A New Rare Mutation (691delCC/insAAA) in Exon 17 of the PYGM Gene Causing McArdle Disease

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Objective: To investigate the genetic effect of a new mutation found in exon 17 of the myophosphorylase (PYGM) gene as a cause of McArdle disease (also known as type 5 glycogenosis).

Patients: A Spanish patient with McArdle disease was screened for 3 common mutations in the PYGM gene (R49X, W797R, and G204S), as previously described. The patient was heterozygous for R49X. To find other mutations, the coding sequence of the entire PYGM gene was sequenced. The carrier status of his relatives was also studied.

Results: A novel rare mutation was found in codon 691 of exon 17. This is an insertion/deletion (indel) and consists simultaneously of a deletion of 2 bases and an insertion of 3 bases (691delCC/insAAA). A restriction analysis was designed to simplify the detection method.

Conclusions: The 691delCC/insAAA is the third indel described in the PYGM gene. Indels represent 0.95% of the total reported mutations in the Human Gene Mutation Database. The molecular origin of this mutation is not fully understood. These findings point again to the allelic heterogeneity of McArdle disease.

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Type 5 glycogenosis, or McArdle disease, is one of the most common metabolic myopathies. It is an autosomal recessive disorder that affects the glycolytic phase of muscle glycogen metabolism. The disease is clinically characterized by exercise intolerance, premature fatigue, myalgia, muscle cramps, and recurrent myoglobinuria. Symptoms typically appear in adolescence or early adulthood.

The genetic cause is a deficiency of myophosphorylase due to inherited mutations in the myophosphorylase (PYGM) gene. Myophosphorylase (α-1,4-glucan orthophosphate glycosyltransferase [Enzyme Commission 2.4.1.1]) is a specific skeletal muscle enzyme that initiates glycogen breakdown. The PYGM gene has been cloned, sequenced, and assigned to chromosome 11q13 (GenBank U94774).

Allelic heterogeneity has been demonstrated by the identification of 41 different mutations in the coding region and splice sites of the gene, most of which are quoted by other researchers. The most common mutation in white persons is R49X, and nearly 70% of Spanish patients harbor the R49X, G204S, or W797R mutation. To our knowledge, the W797R mutation has only been reported in Spanish patients and seems to be the second most frequent mutation in this population.

Single base pair substitutions are the most frequent type of mutations in the PYGM gene, but 5 small deletions and 2 insertions/deletions (indels) have been reported as well. This last mutation is an unusual complex lesion that seems to combine microdeletion and microinsertion.

Herein, we report a new indel in the PYGM gene at codon 691 in exon 17 (691delCC/insAAA) that causes the premature termination of translation 29 amino acids downstream of the mutation site.

METHODS

PATIENTS

A 34-year-old man of nonconsanguineous parents had increased creatine kinase levels in a routine laboratory analysis.

The patient had exercise-related myalgia and early fatigue since infancy. He described a characteristic “second-wind phenomenon,” being able to continue to exercise if he rests briefly after the first signs of muscle pain. He did not have pigmenturia and has never consulted for these symptoms. His family history was negative for neuromuscular diseases.
The results of his physical examination were normal, and he had normal muscular balance. The results of biochemistry showed elevated aspartate aminotransferase (189 IU/L; normal, 0-35 IU/L), alanine aminotransferase (97 IU/L; normal, 0.35 IU/L), creatine kinase (6826 IU/L; normal, 150 IU/L), and uric acid (9.73 mg/dL [0.58 mmol/L]; normal, 2.5-8.0 mg/dL [0.15-0.48 mmol/L]) levels. His electrocardiogram showed no abnormalities. Electromyography showed a reduced amplitude and duration of motor units.

Study of a muscle biopsy revealed typical periodic acid–Schiff–positive amylase-sensitive subsarcolemmal vacuoles and absent myophosphorylase. The diagnosis of type 5 glycogenosis, or McArdle disease, was established.

**MUTATION SCREENING**

After informed consent was obtained, genomic DNA was extracted from peripheral blood leukocytes by the conventional method of phenol-chloroform. Screening for the R49X, G204S, and W797R mutations was performed by polymerase chain reaction–restriction fragment length polymorphism. The patient was heterozygous for the R49X mutation. To search for mutations in the other allele, the coding sequence of the entire *PYGM* gene was amplified by polymerase chain reaction, and products were checked by electrophoresis in 3% agarose gel. After purification with a polymerase chain reaction kit (QIAquick PCR Purification kit; QIAGEN GmbH, Hilden, Germany), a sequence reaction was performed using another kit (ABI Prism BigDye Terminator kit; Applied Biosystems, Foster City, Calif) and running the sequences in an analyzer (ABI Prism 310 Genetic Analyzer; Applied Biosystems).

To simplify the detection of the 691delCC/insAAA mutation, a restriction fragment length polymorphism method was optimized because indel destroys a restriction site for the *Nco* endonuclease (New England Biolabs, Beverly, Mass). Enzyme restriction was performed with 1-hour incubation at 37°C, and products were visualized in a 2.5% agarose gel.

We also analyzed the proband’s family members and 40 healthy control subjects, who all provided informed consent, to demonstrate the pathogenic role of the mutation.

**RESULTS**

The proband was heterozygous for the R49X mutation, the most common defect in the *PYGM* gene in the white population. By sequencing the entire coding region of the *PYGM* gene, we identified an overlap sequence downstream at codon 691 in exon 17 in the forward and reverse directions (Figure 1). This suggests the presence of 2 alleles: one is a wild type, and the other presents a deletion of CC and an insertion of AAA (691delCC/insAAA). The proband’s mother and sister carry this mutation (Figure 2A). The mutation was confirmed by restriction fragment length polymorphism analysis after digestion with *Nco* (Figure 2B).
We have identified a novel rare mutation in exon 17 (691delCC/insAAA) in a Spanish patient with McArdle disease. The pathogenic effect of this mutation is supported by the following: (1) It was the only nucleotide change detected, apart from the R49X mutation, in the entire coding region and splice junctions of the PYGM gene. (2) It predicts a frameshift and premature termination of the myophosphorylase protein 29 amino acids downstream of the mutation, resulting in a 720-amino acid peptide instead of the normal 841-residue protein. (3) This truncated protein has possibly affected the C-terminal catalytic domain that extends from amino acid 482 to amino acid 841.20 This peptide may be unstable and enzymatically inactive, inducing rapid breakdown of the protein. (4) This mutation was not found in the 40 healthy Spanish controls.

Indels are complex mutations that represent just 0.95% of the total mutations in the Human Gene Mutation Database (available at: http://www.hgmd.org)21; to our knowledge, 339 indels of 35657 mutations have been published. Only 2 other mutations of this type were previously found in the PYGM gene.4,15 It has been suggested that most indels can be explained by a 2-step insertion/deletion process.22 To our knowledge, there is not a single model to explain the wide spectrum of microdeletions and microinsertions involved in indel formation. It seems that the most probable pathway of indel formation in the PYGM gene is a first step accounting for the deletion of CC followed by the insertion of AAA. The deletion of 2 base pairs could be mediated by an inverted repeat. This repeat seems to have promoted the formation of a hairpin loop with the subsequent deletion of 2 base pairs looping out of this structure. The second step, insertion, was ambiguous.

The high allelic heterogeneity of McArdle disease has been well demonstrated.3,10 Because the second mutation remains unknown in many compound heterozygotes, there will probably be future new mutations.

Since the submission of this article, we have found the mutation described herein (691delCC/insAAA) in a 27-year-old man with McArdle disease, who also bears the mutation R49X in the other allele. The patient is from Portugal and lives in France; his DNA was sent to us by El Hadi Hammouda, MD (Genéthon, Paris), for genetic studies. He was diagnosed and followed up at the Neuromuscular Unit of the Institut de Myologie, La Salpêtrière, Paris, by Bruno Eymard, MD, and Pascal Lafont, MD. This observation could be of interest because the patient described in this article is from Galicia, northwest of Spain, a region that shares historical and cultural roots with Portugal.

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