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

Juan J. Vilchez, MD; Pia Gallano, PhD; Eduard Gallardo, PhD; Adriana Lasa, PhD; Ricardo Rojas-García, MD; Alba Freixas, BSc; Noemí De Luna, BSc; Francesc Calafell, PhD; Teresa Sevilla, MD; Fernando Mayordomo, MD; Montserrat Baiget, PhD; Isabel Illa, MD

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.

Arch Neurol. 2005;62:1256-1259
the legs (dominant involvement of the posterior compartment of
B) presented a Miyoshi phenotype characterized by pre-
proximal weakness in the upper limbs was detected in
were investigated for familial neuropathy. Progression to
terior compartment, with a steppage gait, and mild proxi-
vealed severe distal weakness, predominantly in the an-
(Figure 2C and D). The initial clinical examination re-
no proximal involvement after 8 years of follow-up, and
observed during the course of the disease. Patient 4 had
reported cramps and exercise-related pain. Varying de-
rees of proximal weakness in the lower extremities were
received informed consent from all patients enrolled in the study.

MUSCLE BIOPSIES

Muscle biopsy specimens were obtained from at least one mem-
ber 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).3
Samples were developed with dianibenozidine (Vector Lab-
ratories, 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 amplifi-
cation analysis of each exon using primers. We used single-
stranded conformation polymorphism analysis to screen each
exon as described.3 The samples showing an altered electro-
phoretic migration were amplified, purified with a polymer-
ase chain reaction purification kit (QIAquick column; Qia-
gen, Studio City, Calif), and analyzed by direct forward and
reverse sequencing with a DNA sequencing kit (Applied Bio-
systems, Foster City, Calif) on a DNA automatic sequencer (ABI
PRISM 310).

DNA samples from the 8 patients and their family mem-
bers and from 60 individuals from a control population in Sueca
underwent genotypic analysis with 4 intragenic and extra-
genic 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 ex-
pectation-maximization algorithm,9 as implemented in com-
puter software (Arlequin 2.1).9

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 predomi-
lar involvement of the posterior compartment of the legs (Figure 2A and B). In addition, patient 4 re-
ported cramps and exercise-related pain. Varying de-
rees 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 pa-
tients presented the distal anterior dysferlinopathy phe-
notype (patients 5, 6, and 7 in families C and D) (Figure 2C and D). The initial clinical examination re-
vealed severe distal weakness, predominantly in the an-
terior compartment, with a steppage gait, and mild proxi-
mal 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 pheno-
type), which rapidly evolved toward an important de-
gree of disability (Figure 2E).

All the patients disclosed high levels of serum creat-
ine kinase (Table). Electromyography revealed the pres-
ence of spontaneous activity and low-amplitude, short-
duration, and polyphasic motor unit potentials in all
patients. Moreover, abundant spontaneous electromyo-
graphic activity at rest and complex repetitive dis-
charges were found in 5 of them (patients 1, 2, 3, 6, and
8) (Table). The results of a muscle biopsy disclosed dys-
rophic features without vacuoles; in addition, endo-
mysial mononuclear infiltration, particularly around ne-
rotic fibers undergoing phagocytosis, was patent in
patients 5, 6, and 8 in a pattern similar to that previ-
ously described.10 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 di-
rectly sequenced, and the same mutation was identified in
homozygosity: a C-to-T transition at nucleotide po-
sition 6086 (codon 1905). This changes arginine into a
stop codon (R1905X), leading to premature termina-
tion of translation. The single-stranded conformation polymorphism undertaken in the family members con-
firmed the heterozygous state of the parents and en-
abled us to perform a carrier diagnosis. The mutation
was not found in any of the 9 patients with dysfer-
linopathy outside of Sueca.

The fact that the mutation was absent in 168 control
chromosomes tested provides strong evidence that this mu-
nation was the cause of the disease and not a coinci-
dental 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.
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.

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 us understand which modifying factors play a role in the different manifestations of the disease. Earlier studies have demon-

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.
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.

Accepted for Publication: February 7, 2005.

Correspondence: Isabel Illa, MD, Department of Neurology, Hospital Universitari de la Santa Creu i Sant Pau, Av Pare Claret, 167, 08025 Barcelona, Spain (illa@hspsantpau.es).

Author Contributions: Study concept and design: Vilchez, Gallardo, Baiget, and Illa. Acquisition of data: Vilchez, Gallano, Lasa, Rojas-García, Freixas, De Luna, Sevilla, and Mayordomo. Analysis and interpretation of data: Vilchez, Gallano, Gallardo, Calafell, and Illa. Drafting of the manuscript: Vilchez, Gallano, Gallardo, Lasa, Freixas, De Luna, Calafell, and Illa. Critical revision of the manuscript for important intellectual content: Gallardo, Rojas-García, Sevilla, Mayordomo, Baiget, and Illa. Statistical analysis: Lasa, Freixas, and Calafell. Obtained funding: Vilchez, Gallano, Gallardo, Baiget, and Illa. Administrative, technical, and material support: De Luna, Sevilla, and Mayordomo.

Study supervision: Vilchez, Rojas-García, and Illa.

Funding/Support: This study was supported by grants FIS 99/0019-02, FIS 01/0979, and P102/0388 from the Fondo de Investigaciones Sanitarias, grant C03/05 from Red INERGEN, and grant C03/06 from Red CIEN, Madrid, Spain; Association Française contre les Myopathies, Paris, France; and grant CTIDIB/2002/328 from Generalitat Valenciana, Valencia, Spain.

Disclaimer: Drs Vilchez and Gallano contributed equally to this work.

Acknowledgment: We thank the patients and their relatives for their collaboration; Jose Segarra from the town hall of Sueca for supplying historical documentation; Encarná García for technical assistance; and Kate Bushby, MD, PhD, for providing the primers used in this study.

REFERENCES