Novel myophosphorylase mutation (p.Arg94Pro) with progressive exercise intolerance

Maryam Nabavi Nouri¹, Anne-Marie Lamhonwah¹,² & Ingrid Tein¹,²,³

¹Division of Neurology, Department of Pediatrics, The Hospital for Sick Children, Toronto, ON, Canada
²Genetics and Genome Biology Program, The Research Institute, The Hospital for Sick Children, Toronto, ON, Canada
³Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

Correspondence
Ingrid Tein, Division of Neurology, Department of Pediatrics, The Hospital for Sick Children, 555 University Ave., Toronto, ON, Canada M5G 1X8. Tel: 416-813-5041; Fax: 416-813-6334; E-mail: Ingrid.tein@sickkids.ca

Key Clinical Message
We present a 16-year-old girl with a unique clinical phenotype characterized by rapidly progressive exercise intolerance, transient exertional weakness, and progressive muscle cramps involving all limbs and bulbar muscles, following a first myoglobinuric episode at age 15 years, arising from homozygosity for a novel missense mutation (c.281G>C) in PYGM.

Keywords
McArdle’s disease, myoglobinuria, myophosphorylase deficiency, novel missense mutation c.281G>C (p.Arg94Pro), progressive exercise intolerance.

Introduction
Genetic defects of the myophosphorylase (PYGM) gene result in McArdle’s disease (GSD type V) (OMIM 232600) characterized by exercise intolerance, premature fatigue, muscle cramps, and recurrent myoglobinuria [1]. Exercise intolerance generally starts in childhood, but overt episodes of muscle cramps and myoglobinuria generally develop later in adolescence. Brief isometric contraction and less intense but sustained dynamic exercise are two primary precipitants. Affected patients have mutations in both alleles of the PYGM gene. Molecular heterogeneity has been demonstrated by identification of different mutations in the coding regions or splice sites of the gene [2, 3]. We describe a novel homozygous missense sequence variant (c.281G>C; p.Arg94Pro) in exon 2 in an adolescent with a rapidly progressive phenotype.

Case Report
This previously healthy 16-year-old East Indian girl achieved normal developmental milestones, but suffered early fatigue on exercise as a young child with mild leg cramps following strenuous activity. Her asymptomatic parents are consanguineous with no family history of muscle disease. The proband presented at 15 years of age with an episode of severe leg cramps following 8 min of high-intensity stepping exercises, associated with palpitations, pigmenturia, and elevated serum CPK of 24,500 U/L.

Following this episode, she noted a significant decline in her exercise tolerance and had onset of muscle cramps at 2–3 min of even mild-to-moderate intensity activity with a need for frequent rests. She also noted a decreased time interval between onset of exercise and painful cramps, even on walking short distances and with activities of daily living. Her muscle cramps, originally localized to her legs, progressed to involve her arms while brushing her hair, muscles of mastication on chewing and talking, and hands on writing. Rest and avoidance of high-intensity activity together with maintenance of activity at a lower pace helped lessen her cramps. She described a second wind phenomenon whereby walking at a low pace for >12 min allowed her to continue walking for 30 min with a reduction in tachycardia and diaphoresis felt at the beginning of exercise. She described transient muscle
weakness in association with muscle cramps on exercise with full recovery on resting. Triggers such as fever, infection and fasting worsened her episodes. Her progressive exercise intolerance limited her life style interfering with school attendance. Her interictal neurologic examination was normal with full-peak power.

Studies revealed normal serum electrolytes, BUN, creatinine, glucose, calcium, phosphorus, and uric acid with an elevated CPK of 5754 U/L 10 months after her first myoglobinuric episode. Permission for the genetic studies and to publish this case was obtained by written informed consent from the proband and her parents. Using patient genomic DNA, the full-coding regions of the PYGM exons were amplified and sequenced as well as ~20 bases of flanking noncoding regions using Sanger Sequencing by Prevention Genetics (Marshfield, WI). The patient’s and parents’ sequences were aligned and compared with the reference sequences. Using parental genomic DNA, the full-coding region of exon 2 was amplified and sequenced as well as ~20 bases of flanking noncoding sequences and then aligned and compared. Our proband was homozygous and her parents heterozygous for a novel missense sequence variant (c.281G>T; p.Arg94Pro) in exon 2. No other PYGM variants were identified. This novel mutation has been submitted to LOVD v.3.0-Leiden Open Variation Database (http://databases.lovd.nl/shared/variants/0000128882).

During forearm ischemic exercise testing, her strength, by hand-held dynamometry, dropped from 60 mm Hg to <10 mm Hg by 20 sec with cessation of exercise by 65 sec due to forearm cramps. Her postexercise lactate remained flat and her ammonia demonstrated an exaggerated compensatory 10-fold rise by 1 min.

Her therapy includes glucose- or sucrose-loading prior to exercise, a low-intensity warm-up period of exercise for 10–15 min prior to any activity to stimulate a second wind, pyridoxine as a cofactor for myophosphorylase, a high-carbohydrate/high-protein diet as well as sustained aerobic exercise with a reduction in isometric exercise and avoidance of known stressors. Despite this, she has suffered breakthrough muscle cramps.

**Discussion**

McArdle’s disease is an autosomal recessive disease caused by genetic defects in myophosphorylase, the skeletal muscle isoform of glycogen phosphorylase [1] which catalyzes and regulates the breakdown of glycogen to glucose 1-phosphate during glycogenolysis. The PYGM gene, on chromosome 11q13.1 [2], has 20 exons encoding for a protein of 842 amino acids. Over 149 different causative mutations have been reported including missense, nonsense, splicing, and frameshift mutations [3].

This patient was homozygous, and her parents were heterozygous for a missense sequence variant c.281G>C (p.Arg94Pro) in exon 2 which in our search of various databases (e.g., Leiden Open Variation Database (LOVD) 3.0; 170 variants described, UCSC Genome Database, ClinVar Database of NCBI; 59 variants) has not been reported. Another mutation affecting the same amino acid (c.280C>T; p.Arg94Trp) has been reported in an individual with McArdle disease [4]. Similarly, a sequence variant has been reported in the UCSC Genome Database which affects the same nucleotide and amino acid but leads to a different substitution (c.281G>A; p.Arg94Gln).

Several lines of evidence suggest that this novel mutation is pathogenic. First, it was the only nucleotide alteration in the coding region and in adjacent exon/intron boundaries of the PYGM gene. Second, p.Arg94 is evolutionarily conserved among myophosphorylase proteins in multiple mammalian and nonmammalian species. Third, parents were heterozygous carriers. Three bioinformatics programs including polyPhen-2 [5], SIFT [6], and Mutation Taster (www.mutationtaster.org) have predicted the c.281G>C (p.Arg94Pro) change to be deleterious. Proline substitution bends the main chain of the protein and is a known breaker of secondary structures [7]. Her flat lactate response and exaggerated ammonia rise during the forearm ischemic exercise test provide physiological support for the pathogenicity of the mutation.

The reasons for clinical variability in McArdle’s are not completely understood but may be due, in part, to epigenetic modulators or metabolic flux through related pathways [8] as well as to the severity of the mutation and resulting residual enzyme activity [9]. Our patient’s phenotype was characterized by rapid progression of exercise intolerance and contractures following a first myoglobinuric episode which may potentially suggest an alteration in the function/stability of her myophosphorylase protein due to this novel mutation.

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**Authorship**

MNN: wrote the first draft of the manuscript and contributed to the analysis and interpretation of the data. AML: contributed to the analysis and interpretation of the data and to the writing and revision of the manuscript for intellectual content. IT: contributed to the design and conceptualization of the study, analysis and interpretation.
Conflict of Interest

Dr. Nabavi Nouri has nothing to disclose. Dr. Lamhounwah has nothing to disclose. Dr. Tein reports grants from United Mitochondrial Disease Foundation, Physician’s Services Incorporated Foundation of Ontario, Foundation for Prader-Willi Research and Myositis Association Canada—all outside the submitted work. The authors have read and comply with the Journal’s position on issues involved in ethical publication.

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