A Novel Missense Mutation (W797R) in the Myophosphorylase Gene in Spanish Patients With McArdle Disease

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Objective: To investigate the degree of genetic heterogeneity of myophosphorylase deficiency (McArdle disease) in Spain through molecular studies of 10 new patients.

Design: The coding sequence of the entire myophosphorylase gene was sequenced in DNA extracted from muscle and blood. Restriction fragment length polymorphism analysis of polymerase chain reaction fragments was used to confirm and simplify detection of a novel mutation.

Setting: A collaborative study between 2 university laboratories in Spain and the United States.

Results: Five of the 10 patients harbored a novel missense mutation in exon 20, converting a tryptophan to an arginine (W797R). Three patients were homozygous for the “common” R49X mutation, and the remaining 2 patients were compound heterozygotes for R49X and a previously described missense mutation, G204S.

Conclusions: The W797R missense mutation is the third novel mutation to be identified among Spanish patients. Its relative frequency suggests that it should be added to the R49X mutation in the molecular screening of McArdle disease in Spain.

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MCARDLE disease (glycogenosis type V), one of the most common metabolic myopathies, is caused by the deficiency of myophosphorylase (α-1,4-glucan orthophosphate glycosyltransferase), a specific skeletal muscle enzyme that initiates glycogen breakdown.1 The disease is characterized clinically by exercise intolerance, myalgia, muscle cramps, and, in some patients, recurrent myoglobinuria. These symptoms typically appear in adolescence or early adulthood, although a rare fatal infantile form of the disease has also been described.1,3

The myophosphorylase gene, located on chromosome 11q13,4,5 has been cloned and sequenced, and a recent revision of its structure6 has made it possible to identify several new mutations,7,8 for a total of 16 different mutations in patients from the United States, Japan, and several European countries, including Spain. The most frequent mutation, at least among patients of Anglo-Saxon origin, is a nonsense mutation in exon 1 (R49X), which causes the substitution of an arginine (CGA) with a stop codon (TGA).5-11 However, this mutation appears to be less frequent in southern European countries (32% frequency in Italian and Spanish patients), suggesting a decreasing north-south gradient.12,13 Also, increasing numbers of private mutations have been reported in specific ethnic groups, which underscores the genetic heterogeneity of this disease.

We now describe molecular studies in a series of Spanish patients with McArdle disease that revealed a new and relatively frequent missense mutation (W797R) in exon 20.

RESULTS

Muscle biopsies showed subsarcolemmal vacuoles containing periodic acid–Schiff-positive, amylase-sensitive material in the majority of fibers. The histochemical reaction for myophosphorylase was absent.

Direct sequencing of the entire coding region of the myophosphorylase gene showed a new missense mutation (T→C) at codon 797, exon 20 (W797R), which resulted in the change of tryptophan (TGG) to arginine (CGG) (Figure 1). To confirm the mutation and develop a rapid detection method, a 903-bp DNA frag-
PATIENTS AND METHODS

We studied 10 patients from 9 families, who had typical symptoms, including exercise intolerance with cramps and myalgia but no overt pigmenturia. Only 1 patient had a positive family history. Laboratory tests showed increased serum creatine kinase levels (range, 138-20 000 U/L; reference range, <80 U/L). Electromyography showed myopathic features, and fore-arm ischemic exercise caused no rise in venous lactate levels. Open muscle biopsy was performed in all cases.

Genomic DNA was extracted by conventional methods from peripheral-blood leukocytes and muscle tissue of patients and 40 healthy controls.14 The coding sequence of the entire myophosphorylase gene was amplified by polymerase chain reaction (PCR) from genomic DNA as described by Kubisch et al.6 The PCR products were purified by electrophoresis in 2% agarose gel and sequenced directly, with the same set of primers as for amplification, by means of a terminator cycle sequencing kit and a genetic analyzer (ABI Prism Big-Dye Terminator Cycle Sequencing Kit and ABI Prism 310 Genetic Analyzer, respectively; Perkin Elmer, Foster City, Calif).

To simplify the detection method of mutation T→C in exon 20, a 903-base pair (bp) genomic DNA fragment was amplified by PCR with primers 18F and 20B.6 The PCR conditions were 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. In the presence of the mutation, the PCR product was cleaved by BsrBI restriction endonuclease (New England Biolabs, Beverly, Mass) into 2 fragments of 664 and 239 bp, after incubation at 37°C overnight. Digestion products were analyzed in 2% agarose gel.

Restriction length fragment polymorphism analysis with the enzyme BsrBI (Figure 2) showed that 2 patients were homozygous for the W797R mutation, while 1 patient was a compound heterozygote for this mutation and R49X. In 2 other patients, the new mutation was identified only in 1 allele. Three patients were homozygous for the common R49X mutation, and 2 siblings were compound heterozygotes for R49X and mutation G204S. The W797R mutation was not detected in a series of 40 normal individuals of the same ethnic origin.

COMMENT

We have identified a novel mutation in exon 20 (W797R) of the myophosphorylase gene in 5 of 10 Spanish patients with McArdle disease. We believe this mutation to be pathogenic for the following reasons: (1) the T→C at codon 797 was the only nucleotide change found in the entire coding region and exon-intron boundaries of the myophosphorylase gene; (2) the tryptophan in amino acid position 797 is highly conserved, not only in human liver and brain isozymes, but also in glycogen phosphorylases of species phylogenetically far removed from humans, such as yeast, potato, and Escherichia coli; (3) substitution of an aromatic amino acid (Trp) with a basic amino acid (Arg) in codon 797 may affect the C-terminal catalytic domain of the enzyme, changing its secondary structure and possibly inducing rapid breakdown of the protein; and (4) this mutation was not found in 40 normal Spanish controls.

McArdle disease is genetically highly heterogeneous, with 16 specific mutations described to date in patients from different countries. The most frequent among these is a nonsense mutation at codon 49 (R49X), observed in a high percentage of patients from the United States (64%) and Great Britain (81%); however, the frequency of this mutation declines to 56% in Germany and 32% in Italy and Spain, suggesting a north-south gradient through the European continent.

The missense mutation G204S located in exon 5 of the gene, which changes a glycine to a serine, is thought to affect the N-terminal regulatory domain, which contains the majority of ligand binding residues. This mutation was found in several American families (12%) and in 2 different Spanish families (1 of which is described here), but always in heterozygous form. A frequent mutation confined to the Japanese population is a deletion...
of codon 708/709, found in 72% of cases.10 A few other mutations have been described in Japanese patients,8,17 but the R49X, which is so common in western countries, has never been found. The remaining mutations are authentic rarities,18-21 described only in isolated cases.

Previous genetic studies in Spanish patients had established the R49X mutation as the most frequent in this population,13,22 although private mutations are also being described.23,24 In our series, the frequency of the R49X mutation was significantly higher than in earlier series, whereas we found none of the other mutations described in the United States, United Kingdom, or Japan. On the other hand, in 5 patients we identified a new pathogenic missense mutation (W797R); considering that 2 of these patients were homozygous, W797R appears to be the second most frequent mutation in Spanish patients with McArdle disease. The fact that 2 patients who were heterozygous for this mutation did not harbor any known mutation in the second allele suggests the existence of more undefined mutations.

Our findings confirm the allelic heterogeneity of myophosphorylase deficiency, a characteristic shared with other glycosogenoses, such as acid maltase and phosphofructokinase deficiencies.25,26

McArdle disease can be easily diagnosed in blood cells, especially in ethnic groups with specific mutations. However, since there are other metabolic myopathies with similar clinical picture and the number of known mutations is relatively large, it seems advisable to perform a muscle biopsy in at least 1 member of the family before embarking on molecular analysis. Finally, the fact that many patients are compound heterozygotes may indicate a high frequency of asymptomatic carriers in the general population.

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