Identification of a Novel Founder Mutation in the DYSF Gene Causing Clinical Variability in the Spanish Population

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Background: Mutations in the dysferlin (DYSF) gene cause 3 different phenotypes of muscular dystrophies: Miyoshi myopathy, limb-girdle muscular dystrophy type 2B, and distal anterior compartment myopathy.

Objective: To present the results of clinical and molecular analysis of 8 patients with dysferlinopathy from 5 unrelated families.

Design: Clinical assessment was performed with a standardized protocol. A muscle biopsy specimen was obtained and studied by immunohistochemistry. Genetic analysis was performed using single-stranded conformation polymorphism and direct sequencing of genomic DNA.

Results: All the patients presented the R1905X mutation in the DYSF gene in homozygosity, and the haplotype analysis at the DYSF locus revealed that it was a novel and founder mutation. A C-to-T transition at nucleotide position 6086 changes an arginine into a stop codon, leading to premature termination of translation. This mutation was expressed as 3 different clinical phenotypes (limb-girdle muscular dystrophy type 2B, Miyoshi distal myopathy, and distal anterior dysferlinopathy), but only 1 phenotype was found in the same family.

Conclusions: The new R1905X DYSF founder mutation produced the 3 possible dysferlinopathy phenotypes without intrafamilial heterogeneity. This homogeneous population in Sueca, Spain, should be helpful in studying the modifying factors responsible for the phenotypic variability.

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PHENOTYPIC HETEROGENEITY is a common feature in patients with dysferlinopathy.1 Weakness is initially observed in the proximal muscles of patients with limb-girdle muscular dystrophy (LGMD) type 2B, whereas the disorder initially involves the gastrocnemius muscle and subsequently the proximal muscles2 in those with Miyoshi myopathy. Furthermore, onset included the tibial anterior muscles in a previously described family, described as distal anterior dysferlinopathy.3

Dysferlin (DYSF) gene mutations have been described throughout, with no hot spots. In contrast, patients from the same family with the identical DYSF gene mutation have exhibited different phenotypes.4,5 Some population clusters have been described in relation to other muscular dystrophies (LGMD type 2A in Basque, Amish, and Reunion Island populations; LGMD type 2B in Libyan Jews; and LGMD type 2C in Gypsies).6 Recently, a novel DYSF gene mutation with a possible founder effect was reported in the Italian population.7

We describe a new mutation of the DYSF gene with a founder effect that clinically manifested the 3 dysferlinopathy phenotypes (Miyoshi myopathy, LGMD type 2B, and distal anterior dysferlinopathy) in 5 unrelated families that showed the same nonsense mutation in the DYSF gene in a homozygous state.

METHODS

PATIENTS

We studied 8 patients from 5 different families diagnosed in the reference neuromuscular center in Valencia, which provides health care for 4 million people. We identified these patients from a cohort of 17 dysferlinopathies seen at this center because they all came from Sueca, Spain, a town of 23,000 inhabitants. Clinical assessment was performed with a standardized protocol, including the Medical Research Council scale, a modified Vignos and...
Archibald disability scale, a serum creatine kinase level, electromyography, and a muscle computed tomographic scan. We received informed consent from all patients enrolled in the study.

**MUSCLE BIOPSIES**

Muscle biopsy specimens were obtained from at least one member of each family. Serial frozen muscle sections were stained using monoclonal antibodies to dysferlin, dystrophin, and α-, β-, γ-, and 8-sarcoglycans (Novocastra, Newcastle, England). Samples were developed with diaminobenzidine (Vector Laboratories, Burlingame, Calif). Histochemistry (using hematoxylin-eosin and modified Gomori trichromic stains) was performed on consecutive sections.

**GENETIC ANALYSIS**

Total genomic DNA from the peripheral blood of the patients was used as a template for polymerase chain reaction amplification analysis of each exon using primers. We used single-stranded conformation polymorphism analysis to screen each exon as described. The samples showing an altered electrophoretic migration were amplified, purified with a polymerase chain reaction purification kit (QIAquick column; Qiagen, Studio City, Calif), and analyzed by direct forward and reverse sequencing with a DNA sequencing kit (Applied Biosystems, Foster City, Calif) on a DNA automatic sequencer (ABI PRISM 310).

DNA samples from the 8 patients and their family members and from 60 individuals from a control population in Sueca underwent genotypic analysis with 4 intragenic and extragenic microsatellite markers (D2S443, Cy172-H32, 104-sat, and D2S291) using the primers listed in the Genome Database.

**STATISTICAL ANALYSIS**

Haplotype frequencies were statistically estimated using an expectation-maximization algorithm, as implemented in computer software (Arlequin 2.1).

**RESULTS**

We identified 8 patients from 5 unrelated families (Figure 1) who had different clinical profiles (Table). Four patients (patients 1, 2, 3, and 4 from families A and B) presented a Miyoshi phenotype characterized by predominant involvement of the posterior compartment of the legs (Figure 2A and B). In addition, patient 4 reported cramps and exercise-related pain. Varying degrees of proximal weakness in the lower extremities were observed during the course of the disease. Patient 4 had no proximal involvement after 8 years of follow-up, and patients 2 and 3 were able to climb stairs; patient 1 was unable to do so and needed help to stand up. Three patients presented the distal anterior dysferlinopathy phenotype (patients 5, 6, and 7 in families C and D) (Figure 2C and D). The initial clinical examination revealed severe distal weakness, predominantly in the anterior compartment, with a steppage gait, and mild proximal weakness in the lower extremities. Initially, patients were investigated for familial neuropathy. Progression to proximal weakness in the upper limbs was detected in patients 5 and 6, whereas weakness remained restricted to the thighs and legs in patient 7. Patient 8 presented with juvenile pelvic-femoral weakness (LGMD phenotype), which rapidly evolved toward an important degree of disability (Figure 2E).

All the patients disclosed high levels of serum creatine kinase (Table). Electromyography revealed the presence of spontaneous activity and low-amplitude, short-duration, and polyphasic motor unit potentials in all patients. Moreover, abundant spontaneous electromyographic activity at rest and complex repetitive discharges were found in 5 of them (patients 1, 2, 3, 6, and 8) (Table). The results of a muscle biopsy disclosed dystrophic features without vacuoles; in addition, endomysial mononuclear infiltration, particularly around necrotic fibers undergoing phagocytosis, was patent in patients 5, 6, and 8 in a pattern similar to that previously described. All specimens lacked dysferlin in the sarcolemma, while a normal pattern of expression was detected for dystrophin and sarcoglycans in immuno-histochemical analyses.

The genetic study showed an abnormal single-stranded conformation polymorphism pattern in exon 51 of the DYSF gene in all patients. This exon was directly sequenced, and the same mutation was identified in homozygosity: a C-to-T transition at nucleotide position 6086 (codon 1905). This changes arginine into a stop codon (R1905X), leading to premature termination of translation. The single-stranded conformation polymorphism undertaken in the family members confirmed the heterozygous state of the parents and enabled us to perform a carrier diagnosis. The mutation was not found in any of the 9 patients with dysferlinopathy outside of Sueca.

The fact that the mutation was absent in 168 control chromosomes tested provides strong evidence that this mutation was the cause of the disease and not a coincidental polymorphism.

In the 5 pedigrees, all the patients were homozygous for the tested markers (D2S443, Cy172-H32, 104-sat, and D2S291) and shared the same 1-5-2-6 haplotype (allele 1 = 223 base pairs, allele 5 = 215 base pairs, allele 2 = 154 base pairs, and allele 6 = 190 base pairs). The maximum frequency of this haplotype calculated in DNA samples from control individuals from Sueca was estimated at 0.02.

**Figure 1.** Pedigrees of the investigated families. Circles indicate females; squares, males; unshaded symbols, unaffected individuals; shaded symbols, individuals with a dysferlin gene mutation; half-shaded symbols, carriers of a dysferlin gene mutation; and symbols with diagonal lines, deceased individuals.
This low frequency is not unusual given that 78 different haplotypes were estimated to be present (at a frequency of >0.05). Consequently, the haplotype studies constitute further evidence that the R1905X mutation occurred just once in a specific founder haplotype.

### Table. Clinical Profile of Patients With Dysferlinopathy From Sueca, Valencia, Spain

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient No./Sex/Age, y</th>
<th>Presenting Symptoms</th>
<th>Initial Muscle Involvement</th>
<th>Duration, y</th>
<th>Progression</th>
<th>CK Level, U/L</th>
<th>EMG Findings</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1/M/15</td>
<td>Difficult to run</td>
<td>Distal posterior compartment</td>
<td>19</td>
<td>Severe in PLL muscles and mild in PUL muscles</td>
<td>6970</td>
<td>Sp activity and CRDs</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>2/F/14</td>
<td>Difficult to walk on her toes</td>
<td>Distal posterior compartment</td>
<td>17</td>
<td>Mild in PLL and PUL muscles</td>
<td>4490</td>
<td>Sp activity and CRDs</td>
<td>MM</td>
</tr>
<tr>
<td>B</td>
<td>3/F/23</td>
<td>Difficult to stand on her toes</td>
<td>Distal posterior compartment</td>
<td>14</td>
<td>Mild in PUL muscles</td>
<td>5090</td>
<td>Sp activity</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td>4/F/20</td>
<td>Difficult to stand on her toes</td>
<td>Distal posterior compartment</td>
<td>8</td>
<td>None</td>
<td>6111</td>
<td>Sp activity</td>
<td>MM</td>
</tr>
<tr>
<td>C</td>
<td>5/F/18</td>
<td>Steppage gait</td>
<td>Distal anterior compartment</td>
<td>20</td>
<td>Mild in PLL and PUL muscles</td>
<td>5400</td>
<td>Sp activity and CRDs</td>
<td>DAT</td>
</tr>
<tr>
<td></td>
<td>6/F/16</td>
<td>Steppage gait</td>
<td>Distal anterior compartment</td>
<td>20</td>
<td>Mild in PUL muscles</td>
<td>5849</td>
<td>Sp activity and CRDs</td>
<td>DAT</td>
</tr>
<tr>
<td>D</td>
<td>7/F/30</td>
<td>Steppage gait</td>
<td>Distal anterior compartment</td>
<td>25</td>
<td>Mild in PUL muscles</td>
<td>1397</td>
<td>Sp activity</td>
<td>DAT</td>
</tr>
<tr>
<td>E</td>
<td>8/F/14</td>
<td>Difficult to get up from a chair</td>
<td>Limb-girdle</td>
<td>10</td>
<td>Severe in PLL and distal LL muscles and mild in PUL muscles</td>
<td>4773</td>
<td>Sp activity and CRDs</td>
<td>LGMD type 2B</td>
</tr>
</tbody>
</table>

Abbreviations: CK, creatine kinase; CRD, complex repetitive discharge; DAT, distal anterior dysferlinopathy; EMG, electromyographic; LGMD, limb-girdle muscular dystrophy; MM, Miyoshi myopathy; PLL, proximal lower limb; PUL, proximal upper limb; Sp, spontaneous.

We describe a novel mutation in the DYSF gene in 8 patients from 5 unrelated families from Sueca. The facts that the 5 families came from the same town and that all the affected members presented the same haplotype in a homozygous state for markers in the DYSF locus indicate that the R1905X mutation has a founder effect. In addition, we demonstrated that this haplotype is found in a low frequency in control individuals from Sueca. Furthermore, the mutation has not been found outside the area. The common ancestor might have lived many centuries ago. Sueca was founded in 1245 by 17 settlers belonging to the Hospital Order (Orden de los Hospitalarios). This order received land from King James I of Aragon as a reward for help in reconquering Valencia from the Moors. To our knowledge, a founder mutation in the DYSF gene has been reported in 12 Libyan inbred Jewish families. However, despite the clinical variability in the Libyan Jewish patients, only the LGMD type 2B phenotype was observed. Recently, another possible founder mutation was described in the Italian population. These researchers describe 2 families with LGMD type 2B bearing the R959W mutation, which was previously reported in the Leiden database in a patient with Miyoshi myopathy.

One feature of the dystrophic patients from Sueca is that an identical mutation is expressed as 3 different clinical phenotypes, but in our patients only 1 phenotype is expressed in the same family. Although the number of patients in each family is small, and this phenotypic expression could be by chance, perhaps the intrafamilial homogeneity observed in our patients, together with the interfamilial heterogeneity, could help understand which modifying factors play a role in the different manifestations of the disease. Earlier studies have demon-
strated that members of the same family with an identical mutation in the DYSF gene present the Miyoshi myopathy or LGMD phenotype, but not the distal anterior dysferlinopathy phenotype.

All these observations support the existence of modifying factors or genes that may account for the clinical variability observed in patients bearing the same mutation. Furthermore, linkage to chromosome 10 has been reported in families displaying a Miyoshi-like phenotype, whereas a third family with similar features did not link either to chromosome 2 or 10. These findings indicate 2 situations: (1) different phenotypes are produced by the same mutation in a given gene, as observed in our patients; or (2) the same phenotype is produced by mutations in different genes. The modifying factors that interact with a gene product to shape the specific phenotype remain to be elucidated. Recently, it has been demonstrated that annexins A1 and A2 interact with dysferlin in skeletal muscle; polymorphisms in these genes could be responsible for the phenotype variability. The families described herein should be helpful in identifying such factors.

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REFERENCES