607.1, and SCV001980710.1.

Ethics Statement

The study was approved by the Institutional Review Board of Columbia University (IRB-AAAR1159, IRB-AAAA7683) and written informed consent was obtained from the research subjects.

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Author Contributions

A.T.-W., A.V.D., and M.G. prepared the original draft. J.J.H. and M.H. oversaw patient care and data collection. A.T.-W., A.V.D, A.B.N., V.J., and M.G. performed data analysis and genetic interpretation. S.D., M.H., V.J., and A.B.N. assisted in critical revision of the manuscript. All coauthors read and approved the manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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