□ 遺伝子解析を行っていない

□ 遺伝子解析を行ったか分からない

Q1-c 先天性筋ジストロフィー

上記 a,b で、☑筋生検を行っていない、☑遺伝子解析を行っていない、ともに☑がある方のみ回答ください（a,b で診断がついている方は、下記の回答は不要です）

＜診断根拠＞

□ CK

□ 骨格筋 MRI/CT

□ 筋電図

□ 脊椎 Xp

□ 心機能低下（不整脈含）

□ 呼吸機能低下

□ 頭部 MRI

□ ほか特徴的な臨床所見

臨床診断の根拠を簡潔にご記入ください。

(　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　)

＜Q2　先天性ミオパチー＞

Q2-a　先天性ミオパチー

- □　筋生検を行った（下記も記入ください）
  - □　ネマリンミオパチー
  - □　セントラルコア病
  - □　マルチミニコア病
  - □　ミオチュブラーミオパチー
  - □　中心核病
  - □　先天性筋線維タイプ不均等症
  - □　先天性全タイプ１線維ミオパチー
  - □　タイプ１線維優位を示す先天性ミオパチー
  - □　還元小体ミオパチー
  - □　cap disease
  - □　非特異的所見

    （congenital myopathy without specific morphologic features, minimal change myopathy 等）
  - □　その他（　　　　　　　　　　　　　　　　　　　　　）

- □　筋生検を行っていない

Q2-b　先天性ミオパチー

- □　遺伝子解析を行った（下記記入ならびに報告書コピーを同封してください）
  - □　ACTA1
  - □　BIN1
  - □　CFL2
  - □　CHST14
  - □　CNTN1
  - □　COL12
  - □　DNM2
  - □　FHL1
  - □　FKBP14
  - □　KBDBD13
  - □　LAMP2
  - □　MTM1
  - □　MTMR14
  - □　MYH7
  - □　NEB
  - □　PLOD
  - □　RYR1
  - □　TNNT1
  - □　TPM2

- □ TPM3
- □ TRIM32
- □ TTN
- □ その他（　　　　　　　　　　　　　　　　　　　）
- □ 遺伝子解析を行ったが変異は見つからなかった

- □ 遺伝子解析を行っていない
- □ 遺伝子解析を行ったか分からない

Q2-c　先天性ミオパチー

上記 a,b で、☑筋生検を行っていない、☑遺伝子解析を行っていない、ともに☑がある方のみ回答ください（a,b で診断がついている方は、下記の回答は不要です）

＜診断根拠＞

- □ CK
- □ 骨格筋 MRI/CT
- □ 筋電図
- □ 脊椎 Xp
- □ 心機能低下（不整脈含）
- □ 呼吸機能低下
- □ 頭部 MRI
- □ ほか特徴的な臨床所見

臨床診断の根拠を簡潔にご記入ください。

（　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　）

＜Q3　筋原線維ミオパチー＞

Q3-a　筋原線維ミオパチー

- □　筋生検を行った（報告書コピーを同封してください）
- □　筋生検を行っていない

Q3-b　筋原線維ミオパチー

- □　遺伝子解析を行った（下記記入ならびに報告書コピーを同封してください）
  - □　BAG3
  - □　CNT2
  - □　CRYAB
  - □　DES
  - □　FLNC
  - □　GAA
  - □　MYOT
  - □　ZASP
  - □　その他（　　　　　　　　　　　　　　　　　　　　）
  - □　遺伝子解析を行ったが変異は見つからなかった

- □　遺伝子解析を行っていない
- □　遺伝子解析を行ったか分からない

Q3-c　筋原線維ミオパチー

上記 a,b で、☑筋生検を行っていない、☑遺伝子解析を行っていない、ともに☑がある方のみ回答ください
（a,b で診断がついている方は、下記の回答は不要です）

＜診断根拠＞

- □　CK
- □　骨格筋 MRI/CT
- □　筋電図
- □　脊椎 Xp
- □　心機能低下（不整脈含）
- □　呼吸機能低下
- □　頭部 MRI
- □　ほか特徴的な臨床所見

臨床診断の根拠を簡潔にご記入ください。
（　　）

<Q4　先天性筋無力症>

Q4-a　先天性筋無力症

- ☐　筋生検を行った（報告書コピーを同封してください）
- ☐　筋生検を行っていない

Q4-b　先天性筋無力症

- ☐　遺伝子解析を行った（下記記入ならびに報告書コピーを同封してください）
  - ☐　AGRN
  - ☐　CHAT
  - ☐　CHRNA1
  - ☐　CHRNB1
  - ☐　CHRND
  - ☐　CHRNDE
  - ☐　CHRNG
  - ☐　COLQ
  - ☐　DOK7
  - ☐　GFPT1
  - ☐　LAMB2
  - ☐　LRP4
  - ☐　MUSK
  - ☐　PLEC
  - ☐　RAPSN
  - ☐　SCN4A
  - ☐　その他（　　　　　　　　　　　　　　　　　　）
  - ☐　遺伝子解析を行ったが変異は見つからなかった
- ☐　遺伝子解析を行っていない
- ☐　遺伝子解析を行ったか分からない

Q4-c　先天性筋無力症

上記 a,b で、☑筋生検を行っていない、☑遺伝子解析を行っていない、ともに☑がある方のみ回答ください（a,b で診断がついている方は、下記の回答は不要です）

<診断根拠>

- ☐　CK
- ☐　骨格筋 MRI/CT
- ☐　筋電図
- ☐　脊椎 Xp
- ☐　心機能低下（不整脈含）
- ☐　呼吸機能低下

□　頭部 MRI

□　ほか特徴的な臨床所見

臨床診断の根拠を簡潔にご記入ください。

（　　）

<Q5　その他>

（診断名（記入）：　　　　　　　　　　　　　　　　）

☐　筋生検を行った　（所見：　　　　　　　　　　　　　　　　　）（報告書コピーを同封してください）

☐　筋生検を行っていない

☐　遺伝子解析を行った　（所見：　　　　　　　　　　　　　　　）（報告書コピーを同封してください）

☐　遺伝子解析を行っていない

☐　遺伝子解析を行ったか分からない

☐　脊髄性筋萎縮症ではない

☐　筋強直性ジストロフィーではない

※　脊髄性筋萎縮症、筋強直性ジストロフィー

⇒　個別の登録システムがございます。そちらに登録をお願いします。

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍
なし

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nishikawa A, **Mori-Yoshimura M**, Segawa K, Hayashi YK, Takahashi T, Saito Y, Nonaka I, Krahn M, Levy N, Shimizu J, Mitsui J, **Kimura E**, Goto J, **Yonemoto N**, **Aoki M**, *et al.*	Respiratory and cardiac function in Japanese patients with dysferlinopathy.	***Muscle Nerve***	53 (3)	394-401	2016
Izumi R, Niihori T, Takahashi T, Suzuki N, Tateyama M, Watanabe C, Sugie K, Nakanishi H, Sobue G, Kato M, Warita H, Aoki Y, **Aoki M.**	Genetic profile for suspected dysferlinopathy identified by targeted next-generation sequencing.	***Neurol Genet***	1	e36	2015
Yamashita S, Mori A, Nishida Y, Kurisaki R, Tawara N, Nishikami T, Misumi Y, Ueyama H, *et al.*	Clinicopathological features of the first Asian family having vocal cord and pharyngeal weakness with distal myopathy due to a *MATR3* mutation.	***Neuropathol Appl Neurobiol***	41	391-398	2015
Azuma Y, Nakata T, Tanaka M, Shen XM, Ito M, Iwata S, Okuno T, Nomura Y, Ando N, Ishigaki K, Ohkawara B, Masuda A, Natsume J, Kojima S, Sokabe M, **Ohno K.**	Congenital myasthenic syndrome in Japan: Ethnically unique mutations in muscle nicotinic acetylcholine receptor subunits	***Neuromuscul Disord***	25	60-69	2015
Selcen D, Ohkawara B, Shen XM, McEvoy K, **Ohno K**, Engel AG.	Impaired Synaptic Development, Maintenance, and Neuromuscular Transmission in LRP4-Related Myasthenia	***JAMA Neurol***	72	889-896	2015
Rahman MA, Azuma Y, Nasrin F, Takeda J, Nazim M, Ahsan KB, Masuda A, Engel AG, **Ohno K**.	SRSF1 and hnRNP H antagonistically regulate splicing of COLQ exon 16 in a congenital myasthenic syndrome	***Sci Rep***	5	13208	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Watanabe N, Horikoshi M, Yamada M, Shimodera S, Akechi T, Miki K, Inagaki M, Yonemoto N, *et al.*	Adding smartphone-based cognitive-behavior therapy to pharmacotherapy for major depression (FLATT project): study protocol for a randomized controlled trial	***Trials***	16	293	2015
Watanabe N, Furukawa TA, Shimodera S, Katsuki F, Fujita H, Sasaki M, Sado M, Perlis ML.	Cost-effectiveness of cognitive behavioral therapy for insomnia comorbid with depression: Analysis of a randomized controlled trial	***Psychiatry Clin Neurosc***	69	335-343	2015

Ⅳ．研究成果の刊行物・別刷

RESPIRATORY AND CARDIAC FUNCTION IN JAPANESE PATIENTS WITH DYSFERLINOPATHY

ATSUKO NISHIKAWA, MD,[1,2,3] MADOKA MORI-YOSHIMURA, MD, PhD,[1] KAZUHIKO SEGAWA, MD, PhD,[4] YUKIKO K. HAYASHI, MD, PhD,[3,5] TOSHIAKI TAKAHASHI, MD, PhD,[6] YUKO SAITO, MD, PhD,[7] IKUYA NONAKA, MD, PhD,[8] MARTIN KRAHN, MD, PhD,[9,10] NICOLAS LEVY, MD, PhD,[9,10] JUN SHIMIZU, MD, PhD,[11] JUN MITSUI, MD, PhD,[11] EN KIMURA, MD, PhD,[12] JUN GOTO, MD, PhD,[11,13] NAOHIRO YONEMOTO, MD, PhD,[12] MASASHI AOKI, MD, PhD,[14] ICHIZO NISHINO, MD, PhD,[3,12] YASUSHI OYA, MD,[1] and MIHO MURATA, MD, PhD[1]

[1] Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[2] Department of Education Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan
[3] Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[4] Department of Cardiology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[5] Department of Pathophysiology, Tokyo Medical University, Tokyo, Japan
[6] Department of Neurology and Division of Clinical Research, Sendai Nishitaga National Hospital, Miyagi, Japan
[7] Department of Laboratory Medicine, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[8] Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[9] Aix-Marseille University, UMR 910 INSERM, Faculté de Médecine Timone, Marseille, France
[10] APHM, Hôpital d'Enfants de la Timone, Département de Génétique Médicale et de Biologie Cellulaire, Marseille, France
[11] Department of Neurology, Division of Neuroscience, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[12] Department of Promoting Clinical Trial and Translational Medicine, Translational Medical Center, National Center of Neurology and Psychiatry, Ogawahigashi, Tokyo, Japan
[13] Department of Neurology, International University of Health and Welfare Mita Hospital, Tokyo, Japan
[14] Department of Neurology, Tohoku University School of Medicine, Miyagi, Japan

Accepted 17 June 2015

ABSTRACT: *Introduction:* We retrospectively reviewed respiratory and cardiac function in patients with dysferlinopathy, including 2 autopsy cases with respiratory dysfunction. *Methods:* Subjects included 48 patients who underwent respiratory evaluation (n = 47), electrocardiography (n = 46), and echocardiography (n = 23). *Results:* Of the 47 patients, 10 had reduced percent forced vital capacity (%FVC), and 4 required noninvasive positive pressure ventilation. %FVC was significantly correlated with disease duration, and mean %FVC was significantly lower in non-ambulatory patients, as well as in those aged $\geq$65 years with normal creatine kinase levels. On electrocardiography, QRS complex duration was prolonged in 19 patients, although no significant association with age, disease duration, or respiratory function was found. Echocardiography indicated no left ventricular dysfunction in any patient. Histopathology of autopsied cases revealed mild cardiomyopathy and moderate diaphragm involvement. *Conclusion:* Patients with dysferlinopathy may develop severe respiratory failure and latent cardiac dysfunction. Both respiratory and cardiac function should be monitored diligently.

Muscle Nerve **53**: 394–401, 2016

Additional Supporting Information may be found in the online version of this article.

Abbreviations: CK, creatine kinase; CPF, cough peak flow; DCM, dilated cardiomyopathy; DYSF, dysferlin; ECG, electrocardiogram; EF, ejection fraction; FVC, forced vital capacity; LGMD2B, limb-girdle muscular dystrophy type 2B; MM, Miyoshi myopathy; NADH-TR, nicotinamide adenine dinucleotide–tetrazolium reductase; NCNP, National Center of Neurology and Psychiatry; NIV, noninvasive positive pressure ventilation

Key words: autopsy; cardiac function; dysferlinopathy; LGMD2B; Miyoshi myopathy; respiratory function

This work was partially supported by grants for Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan, and Intramural Research Grants (23-4/26-7) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry.

Correspondence to: M. Mori-Yoshimura; e-mail: yoshimur@ncnp.go.jp

Published online 18 June 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.24741

Dysferlinopathy is an autosomal recessive muscular dystrophy caused by dysferlin gene mutations (*DYSF*; MIM # 603009). The *DYSF* gene is located on chromosome 2p13 and codes for dysferlin, which is mainly localized in the sarcolemma and is associated with Ca^{2+}-dependent defective membrane repair and T-tubule system development. The 2 major phenotypes of dysferlinopathy are proximal dominant limb-girdle muscular dystrophy type 2B (LGMD2B) and distal dominant Miyoshi myopathy (MM). In patients with dysferlinopathy, dysferlin in the sarcolemma is absent or markedly reduced. Immunohistochemical staining (IHC), immunoblotting (IB), and gene analysis are useful for diagnosis.

Cardiac dysfunction (e.g., dilated cardiomyopathy, cardiac conduction defect) is variably present in patients with certain muscular dystrophies, including dystrophinopathy and sarcoglycanopathy, and respiratory failure is a major complication in many neuromuscular disorders. However, these are not part of the usual manifestations of dysferlinopathy[1]; only a limited number of studies have reported respiratory and cardiac function in patients with dysferlinopathy.[2–11] The majority of patients, including those with long disease duration, reportedly exhibited no respiratory problems, although some non-ambulatory, advanced-stage patients (but not necessarily with long disease duration) showed reduced vital capacity.[2–6] Moreover, dilated cardiomyopathy on echocardiography, and/or abnormalities on electrocardiogram (e.g., ST–T change), were observed in both advanced and non-advanced stage patients.[9–11] However,

Table 1. Patient characteristics.

	Mean	SD	Median	Range	Interquartile range (25th–75th percentile)
Age (years)	42.5	14.7	39.5	20–79	32.8–50.5
Age at onset (years)	22.5	7.5	20.5	10–46	16.0–29.0
Duration from disease onset (years)	20.0	12.3	17.5	3–46	11.0–26.0
Age at gait disturbance	43.8	10.6	45.5	22–60	36.0–50.0
CK (IU/L)	3,596	2,978	3,160	94–14,591	1,249–4,884
BMI	21.5	3.7	21.3	15.3–35.0	18.9–23.0
%VC (%)	92.6	28.3	101.8	23.1–129.0	86.8–112.8
%FVC (%)	92.7	28.9	100.6	20.1–131.5	84.7–112.5
EF (%)	68.0	8.3	66.0	48–84	65.0–73.5
QRS duration (ms)	97.5	12.7	94.0	78–138	88.0–108.0

CK, creatine kinase; BMI, body mass index; VC, vital capacity; FVC, forced vital capacity; EF, ejection fraction.

there are no clinical reports with details regarding respiratory dysfunction associated with dysferlinopathy, and only a few reports have assessed histological changes in respiratory and cardiac muscles in dysferlinopathy patients.[7,8]

Patients with neuromuscular disorders who present with respiratory dysfunction undergo respiratory training using the air stacking technique to increase thoracic capacity and to use assisted cough peak flow (CPF) from an early stage.[12] In addition, medication for cardiomyopathy, such as angiotensin-converting enzyme inhibitors and/or β-blockers, can be effective in delaying progression.[13,14]

If dysferlinopathy patients commonly develop respiratory and cardiac dysfunction, physicians should diligently monitor respiratory and cardiac function to detect early signs to enable respiratory training and prescription of cardiac medication from an early stage. In light of this background, we aimed to analyze factors that contribute to respiratory and cardiac dysfunction in dysferlinopathy.

METHODS

Study Population. We retrospectively reviewed medical charts of 48 patients with dysferlinopathy who visited the National Center Hospital of the National Center of Neurology and Psychiatry (NCNP) from July 1979 to September 2013. Patient diagnoses were confirmed by genetic analysis and/or IHC together with IB,[15] for those who satisfied 1 or more of the following diagnostic criteria: (1) known homozygous or compound heterozygous *DYSF* gene mutations; (2) 1 reported and 1 unreported, but causing aberrant splicing mutations; and (3) absence of dysferlin in the sarcolemma by IHC. All patients who had no or only 1 mutation in a single allele were verified to have absent dysferlin. This study was approved by the ethics committee of the NCNP.

Measurements and Analysis. We collected data on age at the time of examination, age at onset, disease duration, ambulation status, serum creatine kinase (CK) levels, disease phenotype, respiratory function (%FVC), and cardiac function (electrocardiogram and echocardiogram) for analysis. As percent vital capacity changed in parallel with %FVC, we only used %FVC in respiratory assessments. Decrement in %FVC and ejection fraction (EF) were defined as $<80\%$[16] and 55%,[17] respectively. Prolongation in QRS complex duration was defined as >100 ms.[18]

A *t*-test was used for group comparisons of continuous data, and the Fisher exact test was used for binary data. Correlation of data was assessed using the Spearman rank correlation coefficient, which is appropriate for both continuous and discrete variables, including ordinal variables. Fisher exact tests were used to analyze factors that potentially contribute to respiratory dysfunction: age ≥ 65 years (the age at which one is defined as elderly in Japan)[19]; age at onset ≥ 20 years; gender; ambulation status; serum CK levels; and disease phenotype. All analyses were performed using SPSS for Macintosh, version 18 (SPSS, Inc., Chicago, Illinois).

RESULTS

General Characteristics. The analysis included a total of 48 Japanese patients (25 men, 23 women). Age at onset was 22.5 ± 7.4 years, and mean age at the time of data collection was 42.5 ± 14.7 years (Table 1). Of these, 22 and 26 patients were diagnosed with LGMD2B and MM, respectively, 36 were still ambulatory, and 12 were completely wheelchair-bound. Among non-ambulatory patients, mean disease duration at loss of ambulation was 19.4 ± 5.9 years. Mean serum CK was 3,596 ± 2,978 IU/L. More men were included in the MM group, and more women were in the LGMD2B group; age at disease onset was significantly older in the LGMD2B group (see Table S1 in Supplementary Material, available online), although other parameters did not show a statistically significant difference.

Table 2. Genotypes of patients with dysferlinopathy.

Homozygous	
c.493delC	1
c.663+1 G>C	1
c.855+4 T>C	1
c.1284+2 T>C	1
c.1852 G>A	1
c.2233–2235delAAC*	1
c.2551 C>T*	1
c.2997 G>T	2
c.4934 T>A*	1
c.4497delT	3
c.5077 C>T	2
c.5509 G>A	1
c.6135 G>A	1
Compound heterozygous	
c.342+1 G>A / c.6135 G>A	1
c.565 C>G / c.855+4 T>C	1
c.855+4 T>C / c.4200 delC	1
c.937+1 G>A / c.2643+1 G>A	1
c.1004 G>A* / c.4497 delT	1
c.1353+1G>A / c.5526-1 G>A	1
c.1566 C>G / c.2997 G>T	1
c.1556 C>G / c.5509 G>A	1
c.1566 C>G / c. 5698_5699 delAG	1
c2644-2 A>G / c.2997 G>C	1
c.2746_2747dupGG* / c.5309 T>C*	1
c.2997 G>T / c.3373 delG	2
c.2997G>T / c.3827 delT*	2
c.2997G>T / c.4497 delT	1
c.2997 G>T / c.4886+2 T>A*	1
c.2997 G>T / c.6135 G>A	2
c.3373 delG / c.4748_4750 delACAinsT	1
c.4720G>T / c.5036 G>A	1
Heterozygous	
c.2997 G>T	4
Not tested	6
Total	48

*Unreported.

Immunohistochemistry and Gene Analysis. IHC and IB were performed in 34 (71%) and 25 (52%) patients, respectively, revealing completely absent sarcolemmal dysferlin in all patients. *DYSF* gene analysis was performed in 42 (88%) patients. Of these patients, 17 were homozygous and 21 were compound heterozygous, including 7 with 8 unreported mutations (Table 2, and Table S2 online). Gene analysis and IHC were performed in 28 (58%) patients. Although the pathogenicity of these unreported mutations is unclear, they were not detected in 100 healthy Japanese people, and 6 patients with unreported mutations, with the exception of 1, showed abnormalities on IHC. One patient without muscle biopsy had c.2997 G>T and c.4886+2 T>A compound heterozygote. As c.2997 G>T is a well-known causative mutation and c.4886+2 T>A causes aberrant splicing, we considered these mutations to be pathogenic. Only 1 point mutation in a single allele was detected in 4 patients whose diagnosis was confirmed by IHC and IB. In these patients, another mutation may exist in non-translated or promoter regions. The most common mutation was c.2997 G>T.

Respiratory Function. None of the patients had lung or thoracic diseases that could affect their respiratory function. Mean %FVC was 92.7 ± 28.9 [median 100.6, interquartile range (25th–75th percentiles) 84.7–112.5, range 20.1–131.5], and no significant difference was found between the LGMD2B and MM groups (see Table S1 online).

Patients with Respiratory Dysfunction. Age and disease duration were significantly correlated with a reduced %FVC (Fig. 1). Significant factors associated with %FVC <80 identified by Fisher exact tests included age ≥65 years, non-ambulatory status, and CK levels (Table 3).

Decreased %FVC of <80 (range 20.1–71.2) was seen in 21.3% (10 of 47) of patients (see Table S3 online), including 9 patients who were completely wheelchair-bound and 4 who had serum CK levels within the normal range. Their mean age was significantly older (58.4 ± 16.9 vs. 38.7 ± 11.0, $P < 0.001$) than in patients with normal respiratory function. Patients aged <40 years or those who were still ambulatory also had a decreased %FVC. Among these patients, 5 had episodes of respiratory failure, such as hypoventilation coma during respiratory infection requiring nocturnal, or occasional noninvasive positive pressure ventilation

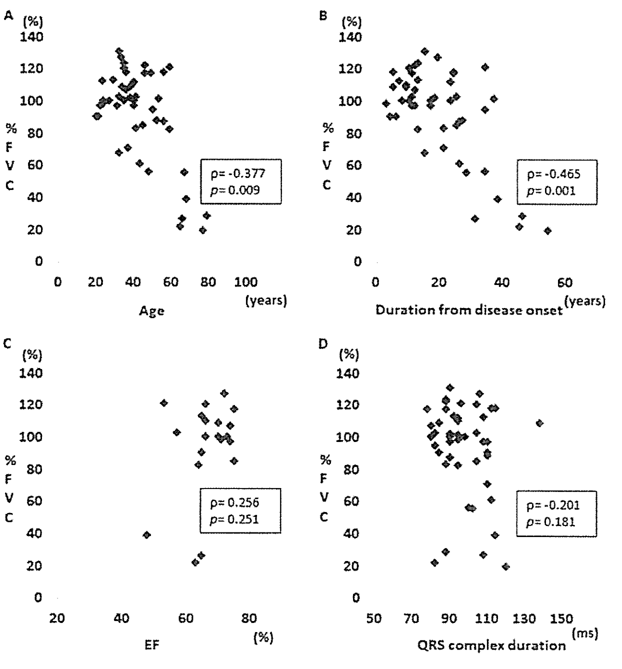

FIGURE 1. Scatterplots of percent forced vital capacity (FVC) and age **(A)**, disease duration from onset **(B)**, ejection fraction **(C)**, and QRS complex duration **(D)**. A significant correlation was found between disease duration and %FVC (ρ = 0.465, P = 0.001).

Table 3. Fisher exact tests for the influence of respiratory factors studied.

	Respiratory function		
	FVC ≥80%	FVC <80%	*P*
Age (years)			<0.001
≥65	0	6	
<65	37	4	
Age at onset (years)			1.00
≥20	21	6	
<20	16	4	
Gender			0.072
Men	16	8	
Women	21	2	
Ambulation status			<0.001
Ambulatory	34	1	
Non-ambulatory	3	9	
CK			0.001
Normal	0	4	
Elevated	37	6	
Phenotype			0.734
MM	21	5	
LGMD2B	16	5	

CK, creatine kinase; MM, Miyoshi myopathy; LGMD2B, limb-girdle muscular dystrophy type 2B; FVC, forced vital capacity.

(NIV); however, they did not share the same mutations, except c.2997G>T. Respiratory function was monitored for ≥10 years in 3 patients (Fig. 2).

Cardiac Function. A history of myocardial infarction was noted in 1 patient and hypertension in another. No other patients had a disease history that could affect their cardiac function. Electrocardiography and echocardiography were performed in 46 and 23 patients, respectively (Tables 4 and 5). Electrocardiographic abnormalities were observed in 24 patients, including 9 patients with %FVC <80. Intraventricular conduction delay (i.e., QRS duration prolongation) was detected in 19 patients, but there was no significant association with age (P = 0.911), disease duration (P = 0.932), or %FVC (P = 0.181). Echocardiographic abnormalities were observed in 7 patients, but they were not clinically significant. Of these, 5 patients had mild valvular regurgitation, including 1 with mild left atrial dilation. Mean EF was 68.0 ± 8.3 (median 66.0, interquartile range 65–73.5, range 48–84), but there was no significant difference between patients with %FVC ≥80% and <80% (69.6 ± 7.5 vs. 58.7 ± 9.3, P = 0.156).

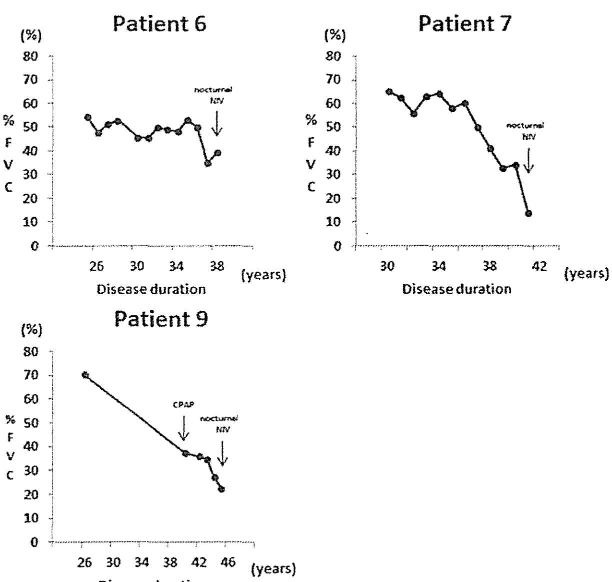

FIGURE 2. Progress of respiratory dysfunction in 3 patients.

Table 4. Electrocardiography findings (*n* = 46).

			%FVC		
QRS duration		Total	≥80%	<80%	Unknown
<100 ms	Normal	22	21	0	1
	Abnormal	5*	3	2	0
≥100 ms	Non-specific ICD	15	10	5	0
	IRBBB	2	1	1	0
	CRBBB	1	1	0	0
	LAH	1	0	1	0

ICD, intraventricular conduction delay; IRBBB, incomplete right bundle-branch block, CRBBB, complete right bundle branch block; LAH, left anterior hemiblock; FVC, forced vital capacity.

**Premature atrial contraction (PAC) / non-specific ST–T abnormality and left axis deviation (LAD) / flattened T wave / PAC / premature ventricular contraction, negative T wave, LAD, atrial fibrillation.*

Autopsy. Patient 6 was found to have asymptomatic nocturnal hypoxemia and was started on nocturnal NIV at age 68. An electrocardiogram at age 69 showed non-specific intraventricular conduction delay (QRS duration 114 ms) and left axis deviation. He died from peritonitis at age 70. Patient 7 required NIV temporarily at age 79, with %FVC of 28.9%. An electrocardiogram at age 79 revealed atrial fibrillation, premature atrial contraction, and non-specific ST–T abnormality, but no prolonged QRS duration. He died from multiple-organ failure

Table 5. Echocardiography findings (*n* = 23).

		%FVC		
	Total	≥80%	<80%	Unknown
Normal	15	13	1	1
Abnormal				
Mild TR	2	2	0	0
Mild TR + PR	1	1	0	0
Mild MR	1	1	0	0
Mild AR	1	0	1	0
Mild LA dilation	1	1	0	0
EF < 55%	2	1 (53%)	1 (48%)	0

TR, tricuspid regurgitation; PR, pulmonary regurgitation; MR, mitral regurgitation; AR, aortic regurgitation; LA, left atrium; EF, ejection fraction; FVC, forced vital capacity.

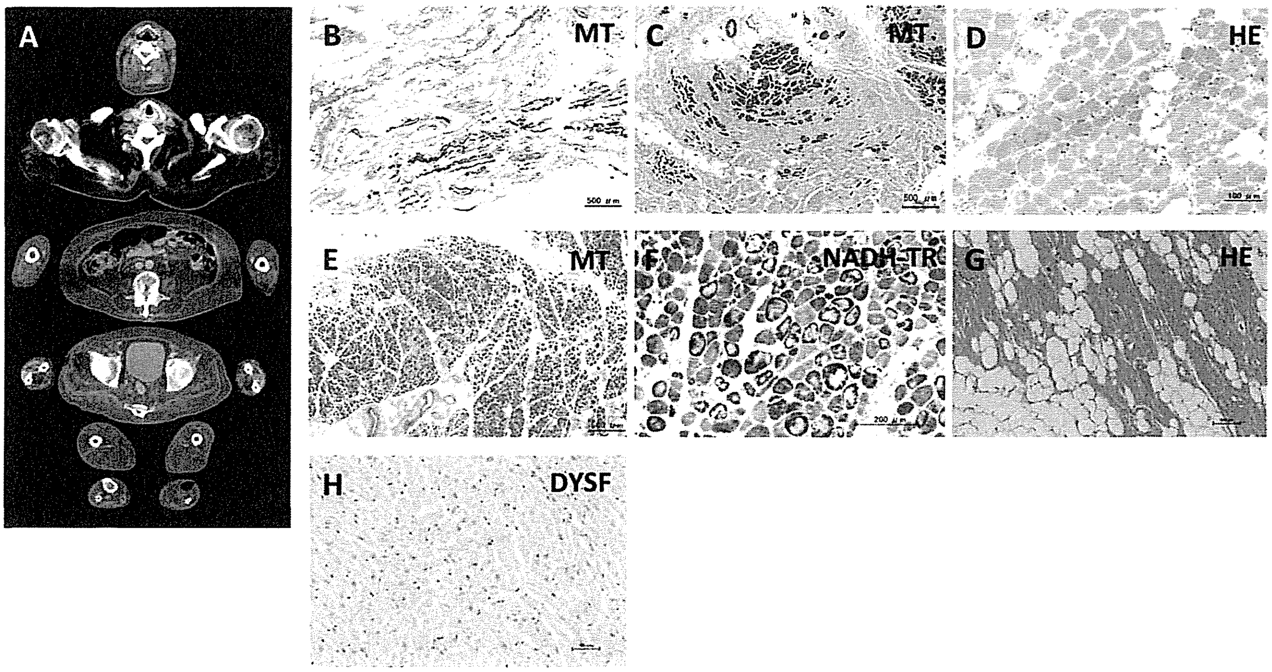

FIGURE 3. Pathological specimens and skeletal muscle computed tomography (CT) from patient 6. Skeletal muscle CT at age 61 years shows marked muscle atrophy and adipose infiltration **(A)**. Almost all fibers were replaced by adipose tissue and fibrous tissue in biceps brachii **(B)** and iliopsoas **(C)**. Flexor digitorum superficialis was relatively preserved **(D)**. The diaphragm exhibited moderate to severe variation in fiber size, adipose tissue infiltration, and endomysial fibrosis **(E, F)**. Cardiac muscle was preserved compared with diaphragm. Minimal variation in fiber size and scattered adipose tissue infiltration was observed **(G)**. Dysferlin was completely deficient in the sarcolemma of cardiac muscle **(H)**. MT, Masson trichrome; HE, hematoxylin and eosin; NADH-TR, nicotinamide adenine dinucleotide–tetrazolium reductase, DYSF, dysferlin.

at age 79. Both patients had complications of diabetes mellitus.

Histopathologically, both patients showed similar findings. Almost all fibers were replaced by adipose tissue and fibrous tissue in limb muscles (Figs. 3B and C and 4A–F). The diaphragm showed moderate to severe fiber size variation, endomysial fibrosis, and adipose tissue infiltration (Figs. 3E and F and 4H–J). The cardiac muscle showed minimal to mild variation in fiber size, endomysial fibrosis, and scattered adipose tissue infiltration (Figs. 3G and H and 4K). Dysferlin was completely absent in cardiac muscle sarcolemma (Figs. 3K and 4L).

DISCUSSION

Respiratory function in patients with dysferlinopathy has attracted little attention so far. Although some reports have described respiratory symptoms or abnormal findings in patients with dysferlinopathy,[2–5,7] there are insufficient data to allow prediction of which patients are susceptible to respiratory dysfunction, or when the signs of respiratory dysfunction may appear. In a previous autopsy case, degeneration of the diaphragm was noted, but no detailed description regarding the degree of severity in each muscle (e.g., limbs, diaphragm) was provided.[7] As appropriate induction of respiratory rehabilitation and NIV can improve outcome and quality of life in patients, an effort should be made to promote early detection of respiratory compromise. Our study showed that age, disease duration, ambulation status, and serum CK levels were associated with respiratory function (Fig. 1). Moreover, patients <40 years of age and those who were still ambulatory also had respiratory insufficiency.

In previous studies, patients with LGMD2B[2,4] or proximodistal dysferlinopathy[3] were reported to have respiratory dysfunction. Because diaphragm and respiratory muscle weakness can cause respiratory dysfunction, we predicted that a proximal-dominant phenotype, LGMD2B, may be associated with an early onset of respiratory failure relative to a distal-dominant type, MM. As average %FVC did not significantly differ between the MM and LGMD2B groups, it may be difficult to predict respiratory dysfunction on the basis of clinical phenotype. However, among the patients with %FVC <80, the decrease in %FVC was greater in LGMD2B patients (see Table S3 online). LGMD2B patients with respiratory dysfunction were older and had longer disease duration compared with MM patients, and thus it is difficult to determine

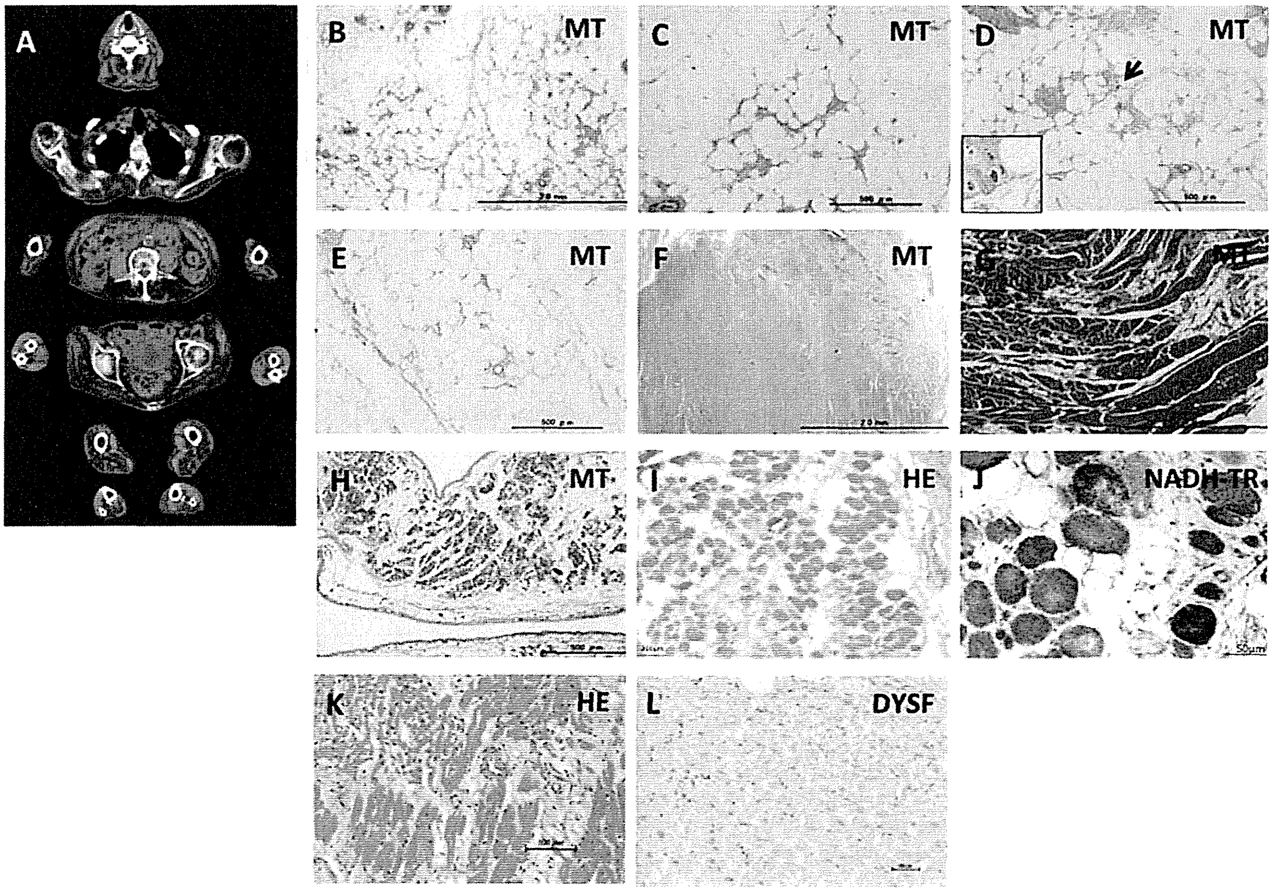

FIGURE 4. Pathological specimens and skeletal muscle computed tomography (CT) from patient 7. Skeletal muscle CT at age 73 shows proximal dominant marked muscle atrophy and adipose infiltration **(A)**. Almost all fibers were replaced by adipose tissue and fibrous tissue in biceps brachii **(B)**, iliopsoas **(C)**, tibialis anterior **(D)**, and rectus femoris **(E, F)**, and only a few muscle fibers remained [**(D)** arrow]. The tongue was well preserved **(G)**. The diaphragm exhibited moderate to severe variation in fiber size, adipose tissue infiltration, and endomysial fibrosis **(H–J)**. Cardiac muscle was preserved compared with diaphragm. Mild endomysial fibrosis and scattered adipose tissue infiltration with muscular degeneration was observed **(K)**. Dysferlin was completely deficient in cardiac muscle sarcolemma **(L)**. MT, Masson trichrome; HE, hematoxylin and eosin; NADH-TR, nicotinamide adenine dinucleotide–tetrazolium reductase; DYSF, dysferlin.

whether the difference in %FVC was attributable to the phenotypic difference. However, it is possible that patients with LGMD2B develop respiratory failure in earlier stages.

Almost all reports of patients with dysferlinopathy who have severe respiratory dysfunction are from Japan. Therefore, we considered the possibility that the severity of the condition is influenced by genetic factors. In this study, however, we found different *DYSF* gene mutations in every patient (except for siblings), and no mutations other than the heterozygous c.2997G>T mutation were common in patients with reduced %FVC. The c.2997G>T mutation has been reported very frequently among Japanese patients,[20] and most of the patients with the c.2997G>T mutation in our study had normal respiratory function; therefore, no specific relationship was suggested between gene mutations and respiratory dysfunction. Because background conditions, such as age and disease duration, vary from patient to patient, the number of patients from a single institution was too small to consider the relationship between gene mutations and disease severity. Multicenter trials and long-term follow-up are necessary to reach a conclusion.

Only a few studies have evaluated cardiac function in patients with dysferlinopathy.[7,11] In our study, electrocardiographic abnormalities were more frequently observed in patients with %FVC <80. As the mean age of patients with %FVC <80 was higher, and electrocardiogram abnormalities such as premature beats or right bundle-branch block reportedly increase with age,[21] there may be some association between abnormal electrocardiogram findings and aging. On the other hand, QRS complex duration was prolonged in some patients regardless of age, disease duration, or %FVC. Prolonged QRS duration is caused by conduction delays at various sites within the ventricles.

Intraventricular conduction delay may be caused by structural changes or by the functional properties of the cardiac conduction system. Shinmura *et al.* reported that the QRS complex duration did not change significantly with age in healthy elderly people.[22] Ilkhanoff *et al.* reported that QRS complex duration >100 ms was significantly associated with incident heart failure.[23] Although follow-up is necessary to determine whether cardiac dysfunction appears in the future in patients with prolonged QRS duration, this may suggest potential cardiac dysfunction and could be used as an indicator for detecting early-stage cardiomyopathy. Moreover, patients with prolonged QRS complex duration were distributed from early to advanced stages in this study and, according to Kuru *et al.*[8] and Wenzel *et al.*,[9] patients with dilated cardiomyopathy (DCM) who maintained walking ability with assistance had high serum CK levels. Therefore, cardiac dysfunction may occur irrespective of stage of disease progression. On echocardiography, valvular regurgitation was observed in some patients with or without respiratory dysfunction, but the degree of regurgitation was mild in all patients. Given that trivial to mild valvular regurgitation on echocardiography has been reported in many healthy people with normal cardiac function,[24] the abnormal findings seen in our study are likely nonpathogenic.

Only a few histopathological assessments have been performed on respiratory and cardiac muscles in patients with dysferlinopathy.[7–9] In our autopsy cases, limb muscles were most severely affected. The diaphragm was also damaged, but to a milder degree compared with limb muscles. As limb muscle weakness tends to be more prominent than respiratory dysfunction, respiratory problems in patients with dysferlinopathy may have been underestimated. Cardiac muscle showed mild changes. Suzuki *et al.*[7] reported that, in an autopsy case with respiratory and cardiac involvement, the diaphragm was more severely affected than the cardiac muscle, suggesting that the myocardium tends to be preserved better than the diaphragm. In our patients, old age, diabetic complications, and/or severe arteriosclerosis may have affected myocardial changes, including cardiac muscle degeneration. However, our findings (i.e., variation in fiber size, endomysial fibrosis, and complete dysferlin deficiency in the sarcolemma) are consistent with those reported previously in patients with DCM,[9] and may be attributed to dysferlinopathy.

We are aware that recruiting patients from the NCNP, an institution highly specialized in muscle disease, is a potential source of selection bias, because these patients may be more severely affected than the general patient population. Therefore, our study may not accurately reflect the general patient population. Investigations of small populations may also underestimate the statistical significance. A broader investigation, such as one that uses an international registry and clinical outcome studies, will be needed in the future.

In conclusion, as patients with dysferlinopathy are prone to respiratory dysfunction, respiratory function should be evaluated regularly, especially in older, advanced-stage patients. Furthermore, as QRS complex duration prolongation on the electrocardiogram could also occur, irrespective of age, disease duration, and %FVC, it is preferable to evaluate cardiac function regularly.

REFERENCES

1. Urtizberea JA, Bassez G, Leturcq F, Nguyen K, Krahn M, Levy N. Dysferlinopathies. Neurol India 2008;56:289–297.
2. Takahashi T, Aoki M, Suzuki N, Tateyama C, Sato H, Hayasaka M, *et al.* Clinical features and a mutation with late onset of limb girdle muscular dystrophy 2B. J Neurol Neurosurg Psychiatry 2013;84:433–440.
3. Nguyen K, Bassez G, Krahn M, Bernard R, Laforêt P, Labelle V, *et al.* Phenotypic study in 40 patients with dysferlin gene mutations: high frequency of atypical phenotypes. Arch Neurol 2007;64:1176–1182.
4. 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. Neuromuscul Disord 2001;11:20–26.
5. Klinge L, Aboumousa A, Eagle M, Hudson J, Sarkozy A, Vita G, *et al.* New aspects on patients affected by dysferlin deficient muscular dystrophy. J Neurol Neurosurg Psychiatry 2010;81:946–953.
6. Nalini A, Gayathri N. Dysferlinopathy: a clinical and histopathological study of 28 patients from India. Neurol India 2008;56:379–385.
7. Suzuki N, Takahashi T, Suzuki Y, Narikawa K, Kudo S, Suzuki H, *et al.* An autopsy case of a dysferlinopathy patient with cardiac involvement. Muscle Nerve 2012;45:298–299.
8. Kuru S, Yasuma F, Wakayama T, Kimura S, Konagaya M, Aoki M, *et al.* A patient with limb girdle muscular dystrophy type 2B (LGMD2B) manifesting cardiomyopathy. Clin Neurol 2004;44:375–378.
9. Wenzel K, Geier C, Qadri F, Hubner N, Schulz H, Erdmann B, *et al.* Dysfunction of dysferlin-deficient hearts. J Mol Med (Berl) 2007;85:1203–1214.
10. Rosales XQ, Moser SJ, Tran T, McCarthy B, Dunn N, Habib P, *et al.* Cardiovascular magnetic resonance of cardiomyopathy in limb girdle muscular dystrophy 2B and 2I. J Cardiovasc Magn Reson 2011;13:39.
11. Choi ER, Park SJ, Choe YH, Ryu DR, Chang SA, Choi JO, *et al.* Early detection of cardiac involvement in Miyoshi myopathy: 2D strain echocardiography and late gadolinium enhancement cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2010;12:31.
12. Bach JR. Noninvasive respiratory muscle aids. In: Bach JR, editors. Management of patients with neuromuscular disorders. Philadelphia: Hanley & Belfus; 2004. p. 211–308.
13. Duboc D, Meune C, Pierre B, Wahbi K, Eymard B, Toutain A, *et al.* Perindopril preventive treatment on mortality in Duchenne muscular dystrophy: 10 years' follow-up. Am Heart J 2007;154:596–602.
14. Rhodes J, Margossian R, Darras BT, Colan SD, Jenkins KJ, Geva T, *et al.* Safety and efficacy of carvedilol therapy for patients with dilated cardiomyopathy secondary to muscular dystrophy. Pediatr Cardiol 2008;29:343–351.
15. Matsuda C, Aoki M, Hayashi Y, Ho MF, Arahata K, Brown RH Jr. Expedited Publication: Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy. Neurology 1999;53:1119–1122.
16. Clinical Pulmonary Functions Committee of Japanese Respiration Society. Clinical respiratory function examinations, 7th ed. [in Japanese]. Tokyo: Medical View; 2008.
17. The Japan Society of Ultrasonic in Medicine. Standard measurement of cardiac function indexes. J Med Ultrason 2006;33:123–127.
18. Josephson ME. Intraventricular conduction disturbances. In: Josephson ME, editor. Clinical cardiac electrophysiology: techniques and interpretations, 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 110–139.
19. Ministry of Internal Affairs and Communications, Statistics Bureau. Available at http://www.stat.go.jp/data/topics/topics051.html/.

20. Takahashi T, Aoki M, Tateyama M, Kondo E, Mizuno T, Onodera Y, *et al.* Dysferlin mutations in Japanese Miyoshi myopathy: relationship to phenotype. Neurology 2003;60:1799–1804.
21. Watanabe T, Kuramoto K. Arrhythmia of the aged. Clin Med Res 1991;68:2654–2660.
22. Shinmura K, Ebihara Y, Kawamura M, Tani M, Nakamura Y. Changes in electrocardiographic findings with aging in a longitudinal study of 500 apparently healthy persons aged 60 years and older. Jpn J Geriatr 1994;31:366–373.
23. Ilkhanoff L, Liu K, Ning H, Nazarian S, Bluemke DA, Soliman EZ, *et al.* Association of QRS duration with left ventricular structure and function and risk of heart failure in middle-aged and older adults: Multi-Ethnic Study of Atherosclerosis (MESA). Eur J Heart Fail 2012; 14:1285–1292.
24. Okura H, Takada Y, Yamabe A, Kubo T, Asawa K, Ozaki T, *et al.* Age and gender specific changes in the left ventricular relaxation: a Doppler echocardiographic study in healthy individuals. Circ Cardiovasc Imaging 2009;2:41–46.

Genetic profile for suspected dysferlinopathy identified by targeted next-generation sequencing

OPEN

Rumiko Izumi, MD, PhD
Tetsuya Niihori, MD, PhD
Toshiaki Takahashi, MD, PhD
Naoki Suzuki, MD, PhD
Maki Tateyama, MD, PhD
Chigusa Watanabe, MD, PhD
Kazuma Sugie, MD, PhD
Hirotaka Nakanishi, MD, PhD
Gen Sobue, MD, PhD
Masaaki Kato, MD, PhD
Hitoshi Warita, MD, PhD
Yoko Aoki, MD, PhD
Masashi Aoki, MD, PhD

Correspondence to Dr. Masashi Aoki: aokim@med.tohoku.ac.jp

See editorial

See related article

Supplemental data at Neurology.org/ng

ABSTRACT

Objective: To investigate the genetic causes of suspected dysferlinopathy and to reveal the genetic profile for myopathies with dysferlin deficiency.

Methods: Using next-generation sequencing, we analyzed 42 myopathy-associated genes, including *DYSF*, in 64 patients who were clinically or pathologically suspected of having dysferlinopathy. Putative pathogenic mutations were confirmed by Sanger sequencing. In addition, copy-number variations in *DYSF* were investigated using multiplex ligation-dependent probe amplification. We also analyzed the genetic profile for 90 patients with myopathy with dysferlin deficiency, as indicated by muscle specimen immunohistochemistry, including patients from a previous cohort.

Results: We identified putative pathogenic mutations in 38 patients (59% of all investigated patients). Twenty-three patients had *DYSF* mutations, including 6 novel mutations. The remaining 16 patients, including a single patient who also carried the *DYSF* mutation, harbored putative pathogenic mutations in other genes. The genetic profile for 90 patients with dysferlin deficiency revealed that 70% had *DYSF* mutations (n = 63), 10% had *CAPN3* mutations (n = 9), 2% had *CAV3* mutations (n = 2), 3% had mutations in other genes (in single patients), and 16% did not have any identified mutations (n = 14).

Conclusions: This study clarified the heterogeneous genetic profile for myopathies with dysferlin deficiency. Our results demonstrate the importance of a comprehensive analysis of related genes in improving the genetic diagnosis of dysferlinopathy as one of the most common subtypes of limb-girdle muscular dystrophy. Unresolved diagnoses should be investigated using whole-genome or whole-exome sequencing. ***Neurol Genet* 2015;1:e36; doi: 10.1212/NXG.0000000000000036**

GLOSSARY

CK = creatine kinase; **DM** = distal myopathy; **HGMD** = Human Gene Mutation Database; **LGMD** = limb-girdle muscular dystrophy; **MLPA** = multiplex ligation-dependent probe amplification; **MMD** = Miyoshi muscular dystrophy; **XHMM** = eXome-Hidden Markov Model.

Dysferlinopathies are a group of autosomal recessive muscular dystrophies caused by mutations in *DYSF*. There are 3 main phenotypes: Miyoshi muscular dystrophy 1 (MMD1; OMIM #254130), limb-girdle muscular dystrophy type 2B (LGMD2B; OMIM #253601), and distal myopathy with anterior tibial onset (OMIM #606768). *DYSF* is located on chromosome 2p13.3-p13.1. It is composed of 55 exons coding for 2,080 amino acid residues, which form the dysferlin protein (approximately 230 kDa).[1] Dysferlin is expressed in the plasma membrane of skeletal muscles[2] and is involved in calcium-mediated membrane fusion events and membrane repair.[3,4] Patients with *DYSF* mutations have very low levels of dysferlin expression in

From the Departments of Neurology (R.I., N.S., M.T., M.K., H.W., M.A.) and Medical Genetics (R.I., T.N., Y.A.), Tohoku University Graduate School of Medicine, Sendai, Japan; Department of Neurology (T.T.), National Hospital Organization Sendai-Nishitaga, National Hospital, Sendai, Japan; Department of Neurology (M.T.), Iwate National Hospital, Ichinoseki, Japan; Department of Neurology (C.W.), Hiroshima-Nishi Medical Center, Hiroshima, Japan; Department of Neurology (K.S.), Nara Medical University, Nara, Japan; and Department of Neurology (H.N.) and Research Division for Neurodegeneration and Dementia (G.S.), Nagoya University Graduate School of Medicine, Nagoya, Japan.

Funding information and disclosures are provided at the end of the article. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was paid by the authors.