RESPIRATORY AND CARDIAC FUNCTION IN JAPANESE PATIENTS WITH DYSFERLINOPATHY

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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 ≥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.

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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

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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

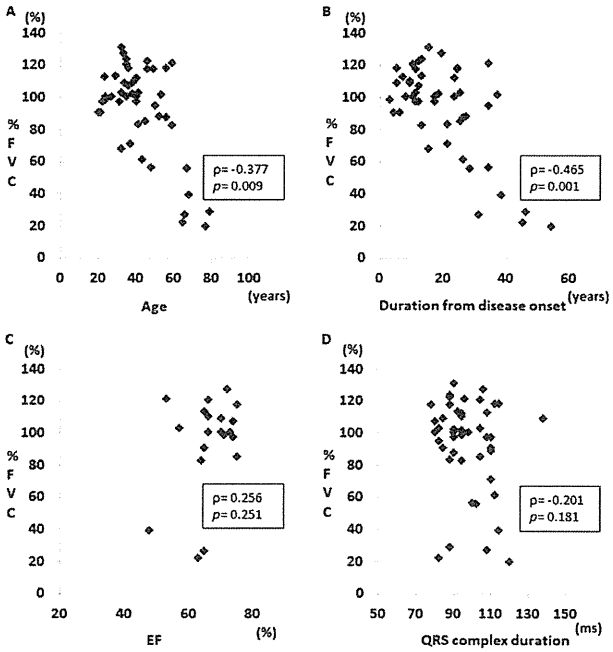

FIGURE 1. Scatterplots of percent forced vital capacity (FVC) and age **(A)**, disease duration from onset **(B)**, ejection fraction **(C)**, and QRS complex duration **(D)**. A significant correlation was found between disease duration and %FVC ($\rho = 0.465$, $P = 0.001$).

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

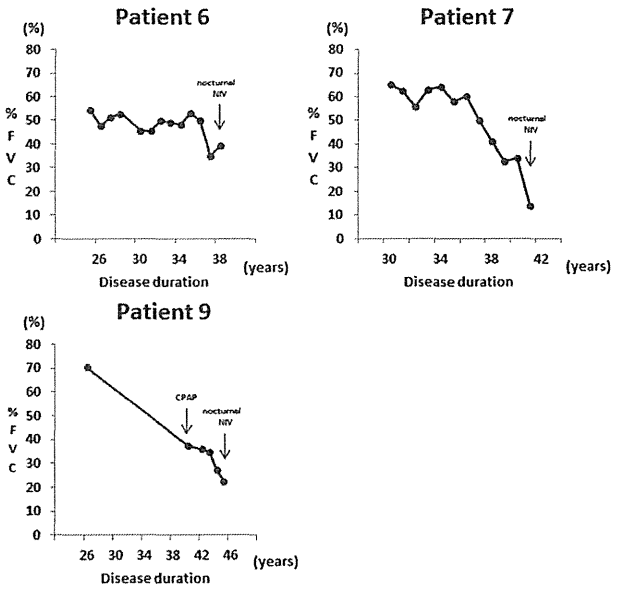

FIGURE 2. Progress of respiratory dysfunction in 3 patients.

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.*

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).

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.

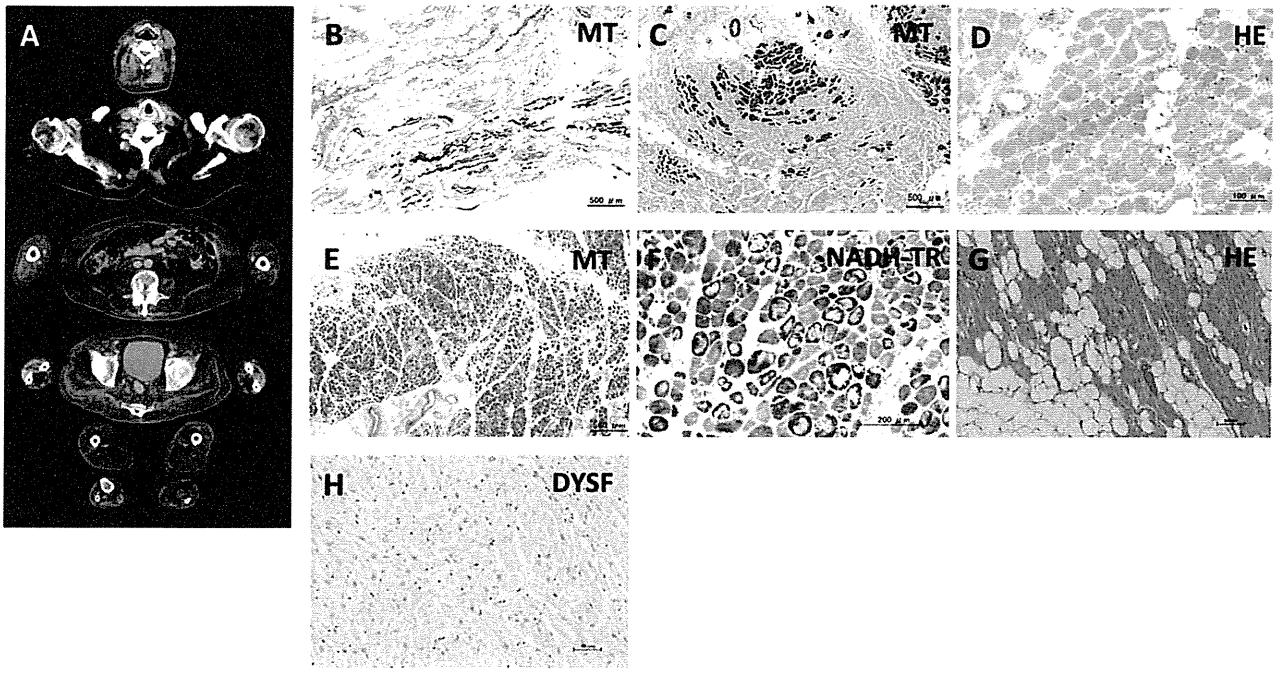

FIGURE 3. Pathological specimens and skeletal muscle computed tomography (CT) from patient 6. Skeletal muscle CT at age 61 years shows marked muscle atrophy and adipose infiltration **(A)**. Almost all fibers were replaced by adipose tissue and fibrous tissue in biceps brachii **(B)** and iliopsoas **(C)**. 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

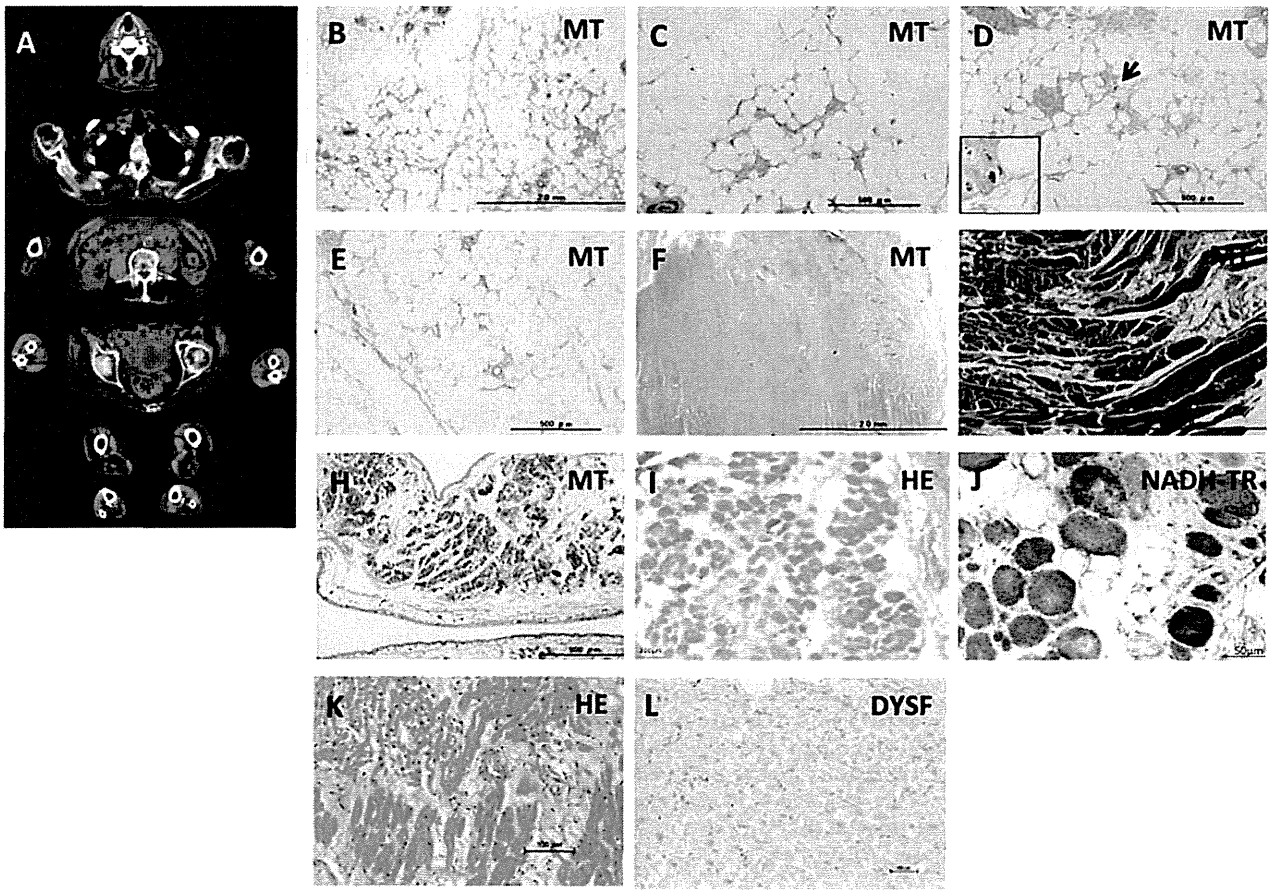

FIGURE 4. Pathological specimens and skeletal muscle computed tomography (CT) from patient 7. Skeletal muscle CT at age 73 shows proximal dominant marked muscle atrophy and adipose infiltration **(A)**. Almost all fibers were replaced by adipose tissue and fibrous tissue in biceps brachii **(B)**, iliopsoas **(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.

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Case report

Successful treatment of paroxysmal tonic spasms with topiramate in a patient with neuromyelitis optica

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ABSTRACT

A 49-year-old woman with neuromyelitis optica (NMO) developed severe quadriplegia and frequent paroxysmal tonic spasms (PTS). Carbamazepine, although initially effective against PTS, caused drug eruption and she was unable to continue. PTS re-emerged after discontinuation of carbamazepine and hindered rehabilitation. Then topiramate was started, and PTS promptly disappeared. The patient became able to resume rehabilitation and her activity of daily life improved significantly.

Carbamazepine and topiramate have a common pharmacological action to block voltage-gated sodium channels. The action may have contributed to inhibition of ephaptic transmission in the demyelinating lesions by NMO and eventually improved PTS.

1. Introduction

Patients with multiple sclerosis (MS) and neuromyelitis optica (NMO) frequently experience various types of pain in the clinical history (Solaro and Uccelli, 2011). Paroxysmal tonic spasms (PTS) is a characteristic symptom (Guillain, 1928; Matthews, 1958; Shibasaki and Kuroiwa, 1974), and diphenylhydantoin and carbamazepine have been known to be effective for the treatment of PTS (Kuroiwa and Shibasaki, 1968).

Herein we report successful treatment of PTS with topiramate in a patient with NMO, and discuss the pathogenesis of PTS and pharmacological action of topiramate.

2. Case report

A 49-year-old woman admitted because of quadriplegia and recurrent painful tonic attacks. She noticed abnormal sensation in the legs and sphincter disturbance 9 months before admission, followed by subacute progression of spastic paraplegia. A nearby orthopedic hospital failed to diagnose her condition. Blurred vision in the left eye, and weakness with dysesthesia over both arms also appeared. She became quadriplegic 1 month before admission. Shortly thereafter, she was intubated due to hypoventilation respiratory failure and transferred to our hospital.

Although intubated, the patient was alert and the respiratory rhythm was regular. The pupils were isocoric, but left pupillary dilatation was observed on the swinging flashlight test. Severe flaccid quadriplegia with slight voluntary movements in the right fingers was observed. Deep tendon reflexes were absent, and plantar responses were neutral bilaterally. Painful attacks of less than one minute duration with tonic flexing of the right arm and extending the left arm and both legs frequently occurred. The attacks were easily induced by voluntary movements.

Serum anti-aquaporin-4 antibody was positive. Cerebrospinal fluid examination revealed normal cell count, slightly elevated protein, negative myelin basic protein and positive oligoclonal bands. A longitudinally extensive spinal cord lesion from C1 to C6, as well as a medullary lesion was detected by T2-weighted MRI.

A diagnosis of NMO with PTS was made, and high dose intravenous methylprednisolone pulse therapy (1000 mg/day for 3 days) was started, followed by maintenance therapy with oral prednisolone (50 mg/day). Clinical courses after admission were indicated in Fig. 1. The patient required mechanical ventilation (MV) on the fourth admission day, and a series of plasma exchange (PE) therapy was started. The respiration improved promptly after introduction of PE, and the patient was weaned from MV after third PE. Weakness in the extremities began to improve gradually 3 weeks after admission, and the right arm became able to move at the shoulder joint during the 5 week of admission. However, PTS did not respond to methylprednisolone pulse therapy or PE. Therefore, carbamazepine of 200 mg bid was started on the eleventh hospital day, but relief of pain was insufficient. Then the

Abbreviations: MV, mechanical ventilation; PE, plasma exchange; PTS, paroxysmal tonic spasms

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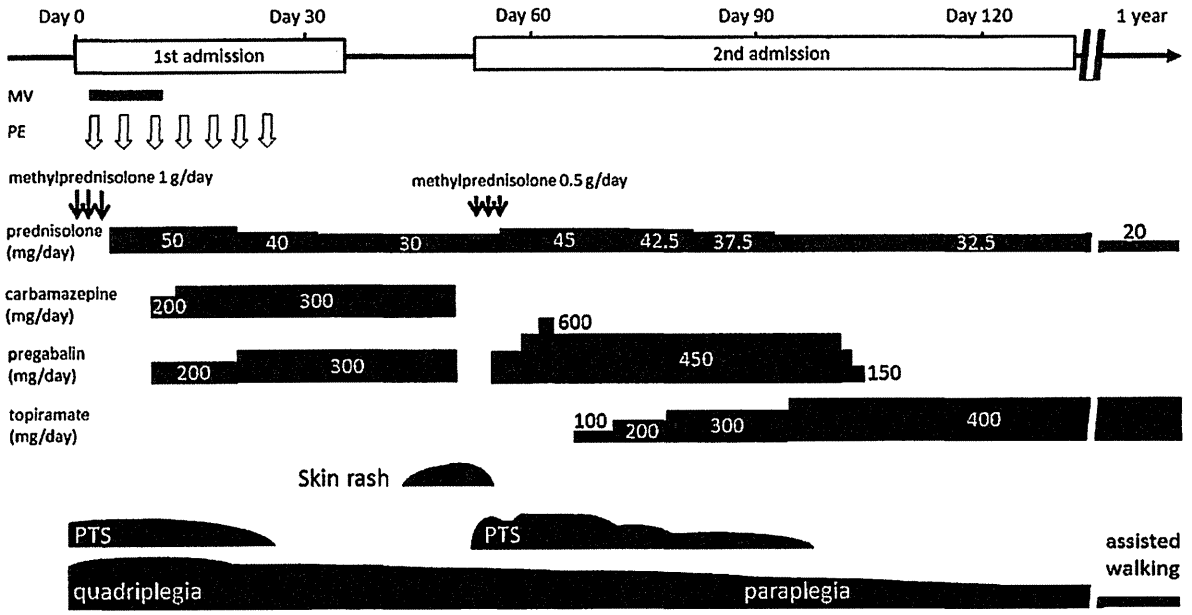

Fig. 1. The clinical courses and treatment after admission.

dose of carbamazepine was increased to 300 mg tid with additional pregabalin, and PTS disappeared. The patient was discharged and transferred to a rehabilitation hospital with oral prednisolone of 30 mg/day at 5 weeks after admission.

However, a skin eruption appeared over the face, trunk and extremities 1 week after hospital discharge. Drug eruption was suspected, and carbamazepine and pregabalin were discontinued. After discontinuation of these drugs, the PTS re-appeared and the patient was readmitted to our hospital at 7 weeks after the first admission.

A diagnosis of drug-induced hypersensitivity was made and prednisolone was increased to 45 mg/day. The skin eruption disappeared quickly, but PTS was so frequent that the patient was unable to continue rehabilitation. Drug lymphocyte stimulation test disclosed the responsible drug as carbamazepine, and only pregabalin was readministered. PTS did not respond to 300 mg/day of pregabalin, and the dosage was increased to 600 mg/day, but pain relief was insufficient. Therefore, 100 mg/day of topiramate was started at 9 weeks after admission and titrated up to 300 mg/day. As topiramate was increased, severity and frequency of PTS gradually decreased. Pregabalin was tapered off, and 400 mg/day of topiramate monotherapy completely inhibited PTS. The patient became able to resume rehabilitation, which contributed significantly to improve her activity of daily living. She was able to feed herself with a spoon, and was able to keep her knees bent in a supine posture at discharge. She became able to use chopsticks and able to walk assisted 1 year later.

3. Discussion

Carbamazepine was initially effective against PTS, but it induced a hypersensitivity syndrome and was discontinued. PTS re-emerged, which was not responsive to pregabalin. PTS may disappear spontaneously without medication in some cases, but painful attacks of our case were persistent and disabling. Then topiramate was started, and PTS promptly disappeared. The patient became able to resume rehabilitation and her activity of daily life improved significantly.

More than half of patients with MS have complication with pain (O'Connor et al., 2008). PTS is reported as a complication for 11% of patients with MS, and baclofen, benzodiazepines and gabapentin have been used, but a large scale clinical study has not yet been performed (Solaro and Messmer Uccelli, 2010). Pain prevalence is more common in NMO than in MS, and NMO-associated pain is often intractable and impairs patients' quality of life (Kanamori et al., 2011).

NMO is a primary astrocytopathy with secondary demyelination, whereas demyelination is caused directly by destruction of oligodendrocytes in MS (Lucchinetti et al., 2014). The different pathomechanism of demyelination is assumed to cause different prevalence and severity of pain.

Ectopic induction of voltage-gated sodium channels at demyelinated axons contributes to generate ephaptic transmission, which is thought to be one of the causes of PTS (Smith and McDonald, 1999). Blockade of voltage-gated sodium channels and calcium channels is a common pharmacological action between carbamazepine and topiramate. On the other hand, pregabalin blocks L-type calcium channels (Gee et al., 1996) but does not have direct effect on blocking sodium channels. Successful treatment of PTS with topiramate might be due to inhibition of ephaptic transmission at demyelinated lesions by blocking ectopic sodium channels. Topiramate may be an effective alternative to carbamazepine should hypersensitivity or other problems arise.

Conflict of interest statement

The authors declare there is no conflict of interest.

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Note

Patient consent has been obtained.

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INTERNAL MEDICINE

□ CASE REPORT □

Early-onset Parkinson's Disease Associated with Chromosome 22q11.2 Deletion Syndrome

Mitsuaki Oki, Shin-ichiro Hori, Shinya Asayama, Reika Wate, Satoshi Kaneko and Hirofumi Kusaka

Abstract

We herein report the case of a 43-year-old man with a 4-year history of resting tremor and akinesia. His resting tremor and rigidity were more prominent on the left side. He also presented retropulsion. His symptoms responded to the administration of levodopa. The patient also had a cleft lip and palate, cavum vergae, and hypoparathyroidism. A chromosome analysis disclosed a hemizygous deletion in 22q11.2, and he was diagnosed with early-onset Parkinson's disease associated with 22q11.2 deletion syndrome. However, the patient lacked autonomic nerve dysfunction, and his cardiac uptake of ^{123}I-metaiodobenzylguanidine was normal, indicating an underlying pathological mechanism that differed to that of sporadic Parkinson's disease.

Key words: Parkinson's disease, 22q11.2 deletion syndrome, ^{123}I-metaiodobenzylguanidine cardiac scintigraphy

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Introduction

Chromosome 22q11.2 deletion syndrome (22q11.2DS) is the second most common chromosomal abnormality next to Down's syndrome. It causes abnormalities in the midline structures such as characteristic facial features, cleft lip and palate, hypoparathyroidism, and cavum vergae. It also causes mental retardation or schizophrenia (1).

Several cases of early-onset Parkinson's disease (PD) associated with 22q11.2DS have been reported, but its pathological mechanism is not yet clear. We herein report a case of early-onset PD associated with 22q11.2DS, review the previous cases in the literature, and discuss the possible pathogenesis.

Case Report

A 43-year-old man presented with resting tremor, disabling akinesia, and gait disturbance. He first noticed a resting tremor in his left arm at 39 years of age, for which a nearby neurologist prescribed pramipexole (0.5 mg tid). His tremor was not relieved, and the medication was discontinued. Short-stepped gait and bradykinesia developed at 41 years of age. He became depressed at 42 years of age, but improved spontaneously over the following 3 months. However, the motor symptoms showed gradual progression, and he eventually required help in getting dressed.

He received an operation for a cleft lip and palate in his infancy. Hypocalcemia was diagnosed when he was 39 years of age, and 1-alpha-calcidol was prescribed. Neither neurological diseases nor consanguinity were reported in his family history. On examination, he was alert and did not show signs of dementia. Although he was not aware of anosmia, his odor identification rate in the Odor Stick Identification Test for Japanese (2) was 25%, indicating decreased olfactory recognition ability. His facial expression was mask-like, and his speech was monotonous. The patient's rigidity was severe in the neck, moderate on the left side and mild in the right extremities. A pill-rolling tremor of 4-6 Hz was noticed predominantly on the left side. Finger- and foot-tapping was decreased predominantly on the left side. His gait was short-stepped, but independent. Retropulsion was noticed. Orthostatic hypotension was not observed in the head-up tilt test. Constipation was denied. No symptoms of rapid eye movement (REM) sleep behavior disorder (RBD)

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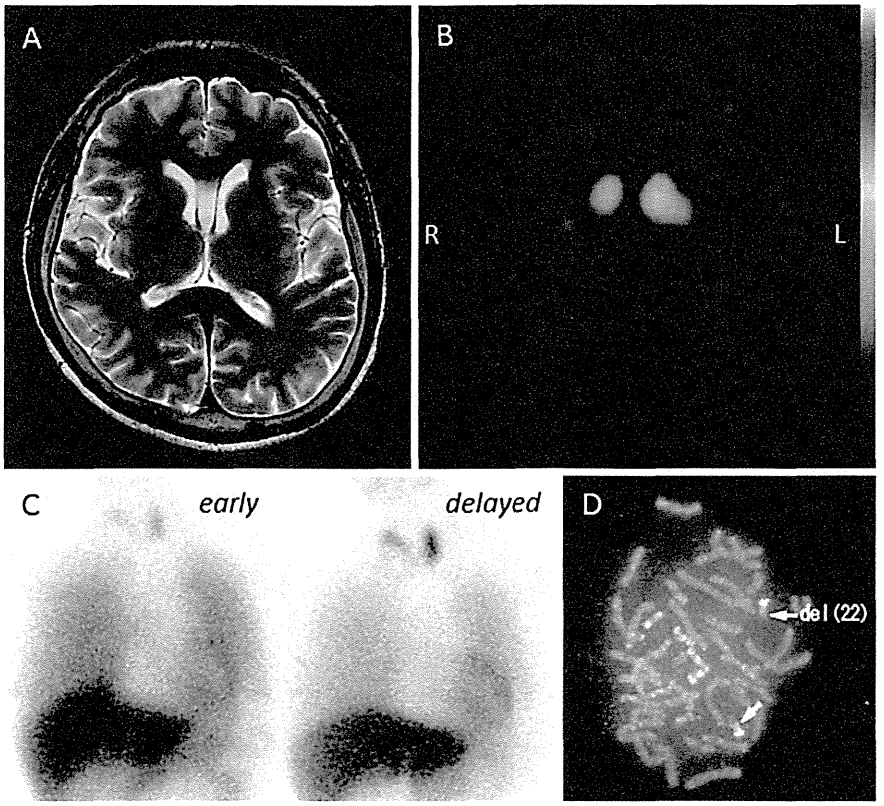

Figure. (A) T2-weighted brain MRI showing cavum vergae. (B) ^{123}I-FP-CIT-SPECT revealed a reduced uptake in both of the putamen, which was more prominent on the right. (C) ^{123}I-metaiodobenzylguanidine scintigraphy showing normal cardiac uptake in the early and delayed phases. (D) A FISH chromosome analysis indicating del(22) (q11.2q11.2)(TUPLE1-).

were detected on the RBD screening questionnaire.

The results of an electrocardiogram, chest X-ray, and routine blood tests were normal. Brain CT and MRI (Figure A) studies showed cavum vergae, but no calcification was detected. The patient's total Unified Parkinson's Disease Rating Scale (UPDRS) part III score improved by 28% after the intravenous infusion of 100 mg levodopa. The ioflupane (^{123}I)-FP-CIT-single photon emission computed tomography (SPECT) (Figure B) showed the reduced uptake of both of the putamen (specific binding ratio: R/L=1.81/2.28). The heart to mediastinum (H/M) ratio obtained by ^{123}I-metaiodobenzylguanidine (MIBG) cardiac scintigraphy (Figure C) was 2.29 in the early phase and 2.60 in the late phase. A fluorescence in situ hybridization (FISH) chromosome study (Figure D) disclosed del(22) (q11.2q11.2) (TUPLE1-), and a genetic analysis revealed no mutations in the *Parkin*, *PINK1*, *LRRK2* or *SYNA* genes.

Discussion

Six cases of PD associated with 22q11.2DS have so far been reported in the literature (3-5). The clinical features of these previously reported cases and our own case are summarized in the Table. In all cases, motor symptoms began before the fifth decade of life and responded well to levodopa therapy. Mental symptoms such as schizophrenia or depression were frequent. An observational study of 159 adults with confirmed 22q11.2DS (5) revealed 4 cases of early-onset PD. Neuropathological examinations were performed in 3 cases; and 2 of these cases showed Lewy pathology with positive α-synuclein immunohistochemistry, whereas the remaining case lacked Lewy pathology.

The age of onset and motor symptoms of our case were similar to those of the 6 previously reported cases. A ^{123}I-FP-CIT-SPECT analysis revealed a reduced uptake in both of the putamen, which was more prominent on the right, in accordance with the laterality of clinical symptoms. Moreover, ^{123}I-MIBG cardiac scintigraphy, which was performed for the first time, showed a normal H/M ratio. Considering that the H/M ratio is decreased in 73% cases of advanced PD (6), the peripheral sympathetic nerve in our case seems to have been relatively spared in comparison to the motor symptoms, suggesting that the underlying pathological mechanism differs from that of sporadic Parkinson's disease.

Among the genes located within the deleted region of 22q11.2, *MIR185*, *DGCR8*, and *COMT* genes are possibly related to the pathological mechanisms of PD (7). The *MIR185* gene, which encodes a microRNA, is predicted to target *LRRK2*. PD patients with an *LRRK2* mutation sometimes lack Lewy bodies (8), have less autonomic impair-

Table. A Summary of the Clinical and Radiological Features of 6 Cases of Parkinson's Disease Associated with Chromosome 22q11.2 Deletion Syndrome Reported in the Literature and of Those of the Present Case.

Reference		2	3	4	4	4	4	This case
Sex		M	M	F	M	M	M	M
Age, years	motor onset	<42	<42	45	48	43	39	39
	PD diagnosis	42	52	55	54	44	48	43
Motor	Tremor	-	NR	+	+	+	+	+
symptoms	Rigidity	+	NR	+	+	+	+	+
	Bradykinesia	+	NR	+	+	+	+	+
	Postural instability	+	NR	+	+	+	+	+
	Laterality	+	NR	+	-	+	+	+
Non-motor	Mood disturbances	+	NR	-	-	+	+	+
symptoms	Cognitive disability	+	+	+	+	+	+	-
	Psychotic disorder	-	+	+	+	-	+	-
Hypoparathyroidism		+	NR	+	+	+	+	+
Cavum vergae		+	NR	NR	NR	+	+	+
levodopa responsiveness		+	NR	+	+	+	+	+
^{123}I-FP-CIT SPECT		NR	Decreased	NR	NR	NR	NR	Decreased
^{123}I-MIBG cardiac scintigraphy		NR	NR	NR	NR	NR	NR	Normal

NR: not reported

ment (9), and show a relatively lower decrease of the H/M ratio, as determined by ^{123}I-MIBG cardiac scintigraphy (10). These clinical features are shared by PD associated with 22q11.2DS. Therefore, the pathological mechanisms behind PD associated with 22q11.2DS may be related to the PD pathogenesis of mutant *LRRK2*.

The authors state that they have no Conflict of Interest (COI).

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PLOS | ONE

RESEARCH ARTICLE

Clinical Features of Autoimmune Autonomic Ganglionopathy and the Detection of Subunit-Specific Autoantibodies to the Ganglionic Acetylcholine Receptor in Japanese Patients

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Abstract

Autoimmune autonomic ganglionopathy (AAG) is a rare acquired channelopathy that is characterized by pandysautonomia, in which autoantibodies to ganglionic nicotinic acetylcholine receptors (gAChR) may play a central role. Radioimmunoprecipitation (RIP) assays have been used for the sensitive detection of autoantibodies to gAChR in the serum of patients with AAG. Here, we developed luciferase immunoprecipitation systems (LIPS) to diagnose AAG based on IgGs to both the α3 and β4 gAChR subunits in patient serum. We reviewed the serological and clinical data of 50 Japanese patients who were diagnosed with AAG. With the LIPS testing, we detected anti-α3 and -β4 gAChR antibodies in 48% (24/50) of the patients. A gradual mode of onset was more common in the seropositive group than in the seronegative group. Patients with AAG frequently have orthostatic hypotension and upper and lower gastrointestinal tract symptoms, with or without anti-gAChR. The occurrence of autonomic symptoms was not significantly different between the seropositive and seronegative group, with the exception of achalasia in three patients from the seropositive group. In addition, we found a significant overrepresentation of autoimmune diseases in the seropositive group and endocrinological abnormalities as an occasional complication of AAG. Our results demonstrated that the LIPS assay was a useful novel tool for detecting autoantibodies against gAChR in patients with AAG.

OPEN ACCESS

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Introduction

The ganglionic nicotinic acetylcholine receptor (gAChR) mediates fast synaptic transmission in all peripheral autonomic ganglia (sympathetic, parasympathetic, and enteric ganglia) in the peripheral autonomic nervous system. AChRs on autonomic neurons are typically composed of two α3 subunits in combination with three other AChR subunits [1]. Although neurons of the autonomic ganglia can express numerous neuronal AChR subunits, including α3, α4, α5, α7, β2, and β4, the properties of the AChR at mammalian ganglionic synapses are most similar to AChRs that are formed by α3 and β4 subunits [2].

Autoimmune autonomic ganglionopathy (AAG) is an acquired immune-mediated disorder that leads to autonomic failure. The disorder is associated, at least in part, with autoantibodies to the gAChR. Antibodies to the gAChR that are found in the serum of 50% of patients with the acute or subacute form of AAG correlate with disease severity, and have been shown to be pathogenic [3,4]. Several studies have reported that these autoantibodies induce the internalization of cell-surface nicotinic gAChRs and thereby impair synaptic transmission [4,5]. Furthermore, it has been demonstrated that antibodies to the α3 subunit of the gAChR or AAG serum have been shown to directly cause autonomic dysfunction in experimental animal models of AAG [6,7]. Although these antibodies are proving to be useful serological markers of AAG, the positivity of gAChR antibodies in acute or subacute panautonomic failure remains around 50%. Cases of idiopathic pure autonomic neuropathy have been reported since 1975 in Japan, and 29 cases of AAG have been reported [8]. However, no assays are available that detect the antibodies to gAChR in Japan, and this has caused difficulty in the diagnosis of AAG. Furthermore, antibodies to non-α3 subunits, including the β4 gAChR subunit, have not been identified in AAG to date.

In this study, we attempted to develop a novel technique to detect the subunit-specific antibodies of gAChR without the use of a radioisotope. Here we established luciferase immunoprecipitation systems (LIPS) using GL^{8990} that can detect antibodies that bind to the α3 or β4 gAChR subunits with high sensitivity. The radioimmunoprecipitation (RIP) assay using [^{125}I] labeled epibatidine has been used as a convenient method to detect autoantibodies to the gAChR [9]. In the RIP, a subunit-specific antibody cannot be detected because of the epibatidine binding the pentamer form of the gAChR. In contrast, LIPS, which is a powerful diagnostic technique for the serological testing of antibodies that are associated with many different human pathogens, is suitable for detecting a subunit-specific antibody [10–13]. In order to provide higher performance on the LIPS, we selected a *Gaussia* luciferase (GL) mutant, called GL^{8990}, in this study. GL is the smallest marine luciferase that has been discovered [14]. GL generates a greater signal intensity from cells in culture (1000-fold) compared with the *Renilla* luciferase (RL) [15]. GL^{8990} (meaning F89W and I90L) is a GL mutant that is generated by site-directed mutagenesis and that emits bioluminescence that is 10 times stronger and/or prolonged than intact GL [16]. Here we performed the LIPS that with the α3 or β4 gAChR subunit fused to a luciferase to detect the respective autoantibodies in human sera. In addition, we extensively reviewed the histories and ongoing clinical and laboratory evaluations of 50 Japanese patients who had been diagnosed with AAG and measured their antibodies to gAChR with the LIPS. This study demonstrated the clinical features of AAG in patients in Japan and provides a tool for precise disease diagnosis.

Table 1. Clinical features of patients with AAG/APD.

	Patients with AAG/APD	Patients with AAG/APD, Anti-gAChR Ab positive	Patients with AAG/APD, Anti-gAChR Ab negative	P value
Number of patients	50	24	26	
Age (yr)	52.5 ± 19.0	51.9 ± 20.4	53.0 ± 18.1	0.838
Age at onset (yr)	48.8 ± 20.1	46.8 ± 20.8	50.7 ± 19.7	0.495
Sex (female, %)	24 (48.0)	13 (54.2)	11 (42.3)	0.413
Duration of the autonomic symptoms (yr)	3.7 ± 6.9	5.1 ± 8.8	2.3 ± 4.1	0.344
Onset (%)	Subacute: 25 (50.0), Gradual: 25 (50.0)	Subacute: 9 (37.5), Gradual: 15 (62.5)	Subacute: 16 (61.5), Gradual: 10 (38.5)	0.095
Antecedent event (%)	11 (22.0)	4 (16.7)	7 (26.9)	0.212
Orthostatic hypotension and/or orthostatic intolerance (%)	42 (84.0)	20 (83.3)	22 (84.6)	0.915
Sicca complex (%)	28 (56.0)	14 (58.3)	14 (53.8)	0.760
Coughing episodes (%)	8 (16.0)	4 (16.7)	4 (15.4)	0.915
Heat intolerance and/or anhidrosis (%)	34 (68.0)	15 (62.5)	19 (73.1)	0.435
Pupil abnormality (%)	20 (40.0)	11 (45.8)	9 (34.6)	0.281
Gastrointestinal tract symptoms (%)	46 (92.0)	22 (91.7)	24 (92.3)	0.951
Bladder dysfunction (%)	29 (58.0)	16 (66.7)	13 (50.0)	0.242
Sexual dysfunction [a] (%)	15 (57.7)	7 (63.6)	8 (53.3)	0.628
Other clinical features [b] (%)	15 (30.0)	8 (33.3)	7 (27.0)	0.633
Complication: endocrine disorder [c] (%)	5 (10.0)	3 (12.5)	2 (7.7)	0.588
Complication: autoimmune disease [d] (%)	11 (22.0)	9 (37.5)	2 (8.0)	0.012
Complication: tumor [e] (%)	5 (10.0)	4 (16.7)	1 (3.8)	0.140

a. We reviewed the 26 male patients only.

b. Numbness, mental symptom, dementia, character change, and back pain

c. Amenorrhea, eating disorder, SIADH (Syndrome of inappropriate secretion of antidiuretic hormone), and panhypopituitarism

d. Still disease, PBC (primary biliary cirrhosis), Hashimoto disease, PMR (polymyalgia rheumatica), SLE (systemic lupus erythematosus), SS (Sjögren's syndrome), Graves' disease, RA (rheumatoid arthritis), fibromyalgia, and other autoantibodies positive

e. Ovarian tumor, pancreas cancer, mediastinal tumor, and paranasal cancer

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Materials and Methods

Patients and serum samples

The series of subjects in this study was comprised of the groups of patients with AAG, healthy controls (HC), and controls with other diseases (DC). Serum samples from 50 patients with AAG were obtained from general and teaching hospitals throughout Japan between January 2012 and February 2014 (mean age, 52.5 ± 19.0 years old, 26 males and 24 females, Table 1). The clinical diagnoses were made in each hospital and the patients' clinical data were provided at the same time. Serum samples from patients showing limb muscle weakness or severe sensory disturbance were excluded from the study. The control groups consisted of 73 HC (mean age, 38.3 ± 11.1 years old, 31 males and 42 females) and 34 subjects with other diseases (DC: for details see S1 Table. Detailed clinical characteristics of OND patients; mean age, 56.3 ± 20.4 years old, 19 males and 15 females).

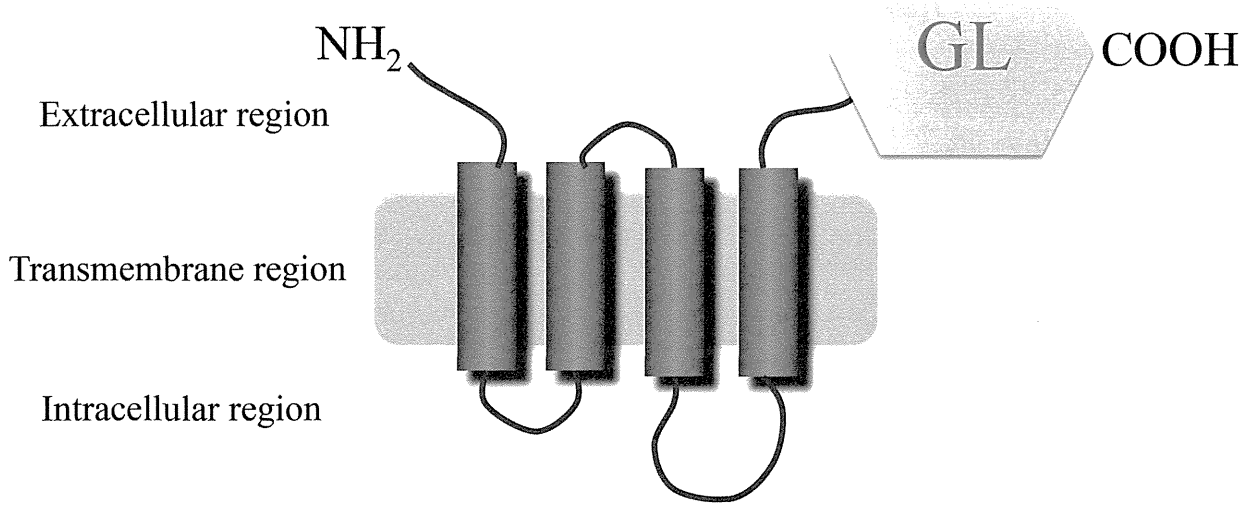

Fig 1. Schematic representation of the acetylcholine receptor (AChR) α3-*Gaussia* luciferase (GL) [8990]. For the ganglionic AChR (gAChR)-LIPS assay, human embryonic kidney (HEK) 293 cells were transfected with an expression plasmid for the gAChRα3 or β4-GL reporter.

doi:10.1371/journal.pone.0118312.g001

Ethics

All of subjects gave their written, informed consent to participate in the present study. The study was approved by the Ethics Committee of Nagasaki Kawatana Medical Center (Nagasaki, Japan).

LIPS assay for the detection of autoantibodies to gAChR

To generate luciferase reporters for the gAChR α3 and β4 subunits (termed gAChRα3-GL and gAChRβ4-GL, respectively) of the human gAChR, full-length human AChR α3 (P32297, Promega Corporation, Madison, WI, USA) or β4 (P30296, Promega Corporation) was fused to a *Gaussia* luciferase (GL) mutant (GL^{8990}) (Fig. 1). Human embryonic kidney (HEK) 293F cells (Life Technologies Corportion, Graind Island, NY, USA) were transfected with the expression plasmid encoding the gAChRα3-GL or the gAChRβ4-GL with FuGENE6 (Promega Corporation). Two days later, the transfected cells were solubilized with a Tris-based saline containing 1% Triton™ X-100. To detect the α3 or β4 gAChR antibodies, 100 μL of the soluble fraction, containing gAChR α3-GL or gAChR β4-GL, was incubated with 15 μL of human serum for 1 hour at 4°C. Subsequently, the fraction was mixed with 15 μL of protein G-sepharose (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and 600 μL phosphate-buffered saline (PBS) with 3% bovine serum albumin and 0.05% Tween 20 and incubated for several hours at 4°C. Following centrifugation and washes with PBS containing 0.05% Tween 20 twice, the bioluminescence activities of the luciferase reporters in the protein G-sepharose were measured with a BioLux GL assay kit (New England Biolabs, Ipswich, MA, USA) and a Lumat LB 9507 luminometer (BERTHOLD TECHNOLOGIES GmbH & Co. KG, Bad Wildbad, Germany) (Fig. 2). The luminometer output was measured in relative luminescence units (RLU). In order to confirm the accuracy of the LIPS assay for the gAChR antibodies, we used commercially available antibodies to human gAChR α3 and β4 (H-100 and S-15; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as positive controls.

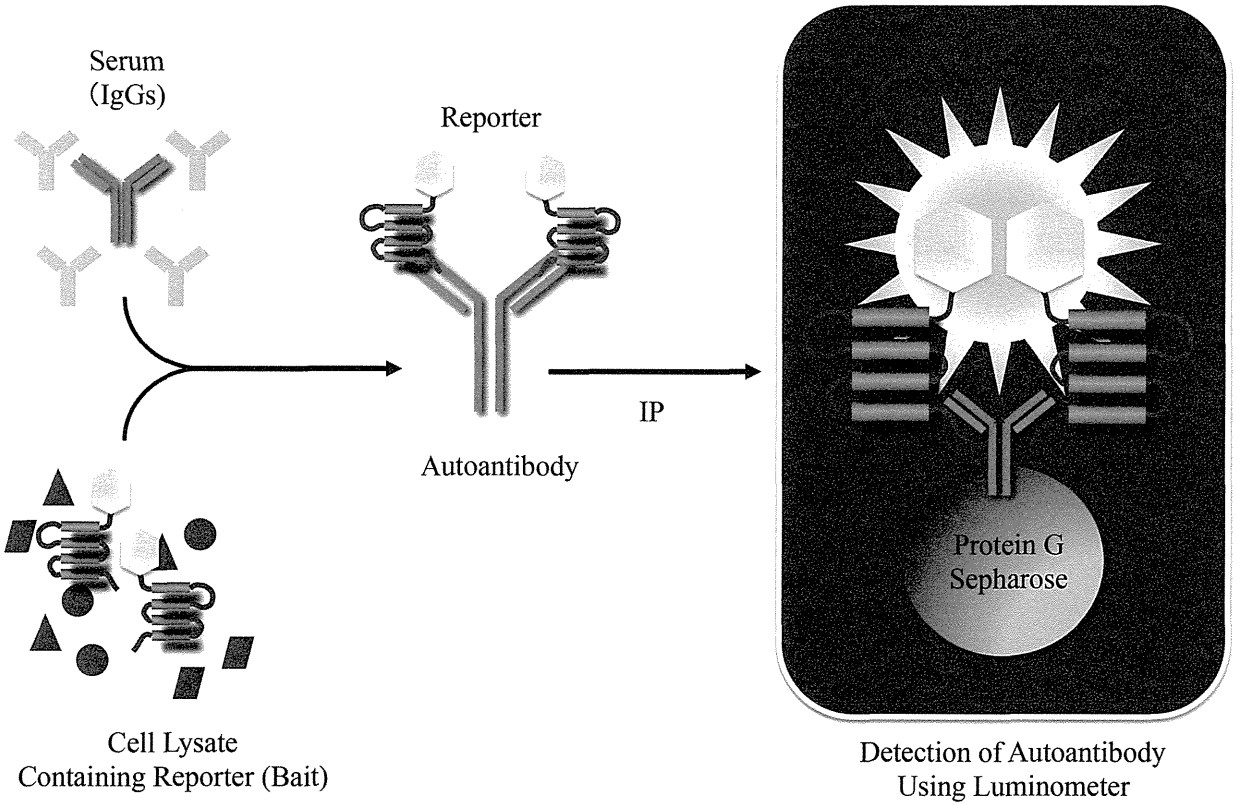

Fig 2. The Luciferase Immunoprecipitation Systems (LIPS). The soluble fractionated component from the solubilized HEK 293F cells, including the gAChRα3 or β4-GL, reacted with human serum, and the specific luciferase activities of the gAChRα3 or β4-GL were found with the luminometer. The *in vitro* LIPS assay can quantitatively evaluate an interaction between an antigen and an antibody with high sensitivity and without a radioisotope.

doi:10.1371/journal.pone.0118312.g002

Based on the data for anti-gAChRα3 and β4 antibodies from the 73 HC, the cut-off values were calculated as the mean plus 3 standard deviations from the mean (SD). In this study, the antibody levels were expressed as an antibody index (A.I.) that was calculated as follows:

A.I. = [measurement value of the sample serum (RLU)]/[the cut-off value (RLU)].The normal value that was established in this study from healthy individuals was <1.0 A.I.

The RIP assay for the detection of autoantibodies to gAChR

Sera from eight of the 50 patients with AAG had been examined previously for antibodies to the gAChR with conventional RIP in the laboratory of Dr. Vernino. These assays for antibodies against the AChR were performed as previously described. In brief, the antibodies were detected with an immunoprecipitation assay in which the AChR antigen was solubilized from a human neuroblastoma cell line (IMR-32) and complexed with iodine I^{125}-labeled epibatidine [3,4,17].

Our assay for the anti-gAChR antibodies may have a different sensitivity and specificity compared with the RIP assay; therefore, we were able to compare the results of the two different assays on these eight samples.

Clinical assessment of autonomic function

Patients with generalized or restricted autonomic dysfunction were identified in each participating hospital in Japan. All of the patients with AAG had dysfunction in at least one autonomic domain, and they underwent a baseline assessment, which included a determination of the gAChR α3 and β4 antibody levels. Subacute onset was defined as the reaching of the peak of autonomic failure within 3 months, and chronic was defined as reaching of the peak after 3 months. Comprehensive clinical, hematologic, biochemical, neurological, and serologic assessments of all patients were performed at baseline. In addition, cerebrospinal fluid analysis was also conducted.

We inquired about the presence or absence of each of the following functions that are controlled by the autonomic system: syncope or orthostatic hypotension for orthostatic intolerance; sicca complex, dryness of the skin, or hypohidrosis/anhidrosis for heat intolerance; pupillary dysfunction; diarrhea or constipation for dysfunction of the gastrointestinal system; dysuria or urinary retention needing catheterization for bladder dysfunction; and sexual dysfunction. However, we were not able to assess the extent of each autonomic symptom rigorously with the composite autonomic scoring scale. Patients with known causes of autonomic failure, including multiple system atrophy, diabetes, and amyloidosis were excluded.

Each patient went through autonomic testing, which involved the Schellong test, head-up tilt test, measurement of the coefficient of variation in R-R intervals (CV_{R-R}), noradrenaline (NA) infusion test, pupillary response to local instillation, assessment of the plasma levels of catecholamines, sweat testing, quantitative sudomotor axon reflex test (QSART), [^{123}I] metaiodobenzylguanidine (^{123}I-MIBG) myocardial scintigraphy, and cystometry. The physiologic analog of noradrenaline, ^{123}I-MIBG, traces the uptake and transport in both noradrenaline presynaptic sympathetic nerve terminals and in subsequent vesicular storage [18]. Postganglionic presynaptic cardiac sympathetic nerve endings can be noninvasively assessed by MIBG scintigraphy because a reduction in cardiac MIBG uptake (H/M ratio) indicates postganglionic sympathetic dysfunction. Cardiac MIBG uptake is reduced in patients with Lewy body diseases such as Parkinson's disease, as well as dementia with Lewy bodies [19,20]. In the standard procedure, the H/M ratio is calculated on early and delayed anterior chest planar images by drawing a region of interest including the heart (H) and the other one over the upper mediastinum (M). However, we were unable to unify the itemss of autonomic testing facilities among the different hospitals.

Statistical analysis

Commercially available statistics software was used for the data analysis (SigmaPlot, Systat Software, Inc., San Jose, CA, USA). The A.I. data that were normally distributed were analyzed with one-way analysis of variance. For the data that were not normally distributed, a one-way analysis of variance of ranks was employed. The significance level was set at $P<0.05$.

Results

Establishment of the LIPS assay for the detection of antibodies to the gAChR

The RIP is a very useful tool for obtaining information about the total amount of antibodies to gAChR, but it cannot distinguish subunit-specific antibodies [3]. In order to detect subunit-specific antibodies for the gAChR, we prepared two subunit-specific luciferase reporters, termed gAChRα3-GL and gAChRβ4-GL. In order to confirm that these subunit-specific luciferase reporters work as a bait in the LIPS, we examined a LIPS assay that used ready-made