Phenotypic Features and Genetic Findings in 2 Chinese Families With Miyoshi Distal Myopathy

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Background: Miyoshi distal myopathy (MM) and limb girdle muscular dystrophy type 2B (LGMD2B) were found to map to the same mutant gene encoding for dysferlin on chromosome 2p13. Most reported cases were large inbred kindreds whose members demonstrated both MM and LGMD2B phenotypes.

Objective: To investigate the clinical, neurophysiological, histopathological, and genetic features in 4 patients with MM from 2 unrelated Chinese families demonstrating linkage to the dysferlin locus.

Results: All patients were characterized by early adult onset, preferential atrophy, and weakness of calf muscles, marked elevation of serum creatine kinase levels, and absence of dysferlin staining. Magnetic resonance imaging showed fatty and fibrotic tissue signals in the affected muscles. Genetic analysis revealed novel compound heterozygous mutations, 1310+1G to A and G to T transition at nucleotide 1650 (G426V) in one family and another novel compound heterozygous mutation, a deletion of C at nucleotide 477 and a C to TG transition at nucleotide 6576 (P2068L), in the other family.

Conclusion: Miyoshi distal myopathy in these 2 Chinese families demonstrated a homogenous phenotype and compound heterozygous mutations. Among the 4 mutations, 3 were novel mutations that, to our knowledge, have not been reported previously.

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Miyoshi distal myopathy (MM) is a rare progressive muscular dystrophy. The disease gene has been mapped to chromosome 2p12-14, with its product named “dysferlin.” The clinical features of this disorder include autosomal recessive inheritance, onset between 15 and 25 years of age, early and predominant involvement of the gastrocnemius muscle with sparing of the anterior tibialis muscle, high serum creatine kinase levels, and absence of dysferlin staining in muscle specimens. Phenotypic expressions of the dysferlin gene mutations are heterogeneous because identical gene mutations in a large inbred family can produce both limb girdle muscular dystrophy type 2B (LGMD2B) and MM. Modifier gene(s) or additional factors must be responsible for the differences in the phenotype. Miyoshi distal myopathy seems to be widely distributed throughout the world but, to our knowledge, has not been reported in Taiwan. In this study, we investigated the clinical, neurophysiological, histopathological, and genetic features of 4 patients with MM from 2 unrelated Chinese families.

METHODS

We examined 4 patients with MM from 2 unrelated Chinese families (families A and B) living in Taipei, Taiwan (Figure 1). Their clinical and laboratory features are listed in Table 1.

FAMILY A

Three siblings (2 sisters and 1 younger brother) aged 23 (AII:1), 22 (AII:2), and 19 years (AII:3), respectively, from a nonconsanguineous family started to experience difficulty in toe walking from the age of 17 through 18 years. Difficulty in climbing stairs and in rising from a squatting position slowly progressed for 2 to 5 years. On physical examination, there was muscle wasting and bilateral weakness in the lower legs in the 2 sisters, which was not apparent in the brother. A moderate to severe degree of weakness in the gastrocnemius and soleus muscles and a mild degree of weakness in the hamstring and quadriceps femoris muscles were noted in all 3 patients. However, the anterior tibialis muscles were relatively spared. The tendon reflexes were all symmetric, but
they were decreased or absent in the ankle. Magnetic resonance imaging (MRI) demonstrated a mild to moderate degree of increased signal intensity in the posterior compartment of the distal legs, especially in the gastrocnemius and soleus muscles, with the anterior tibialis relatively spared (Figure 2A-C).

FAMILY B

A 17-year-old male patient (BII:2) from a nonconsanguineous family had developed difficulty running since the age of 15 years. One year later he had weakness in both legs when climbing stairs. His symptoms progressed to having difficulty in rising from a squatting position 6 months prior to admission to the hospital. No other family members had similar symptoms. Findings from neurological examination revealed mild bilateral weakness in the hamstring muscles and moderate bilateral weakness in the gastrocnemius muscles. The ankle jerks were absent and other tendon reflexes were relatively preserved. Magnetic resonance imaging showed a mild degree of increased signal intensity in the hamstring and gastrocnemius muscles (Figure 2D) bilaterally.

IMMUNOHISTOCHEMICAL STAINING

Frozen muscle sections (8-µm thick) were placed on slides (Dako, Kyoto, Japan) and allowed to air dry for 2 hours. Dysferlin immunohistochemistry was performed using mouse antihuman monoclonal antibodies, including DY1 at a 1:20 dilution, DY2 at 1:20, and DY3 at 1:20 (Novocastra, Newcastle, England), to identify the midrod, carboxyl (C)-terminus, and amino (N)-terminus, respectively. Analysis of the sarcoglycan complex was performed using mouse antihuman monoclonal antibodies, including α-sarcoglycan (1:200 dilution), β-sarcoglycan (1:200 dilution), γ-sarcoglycan (1:100 dilution), and δ-sarcoglycan (1:50 dilution) (Novocastra). Immunohistochemical studies using mouse monoclonal antibodies (Novocastra) to merosin (1:100 dilution), emerin (1:40 dilution), dystroglycans (1:20 dilution), and dysferlin (1:40 dilution) were also performed.

HAPLOTYP ANALYSES

Blood samples were collected from 4 patients and 3 unaffected family members after obtaining informed consent. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. Haplotype analyses were performed using 3 highly informative microsatellite markers D2S292, D2S286, and D2S169, spanning the dysferlin gene on chromosome 2p, as described previously.2

ANALYSIS OF THE NUCLEOTIDE SEQUENCE OF GENOMIC DNA

DNA was prepared from leukocytes according to standard procedures. We screened all 55 exons of the dysferlin gene. Polymerase chain reaction of dysferlin exons, including adjacent intronic regions, was performed, using primers and conditions described at the Neurology Web site.5 Genomic DNA (100 ng) was added in a 25-µL polymerase chain reaction containing 10 mM Tris, pH 8.3; 50 mM potassium chloride; 0.1% Triton X-100; 0.2mM deoxyribonucleotide triphosphate; 0.4µM of each primer, and 0.5 U of Taq polymerase (Promega Corp, Madison, Wis). Cycling conditions were as follows: an initial 6-minute denaturation at 94°C followed by 35 cycles of 30-second denaturation at 94°C; 30-second annealing at 52°C; and 30-second extension at 72°C; with a final 10-minute extension step at 72°C. Polymerase chain reaction products were sequenced directly using the DYEnamic ET Dye Terminator Kit and MEGA BACE Analyzer (Molecular Dynamics, Division of Amersham Pharma Biotech, Buckinghamshire, England).

Table 1. Clinical and Laboratory Findings of 2 Chinese Families With Miyoshi Distal Myopathy

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient Age at Symptom Onset, y</th>
<th>Patient Age at Diagnosis, y</th>
<th>MRC Scale Score</th>
<th>Serum CPK Level, U/L</th>
<th>EMG, Fibs/PSWs</th>
<th>MRI Fatty Infiltration</th>
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<td>4</td>
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<td>5</td>
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<tr>
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<td>19</td>
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<td>5</td>
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<td>15</td>
<td>17</td>
<td>4+ 5</td>
<td>5+ 5−</td>
<td>5</td>
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</tr>
</tbody>
</table>

Abbreviations: CPK, creatine kinase; EMG, electromyography; Fibs, fibrillations; G, gastrocnemius; H, hamstring; MRC, Medical Research Council muscle power scale; MRI, magnetic resonance imaging; NA, not available; PSWs, sharp positive waves; Q, quadriceps; TA, tibialis anterior; 1+, mild; 2+, moderate; 3+, severe; −, absent. Note that muscle power deficit correlates with degrees of fatty infiltration. All tested anterior tibialis muscles on the EMG showed abnormal Fibs/PSWs although no abnormality was found in MRI and MRC muscle power test.
RESULTS

IMMUNOHISTOCHEMICAL STAINING

In muscle biopsy specimens from cases AII:3 and BII:2, immunohistochemical stains of DY (Figure 3A and E), sarcoglycan (Figure 3B and F), dystroglycan, emerin, and merosin (Figure 3C and G) were distributed in a normal pattern. However, dysferlin was totally absent or markedly reduced (Figure 3D and H).

HAPLOTYPE ANALYSES

These markers in all patients from both families were heterozygous, indicating that all patients were from non-consanguineous families (Figure 1).

DETECTION OF DYSFERLIN GENE MUTATION

All dysferlin exons from cases AII:1 through AII:3 and BII:2 were amplified and sequenced. Four heterozygous mutations were found. Mutations 1310+1G to A, G426V, and P2068L were detected by direct sequencing, whereas 477delC was revealed by cloning and sequencing. The 3-point mutations were further confirmed by restriction enzyme digestion (Table 2 and Figure 4A and B). Both 1310+1G to A and G426V mutations were found in all patients in AII:1 through AII:3. Both 477delC and P2068L were detected in BII:2.

COMMENT

The clinical and laboratory findings of the 4 cases were consistent with the diagnosis of MM. They showed distal weakness beginning from 15 through 17 years of age that progressed for 2 through 6 years before the diagnosis of MM was made. The preferential involvement of the posterior compartment muscles, hereditary pattern, and the age at onset of the symptoms may differentiate MM from other distal myopathies, such as late distal hereditary myopathy (or Welander distal myopathy), non-Scandinavian late-onset autosomal dominant distal myopathy, Nonaka distal myopathy, and infantile- and juvenile-onset distal myopathies.

In this study, patients with MM tended to have a longer disease duration, more severe muscle weakness, and a lower creatine kinase level. We found that the myopathic changes shown by electromyography varied greatly among different families. Clinically, muscle weakness was generally more severe in cases AII:1 through AII:3 than in case BII:2. However, electromyography in the upper and lower limb muscles showed a more diffuse, severe degree of spontaneous activities in case BII:2 than cases AII:1 through AII:3.

Recently, MRI has become a useful tool to investigate the distribution of muscle degeneration in neuromuscular disorders. In our patients, gastrocnemius and soleus muscles demonstrated extensive fatty infiltration by MRI, whereas the quadriceps and anterior tibialis muscles were spared, compatible with typical findings of MM. Moreover, a mild degree of abnormality in the proximal hamstring muscles was found before the clinical muscle weakness developed. These findings suggested that MRI might have detected muscle abnormalities in the early stage before clinical signs and symptoms became apparent.

A biopsy specimen of the quadriceps muscle from case AII:3 showed a mild myopathic pathologic condition. In contrast, a severe dystrophic pattern was found in the symptomatic hamstring muscle from case BII:2. The results of muscle pathologic study were consistent with...
those of a previous study. Since muscle involvement in MM varies greatly, the muscle biopsy specimen obtained in a limited area can only establish the diagnosis but cannot predict the full spectrum of disease severity in each individual. Our immunohistochemical study also showed that dysferlin protein levels were markedly reduced in muscle from patients with MM. By contrast, DY expression seemed to be normal in these patients, suggesting that dysferlin was localized at the muscle plasma membrane but might not be associated with the integrity of the DY complex. Membrane repair assays with 2-photon laser-scanning microscopy demonstrated that wild-type muscle fibers efficiently resealed their sarcolemma in the presence of calcium. Interestingly, dysferlin-deficient muscle fibers were defective in calcium-dependent sarcolemma resealing. Bansal et al concluded that membrane repair was an active process in skeletal muscle fibers and that dysferlin had an essential role in this process. In Nicholson and colleagues, muscular dystrophy, a correlation between increasing DY abundance and milder phenotype, has been reported. We did not perform the Western blot analysis with dysferlin monoclonal antibody because of lack of enough skeletal muscle samples. However, there is no correlation between dysferlin abundance and clinical severity.

Analysis of the dysferlin nucleotide sequence of the genomic DNA revealed 4 mutations, 3 (G426V, 477delC, and P2068L) of which have not been previously reported. The novel P2068L lay in the seventh caveolin-3 scaffolding-binding motifs. Amino acid sequence analysis of the dysferlin protein revealed 7 sites that corresponded to caveolin-3 scaffold-binding motifs, and 1 site with a potential target to bind the WW domain of the caveolin-3 protein. One function of dysferlin may interact with caveolin-3 to serve signaling functions of caveolae. Both G426V and P2068L mutations predicted the replacement of 1 evolutionarily conserved amino acid between human and mouse dysferlin. In each case, 100 control chromosomes were screened for mutations, and none was found. The 477delC (a single C-base deletion at codon 35) produced 5 additional, unrelated amino acids at codon 37 followed by early termination. This novel single-nucleotide deletion probably arose by slippage replication. The 1310+1G to A substitution affecting exon 10 splicing donor site has been reported, which predicts blockage of dysferlin expression through false splicing. This mutation has not been detected in 100 normal control chromosomes.

**CONCLUSIONS**

Miyoshi distal myopathy does occur in Chinese patients with typical clinical, electrophysiological, histopathological, and molecular features. Although identical mutations in the dysferlin gene could produce more than 1
phenotype of myopathy,3,4 we did not find a phenotype other than MM in these 2 families. Our findings suggest that a combination of clinical, laboratory, electrophysiological, radiological, histopathological, and molecular analyses is necessary to precisely evaluate the full spectrum of disease severity and progression.

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REFERENCES


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**Correction**

Error in Figure Reproduction. In the article titled “Number Needed to Treat Estimates Incorporating Effects Over the Entire Range of Clinical Outcomes,” published in the July issue of the ARCHIVES (2004;61:1066-1070) Figure 2 was not printed in color. The figure is reproduced here with its legend.

**Figure 2.** Joint outcome distribution tables for model 100 patient population. Outcome under placebo therapy is indicated in rows, under thrombolytic therapy in columns. A, Distribution at start of expert session, with all patients along diagonal in placebo outcome array. B, Distribution at end of one expert’s session, with individual patients redistributed to yield thrombolytic therapy outcome distribution. Patients shifted left, in cells shaded green, have improved because of therapy; patients shifted right, in cells shaded orange, have worsened because of therapy. For example, values in the modified Rankin Scale (mRS) score row 4 indicate that of 20 patients destined for mRS outcome strata 4 under placebo therapy, 3 attain mRS outcome stratum 1 with thrombolysis (cell row mRS 4, column mRS 1), 1 attains mRS outcome stratum 2 (cell row mRS 4, column mRS 2), 4 attain mRS outcome stratum 3 (cell row mRS 4, column mRS 3), 11 attain mRS outcome stratum 4 (cell row mRS 4, column mRS 4) and 1 attains mRS outcome stratum 6 (cell row mRS 4, column mRS 6). Adding all left-shifted (green cell) patients indicates that 35 of 100 patients had better outcome as a result of treatment, yielding individual expert estimate for the number needed to treat (NNT) for benefit of 2.9. Adding all right-shifted (orange cell) patients indicates that 4 per 100 patients have worsened because of therapy, yielding an individual expert estimate for the number needed to harm (NNH) of 25. tPA indicates tissue plasminogen activator.