Clinical Heterogeneity in Dysferlinopathy

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Abstract

Objective To clarify the clinical heterogeneity and genotype-phenotype correlation in dysferlinopathy.

Methods We evaluated clinical parameters of 74 dysferlinopathy patients with known dysferlin gene mutations who were previously reported in the literature.

Results The age at onset varied from 12 to 59 years (mean 21.7 years). Based on the initial distribution of muscle involvement, clinical phenotypes were divided into four subtypes: limb-girdle type, Miyoshi's type, distal anterior compartment type, or scapuloperoneal type. These phenotypic differences were prominent at the early stages, but were difficult to recognize later in the progression of the disease. Patients with missense mutations had significantly more severe functional status at examination and higher creatine kinase levels than those with frameshift or nonsense mutations.

Conclusion Dysferlinopathy exhibited marked heterogeneity in the age at onset, initial distribution of muscle involvement, and rate of disease progression. As this heterogeneity was observed even within the same family, some additional factors distinct from dysferlin might be involved. (Internal Medicine 41: 532–536, 2002)

Key words: limb-girdle muscular dystrophy 2B, Miyoshi myopathy, dysferlin, autosomal recessive, phenotype, genotype

LGMD1, is classified into five subtypes, LGMD1A to -1E. The autosomal recessive type, LGMD2, is classified into nine subtypes, LGMD2A to -2I. Recently, two clinically distinct forms of muscular dystrophy, LGMD2B (4) and Miyoshi myopathy (5–7), have been shown to be linked to the same genetic interval on chromosome 2p13 (8, 9), and a single gene named 'dysferlin', responsible for both LGMD2B and Miyoshi myopathy, was cloned (10, 11). The dysferlin protein, homologous to *Caenorhabditis elegans* fer-1 (12), is a 230 kDa transmembrane protein consisting of 2,080 amino acids with multiple C2 domains. Recent immunohistochemical studies revealed that dysferlin is localized at the muscle plasma membrane, but may not be associated with dystrophin-glycoprotein complex (13–15). There have been more than 20 different dysferlin gene mutations identified, and these are widely distributed over the coding sequence without any mutational hot spots (10, 11, 16–25). When a single gene 'dysferlin' was found to cause multiple clinical phenotypes, including LGMD2B and Miyoshi myopathy, the concept of 'dysferlinopathy' was proposed (10). To clarify the clinical heterogeneity and genotype-phenotype correlation in dysferlinopathy, we evaluated various clinical, laboratory, and pathologic findings of the previously reported dysferlinopathy patients with known dysferlin gene mutations.

For editorial comment, see p 505.

Introduction

Limb-girdle muscular dystrophy (LGMD) encompasses a genetically and clinically heterogeneous group of disorders characterized by proximal muscle weakness, decreased deep tendon reflexes, and marked by elevated serum creatine kinase (CK) levels (1, 2). Owing to the recent progress in positional cloning technology, the modern genetic classification of LGMD is based on either the responsible gene itself or the gene locus (3). The autosomal dominant type of LGMD, designated

Patients

In this study, only subjects who had been reported in the literature as having known dysferlin gene mutations were included. We omitted the sporadic patients whose clinical description was scanty. The selected pedigrees were as follows: 1) codon 1,837 Asp to Asn missense mutation in Oita, Japan (24); 2) codon 791 Pro to Arg missense mutation in Canada (16, 26); 3) codon 67 Val to Asp missense mutation in Russia (20, 27); 4) codon 573 Glu stop codon mutation in Okinawa, Japan (23); 5) codon 1,624 G deletion in Libyan Jews (19); 6) codon 1,865 G deletion in Spain (22); and 7) codon 422 23-bp

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insertion in Israel (21, 28). A total of 19 pedigrees comprising 74 patients with 7 different dysferlin gene mutations, including our own patients (24), were analyzed. The dysferlin gene mutations comprised three missense mutations (16, 20, 24), one nonsense mutation (23), and three frameshift mutations (19, 21, 22).

Methods

In all patients, we evaluated various clinical parameters including clinical history, neurologic symptoms, laboratory data, electromyography (EMG), muscle CT scan, and muscle biopsy. We also analyzed the functional status according to the scale of Walton and Gardner-Medwin (29). Briefly, this scale consists of grade 0 to grade 10. At the onset of symptoms, the patient is classified as being at grade 1. Grade 4 is defined as walking without assistance, grade 7 as unable to walk, and grade 10 as confined to bed. For some patients the definitions in the above grading scale placed them between grades, so we scored them as having the lower grade plus a half step. To obtain a measure of the rate of disease progression, the change in grade per year was calculated as present grade divided by disease duration in years. It should be remembered that the calculated figure is only a general estimate, as the functional scale is not always linear (19).

Statistical analyses

In the literature we drew from, we compared clinical and laboratory parameters between two major clinical subtypes: the limb-girdle type and Miyoshi's type. To study the genotype-phenotype correlation, we also divided the patients into two genetic groups: patients with frameshift or nonsense mutations and those with missense mutations. We compared clinical parameters between the two groups and statistical analyses were performed. Data for age, age of onset, and serum CK levels were expressed as mean $\pm$ SD and analyzed by using the unpaired *t*-test. Sex ratios were analyzed by using the chi-square test. Functional status and the rate of disease progression were expressed as mean $\pm$ SD and analyzed by using the median test. P values of less than 0.05 were considered significant.

Results

Clinical features

The birthplaces of the reported dysferlinopathy patients were distributed worldwide and a founder effect was evident in some pedigrees (19, 23, 24, 26, 28). Genealogical analysis clearly indicated that all pedigrees showed autosomal recessive inheritance. The age at onset varied from 12 years to 59 years, with a mean of 21.7 years ($n=60$) (Fig. 1). The disease duration ranged from 1 year to 45 years, mean 18.8 years. A female predominance was found in this study (number of men: number of women=29:45, sex ratio=0.64).

We were able to divide the clinical phenotypes into four subtypes according to the initial distribution of muscle involvement: the limb-girdle type (also called LGMD2B; 42 patients, 56.8%), Miyoshi's type (also called Miyoshi myopathy; 24

Figure 1. Distribution of age at onset in dysferlinopathy patients ($n=60$).

patients, 32.4%), the distal anterior compartment type (5 patients, 6.8%), and the scapuloperoneal type (1 patient, 1.4%). Five pedigrees showed several phenotypes within the same family (19, 20, 23, 24, 26), whereas two pedigrees had only one phenotype (21, 22). Extraocular and facial muscles were spared in all but one patient (23). Distal types in the Russian pedigree involved the anterior and posterior muscular compartments equally, which we classified as an unknown type (27). The clinical onset of the limb-girdle type started as weakness of the pelvic-girdle musculature, especially in the posterior compartment of the proximal limbs including the iliopsoas, hamstrings, and thigh adductor muscles (Fig. 2A) (24, 28). Some patients had calf pseudohypertrophy (17 patients, 40.5%) at an early stage that gradually disappeared in about two years (Fig. 2B). In 5 patients in the Libyan pedigree (19), a transient calf myalgia was noted. Six patients experienced myoglobinuria (24, 28).

The muscle involvement of Miyoshi's type started at the posterior distal lower limbs, especially the gastrocnemius and soleus muscles, so that the patients were unable to stand on tiptoes but could stand on their heels (19). The distal upper limbs were also involved, but the intrinsic hand muscles were spared. As the disease progressed, proximal muscles became affected until there was diffuse muscle involvement, and contractures of the foot, knee, and hand joints were occasionally seen (11 patients, 14.9%) (Fig. 2C) (24, 28). In contrast, all patients in the Spanish pedigree had selective involvement of the anterior compartment of the lower limbs throughout the disease course (22). The scapuloperoneal type was found in only one patient in Okinawa, Japan (23). Interestingly, transient clinical improvement was also observed in another patient in that Okinawa family.

Clinical analyses between patients with limb-girdle type and those with Miyoshi's type revealed no statistically significant differences except for a higher frequency of females in

Table 1. Clinical Analysis of Dysferlinopathy Patients with Limb-girdle Type vs. Miyoshi's Type

	Limb-girdle type (n=41)	Miyoshi's type (n=26)	Total (n=74)	P
Age (years)	39.1±16.2	38.2±15.6	38.9±15.7	NS
Sex; women (%)	20 (48.8)	20 (76.9)	45 (64.0)	0.02
Age at onset (years)	21.4±5.6	22.5±9.8	21.7±7.5	NS
Serum CK (times)	47.5±30.6	37.1±40.0	40.7±34.0	NS
Functional status	5.2±1.9	5.3±1.9	5.3±1.9	NS
Rate of disease progression	0.37±0.32	0.43±0.42	0.39±0.36	NS

Serum CK levels are expressed as times higher than the upper limit of the normal. P values show statistical significance in age, age at onset, serum CK level (unpaired *t*-test), sex ratios (chi-square test), functional status, and the rate of disease progression (median test) between limb-girdle type and Miyoshi's type. NS: not significant ($p>0.05$).

Miyoshi's type (women, 64%; $p=0.02$) (Table 1).

To evaluate the genotype-phenotype correlation, we compared various clinical parameters between patients with frameshift or nonsense mutations and those with missense mutations. The functional status score at examination of patients with missense mutations (6.5 ± 1.3) was significantly higher than that of patients with frameshift or nonsense mutations (4.9 ± 1.9 , $p=0.002$) (Table 2). However, other clinical symptoms, including the rate of disease progression, showed no significant differences.

Laboratory findings

Serum CK levels in every patient were more than 20 times higher than the upper limit of the normal (mean 40.7 ± 34.0 times higher than normal, $n=25$), and tended to decrease as the disease progressed. Patients with missense mutations had significantly higher serum CK levels (53.5 ± 37.1 times higher than normal) than those with frameshift or nonsense mutations (24.4 ± 21.8 times higher than normal, $p=0.03$) (Table 2). Nerve conduction studies were all normal. Needle electromyography showed motor units were usually of small amplitude at short duration, which was compatible with a myopathic pattern. Only one patient with Miyoshi's type had mixed myopathic and neuropathic change (26). In patients with the limb-girdle type, muscle CT scans clearly demonstrated the early involvement of the posterior distal limbs, including the gastrocnemius and soleus muscles, as well as pelvic girdle muscles (24, 28). In the distal type, the degree of involvement of the anterior or posterior compartment was variable case by case, but most

Figure 2. Clinical features of cases from our clinical practice (Asp 1,837 Asn mutation of the dysferlin gene). A) A 61-year-old woman with the limb-girdle type. Although muscle weakness was predominantly in the proximal musculature, the distal legs showed calf wasting. B) A 47-year-old woman with the limb-girdle type. Note mild calf pseudohypertrophy. C) A 58-year-old woman with Miyoshi's type about 30 years after onset, confined to bed with ankle and knee joint contractures.

Table 2. Clinical Analysis of Dysferlinopathy Patients with Frameshift/nonsense Mutation vs. Missense Mutation

	Patients with frameshift/nonsense mutation (n=45)	Patients with missense mutation (n=24)	P
Age (years)	39.8±13.7	37.3±19.1	NS
Sex; women (%)	26 (57.8)	15 (62.5)	NS
Age at onset (years)	21.7±8.0	21.4±6.3	NS
Serum CK (fold)	24.4±21.8	53.5±37.1	0.03
Functional status	4.9±1.9	6.5±1.3	0.002
Rate of disease progression	0.35±0.28	0.50±0.51	NS

Serum CK levels are expressed as times higher than the upper limit of the normal. P values show statistical significance in age, age at onset, serum CK level (unpaired *t*-test), sex ratios (chi-square test), functional status, and the rate of disease progression (median test) between each category group. NS: not significant ($p>0.05$).

patients showed posterior involvement dominantly (19, 24).

Muscle biopsies revealed chronic myopathic or dystrophic changes in all patients. Although some patients had lobulated fibers (24, 27) or ragged-red fibers (27), there were no other specific pathologic changes such as rimmed vacuoles. In muscle biopsies of two patients with distal onset, focal lymphocytic infiltrates were seen (27). Electron microscopic examination was reported in only one family (27), demonstrating prominent disorganization and disruption of myofibrils, alteration of the sarcoplasmic reticulum, numerous subsarcolemmal autophagic vacuoles, and degenerated mitochondria. Immunohistochemical studies with an anti-dysferlin antibody were performed in at least 6 cases, which confirmed that dysferlin was absent from or weak in the sarcolemma of the biopsied muscles (16, 19, 21, 22).

Cardiovascular tests including electrocardiograms and echo cardiographs were all normal, indicating no involvement of the cardiac muscles.

Discussion

We evaluated the various clinical parameters of 74 dysferlinopathy patients with known dysferlin gene mutations, all having been previously reported in the literature. In addition to the classical phenotypes of limb-girdle type and Miyoshi's type, we defined two other phenotypes, the distal anterior compartment type and the scapuloperoneal type (22, 23). These phenotypic differences were prominent at the early stage, but were difficult to recognize as the disease progressed. We could not detect any specific genotype-phenotype correlation; however, all patients with codon 1,865delG mutation had the distal anterior compartment type (22) and some women patients with codon 1,624delG mutation developed transient calf myalgia mimicking polymyositis (19). As it remains obscure whether these clinical findings were specific to the above gene mutations, further study will be needed to clarify this question.

There were three types of dysferlin gene mutations reported

in the literature: missense, nonsense, and frameshift. Nonsense and frameshift mutations are predicted to lead to premature truncation of the dysferlin protein, while missense mutations preserve the overall structure (10). We therefore compared two groups of patients with the different types of gene mutations. Statistical analyses indicated that patients with missense mutations had a significantly more severe functional status at examination and higher creatine kinase levels than those with frameshift or nonsense mutations. However, the rate of disease progression between them showed no statistical significance, and further studies will be needed to clarify whether clinical symptoms are more severe in patients with missense mutations than in those with frameshift/nonsense mutations.

In the present study, dysferlinopathy was more prevalent in women, especially among patients with Miyoshi's type. However, this result may be a result of sampling bias, because other reports of Miyoshi myopathy showed no woman predominance (5–7).

Although LGMD2B and Miyoshi myopathy had been initially reported as separate clinical entities, these diseases have many similarities, including mode of inheritance, marked elevated serum CK level, and muscle pathology (5, 9). In Japan, about 16% of patients with Miyoshi myopathy develop proximal muscle involvement relatively early, followed by a rapid progression, so the name of 'distal-limb-girdle type muscular dystrophy' had been proposed (30, 31). However, in the present study, this profile seemed to be only a part of the clinical heterogeneity of dysferlinopathy. Limb-girdle type and Miyoshi's type had no clinical differences except for the initial distribution of muscle involvement. In addition, there was no statistically significant difference in the rate of disease progression between them, in contrast to the previous description of more rapid progression of limb-girdle type than Miyoshi's type (31).

The present study has shown dysferlinopathy to have a wider clinical spectrum than previously thought, and the final diagnosis should be made by dysferlin protein analysis of the biopsied muscles or molecular analysis of the dysferlin gene. However, several characteristics can help in the clinical diag-

nosis of dysferlinopathy: 1) autosomal recessive inheritance or sporadic; 2) variable age at onset, although late teenage or early adult onset is common; 3) weakness starting in the lower limbs, with the gastrocnemius muscle usually weaker than the tibialis anterior, although the degree of weakness between them is variable in each case; 4) mixed proximal and distal muscle weakness at onset; 5) transient calf myalgia or pseudohypertrophy; 6) slow progression without loss of walking ability before age 30 years; and 7) variable clinical phenotypes in the same family.

The muscle biopsy findings reported in dysferlinopathy ranged from mild myopathic changes to severe dystrophic changes with extensive fibrosis and fatty replacement (19, 22–24, 27, 28). No specific pathologic changes were found, and only two patients (6.5%) had inflammatory changes (27). In this regard, several reports also mentioned that muscle biopsies show superimposed evidence of inflammation only infrequently (18, 25, 32). However, the reason why some patients with dysferlinopathy, like those with facioscapulohumeral muscular dystrophy, have inflammation in the muscles is obscure.

In conclusion, dysferlinopathy exhibited marked clinical heterogeneity in the age at onset, initial distribution of muscle involvement, and the rate of disease progression. As this heterogeneity was observed both within and among families, it seems to be related to an unknown modifying factor distinct from the dysferlin gene.

References

- 1) Bushby KM. Invited review. Making sense of the limb-girdle muscular dystrophies. *Brain* **122**: 1403–1420, 1999.
- 2) Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. *Muscle Nerve* **23**: 1456–1471, 2000.
- 3) Bushby KM, Beckman JS. Report of the 30th and 31st ENMC International Workshop: the limb-girdle muscular dystrophies and proposal for a new nomenclature. *Neuromusc Disord* **5**: 337–343, 1995.
- 4) Bashir R, Strachan T, Keers S, et al. A gene for autosomal recessive limb-girdle muscular dystrophy maps to chromosome 2p. *Hum Mol Genet* **3**: 455–457, 1994.
- 5) Miyoshi K, Kawai H, Iwasa M, Kusaka K, Nishino H. Autosomal recessive distal muscular dystrophy as a new type of progressive muscular dystrophy. Seventeen cases in eight families including an autopsied case. *Brain* **109**: 31–54, 1986.
- 6) Barohn RJ, Miller RG, Griggs RC. Autosomal recessive distal dystrophy. *Neurology* **41**: 1365–1370, 1991.
- 7) Linssen WH, Notermans NC, Van der Graaf Y, et al. Miyoshi-type distal muscular dystrophy. Clinical spectrum in 24 Dutch patients. *Brain* **120**: 1989–1996, 1997.
- 8) Bejaoui K, Hirabayashi K, Hentati F, et al. Linkage of Miyoshi myopathy (distal autosomal recessive muscular dystrophy) locus to chromosome 2p12–14. *Neurology* **45**: 768–772, 1995.
- 9) Bashir R, Keers S, Strachan T, et al. Genetic and physical mapping at the limb-girdle muscular dystrophy locus (LGMD2B) on chromosome 2p. *Genomics* **33**: 46–52, 1996.
- 10) Liu J, Aoki M, Illa I, et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nature Genet* **20**: 31–36, 1998.
- 11) Bashir R, Britton S, Strachan T, et al. A gene related to *Caenorhabditis elegans* spermatogenesis factor *fer-1* is mutated in limb-girdle muscular dystrophy type 2B. *Nature Genet* **20**: 37–42, 1998.
- 12) Achanzar WE, Ward S. A nematode gene required for sperm vesicle fusion. *J Cell Sci* **110**: 1073–1081, 1997.
- 13) Anderson LV, Davison K, Moss JA, et al. Dysferlin is a plasma membrane protein and is expressed early in human development. *Hum Mol Genet* **8**: 855–861, 1999 (published erratum appears in *Hum Mol Genet* **8**: 1141, 1999).
- 14) Matsuda C, Aoki M, Hayashi YK, Ho MF, Arahata K, Brown RH. Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy. *Neurology* **53**: 1119–1122, 1999.
- 15) Piccolo F, Moore SA, Ford GC, Campbell KP. Intracellular accumulation and reduced sarcolemmal expression of dysferlin in limb-girdle muscular dystrophies. *Ann Neurol* **48**: 902–912, 2000.
- 16) Weiler T, Bashir R, Anderson LV, et al. Identical mutation in patients with limb girdle muscular dystrophy type 2B or Miyoshi myopathy suggests a role for modifier gene(s). *Hum Mol Genet* **8**: 871–877, 1999.
- 17) Matsumura T, Aoki M, Nagano A, et al. Molecular genetic analysis of dysferlin in Japanese patients with Miyoshi myopathy. *Proc Jpn Acad* **75**: 207–212, 1999.
- 18) McNally EM, Ly CT, Rosenmann H, et al. Splicing mutation in dysferlin produces limb-girdle muscular dystrophy with inflammation. *Am J Med Genet* **91**: 305–312, 2000.
- 19) Argov Z, Sadeh M, Mazor K, et al. Muscular dystrophy due to dysferlin deficiency in Libyan Jews. Clinical and genetic features. *Brain* **123**: 1229–1237, 2000.
- 20) Illarioshkin SN, Ivanova-Smolenskaya IA, Greenberg CR, et al. Identical dysferlin mutation in limb-girdle muscular dystrophy type 2B and distal myopathy. *Neurology* **55**: 1931–1933, 2000.
- 21) Mahjneh I, Marconi G, Bushby K, Anderson LV, Tolvanen-Mahjneh H, Somer H. Dysferlinopathy (LGMD2B): a 23-year follow-up study of 10 patients homozygous for the same frameshifting dysferlin mutations. *Neuromusc Disord* **11**: 20–26, 2001.
- 22) Illa I, Serrano-Munuera C, Gallardo E, et al. Distal anterior compartment myopathy: A dysferlin mutation causing a new muscular dystrophy phenotype. *Ann Neurol* **49**: 130–134, 2001.
- 23) Nakagawa M, Matsuzaki T, Suehara M, et al. Phenotypic variation in a large Japanese family with Miyoshi myopathy with nonsense mutation in exon 19 of dysferlin gene. *J Neurol Sci* **184**: 15–19, 2001.
- 24) Ueyama H, Kumamoto T, Nagao S, et al. A new dysferlin gene mutation in two Japanese families with limb-girdle muscular dystrophy 2B and Miyoshi myopathy. *Neuromusc Disord* **11**: 139–145, 2001.
- 25) Aoki M, Liu J, Richard I, et al. Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. *Neurology* **57**: 271–278, 2001.
- 26) Weiler T, Greenberg CR, Nylen E, et al. Limb-girdle muscular dystrophy and Miyoshi myopathy in an Aboriginal Canadian kindred map to LGMD2B and segregate with the same haplotype. *Am J Hum Genet* **59**: 872–878, 1996.
- 27) Illarioshkin SN, Ivanova-Smolenskaya IA, Tanaka H, et al. Clinical and molecular analysis of a large family with three distinct phenotypes of progressive muscular dystrophy. *Brain* **119**: 1895–1909, 1996.
- 28) Mahjneh I, Vannelli G, Bushby K, Marconi GP. A large inbred Palestinian family with two forms of muscular dystrophy. *Neuromusc Disord* **2**: 277–283, 1992.
- 29) Walton JN, Gardner-Medwin D. Progressive Muscular Dystrophy and the Myotonic Disorders. in: Walton JN, Ed. *Disorders of Voluntary Muscle*. 4th ed.: Churchill Livingstone, Edinburgh, 1981: 452.
- 30) Nishida Y, Ishimoto S, Kobayashi T, Goto I, Kuroiwa Y. Two sisters with autosomal recessive muscular dystrophy (Miyoshi) with early involvement of limb girdle muscles. *Rinsho Shinkeigaku (Clin Neurol)* **27**: 756–759, 1987 (in Japanese, Abstract in English).
- 31) Miyoshi K. Reported cases of Miyoshi distal muscular dystrophy (1967–1994) and muscular atrophy of limb-girdle type seen in the early stage of the disease. *Shinkeinaika (Neurol Med)* **44**: 61–65, 1996 (in Japanese).
- 32) Selcen D, Stilling G, Engel AG. The earliest pathologic alterations in dysferlinopathy. *Neurology* **56**: 1472–1481, 2001.