Phenotypic Study in 40 Patients With Dysferlin Gene Mutations

High Frequency of Atypical Phenotypes

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Objective: To describe the phenotypic spectrum of dysferlin (DYSF) gene mutations (which cause dysferlinopathies, autosomal recessive muscular dystrophies) in patients with a dysferlin protein deficiency.

Design: Clinical, biological, and pathological data from 40 patients were reviewed. The diagnosis of dysferlinopathy was based on the absence or strong reduction of dysferlin in muscle, and confirmed by mutational screening of the DYSF gene.

Setting: Two French neuromuscular diseases centers (in Paris and Marseilles).

Results: Two main dysferlinopathy phenotypes are well recognized: Miyoshi myopathy and limb-girdle muscular dystrophy type 2B. Typical Miyoshi myopathy and limb-girdle muscular dystrophy type 2B were found in 20 (50%) patients only. Unusual phenotypes included a mixed phenotype, referred to as “proximodistal,” combining distal and proximal onset in 14 (35%) patients, pseudometabolic myopathy in 4 (10%), and asymptomatic hyperCKemia (an increased serum creatine kinase level) in 2 (5%). The disease may worsen rapidly, and 10 (25%) patients were initially misdiagnosed as having polymyositis. We suggest a relationship between proximodistal phenotype, inflammation, and severity.

Conclusion: In addition to typical Miyoshi myopathy and limb-girdle muscular dystrophy type 2B, dysferlinopathies are a clinically heterogeneous group of disorders ranging from asymptomatism to severe functional disability.

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DYSFERLINOPATHIES ARE AUTOSOMAL RECESSIVE MUSCULAR DYSTROPHIES CAUSED BY MUTATIONS IN THE DYSFERLIN (DYSF) GENE (Mendelian Inheritance in Man 603009).1,2 Highly decreased dysferlin expression, demonstrated by Western blot (WB) and immunohistochemistry (IHC) of muscle samples, remains the mandatory criterion for positive diagnosis and indicates gene screening.3 Dysferlin deficiency causes 2 main phenotypes: a distal Miyoshi myopathy (MM) and limb-girdle muscular dystrophy type 2B (LGMD2B).4 Both MM and LGMD2B show onset in young adults, slow course of the disease, and massive increase in serum creatine kinase (CK) level. However, MM affects the posterior compartment of the legs, while LGMD2B affects the pelvic girdle at onset.5 All attempts to correlate phenotypes with DYSF gene mutations have been unsuccessful because identical mutations may lead to either MM or LGMD2B.6,7 Screening for DYSF gene mutations is a challenging task because of the large size of the gene and the absence of a mutational hot spot.8,9 Thus, few large series10,11 of patients with DYSF gene mutations have been studied on a phenotypic basis. Herein, we describe a cohort of 40 patients with dysferlinopathy and focus on the clinical, biological, and histological phenotypes.

METHODS

We retrospectively reassessed clinical data from 40 patients with a dysferlin deficiency followed up in 2 main French centers (Institute of Myology, La Pitié-Salpêtrière, Paris, and the Department of Neuromuscular Diseases, Marseilles) based on the following inclusion criteria: (1) complete or nearly complete dysferlin protein loss in muscle samples (cutoff, <20% of normal expression), evidenced by WB and IHC or WB alone in the proband (N=37,
including 32 probands and 5 affected siblings with no protein study), or linkage to the DYSF locus on 2p13 in the absence of dysferlin protein analysis (n=3) and (2) mutations identified in the DYSF gene (n=40).

Altogether, 36 patients had 2 mutations, either 1 homozygous (n=14) or 2 compound heterozygous (n=22), and 4 patients had only 1 heterozygous mutation identified in the DYSF gene. We did not exclude these 4 patients because dysferlin was completely absent in muscle, whereas levels of other proteins (dysferlin, sarcoglycans, and calpain 3) were normal. All patients with no protein study in muscle (n=8), including the 3 affected siblings and the 3 DYSF gene–linked patients, harbored 2 causative mutations in the DYSF gene. The mutations of 29 patients were previously reported, and 11 patients with recently identified mutations in the DYSF gene were added in the study.

To estimate the rate of disease progression, we determined the Walton score13–disease duration ratio at onset and at last examination and considered the slope of the line between the 2 ratios. The CK levels were normalized as x-fold the upper limit of normal values according to the local reference range.

Monoclonal antibodies (NCL-hamlet as antidysferlin, Calp3d/2C4, and Calp3e/12A2 [Novocastra Laboratories, Newcastle upon Tyne, England] as anticalpain 3) were used for WB and/or IHC of normal values according to the local reference range.

The initial mode of onset was the main criterion to classify patients within phenotypic groups (Figure 1B). Ten patients (25%) presented with an MM, weakness, and atrophy, affecting initially and solely the distal posterior compartments of the legs. Ten patients (25%) started with a proximal weakness of the lower limbs, isolated or associated with a proximal weakness of the upper limbs (LGMD2B). In 14 patients (35%), it was not possible to distinguish between an MM and an LGMD2B phenotype because they presented simultaneously with proximal weakness of the lower limbs and calf atrophy. We called this mixed phenotype “proximodistal” (PD). In addition, 4 patients (10%) presented with distal leg painful swelling without any weakness or atrophy, affecting 1 (n=3) or both (n=1) calves, suggestive of thrombophlebitis or metabolic myopathy. We classified this phenotype as “pseudometabolic.” Two asymptomatic patients (5%) had isolated hyperCKemia (an increased serum CK level) fortuitously discovered on routine blood analysis. The mean ± SD age of onset was 22.7 ± 8.2 years (range, 12-45 years) (Figure 1C).

No difference was found in age of onset between the 2 major ethnic groups (European and North African), and between the 3 major phenotypic groups (MM, LGMD2B, and PD). The 2 asymptomatic patients were a 28-year-old woman and a man referred at the age of 50 years for CK elevation (15-30 xN) with still no muscle weakness or atrophy at the age of 58 years despite skiing and jogging. Magnetic resonance imaging showed mild signal abnormalities in gastrocnemius muscles. The results of WB and IHC showed a complete loss of dysferlin. Two missense mutations were identified in the DYSF gene (patient 39 of family 34) (Table 1).

Symptoms and signs at onset are summarized in Table 2. First symptoms occurred after a period of intensive sports practice in 7 patients. Difficulties in standing on tiptoes were more prevalent at onset in patients...
<table>
<thead>
<tr>
<th>Family</th>
<th>Geographic Origin</th>
<th>Phenotype</th>
<th>Nucleotide Change</th>
<th>Protein Change</th>
<th>State</th>
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**Abbreviations:** Comp hetero, compound heterozygous; Hetro, heterozygous; Homo, homozygous; HyperCK, hyperCKemia (increased serum creatine kinase level); LGMD2B, limb-girdle muscular dystrophy type 2B; LMDD, Leiden muscular dystrophy database (available at: http://www.dmd.nl/); MM, Miyoshi myopathy; PD, proximodistal; PM, pseudomemetic.

* Three truncating and 2 missense mutations are novel. In 4 patients, only 1 heterozygous mutation was identified.
with MM than in those with LGMD2B or PD disease. The other symptoms at onset showed no difference. Selective biceps brachia atrophy with a particular bowl shape was present in 11 of 28 patients (39%) (Figure 2).

Ten patients (25%) were misdiagnosed as having polymyositis before dysferlin protein studies because of histological inflammatory muscle infiltrates associated with rapid progression and/or pain. All patients received corticosteroids, and some also received immunosuppressive drugs. Misdiagnosis of polymyositis was more frequent in the PD group (6 of 14 patients [43%]) vs the MM group (1 of 10 patients [10%]) and the LGMD2B group (1 of 10 patients [10%]) (P = .08). Among the 4 pseudometabolic patients, 2 were considered as having polymyositis and 1 as having focal myositis.

At last examination, the mean ± SD age was 36.0 ± 11.8 years (range, 19-59 years) and the mean ± SD disease duration was 13.3 ± 9.4 years (range, 3-41 years). Most patients (30 [75%]) showed distal and proximal lower limb weakness and wasting. In the MM group, the disease consistently progressed proximally in the lower limbs. Calf atrophy was present in 30 of 38 patients (79%). Contractures affected the triceps surae in 20 of 32 patients (62%) and more rarely the hamstrings, biceps brachia, and finger flexors (n = 6). No patient had early contractures, scapular winging, or facial, bulbar, or cardiac involvement (by electrocardiography and echocardiography). Respiratory vital capacity at last examination was normal in 25 of 27 patients (mean, 93% of average value) and reduced in 2 patients (2.4 L [47% of average value] and 3.9 L [75% of average value]).

Of the 40 patients, 11 (28%) needed a wheelchair permanently and 15 (38%) used it outdoors only. The mean ± SD age at the first use of wheelchair was 33.6 ± 11.7 years (range, 18-58 years) with a mean ± SD disease duration of 15.4 ± 8.4 years (range, 3-41 years). Most patients (30 [75%]) showed distal and proximal lower limb weakness and wasting. The MM group (1 of 10 patients [10%]) and the LGMD2B group (1 of 10 patients [10%]) (P = .08). Among the 4 pseudometabolic patients, 2 were considered as having polymyositis and 1 as having focal myositis.

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The rate of disease progression tended to be higher in the PD group than in the MM or LGMD2B group, and higher in patients misdiagnosed as having polymyositis, although the differences were not significant (P = .07). Among the 8 patients with rapid progression, 4 had PD disease, 3 were misdiagnosed as having polymyositis, and 1 combined both. The latter case is a man from Morocco, who first experienced difficulties in practicing the long jump at the age of 16 years. Weakness progressed rapidly over 5 years because he was not able to lift his arms at the age of 19 years and lost ambulation at the age of 21 years. Weakness and atrophy were pronounced in the calves and thighs. Neck extensors and abdominal muscles were severely affected, and biceps brachia were bowl shaped (Figure 2). The last examination, at the age of 23 years, showed no involvement of cardiac muscles (left ventricular ejection fraction, normal), but a mild restrictive ventilatory defect (vital capacity, 3.95 L [75% of average value]). The CK levels were 30 xN. Two gastrocnemius biopsies performed in Morocco had shown inflammation suggestive of polymyositis, and the patient received corticosteroids and immunosuppressive drugs without benefit. A third deltoid

Table 2. Frequency of Symptoms and Signs at Onset Depending on the Phenotype

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<tr>
<th>Phenotype</th>
<th>Difficulties</th>
<th>Difficulties</th>
<th>Difficulties</th>
<th>Difficulties</th>
<th>Swelling of the Calves</th>
<th>Muscle Pain</th>
<th>Steppage Gait</th>
<th>Waddling Gait</th>
<th>Asymmetry</th>
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<tbody>
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<td>MM (n = 10)</td>
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<td>6</td>
<td>1</td>
<td>5</td>
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<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
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<td>LGMD2B (n = 10)</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>PD (n = 14)</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
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</tbody>
</table>

Abbreviations: See Table 1.

aData are given as number of patients.
biopsy at the age of 23 years showed constant inflammation and complete dysferlin deficiency. A homozygous frame shift mutation was identified in the DYSF gene (Patient 24 of family 19) (Table 1).

We looked at the intrafamilial variability in 4 families (9 patients). The age of onset and progression of the disease and the presence of inflammatory infiltrates were heterogeneous, but mode of onset was rather homogeneous. We did not observe proximal and distal onset in the same family (Table 3).

**SERUM CK LEVEL.**

The mean±SD minimal level of CK was 19.2±12.9 xN at a mean±SD age of 34.8±11.9 years. The mean±SD maximal level was 54.8±47.9 xN at a mean±SD age of 28.8±8.9 years. The CK levels tended to decrease during the disease course (Figure 3A). We found no difference in CK levels between the 3 major phenotypes and no relationship between the CK levels and disease progression. The intraindividual variability of the CK levels during the disease course is illustrated in Figure 3B.

**PATHOLOGICAL FEATURES AND PROTEIN STUDIES.**

A total of 32 muscle samples, corresponding to the 32 patients first included, were available. The mean±SD age at biopsy was 31.8±13.0 years, while the mean±SD disease duration was 10.3±10.0 years. Samples were retrieved from deltoid (n=19), quadriceps (n=10), biceps brachia (n=2), and gastrocnemius (n=1) muscles. All exhibited necrosis and regeneration, and 19 of 32 (59%) showed different extent of fibrosis. However, fibrosis correlated with neither the phenotype nor the rate of disease progression. Inflammation was observed in 11 of 32 samples (34%) and was more frequent in the PD phenotype (6 of 14 patients) than in the MM (2 of 10 patients) or LGMD2B (1 of 10 patients) phenotype, although not significant (P = .08). There was no difference in age of onset, CK levels, or muscle pain in patients with inflammation vs those without inflammation. However, the rate of progression was higher in patients with inflammation and the difference was slightly significant (P = .045). Study of HLA class I expression on the sarcolemma was not systematically performed. In only 1 patient (Patient 31 of family 26), we observed a diffuse sarcolemmal HLA class I expression in nonnecrotic/regenerating fibers.

Western blot was performed in all of the 32 samples and IHC in 22 of the 32 samples. By WB, dysferlin was absent in 21 of 32 samples (66%) or strongly decreased in 11 of 32 samples (34%). By IHC, dysferlin was absent in 19 of 22 samples (86%) or decreased in 3 of 22 samples (14%). The cases with decreased dysferlin by IHC had no dysferlin band by WB. No other discrepancies were observed between WB and IHC findings. A secondary calpain 3 reduction was observed in 6 of 30 biopsy specimens (20%). Neither residual dysferlin expression nor secondary calpainopathy correlated with the phenotype or the rate of disease progression.

**IMAGING STUDIES.**

A muscle computed tomographic scan (n=29), which showed hypodensity and atrophy, was in agreement with clinical features, confirming distal, proximal, or PD involvement. All the patients with pseudometabolic onset...
or asymptomatic hyperCKemia showed slight to severe leg posterior compartment abnormalities suggestive of muscular dystrophy.

**COMMENT**

Dysferlinopathies are muscular dystrophies classically characterized by early adulthood onset, slow course, massive CK elevation, and, in some cases, inflammation in muscle. The present series reports some unusual findings. First, the age of onset was highly variable. One asymptomatic 58-year-old man had isolated hyperCKemia. Although late onset at the age of 45 years has been reported recently and hyperCKemia is known to be a prelude of dysferlinopathies, prolonged asymptomatic in adulthood is unusual and extends the severity spectrum toward the mildest. In a series of patients with MM, 8 of 24 required a wheelchair after a 10-year disease duration. In agreement, we found a similar ratio, and we confirmed that LGMD2B does not have a worse course than MM. Conversely, we observed few patients with a severe course of the disease. The most severe case (Patient 24 of family 19) worsened over 5 years from a PD onset to complete loss of ambulation, also with severe upper limb and axial weakness. This unexpected finding in dysferlinopathies extends the spectrum toward the most severe.

In addition, the classic MM and LGMD2B phenotypes accounted for only 20 (50%) cases. Despite systematic clinical examination at onset of symptoms, 14 (35%) patients could not be classified in either group. Instead, they showed a mixed phenotype with proximal and distal lower limb weakness. This phenotype may be an MM with a fast proximal progression. However, those patients did not match the criteria for MM or LGMD2B, as described initially.

The notion of a mixed phenotype recently emerged under the term of distal limb-girdle dystrophy. We suggest that this phenotype is frequent and should be added to the classic MM and LGMD2B phenotypes. We did not observe in our series distal myopathy with anterior tibial onset. Painful calf swelling at onset, as reported to the classic MM and LGMD2B phenotypes. We did not observe that this phenotype is frequent and should be added as described initially. We did not observe co-occurrence of LGMD2B and MM in a single family. Both LGMD2B and MM have been reported in different branches of large families, but not in the same sibship, probably because of modifier genes. In addition, we observed large intrafamilial variation of CK levels during the disease course, which makes it difficult to correlate with the phenotype. Surprisingly, we observed a pseudodominant inheritance in 1 family: the Algerian affected father carried 2 heterozygous DYSF gene mutations (Patient 15 of family 13). 1 being transmitted to his affected daughter (Patient 16 of family 13), while she inherited the other mutation from her unaffected and unrelated French mother. This finding is unexpected in such a rare condition.

Mutations identified in our patients were diverse, spreading along the coding sequence without any mutational hot spot. Some mutations were recurrent in patients of different ethnic origins. The 4 prevalent mutations reported in the Japanese population were not found. Missense mutations have been suggested to cause more severe manifestations of disease with higher CK levels. In our series, the most severe case (Patient 24 of family 19) carries 1 homozygous truncating mutation, previously found in the homozygous state in classic MM and in distal myopathy with anterior tibial onset. Conversely, the patient with less severe manifestations of disease (Patient 39 of family 34) carries 2 new missense mutations. Therefore, missense mutations are not always associated with severe forms. Genotype-phenotype correlations are not obvious, except that in the Italian population, patients with MM have symptoms earlier than patients with LGMD2B. In our series, we found no relationship between age of onset and phenotype. In 4 patients, we identified only 1 DYSF gene mutation. Several explanations might be proposed: (1) incomplete sensitivity (95%) of the screening method and (2) large genomic rearrangements and intronic variations that remain undetected by this method.

Most described patients with dysferlinopathy carry point mutations. Such large rearrangements, yet undescribed in the DYSF gene, may account for the rare patients with no or only 1 mutation routinely detected.

In summary, the present study provides novel aspects of the phenotypic spectrum of dysferlinopathies, particularly regarding the mode of onset and severity and the high frequency of the mixed PD phenotype.

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