Dysferlin Gene Mutation Spectrum in a Large Cohort of Chinese Patients with Dysferlinopathy

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Abstract

Background: Dysferlinopathy is caused by mutations in the dysferlin (DYSF) gene. Here, we described the genetic features of a large cohort of Chinese patients with this disease.

Methods: Eighty-nine index patients were included in the study. DYSF gene analysis was performed by Sanger sequencing in 41 patients and targeted next generation sequencing (NGS) in 48 patients. Multiplex ligation-dependent probe amplification (MLPA) was performed to detect exon duplication/deletion in patients with only one pathogenic mutation.

Results: Among the 89 index patients, 79 patients were demonstrated to carry two disease-causing (73 cases) or possibly disease-causing mutations (6 cases), including 26 patients with homozygous mutations. We identified 105 different mutations, including 59 novel ones. Notably, in 13 patients in whom only one pathogenic mutation was initially found by Sanger sequencing or NGS, 3 were further identified to carry exon deletions by MLPA. The mutations identified in this study appeared to cluster in the N-terminal region. Mutation types included missense mutations (30.06%), nonsense mutations (17.18%), frameshift mutations (30.67%), in-frame deletions (2.45%), intronic mutations (17.79%), and exonic rearrangement (1.84%). No genotype-phenotype correlation was identified.

Conclusions: DYSF mutations in Chinese patients clustered in the N-terminal region of the gene. Exonic rearrangements were found in 23% of patients with only one pathogenic mutation identified by Sanger sequencing or NGS. The novel mutations found in this study greatly expanded the mutational spectrum of dysferlinopathy.

Key words: Dysferlin Gene; Dysferlinopathy; Exonic Rearrangements; Mainland China; Novel Mutation

Introduction

Dysferlinopathy is a group of autosomal recessive muscular dystrophies caused by mutations in dysferlin (DYSF) gene showing marked clinical heterogeneity. The most common phenotypes of dysferlinopathy are proximal limb weakness (limb girdle muscular dystrophy type 2B [LGMD2B]) and distal myopathy (Miyoshi myopathy [MM]). However, other atypical symptoms such as hyperCKemia, distal anterior compartment myopathy, and proximodistal myopathy (PDM) are not rare and can have a congenital onset. Clinical variability is also observed within a single family.

Western blot analysis and immunohistochemistry are important tools in the initial diagnosis of primary dysferlinopathy due to their low cost and convenience in clinical practice. However, severe reduction of DYSF can also be observed in other skeletal muscle diseases, such as calpainopathy, caveolinopathy, and anoctaminopathy, which are classified as secondary dysferlinopathies. In addition, false-negative results can occur in western blot analysis when DYSF has accumulated in the cytoplasm. Therefore, gene analysis is necessary and still remains the “gold standard” for diagnosis.

The DYSF gene is located on chromosome 2p13, which spans a genomic region of more than 230 kbp and comprises 55 exons. The DYSF gene was found to have exon rearrangements in 23% of patients with only one pathogenic mutation identified by Sanger sequencing or NGS. The novel mutations found in this study greatly expanded the mutational spectrum of dysferlinopathy.
cell adhesion,[16] and angiogenesis.[17] To date, 510 different mutations in this gene have been reported in the Leiden muscular dystrophy database worldwide (Leiden Muscular Dystrophy pages © www.dmd.nl). Most of these mutations are private and there are no hotspots,[18] which makes screening of the entire coding sequence of the \textit{DYSF} gene necessary. In addition, most of the reported mutations are point mutations, small deletion/insertions, and intronic mutations.[18] Exonic rearrangements had been reported on rare occasions and were identified as the second disease-causing mutation in 5 of 12 patients by multiplex ligation-dependent probe amplification (MLPA).[19] Given the high frequency of patients with only one pathogenic mutation (the proportion varied from 12.5% to 34.0% in previous studies),[4,5,18] it is necessary to carry out MLPA testing in these patients as a supplementary tool in the routine screening for \textit{DYSF} gene mutations.

To date, no more than 60 Chinese dysferlinopathy patients with genetic diagnoses had been reported.[4,5,18] To better characterize the genetic spectrum of Chinese patients with dysferlinopathy, we described the genetic and clinical findings in the largest cohort of Chinese dysferlinopathy patients. In addition, we performed MLPA assay of \textit{DYSF} gene in patients with only one pathogenic mutation to confirm the existence of exonic rearrangements in Chinese patients.

\section*{Methods}

\subsection*{Patient selection criteria and clinical evaluation}

Eighty-seven patients were included in this study based on the clinical suspicion of primary dysferlinopathy and absent/severely reduced dysferlin expression as evidenced by immunohistochemical analyses of muscle specimen. Written informed consent was obtained from all participants according to the Declaration of Helsinki. All these patients underwent muscle biopsy at Department of Neurology, Peking University First Hospital after providing written informed consent. Immunohistochemical analyses were performed using primary antibodies for \textit{DYSF}, sarcoglycans, and dystrophin (all from Novocastra Laboratories, Newcastle, UK). Two patients were included with a clinical suspicion of dysferlinopathy without a muscle biopsy. All patients underwent detailed neurological interviews and physical examinations by experienced neurologists (Zhao-Xia Wang, Wei Zhang, or Yun Yuan) at Department of Neurology, Peking University First Hospital. Patients were classified into different phenotypes according to their initial pattern of muscle involvement: hyperCKemia when there are no clinical symptoms, LGMD2B when the proximal leg is first involved, MM when the distal part of leg is first involved, and proximodistal phenotype when there is proximal and distal weakness simultaneously at disease onset. Functional status was evaluated with a modified 0–9 grading system proposed by Gardner-Medwin and Walton (GM-W scale).[24]

\subsection*{Mutation analysis}

Genomic DNA was extracted from peripheral blood cells or skeletal muscle specimens of the patients. In 41 patients, all 55 exons and the intron/exon boundary of the \textit{DYSF} gene were amplified by PCR as previously described.[25] The PCR products were directly sequenced using an ABI 3730XL automatic sequencing machine (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). The sequences were read by Chromas software (http://technetium.com.au/wp/chromas) and compared to the human \textit{DYSF} sequence (NM_003494.3). In 48 patients, next generation sequencing (NGS) was applied with a neuromuscular disease panel (Agilent, Santa Clara, CA, USA) of 420 genes known to be associated with inherited muscular diseases. The exons and 10 bp of flanking splice sites were captured and subsequently sequenced on an Illumina HiSeq 2500 Sequencer (Illumina, San Diego, CA, USA). The reads were aligned by SOAPaligner for single-nucleotide polymorphism calling and other analyses. The sequencing files were mapped to reference sequences with Burrows-Wheeler Aligner and Picard tools, and then called with control samples with the GATK 3.0 HaplotypeCaller (Broad Institute, USA). Sanger sequencing with specific primers was conducted to confirm the mutations detected by NGS. In patients in whom only one mutation was detected, we further performed MLPA assay using a commercially available MLPA kit (SALSA MLPA probemix P268-A2 DYSF; MRC-Holland BV, Amsterdam, The Netherlands) which covered 40 of the 55 exons.

\subsection*{Interpretation of mutations found in this study}

The mutations found in patients were determined to be disease-causing by the following criteria: (1) mutations reported in literature, in the HGMD database, Leiden Muscular Dystrophy pages database (www.dmd.nl), or the UMD-DYSF mutations database (www.umd.be/DYSF); (2) novel null mutations, including nonsense mutations, frameshift mutations, canonical ±1 or 2 splice sites, and single exon or multiexon deletions; (3) novel missense mutations predicted to be disease-causing by a combination of four predictive software programs, including UMD-predictor (predicted as pathogenic/probably pathogenic),[26] Mutation Taster (predicted as disease-causing),[27] PolyPhen-2 (predicted as probably/possibly damaging),[28] and SIFT software (J. Craig Venter Institute, USA) (predicted as deleterious); (4) novel intronic mutations predicted as disease-causing by MutationTaster (predicted as disease-causing)[27] and Human Splicing Finder (http://www.umd.be/HSF3).[29]

\subsection*{Statistical analysis}

All values were calculated using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Values are presented as the mean ± standard error (SE) unless otherwise stated. Mann-Whitney U-test was used to test the significance of differences in the GM-W scale between different types of genetic mutations. Student’s t-test was used to test the significance of differences in age of onset, disease duration, and serum creatine kinase (CK) level between the different types of genetic mutations. The difference in the clinical phenotypes between the two groups was analyzed by Chi-square test. A value of $P \leq 0.05$ was considered statistically significant (two-tailed).
**Results**

**Geographic and clinical data**

The patients in this study came from 27 provinces of mainland China, including 51 men and 38 women. A total of 87 patients were of Han ethnicity, one patient was of Hui ethnicity, and one patient was of Uygur ethnicity. Sixteen patients had a family history of muscle diseases. The mean age of onset was 21.1 ± 7.3 years (range 10–49 years). The mean disease duration was 7.4 ± 5.7 years (range 1 month to 25 years). Forty-five patients presented with LGMD2B, 31 with MM, 7 with PDM, and 6 were clinically asymptomatic and diagnosed with hyperCKemia. The median GM-W scale score was 4, ranging from 0 to 9. Eight patients were wheelchair-dependent. Serum CK ratio (defined as CK level/upper limit of normal range) varied widely, ranging from 10 to 187. Myopathic changes were found in 58 of the 63 patients who underwent examination by electromyography (EMG), and neurogenic changes were found in one patient. The remaining four patients showed normal EMG results. The detailed clinical data are provided in Supplementary Table 1.

**Analysis of mutations identified in this study**

Among the 89 index patients, 79 were demonstrated to carry two disease-causing (73 cases) or possibly disease-causing mutations (six cases), including 26 patients with homozygous mutations and 53 patients with compound heterozygous mutations. In the remaining 10 patients, only one heterozygous mutation was found. Notably, among 13 patients who were initially found to carry only one pathogenic mutation by Sanger sequencing or NGS, three were further identified to carry exonic deletions with MLPA [Figure 1]. In these patients, we identified 105 different mutations, including 98 disease-causing and seven possibly disease-causing mutations [Supplementary Table 2 and Figure 2]. Fifty-nine novel mutations were found, 52 of which were identified as disease-causing [Supplementary Table 2]. Seven novel missense mutations were determined as possibly disease-causing because of inconsistent results of different predicting softwares.

The allele frequencies of disease-causing mutations found in this study were as follows [Table 1]: missense mutations (30.06%), nonsense mutations (17.18%), frameshift mutations (30.67%), in-frame deletions (2.45%), intronic mutations (17.79%), and exonic rearrangement (1.84%). These mutations span the whole length of the DYSF gene. However, the C2B and C2C domain demonstrated the highest frequency of mutations in this study [Table 2 and Figure 2].

Ten recurrent mutations, which were found in more than three unrelated patients, are listed in Table 3. Four of these mutations (c.863A>T, c.1375dupA, c.1667T>C, and c.3988C>T) have only been reported in the Chinese population.

**Genotype-phenotype correlation**

We divided the patients in this study into two groups: (1) patients with at least one missense mutation or in-frame deletion/insertion and (2) patients with no missense mutation or in-frame deletion/insertion. No statistically significant difference was detected between these two groups regarding age of onset, disease duration, phenotype, GM-W scale, and serum CK level ($P > 0.05$).

**Discussion**

The patients enrolled in this study came from 27 of the 34
provinces of China, which enabled us to better characterize the genetic spectrum of patients with dysferlinopathy in mainland China.

As previously reported, the mutations found in this study span the whole length of the DYSF gene, and no mutational hot spots were identified. However, we found that DYSF...
mutations in Chinese patients clustered in the N-terminal region of the gene, especially in and around the C2C and C2B domains. In contrast, previously reported DYSF mutations were distributed evenly along the DYSF gene.\[18\] N-terminal clustering was only observed in a group of South Korean patients with dysferlinopathy.\[5\] Interestingly, four of the ten recurrent mutations found in this study (c.799_800delTT, c.863A>T, c.1180+5G>A, and c.1375dupA) were located in the C2B and C2C domains. The N-terminal clustering of DYSF mutations in Chinese patients was partly attributable to these recurrent mutations. All patients with the c.1375dupA mutation originated from northern China, indicating a potential founder effect of this mutation.

The dysferlin protein consists of seven C2 domains (C2A–C2G) \[30\] which are highly conserved and functions in calcium-dependent phospholipid binding. The affinity of calcium- and phospholipid-binding for each domain varies greatly. For example, the C2B domain was predicted to have no calcium-binding capacity.\[30\] In line with this hypothesis, quantitative study revealed that the C2B domain is one of the domains with lowest affinity for calcium-dependent membrane binding.\[31\] In addition, by constructing mini-dysferlin molecules, Azakir \textit{et al.}\[32\] found that the deletions of the dysferlin C2B domains have no impact on the sarcolemmal localization of dysferlin and the membrane repair of injured muscle cell. However, in this study, the C2B domain was the second most frequently affected domain, which was partly attributed to the recurrent mutation c.863A>T found in Chinese patients. To date, a very few missense mutations affecting the calcium-binding residues have been identified. c.863A>T results in a substitution of aspartate 288 by valine, which was predicted to be a key calcium-binding residue in this domain.\[30\] The recurrence of the missense mutation c.863A>T implied the importance of the C2B domain for the function of dysferlin.

The proportion of different types of mutations in Chinese patients with dysferlinopathy varied among previous studies.\[14,21,23\] We found that there were fewer missense mutations in the current study than previously reported,\[18\] which might be due in part to the exclusion of the possibly disease-causing missense mutations in this study. Interestingly, the missense mutations identified in this study were located mainly in the C2B domain and inner DysF domain. The c.863A>T was found in five of eight patients carrying missense mutations in the C2B domain. Of all nine patients carrying missense mutations in the Inner DysF domain, eight patients were carrying mutations disrupting the arginine/tryptophan (R/W) stacks.\[13\]

In this study, we first determined the existence of exonic rearrangements in Chinese patients. Only a few reports have described exonic rearrangements in patients with dysferlinopathy.\[10,19,34\] Genomic deletions/duplications were found in five of 12 patients with one pathogenic mutation using the MLPA method.\[10\] In this study, the frequency of exonic rearrangements was three in 13 patients with one pathogenic mutation. At present, there is no information available on the percentage of defects in the DYSF gene caused by deletions/duplications of complete exons. In our cohort, the allele frequency was estimated to be 3/178. However, we did not perform the MLPA test in patients with compound heterozygous or homozygous mutations. In addition, the MLPA kit we used only covers 40 of the 55 exons in the DYSF gene, so the frequency might be higher than expected. Given the high frequency of exonic rearrangements in patients with one disease-causing mutation, further MLPA analysis in these patients is recommended. In addition, in patient 22, we first identified a single exonic deletion by NGS using a copy number variation (CNV) calling algorithm, which incorporates read-depth statistics, allele zygosity analysis, and breakpoints detection. CNV calls were further confirmed by MLPA assays, providing a conclusive molecular diagnosis that would not be possible by routine Sanger sequencing alone.

In this study, we identified 59 novel mutations, 52 of which were determined to be disease-causing. However, confirmation of the seven novel missense mutations was
impossible using a bioinformatic approach because of conflicting results among different software programs. The clinical diagnoses of dysferlinopathy in patients (Six patients in total: P21, 74, 75, 77, 78 and 79, Supplementary Table 1 and Supplementary Table 2) carrying these mutations were confirmed based on the typical history and pathological study (especially the immunohistochemistry staining of dysferlin). In five of these patients (P21, 74, 75, 77, and 79), mutations of other muscular dystrophies related genes were ruled out by NGS panel based on 420 different genes, and exonic rearrangements were ruled out by the CNV calling algorithm [Supplementary Table 2]. Therefore, the pathogenicity of these mutations could not be ruled out. Patient 78 had a family history of dysferlinopathy, and negative dysferlin expression was confirmed in her and her affected brother. As the mutation c.5216C>A was also identified in the siblings by Sanger sequencing, it is quite possible that c.5216C>A was disease-causing. Further study at the mRNA level is needed to achieve definitive genetic diagnoses in these patients.

The novel mutations identified in this study, accounting for about 10% of all mutations reported to date, greatly expand the genetic spectrum of dysferlinopathy.

In conclusion, DYSF mutations in Chinese patients clustered in the N-terminal region of the DYSF gene. Exonic rearrangements were found in 23% of patients with only one pathogenic mutation identified by Sanger sequencing or NGS. Novel mutations found in this study greatly expand the mutational spectrum of dysferlinopathy.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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### Supplementary Table 1: Clinical information of patients in this study

| Patient number | Gender/age at diagnosis (years) | Age of onset (years) | Family history | Phenotype      | GM-W scales at diagnosis | CK (×N) | Dysferlin ICH | Calf atrophy | EMG |
|----------------|---------------------------------|----------------------|----------------|----------------|--------------------------|---------|---------------|-------------|-----|
| 1              | Female/34                       | 33                   | MM             | HyperCKemia    | 2                        | 12      | -             |             | NA  |
| 2              | Female/30                       | 26                   | LGMD2B         |                | 3                        | 42      | -             | Normal      |     |
| 3              | Male/13                         | 13                   | HyperCKemia    |                | 0                        | 20      | -             | Myopathic   |     |
| 4              | Male/16                         | 14                   | LGMD2B         |                | 1                        | 118     | -             | Myopathic   |     |
| 5              | Female/28                       | 14                   | LGMD2B         |                | 4                        | 65      | -             | +           | Myopathic |
| 6              | Male/21                         | 15                   | MM             |                | 3                        | 106     | -             | +           | NA  |
| 7              | Female/32                       | 12                   | +               | LGMD2B         | 8                        | 157     | -             | +           | NA  |
| 8              | Male/35                         | 20                   | LGMD2B         |                | 9                        | NA      | -             | +           | Myopathic |
| 9              | Male/37                         | 18                   | MM             |                | 5                        | 29      | -             | +           | Myopathic |
| 10             | Female/49                       | 41                   | LGMD2B         |                | 7                        | 15      | -             | +           | Myopathic |
| 11             | Male/24                         | 18                   | MM             |                | 4                        | 52      | -             | +           | Myopathic |
| 12             | Female/28                       | 20                   | MM             |                | 4                        | 27      | Reduced       | +           | Myopathic |
| 13             | Male/18                         | 15                   | HyperCKemia    |                | 1                        | 29      | -             |             | NA  |
| 14             | Male/16                         | 14                   | LGMD2B         |                | 4                        | 71      | -             | Myopathic   |     |
| 15             | Female/25                       | 24                   | LGMD2B         |                | 3                        | 64      | -             | Myopathic   |     |
| 16             | Female/18                       | 13                   | LGMD2B         |                | 4                        | 47      | -             | Myopathic   |     |
| 17             | Male/22                         | 16                   | MM             |                | 4                        | 59      | -             | +           | Myopathic |
| 18             | Female/36                       | 24                   | LGMD2B         |                | 7                        | 24      | -             | +           | Myopathic |
| 19             | Male/25                         | 22                   | LGMD2B         |                | 4                        | 40      | -             | Myopathic   |     |
| 20             | Male/28                         | 20                   | LGMD2B         |                | 4                        | 43      | -             | +           | Myopathic |
| 21             | Female/36                       | 29                   | PDM            |                | 4                        | 15      | -             | +           | NA  |
| 22             | Female/33                       | 30                   | +               | LGMD2B         | 4                        | 32      | -             |             | NA  |
| 23             | Female/28                       | 25                   | LGMD2B         |                | 5                        | 23      | -             | +           | Myopathic |
| 24             | Male/26                         | 25                   | PDM            |                | 4                        | 71      | -             | +           | Myopathic |
| 25             | Male/25                         | 16                   | MM             |                | 4                        | 34      | -             | +           | Myopathic |
| 26             | Male/25                         | 12                   | LGMD2B         |                | 5                        | NA      | Reduced       | +           | NA  |
| 27             | Female/19                       | 17                   | MM             |                | 2                        | 80      | -             | +           | Myopathic |
| 28             | Male/15                         | 15                   | HyperCKemia    |                | 1                        | 187     | Reduced       | NA          |     |
| 29             | Male/21                         | 14                   | MM             |                | 2                        | 71      | -             | +           | Myopathic |
| 30             | Male/35                         | 31                   | MM             |                | 4                        | 34      | -             | +           | Myopathic |
| 31             | Female/51                       | 49                   | LGMD2B         |                | 3                        | 11      | -             | +           | Myopathic |
| 32             | Female/49                       | 24                   | LGMD2B         |                | 7                        | 10      | -             | +           | Myopathic |
| 33             | Male/22                         | 22                   | PDM            |                | 1                        | 122     | -             | +           | NA  |
| 34             | Male/26                         | 23                   | MM             |                | 8                        | 16      | -             | Myopathic   |     |
| 35             | Male/45                         | 31                   | +               | LGMD2B         | 5                        | NA      | -             | +           | NA  |
| 36             | Male/36                         | 25                   | LGMD2B         |                | 5                        | 38      | -             | +           | Myopathic |
| 37             | Male/23                         | 21                   | +               | PDM            | 2                        | 73      | -             | +           | Myopathic |
| 38             | Male/34                         | 29                   | MM             |                | 8                        | 20      | -             | +           | Myopathic |
| 39             | Female/35                       | 29                   | LGMD2B         |                | 5                        | 33      | -             | Myopathic   |     |
| 40             | Female/37                       | 28                   | LGMD2B         |                | 7                        | 17      | Reduced       | +           | Myopathic |
| 41             | Male/22                         | 20                   | LGMD2B         |                | 2                        | 13      | -             | Myopathic   |     |
| 42             | Female/25                       | 14                   | +               | MM             | 4                        | 57      | -             | +           | Myopathic |
| 43             | Female/32                       | 26                   | LGMD2B         |                | 2                        | 31      | -             | +           | Myopathic |
| 44             | Female/38                       | 28                   | MM             |                | 5                        | 21      | -             | +           | Myopathic |
| 45             | Male/24                         | 19                   | MM             |                | 4                        | 40      | -             | +           | Myopathic |
| 46             | Female/25                       | 22                   | MM             |                | 1                        | 21      | -             | +           | Myopathic |
| 47             | Male/24                         | 22                   | LGMD2B         |                | 3                        | 30      | -             | Myopathic   |     |
| 48             | Male/15                         | 15                   | LGMD2B         |                | 1                        | 31      | -             |             | NA  |
| 49             | Male/25                         | 18                   | MM             |                | 3                        | 51      | Reduced       | +           | Myopathic |
| 50             | Male/17                         | 16                   | +               | MM             | 1                        | 116     | -             |             | NA  |
| 51             | Male/16                         | 15                   | PDM            |                | 1                        | 120     | -             | +           | Normal   |
| 52             | Male/16                         | 10                   | +               | LGMD2B         | 2                        | 118     | -             | +           | Myopathic |
| 53             | Male/36                         | 14                   | MM             |                | 5                        | 29      | -             | +           | Myopathic |

Contd...
## Supplementary Table 1: Contd...

| Patient number | Gender/age at diagnosis (years) | Age of onset (years) | Family history | Phenotype | GM-W scales at diagnosis | CK (×N) | Dysferlin ICH | Calf atrophy | EMG |
|----------------|-------------------------------|----------------------|----------------|-----------|--------------------------|---------|---------------|-------------|-----|
| 54             | Male/42                       | 32                   | MM             | NA        | 24                       | −       | +             | Myopathic   |     |
| 55             | Male/27                       | 16                   | MM             | 5         | 37                       | −       | +             | NA          |     |
| 56             | Male/26                       | 22                   | LGMD2B         | 2         | 26                       | −       | +             | Myopathic   |     |
| 57             | Female/25                     | 23                   | LGMD2B         | NA        | 22                       | −       | −             | Myopathic   |     |
| 58             | Female/43                     | 15                   | LGMD2B         | 8         | 10                       | −       | +             | Myopathic   |     |
| 59             | Male/27                       | 16                   | MM             | 5         | 76                       | −       | +             | Myopathic   |     |
| 60             | Male/19                       | 19                   | HyperCKemia    | 0         | 122                      | −       | −             | Normal      |     |
| 61             | Male/33                       | 25                   | MM             | 2         | 22                       | −       | +             | NA          |     |
| 62             | Male/25                       | 16                   | MM             | 1         | 38                       | −       | +             | Myopathic   |     |
| 63             | Female/31                     | 18                   | LGMD2B         | 4         | NA                       | −       | +             | NA          |     |
| 64             | Female/28                     | 14                   | MM             | 5         | NA                       | −       | +             | NA          |     |
| 65             | Male/41                       | 39                   | LGMD2B         | 5         | 29                       | −       | +             | Myopathic   |     |
| 66             | Male/24                       | 14                   | MM             | 2         | 36                       | −       | +             | Myopathic   |     |
| 67             | Female/26                     | 23                   | LGMD2B         | 5         | 20                       | −       | +             | Myopathic   |     |
| 68             | Male/36                       | 30                   | LGMD2B         | 4         | 71                       | −       | +             | Myopathic   |     |
| 69             | Male/26                       | 17                   | LGMD2B         | 8         | NA                       | NA      | +             | NA          |     |
| 70             | Male/22                       | 14                   | LGMD2B         | 4         | 55                       | NA      | +             | NA          |     |
| 71             | Female/30                     | 28                   | LGMD2B         | 5         | NA                       | −       | −             | Myopathic   |     |
| 72             | Male/29                       | 18                   | LGMD2B         | 5         | 35                       | −       | +             | NA          |     |
| 73             | Female/23                     | 18                   | PDM            | 5         | 49                       | Reduced | +             | NA          |     |
| 74             | Female/32                     | 29                   | LGMD2B         | 4         | 13                       | −       | −             | Neurogenic  |     |
| 75             | Female/33                     | 26                   | MM             | 5         | 16                       | −       | +             | Myopathic   |     |
| 76             | Male/32                       | 26                   | MM             | 2         | 43                       | −       | +             | Myopathic   |     |
| 77             | Female/14                     | 14                   | LGMD2B         | 1         | 93                       | −       | −             | NA          |     |
| 78             | Female/28                     | 15                   | MM             | 4         | NA                       | −       | +             | NA          |     |
| 79             | Male/13                       | 13                   | HyperCKemia    | 1         | 47                       | −       | −             | NA          |     |
| 80             | Male/45                       | 42                   | LGMD2B         | 2         | 38                       | −       | −             | Myopathic   |     |
| 81             | Male/30                       | 23                   | MM             | 4         | 79                       | Reduced | +             | Myopathic   |     |
| 82             | Female/30                     | 19                   | LGMD2B         | 4         | 24                       | −       | −             | Myopathic   |     |
| 83             | Female/36                     | 26                   | LGMD2B         | 4         | 17                       | Reduced | +             | NA          |     |
| 84             | Female/18                     | 16                   | HyperCKemia    | 0         | 48                       | −       | −             | Normal      |     |
| 85             | Female/23                     | 15                   | LGMD2B         | 8         | 35                       | −       | +             | Myopathic   |     |
| 86             | Female/37                     | 15                   | MM             | 8         | 18                       | −       | +             | Myopathic   |     |
| 87             | Female/26                     | 21                   | MM             | 4         | 66                       | Reduced | +             | Myopathic   |     |
| 88             | Male/26                       | 15                   | LGMD2B         | 4         | 44                       | −       | +             | NA          |     |
| 89             | Male/31                       | 23                   | LGMD2B         | NA        | 31                       | −       | +             | NA          |     |

NA: Not available; PDM: Proximodistal myopathy; MM: Miyoshi myopathy; LGMD2B: Limb girdle muscular dystrophy type 2B; GM-W: Gardner-Medwin and Walton; CK: Creatine kinase; ICH: Immunohistochemistry; EMG: Electromyography; + (Family history): patients with a family history of skeletal muscle diseases; − (Dysferlin ICH): positive staining of dysferlin on muscle biopsy; + (Calf atrophy): patients with calf atrophy.
## Supplementary Table 2: Dysferlin gene mutations found in this study

| Patient number | Mutation name | Protein change | Exon/intron | Domain | State | DNA sequencing | Mutation type |
|----------------|---------------|----------------|-------------|--------|-------|----------------|---------------|
| 1              | c.863A>T      | p.D288V        | 9           | C2B    | Homozygous | NGS            | Missense      |
| 2              | c.1375dupA    | p.M459fsX15    | 15          | C2C    | Heterozygous | Frameshift   | Missense      |
| 3              | c.3036G>C*    | p.W1012C       | 29          | InnerDysF-C | Heterozygous |             | Missense      |
| 4              | c.2643+5G>C*  | Abl.spl        | 25          | InnerDysF-N | Heterozygous | NGS            | Splice site   |
| 5              | c.3827T>C     | p.L1276P       | 34          | Heterozygous |              |               | Missense      |
| 6              | c.3059C>T     | p.P1020L       | 28          | InnerDysF-C | Heterozygous | NGS            | Missense      |
| 7              | c.3212+1G>A*  | Abl.spl        | IVS31       | Heterozygous |              |               | Splice site   |
| 8              | c.5302C>T     | p.R1768W       | 47          | Homozygous | NGS            | Missense      |
| 9              | c.1667T>C     | p.L556P        | 19          | Heterozygous | Sanger        | Missense      |
| 10             | c.4894C>T     | p.R1586X       | 43          | C2F    | Heterozygous | Frameshift   | Non-sense     |
| 11             | c.4524T>C     | p.R1749C       | 47          | Heterozygous |              | Missense      |
| 12             | c.5245C>T     | p.R1586X       | 43          | C2F    | Heterozygous | Frameshift   | Non-sense     |
| 13             | c.5525G>A     | p.G1842D       | 49          | C2G    | Homozygous | NGS            | Missense      |
| 14             | c.1535_1553del* | p.F514Pfs*107  | 18          | Heterozygous | Sanger        | Missense      |
| 15             | c.3321_3323del | p.Abl.spl      | IVS17       | Homozygous | NGS            | Missense      |
| 16             | c.2974T>C     | p.W992R        | 28          | InnerDysF-N | Heterozygous |              | Missense      |
| 17             | c.1180+5G>A   | p.M459fs*15    | 15          | C2C    | Heterozygous | Frameshift   | Missense      |
| 18             | c.3601C>T     | p.Q1200X       | 33          | C2D    | Homozygous | NGS            | Missense      |
| 19             | c.4509T>G     | p.L527R        | 42          | Heterozygous |              | Missense      |
| 20             | c.1930+2T>G*  | Abl.spl        | IVS20       | Homozygous | NGS            | Missense      |
| 21             | c.3531_3533delCAT | p.1178del     | 33          | C2D    | Homozygous | NGS            | In frame deletion |
| 22             | c.4167+1G>A   | Abl.spl        | IVS38       | C2E    | Heterozygous | Frameshift   | Missense      |
| 23             | c.4988_4989delTC* | p.V1663Gfs*47  | 45          | C2F    | Homozygous | NGS            | Frameshift   |
| 24             | c.1523-2A>G*  | Abl.spl        | IVS17       | Homozygous | NGS            | Missense      |
| 25             | c.3053C>T     | p.R1586X       | 43          | C2F    | Heterozygous | Frameshift   | Non-sense     |
| 26             | c.1375dupA    | p.M459fsX15    | 15          | C2C    | Heterozygous | Frameshift   | Missense      |
| 27             | c.3827T>C     | p.L1276P       | 34          | Heterozygous |              | Missense      |
| 28             | c.3059C>T     | p.P1020L       | 28          | InnerDysF-C | Heterozygous | NGS            | Missense      |
| 29             | c.1393C>T     | p.D465H        | 15          | C2C    | Homozygous | NGS            | Missense      |
| 30             | c.313dupC     | p.L105PfsX43   | 4           | Homozygous | NGS            | Frameshift   | Missense      |
| 31             | c.3601C>T     | p.Q1201X       | 33          | C2D    | Heterozygous | NGS            | Non-sense     |

Contd...
| Patient number | Mutation name | Protein change | Exon/intron | Domain | State | DNA sequencing | Mutation type |
|---------------|---------------|----------------|-------------|--------|-------|--------------|---------------|
| 32            | c.863A>T      | p.D288V        | 9           | C2B    | Heterozygous | NGS          | Missense      |
| 33            | c.965T>C      | p.L322P        | 11          | FerI   | Heterozygous | NGS          | Missense      |
| 34            | c.2940delG    | p.L981F6X76    | 28          | InnerDys-F-N | Heterozygous | NGS          | Frameshift    |
| 35            | c.252delC     | p.K85Rfs*66    | 4           | C2E    | Heterozygous | Sanger        | Frameshift    |
| 36            | c.2997G>T     | p.W999C        | 28          | InnerDys-F-N | Heterozygous | NGS          | Missense      |
| 37            | c.937+1G>A    | Abl.spl        | IVS10       | C2D    | Heterozygous | Sanger        | Splice site   |
| 38            | c.5077C>T     | p.R1693W       |             |        |        |               | Missense      |
| 39            | c.1667T>C     | p.L556P        | 19          |        |        |               | Missense      |
| 40            | c.0494delC    | p.C1398fs      | 39          | C2E    | Heterozygous | NGS          | Frameshift    |
| 41            | c.826T>2C>G   | Abl.spl        | IVS44       | C2F    | Heterozygous | Sanger        | Splice site   |
| 42            | c.5077C>T     | p.R1693W       | 46          |        |        |               | Missense      |
| 43            | c.1707C>T     | p.L555P        | 19          |        |        |               | Missense      |
| 44            | c.1707C>T     | p.L555P        | 19          |        |        |               | Missense      |
| 45            | c.1707C>T     | p.L555P        | 19          |        |        |               | Missense      |
| 46            | c.1707C>T     | p.L555P        | 19          |        |        |               | Missense      |
| 47            | c.799_800delTT| p.F267LfsX5    | 8           | C2B    | Heterozygous | Sanger        | Frameshift    |
| 48            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 49            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 50            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 51            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 52            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 53            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 54            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 55            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 56            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 57            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 58            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 59            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 60            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 61            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 62            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |
| 63            | c.1823T>C     | p.D288V        | 9           | C2B    | Heterozygous | Sanger        | Missense      |

*Contd...*
| Patient number | Mutation name | Protein change | Exon/intron | Domain | State | DNA sequencing | Mutation type |
|----------------|---------------|----------------|-------------|--------|-------|---------------|--------------|
| 64             | c.339delA†    | p.A115Pfs*36   | 4           | C2D    | Heterozygous | Sanger + MLPA | Frameshift    |
|                | Exon 33 deletion |                |             |        |       |               |              |
| 65             | c.1180+4delC† | Abl.spl        | IVS12       | C2C    | Heterozygous | Sanger        | Splice site   |
|                | p.Q570X       |                |             |        |       |               |              |
| 66             | c.1906C>T     | p.Q636X        | 20          |        | Heterozygous | NGS           | Nonsense      |
|                | Abl.spl       |                |             |        |       |               |              |
| 67             | c.610C>T      | p.R204X        | 6           |        | Heterozygous | Sanger        | Nonsense      |
|                | c.1134_1166del | p. 359A_368Adel | 12          | C2C    | Heterozygous | In frame deletion |              |
| 68             | c.4989_4990insCGGT† | p.1664Rfs*48 | 45          |        | Homozygous   | Sanger        | Frameshift    |
| 69             | c.265C>T      | p.R89X         | 4           |        | Heterozygous | NGS           | Nonsense      |
|                | c.6080G>A†    | p.W2027X       | 54          |        | Heterozygous | Sanger        | Nonsense      |
| 70             | c.792+1G>A†   | Abl.spl        | IVS7        | C2B    | Heterozygous | Sanger        | Splice site   |
|                | c.965T>C      | p.L322P        | 11          |        | Heterozygous | Sanger        | Missense      |
| 71             | c.5740G>A†    | p.D1914N       | 51          |        | C2G    | Heterozygous | Sanger + MLPA |
| 72             | c.567delA†    | p.P190Lfs*37   | 6           |        | Heterozygous | NGS + MLPA   | Frameshift    |
|                | Exon 2 deletion |                |             | C2A    | Heterozygous | Sanger        | Exon deletion |
| 73             | c.1165G>A†    | p.E389K        | 12          | C2C    | Heterozygous | Sanger        | Missense      |
|                | c.2997G>T     | p.W999C        | 28          | InnerDysF-N | Heterozygous | NGS | Missense      |

**Patients with two disease-causing mutations**

| Patient number | Mutation name | Protein change | Exon/intron | Domain | State | DNA sequencing | Mutation type |
|----------------|---------------|----------------|-------------|--------|-------|---------------|--------------|
| 74             | c.2997G>T     | p.W999C        | 28          | InnerDysF-N | Heterozygous | NGS | Missense      |
|                | c.5639C>G*†   | p.A1880G       | 50          | C2G    | Heterozygous | Sanger        | Missense      |
| 75             | c.3702T>G†  | p.Y1234X       | 33          | C2D    | Heterozygous | NGS           | Nonsense      |
|                | c.5511C>A*†  | p.D1837E       | 49          | C2G    | Heterozygous | Sanger        | Missense      |
| 76             | c.3032C>G*†  | p.Y1839F       | 49          | C2G    | Heterozygous | Sanger + MLPA | Missense      |
|                | Abl.spl       |                | IVS28       |        |        |               |              |
| 77             | c.1375dupA    | p.M459NfsX15   | 15          | C2C    | Heterozygous | NGS           | Frameshift    |
|                | c.5197A>G*†  | p.I1733V       | 46          |        |        | Heterozygous | Sanger        | Missense      |
| 78             | c.4497delT    | p.F1499LfsX4   | 41          |        | Heterozygous | Sanger        | Frameshift    |
|                | c.5216C>A*†  | p.I1739Q       | 41          |        |        | Heterozygous | Sanger        | Missense      |
| 79             | c.5792G>C*†  | p.R1931P       | 52          |        | Heterozygous | NGS           | Missense      |
|                | c.5511C>A*†  | p.D1837E       | 49          | C2G    | Heterozygous | Sanger        | Missense      |
|                | c.5516A>T*†  | p.Y1839F       | 49          | C2G    | Heterozygous | Sanger        | Missense      |
| 80             | c.3112C>T     | p.R1038X       | 29          | InnerDysF-C | Heterozygous | NGS + MLPA   | Nonsense      |
| 81             | c.4513T>A†   | p.Y1505N       | 42          |        | Heterozygous | Sanger + MLPA | Missense      |
| 82             | c.1464delT    | p.G489EfsX4    | 16          |        | Heterozygous | Sanger + MLPA | Frameshift    |
| 83             | c.610C>T     | p.R204X        | 6           |        | Heterozygous | Sanger + MLPA | Nonsense      |
| 84             | c.567delA†    | p.P190Lfs*37   | 6           |        | Heterozygous | Sanger + MLPA | Frameshift    |
| 85             | c.1464delT    | p.G489EfsX4    | 16          |        | Heterozygous | Sanger + MLPA | Frameshift    |
| 86             | c.3725G>A     | p.R1241H       | 34          | C2D    | Heterozygous | Sanger + MLPA | Missense      |
| 87             | c.610C>T     | p.R204X        | 6           |        | Heterozygous | Sanger + MLPA | Nonsense      |
| 88             | c.5805C>A†   | p.P1925T       | 52          |        | Heterozygous | Sanger + MLPA | Missense      |
| 89             | c.4063_4064insT | p.P13555fsX27 | 38          | C2E    | Heterozygous | Sanger + MLPA | Frameshift    |

*Mutations with undetermined pathogenicity; †Novel mutations. NGS: Next generation sequencing; Sanger: Sanger sequencing; MLPA: Multiplex ligation-dependent probe amplification.