Original Article

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

Su-Qin Jin, Meng Yu, Wei Zhang, He Lyu, Yun Yuan, Zhao-Xia Wang
Department of Neurology, Peking University First Hospital, Beijing 100034, China

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.[1-6] The most common phenotypes of dysferlinopathy are proximal limb weakness (limb girdle muscular dystrophy type 2B [LGMD2B])[1] and distal myopathy (Miyoshi myopathy [MM]).[2] However, other atypical symptoms such as hyperCKemia,[7] distal anterior compartment myopathy,[8] and proximodistal myopathy (PDM)[3] are not rare and can have a congenital onset.[9] 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.[10] 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.[11] In addition, false-negative results can occur in western blot analysis when DYSF has accumulated in the cytoplasm.[12] Therefore, gene analysis is necessary and still remains the "gold standard" for diagnosis.[13]

The *DYSF* gene is located on chromosome 2p13, which spans a genomic region of more than 230 kbp and comprises 55 exons.[1,2] It encodes a transmembrane protein DYSF which has been linked to membrane repair,[14] Ca^{2+} signaling,[15]

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Address for correspondence: Dr. Yun Yuan, Department of Neurology, Peking University First Hospital, 8 Xishiku St., Xicheng District, Beijing 100034, China
E-Mail: yuanyun2002@126.com

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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 *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 *DYSF* gene mutations.

To date, no more than 60 Chinese dysferlinopathy patients with genetic diagnoses had been reported.[4,20-23] 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 *DYSF* gene in patients with only one pathogenic mutation to confirm the existence of exonic rearrangements in Chinese patients.

Methods

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 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]

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 *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://technelsium.com.au/wp/chromas) and compared to the human *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.

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]

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).

Table 1: Classification of disease-causing mutations of dysferlin gene in this study

Mutation types	Mutations, *n*	Allele frequency (%)
Point mutations	77	47.24
Missense	49	30.06
Nonsense	28	17.18
Deletion/insertion	54	33.13
Frameshift	50	30.67
In-frame	4	2.45
Intronic mutation	29	17.79
Exonic rearrangement	3	1.84
Total	163	100.00

Table 2: Distribution of dysferlin gene mutations in this study

Domains	Proportion of mutations in this domain (%)
C2 domain A	3.70
C2 domain B	9.88
Ferlin family domain FerI	1.23
C2 Domain C	13.58
Ferlin family domain FerA	1.23
Ferlin family domain FerB	0.00
Outer DysF domain, N-terminal	0.62
Inner DysF domain, N-terminal	4.94
Inner DysF domain, C-terminal	4.32
Outer DysF domain, C-terminal	1.23
C2 domain D	6.17
C2 domain E	5.56
C2 domain F	5.56
C2 domain G	3.70
Transmembrane domain	0.00
Total	61.72

Table 3: Recurrent mutations of dysferlin gene identified in this study

Mutations	Protein changes	Domains	Patient number
c.610C>T	p.R204X		41, 67, 83, 87
c.799_800delTT	p.F267LfsX5	C2B	36, 47, 50
c.863A>T	p.D288V	C2B	1, 32, 42, 50, 52
c.1180+5G>A	Abl.spl	C2C	17, 31, 44, 48, 58
c.1375dupA	p.M459NfsX15	C2C	2, 11, 54, 55, 57, 77
c.1464delT	p.G489EfsX4		55,82,85
c.1667T>C	p.L556P		6, 43, 52
c.2997G>T	p.W999C	InnerDysF-N	35, 73, 74
c.3988C>T	p.Q1330X		6, 58, 61
c.4756C>T	p.R1586X	C2F	12, 25, 60

Discussion

The patients enrolled in this study came from 27 of the 34

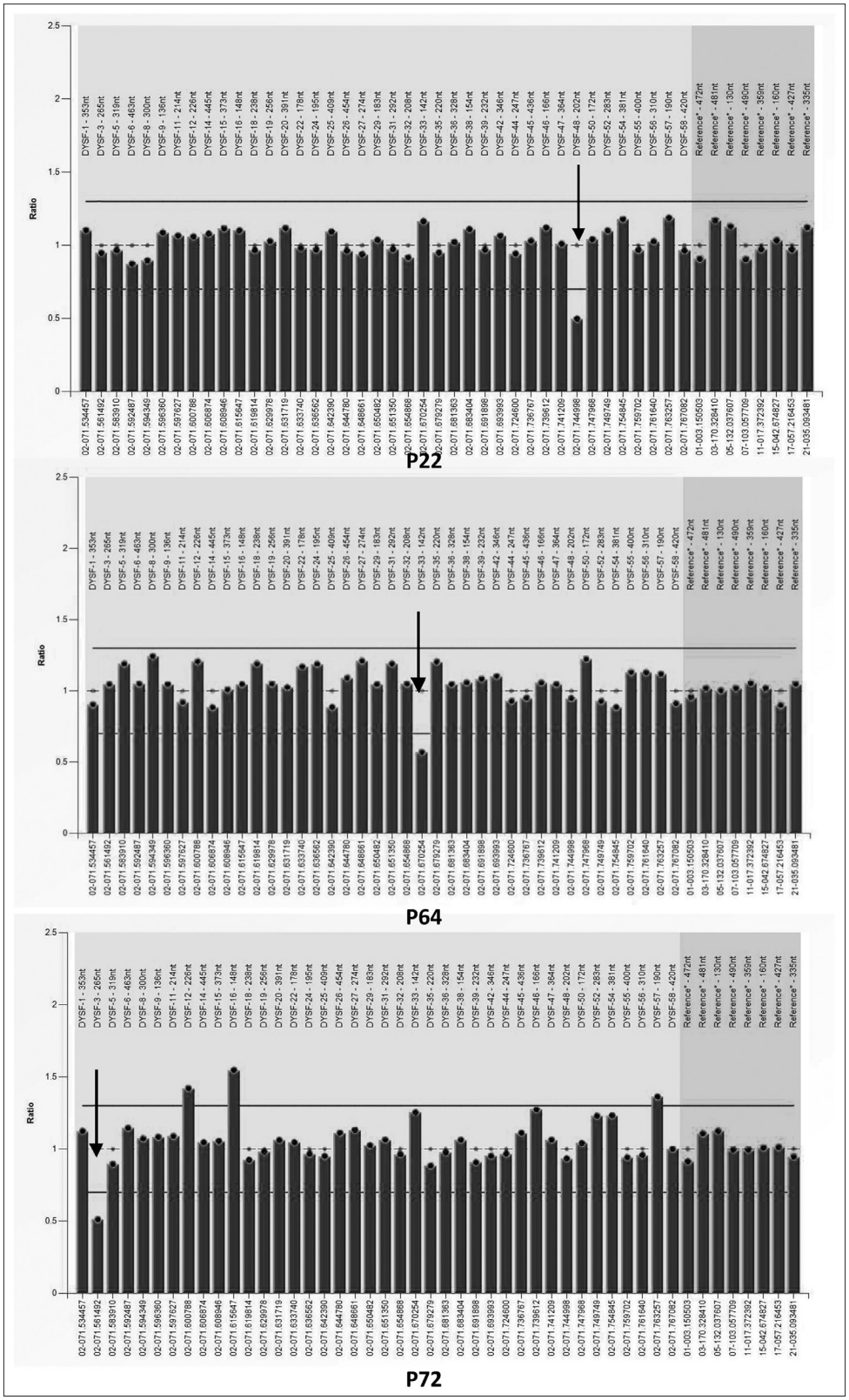

Figure 1: Multiplex ligation-dependent probe amplification results of patients with exon deletions of dysferlin gene. Deletion of exon 48, exon 33, and exon 2 were identified in patient 22, 64, and 72, respectively (arrows).

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) [Figure 2],[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 *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.[4,21,23] 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.[33]

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

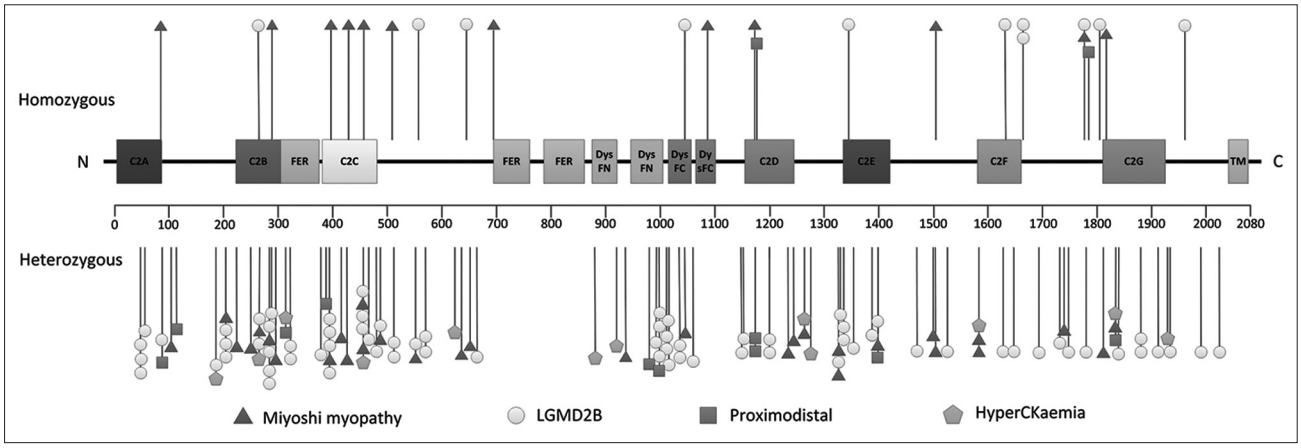

Figure 2: Positioning of dysferlin gene mutations identified in this study along the dysferlin protein sequence. Different domains are indicated by rectangles. Vertical lines above the protein indicate homozygous mutations while the vertical lines below the protein denote the heterozygous mutations.

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.

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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	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	+	PDM	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
			Patients with two disease-causing mutations				
1	c.863A>T	p.D288V	9	C2B	Homozygous	NGS	Missense
2	c.1375dupA	p.M459NfsX15	15	C2C	Heterozygous	NGS	Frameshift
	c.3036G>C†	p.W1012C	29	InnerDysF-C	Heterozygous		Missense
3	c.2643+5G>C†	Abl.spl	25	InnerDysF-N	Heterozygous	NGS	Splice site
	c.3827T>C†	p.L1276P	34		Heterozygous		Missense
4	c.3059C>T†	p.P1020L	28	InnerDysF-C	Heterozygous	NGS	Missense
	c.3442+1G>A†	Abl.spl	IVS31		Heterozygous		Splice site
5	c.5302C>T	p.R1768W	47		Homozygous	NGS	Missense
6	c.1667T>C	p.L556P	19		Heterozygous	Sanger	Missense
	c.3988C>T	p.Q1330X	37		Heterozygous		Nonsense
7	c.4894G>T	p.E1632X	45	C2F	Homozygous	NGS	Nonsense
8	c.5414dupC†	p.R1806Tfs*22	48		Homozygous	NGS	Frameshift
9	c.1523-2A>G†	Abl.spl	IVS17		Homozygous	NGS	Splice site
10	c.3115C>T†	p.R1039W	29	InnerDysF-C	Heterozygous	NGS	Missense
	c.5245C>T†	p.R1749C	47		Heterozygous		Missense
	c.5525G>A	p.G1842D	49	C2G	Heterozygous		Missense
11	c.1375dupA	p.M459Nfs*15	15	C2C	Homozygous	NGS	Frameshift
12	c.895G>C	p.G299R	9	C2B	Heterozygous	Sanger	Missense
	c.4756C>T	p.R1586X	43	C2F	Heterozygous		Nonsense
13	c.1874A>T†	p.D625V	20		Heterozygous	NGS	Missense
	c.2762C>T†	p.S921L	26	OuterDysF-N	Heterozygous		Missense
	c.3785delG†	p.G1263Afs*82	34		Heterozygous		Frameshift
14	c.1535_1553del†	p.F514Pfs*107	18		Heterozygous	Sanger	Frameshift
	c.4167+1G>A	Abl.spl	IVS38	C2E	Heterozygous		Splice site
15	c.4988_4989delTC†	p.V1663Gfs*47	45	C2F	Homozygous	NGS	Frameshift
16	c.1523-2A>G†	Abl.spl	IVS17		Heterozygous	NGS	Splice site
	c.2974T>C	p.W992R	28	InnerDysF-N	Heterozygous		Missense
17	c.1180+5G>A	Abl.spl	IVS12	C2C	Heterozygous	NGS	Splice site
	c.4194delC	p.C1398fs	39	C2E	Heterozygous		Frameshift
18	c.3601C>T	p.Q1200X	33	C2D	Heterozygous	NGS	Nonsense
	c.4580T>G†	p.L1527R	42		Heterozygous		Missense
19	c.1930+2T>G†	Abl.spl	IVS20		Homozygous	NGS	Splice site
20	c.5884 C>T	p.Q1962X	52		Homozygous	NGS	Nonsense
21	c.3531_3533delCAT†	p. 1178Idel	33	C2D	Homozygous	NGS	In frame deletion
	c.5355G>A*,†	p.M1785I	48		Homozygous		Missense
22	Exon 48 deletion		40		Heterozygous	NGS + MLPA	Exonic deletion
	c.4024C>G	p.R1342G	38	C2E	Heterozygous		Missense
23	c.4022T>C	p.L1341P	38	C2E	Homozygous	NGS	Missense
24	c.265C>T	p.R89X	4		Heterozygous	NGS	Nonsense
	c.5509G>A	p.D1837N	49	C2G	Heterozygous		Missense
25	c.1284+2T>C	Abl.spl	IVS13	C2C	Heterozygous	NGS	Splice site
	c.4756C>T	p.R1586X	43	C2F	Heterozygous		Nonsense
26	c.144+1G>A†	Abl.spl	IVS2	C2A	Heterozygous	NGS	Splice site
	c.1393G>C†	p.D465H	15	C2C	Heterozygous		Missense
27	c.3258_3259delAG†	p.D1087Cfs*26	30	OuterDysF-C	Homozygous	NGS	Frameshift
28	c.796_797delCT	p.L266FfsX6	8	C2B	Heterozygous	NGS	Frameshift
	c.1377_1379del	p.R460del	15	C2C	Heterozygous		In frame deletion
29	c.4509+2_c.4509+6delTAAGG†	Abl.spl	IVS41		Homozygous	Sanger	Splice site
30	c.313dupC	p.L105PfsX43	4		Heterozygous	NGS	Frameshift
	c.5438T>C†	p.L1813P	49	C2G	Heterozygous		Missense
31	c.1180+5G>A	Abl.spl	IVS12	C2C	Heterozygous	Sanger	Splice site
	c.3601C>T	p.Q1201X	33	C2D	Heterozygous		Nonsense

Contd...

Supplementary Table 2: Contd...

Patient number	Mutation name	Protein change	Exon/intron	Domain	State	DNA sequencing	Mutation type
			Patients with two disease-causing mutations				
32	c.863A>T	p.D288V	9	C2B	Heterozygous	NGS	Missense
	c.965T>C	p.L322P	11	FerI	Heterozygous		Missense
33	c.2940delG	p.L981FfsX76	28	InnerDysF-N	Heterozygous	NGS	Frameshift
	c.4200dupC	p.I1401HfsX8	39	C2E	Heterozygous		Frameshift
34	c.252delC†	p.K85Rfs*66	4	C2A	Homozygous	Sanger	Frameshift
35	c.1992C>A†	p.Try664X	21		Heterozygous	Sanger	Nonsense
	c.2997G>T	p.W999C	28	InnerDysF-N	Heterozygous		Missense
36	c.799_800delTT	p.F267LfsX5	8	C2B	Homozygous	Sanger	Frameshift
37	c.937+1G>A	Abl.spl	IVS10		Heterozygous	Sanger	Splice site
	c.3521-1G>T†	Abl.spl	IVS32	C2D	Heterozygous		Splice site
38	c.2083delG†	p.A695Pfs*2	22	FerA	Homozygous	NGS	Frameshift
39	c.176delT†	p.L59Rfs*92	3	C2A	Heterozygous	NGS	Frameshift
	c.5975delT†	p.V1992Efs*20	53		Heterozygous		Frameshift
40	c.4194delC	p.C1398fs	39	C2E	Heterozygous	NGS	Frameshift
	c.4886+2T>G†	Abl.spl	IVS44	C2F	Heterozygous		Splice site
41	c.610C>T	p.R204X	6		Heterozygous	NGS	Nonsense
	c.3516_3517delTT	p.S1173X	32	C2D	Heterozygous		Frameshift
42	c.863A>T	p.D288V	9	C2B	Heterozygous	Sanger	Missense
	c.5077C>T	p.R1693W	46		Heterozygous		Missense
43	c.1667T>C	p.L556P	19		Homozygous	NGS	Missense
44	c.1180+5G>A	Abl.spl	IVS12	C2C	Homozygous	Sanger	Splice site
45	c.3516_3517delTT	p.S1173X	32	C2D	Homozygous	Sanger	Frameshift
46	c.5444G>T	p.C1815F	49	C2G	Homozygous	NGS	Missense
47	c.799_800delTT	p.F267LfsX5	8	C2B	Heterozygous	Sanger	Frameshift
	c.3181C>T	p.Q1061RfsX59	30		Heterozygous		Nonsense
48	c.1180+5G>A	Abl.spl	IVS12	C2C	Heterozygous	Sanger	Splice site
	c.4941_4942del CT†	p.Y1648*	45	C2F	Heterozygous		Frameshift
49	c.5302C>T	p.R1768W	47		Homozygous	Sanger	Missense
50	c.799_800delTT	p.F267LfsX5	8	C2B	Homozygous	NGS	Frameshift
	c.863A>T	p.D288V	9	C2B	Heterozygous		Missense
51	c.2997G>A	p.W999X	28	InnerDysF-N	Heterozygous	NGS	Nonsense
	c.4411_4433del	p.E1472Qfs*21	41		Heterozygous		Frameshift
52	c.863A>T	p.D288V	9	C2B	Heterozygous	Sanger	Missense
	c.1667T>C	p.L556P	19		Heterozygous		Missense
53	c.1254delC	p.F419Lfs*41	13	C2C	Heterozygous	NGS	Frameshift
	c.1956G>A†	p.W652X	21		Heterozygous		Nonsense
54	c.1375dupA	p.M459NfsX15	15	C2C	Heterozygous	NGS	Frameshift
	c.3137G>A	p.R1046H	29	InnerDysF-C	Heterozygous		Missense
55	c.1375dupA	p.M459NfsX15	15	C2C	Heterozygous	Sanger	Frameshift
	c.1464delT	p.G489EfsX4	16		Heterozygous		Frameshift
56	c.3137G>A	p.R1046H	29	InnerDysF-C	Homozygous	Sanger	Missense
57	c.144+1G>A†	Abl.spl	IVS2	C2A	Heterozygous	Sanger	Splice site
	c.1375dupA	p.M459NfsX15	15	C2C	Heterozygous		Frameshift
58	c.3988C>T	p.Q1330X	37		Heterozygous	Sanger	Nonsense
	c.1180+5G>A	Abl.spl	IVS12	C2C	Heterozygous		Splice site
59	c.1284+1G>A†	Abl.spl	IVS13	C2C	Homozygous	Sanger	Splice site
60	c.937+1G>A	Abl.spl	IVS10		Heterozygous	Sanger	Splice site
	c.4756C>T	p.R1586X	43	C2F	Heterozygous		Nonsense
61	c.680T>C†	p.I227T	7	C2B	Heterozygous	Sanger	Missense
	c.3988C>T	p.Q1330X	37		Heterozygous		Nonsense
62	c.755C>T	p.T252M	7	C2B	Heterozygous	Sanger	Missense
	c.3789_3796del†	p.S1264Vfs*9	34		Heterozygous		Frameshift
63	c.1708C>T†	p.Q570X	19		Heterozygous	Sanger	Nonsense
	c.4011delG†	p.A1338Hfs*7	38	C2E	Heterozygous		Frameshift

Contd...

Supplementary Table 2: Contd...

Patient number	Mutation name	Protein change	Exon/intron	Domain	State	DNA sequencing	Mutation type
Patients with two disease-causing mutations							
64	c.339delA†	p.A115Pfs*36	4		Heterozygous	Sanger + MLPA	Frameshift
	Exon 33 deletion		33	C2D	Heterozygous		Exonic deletion
65	c.1180+4delC†	Abl.spl	IVS12	C2C	Heterozygous	Sanger	Splice site
	c.1708C>T†	p.Q570X	19		Heterozygous		Nonsense
66	c.1906C>T†	p.Q636X	20		Heterozygous	NGS	Nonsense
	c.2810+5G>A†	Abl.spl	IVS20		Heterozygous		Splice site
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.V1664Rfs*48	45		Homozygous	Sanger	Frameshift
69	c.265C>T	p.R89X	4		Heterozygous	NGS	Nonsense
	c.6080G>A†	p.W2027X	54		Heterozygous		Nonsense
70	c.792+1G>A†	Abl.spl	IVS7	C2B	Heterozygous	NGS	Splice site
	c 965T>C	p.L322P	11	FerI	Heterozygous		Missense
71	c.5740G>A†	p.D1914N	51	C2G	Heterozygous	Sanger + MLPA	Missense
72	c.567delA†	p.P190Lfs*37	6		Heterozygous	NGS + MLPA	Frameshift
	Exon 2 deletion		3	C2A	Heterozygous		Exonic deletion
73	c.1165G>A	p.E389K	12	C2C	Heterozygous	Sanger	Missense
	c.2997G>T	p.W999C	28	InnerDysF-N	Heterozygous		Missense
Patients with one disease-causing mutation							
74	c.2997G>T	p.W999C	28	InnerDysF-N	Heterozygous	NGS	Missense
	c.5639C>G*,†	p.A1880G	50	C2G	Heterozygous		Missense
75	c.3702T>G†	p.Y1234X	33	C2D	Heterozygous	NGS	Nonsense
	c.5511C>A*,†	p.D1837E	49	C2G	Heterozygous		Missense
	c.5516A>T*,†	p.Y1839F	49	C2G	Heterozygous		Missense
76	c.3032-3C>G†	Abl.spl	IVS28	InnerDysF-N	Heterozygous	NGS	Splice site
	c.5639C>G†	p.A1880G	50	C2G	Heterozygous		Missense
77	c.1375dupA	p.M459NfsX15	15	C2C	Heterozygous	NGS	Frameshift
	c.5197A>G*,†	p.I1733V	46		Heterozygous		Missense
78	c.4497delT	p.F1499LfsX4	41		Heterozygous	Sanger	Frameshift
	c.5216C>A*,†	p.P1739Q	41		Heterozygous		Missense
79	c.5792G>C*,†	p.R1931P	52		Heterozygous	NGS	Missense
	c.5511C>A*,†	p.D1837E	49	C2G	Heterozygous		Missense
	c.5516A>T*,†	p.Y1839F	49	C2G	Heterozygous		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.5803C>A†	p.P1935T	52		Heterozygous	Sanger + MLPA	Missense
89	c.4063_4064insT	p.P1355SfsX27	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.