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Clinical Neurology and Neurosurgery

Case series

Clinicopathological features of centronuclear myopathy in Japanese populations harboring mutations in dynamin 2

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ABSTRACT

Background: Missense mutations in dynamin 2 gene (DNM2) are associated with autosomal dominant centronuclear myopathy (CNM) with characteristic histopathological findings of centrally located myonuclei in a large number of muscle fibers.
Methods: To identify Japanese CNM caused by DNM2 mutations (DNM2-CNM), we sequenced DNM2 in 22 unrelated Japanese patients who were pathologically diagnosed with CNM. The clinical and pathological findings of DNM2-CNM in patients were reviewed.
Results: We identified 3 different heterozygous missense mutations (p.E368K, p.R369W, and p.R465W) in 4 probands from 4 families. Clinically, calf muscle atrophy and pes cavus are features that are highly suggestive of DNM2-CNM among all CNMs. Pathologically, all 4 DNM2-CNM patients showed a radial distribution of myofibrils in scattered fibers, type 1 fiber atrophy, type 1 fiber predominance, and type 2C fibers. None of the non-DNM2-CNM patients exhibited all the 4 abovementioned pathological features, although some patients showed radial distribution without type 1 fiber atrophy and/or type 2C fibers.
Discussion: These results indicate that the clinicopathological features of DNM2-CNM are rather homogeneous and can be distinguished from the features of non-DNM2-CNM.

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1. Introduction

Centronuclear myopathy (CNM) is a rare congenital myopathy named after its characteristic feature of centrally located nuclei in majority of the muscle fibers [1]. In autosomal dominant (AD) cases, muscular weakness and atrophy often begin in childhood or early adolescence [2,3]. CNM progresses slowly, and patients usually follow a mild course and can often expect a normal life-span. In muscle biopsy, a radial alignment of intermyofibrillar networks [1] is seen in nicotinamide adenosine dinucleotide-tetrazolium reductase (NADH-TR) preparations due to the presence of central nuclei; type 1 fiber atrophy is also often observed. Several families with CNM are found in Europe, the United States, Central Africa, Argentina, and Japan [2–6].

Thus far, 4 causative genes have been reported for CNM: myotubularin (MTM1), dynamin 2 (DNM2) [7], hJUMPY [8], and amphiphysin 2 (BIN1) [9]. Among them, DNM2 mutations have been

identified among patients in France, French Guiana, the United States, Belgium, Germany, Great Britain, Argentina, and Central Africa [6,10,11]. DNM2 encodes a protein involved in endocytosis, membrane trafficking, actin assembly, and centrosome cohesion [12–14]. Thus, DNM2 mutations cause a reduction of dynamin in transfected fibroblasts, leading to defects in centrosomal function.

Patients with CNM·that is caused by mutation in the middle domain of DNM2 (DNM2-CNM) present with a homogenous mild phenotype characterized by slowly progressing muscle weakness without cardiac or respiratory involvement [10]. Muscle computed tomography (CT) and MRI studies clearly show a relatively diffuse involvement in lower-leg muscles, while a selective pattern appears in thigh muscles [10,15,16]. Subtle mental impairment or peripheral nerve involvement was described in a previous report [17]. Mutations in the PH domain lead to an intermediate phenotype with mild respiratory failure and relatively severe weakness as compared to DNM2-CNM caused by middle-domain mutations [6]. Another study reported a more severe infantile form with hypotonia, weak suckling, and respiratory failure due to mutation in the PH domain of DNM2 [11]. Although no DNM2 mutations have

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been identified among Japanese patients, there have been reports of patients with evidently similar clinicopathological features [4,5], suggesting the possibility of the presence of DNM2-CNM in the Japanese population. We therefore aimed at detecting *DNM2* mutations among Japanese CNM patients.

2. Materials and methods

2.1. Patients

We retrospectively recruited patients who were diagnosed with CNM or myotubular myopathy at the National Center of Neurology and Psychiatry and analyzed their samples from a total of 9639 muscle biopsies obtained between 1978 and 2006. Inclusion criteria were the presence of more than 6% centrally nucleated fibers and the absence of characteristic findings indicating other muscle diseases upon muscle biopsy. Our cohort consists of 22 unrelated patients aged 1–72 years: 2 had an AD family history; 5 had affected siblings, and consanguinity was documented in one of the patient's families; and 8 were sporadic cases. No record of family history was available for 7 patients. Direct sequence analysis previously performed on these patients excluded CTG expansion in the *DMPK* gene and *MTM1* mutations. Their clinical history was carefully reviewed. Additional medical information from affected family members was obtained by the attending neurologist, when possible.

2.2. Sequence analysis of DNM2

'All 22 patients and 4 members of 1 family were examined for *DNM2* sequence variants. DNA was extracted from blood or muscle samples using standard protocols. We sequenced all the exons and the exon–intron boundaries of *DNM2*. Both strands of PCR products were sequenced directly using BigDye Terminator v1.1 Sequencing Standard Kit (Applied Biosystems) with an automated ABI 3100 DNA sequencer with custom-made primers (Supplementary Table).

3. Results

3.1. Genetic diagnosis

Among 22 patients, we identified 3 mutations in 4 probands: c.1102G>A (p.E368K), c.1105C>T (p.R369W), and c.1393C>T (p.R465W), all of which were previously reported [6]. We further confirmed the mutations in affected family members of 2 patients (Table 1). We did not identify mutations from the families with consanguinity.

3.2. Clinical features

The clinicopathological features of patients with *DNM2* mutations are shown in Table 1. Clinical information for Patient 1-1 was not available. He was autopsied at the age of 17 years, at which point the gastrocnemius muscle was taken as a sample for analysis (Fig. 1A). The inheritance pattern was compatible with AD transmission in families 2 and 3, while it was sporadic in Patient 4-1.

Patients 2-1 and 3-2 were previously reported to have AD CNM or myotubular myopathy (Fig. 2A) [4,5]. In brief, Patient 2-1 noticed an ankle contracture at the age of 10 years and started having difficulty in climbing stairs at the age of 30 years. Achilles tendon elongation was performed at the age of 37 years, during which this patient was found to have atrophy of facial and distal muscles, and diminished tendon reflexes. He had mild ptosis, but ophthalmoplegia was not observed. Creatine kinase (CK) levels were within the normal range. nEMG was myogenic. Muscle biopsy of the

rectus femoris at the age of 42 years showed 68% centrally nucleated fibers and a scattered radial distribution (Fig. 1B). CT of the patient's hamstring, soleus, and gastrocnemius muscles showed atrophy and fatty changes. There was no cardiac or respiratory involvement. Nerve conduction velocities were normal except for low-median compound action potentials that could be explained by muscle atrophy. Patient 2-2 exhibited ankle contracture, *pes cavus* due to plantaris muscle atrophy, and distal atrophy since 10 years of age and also underwent Achilles tendon elongation for ankle contracture in his second decade. No ptosis or ophthalmoplegia was observed.

Patient 3-2 noticed progressive lower-leg weakness, atrophy, and ankle contracture when he was 15 years old and he underwent achillotenotomy at 18 years of age. He developed dyspnea at the age of 54 years that necessitated a tracheotomy at the age of 55 years. Neurological findings at the age of 55 years revealed mild ptosis, distal muscle atrophy and weakness, and mild facial muscle involvement including ptosis. CK level was 48 IU/L. nEMG was myogenic. Sural nerve biopsy was unremarkable. Muscle biopsy of the peroneus brevis showed centrally placed nuclei in 40% of the fibers (Fig. 1C). The patient unfortunately died at the age of 58 years, and the primary cause of death was undetermined. His children (Patients 3-6, 3-7, 3-8, and 3-9 (Fig. 2B)) were found to have *pes cavus* caused by plantar muscle atrophy and were slow runners in their childhood.

At the age of 20 years, Patient 3-6 could not appose his palms when his wrists were extended and at the age of 35 years, he had difficulty in walking. He developed bilateral ankle contracture, because of which he had to stand and walk tiptoed. When he was 50 years old, neurological examination showed distal muscle weakness and atrophy with ankle- and finger-joint contractures (Fig. 3A–D). His deep tendon reflexes were also diminished. He lost his left eye in an accident during his childhood, but neither ophthalmoplegia in his right eye nor ptosis was observed. No peripheral nerve involvement was found on normal nerve conduction study. nEMG was myogenic. Results of echocardiography, Holter ECG, and pulmonary function tests were normal. Muscle biopsy of the biceps brachii at the age of 50 years was compatible with the CNM diagnosis (Fig. 1D–G).

The daughters of Patient 3-6 (Patients 3-10 and 3-11) followed a similar clinical course. They did not have ophthalmoplegia nor ptosis (Fig. 3G). Muscle CT showed marked atrophy in the posterior compartment of the lower extremities (gluteus maximus, hamstrings, gastrocnemius, and soleus) and thigh abductors, while only moderate atrophy and fatty changes were observed in the paraspinal muscles (Fig. 3E). Patient 3-11 had muscle involvement limited to the biceps femoris, gastrocnemius, and soleus as shown on CT at the age of 19 years (Fig. 3F). Both Patients 3-10 and 3-11 showed myogenic changes on nEMG, and the findings of nerve conduction studies were normal.

Patient 4-1 had no obvious family history (Fig. 3C). He noticed ankle contracture at the age of 30 and had gait disturbance at the age of 40 years. He underwent muscle biopsy at the age of 55 years. He was ambulant but did not use a cane. nEMG was actively myogenic, and the results of nerve conduction studies were normal.

In all patients, *pes cavus* caused by plantar muscle atrophy was the earliest sign that appeared before the age of 10 years. Atrophy of calf and posterior thigh muscles was seen during the second decade, but could be detected by muscle CT even in early stages (Fig. 3E and F). The clinical course was relatively benign, except for that of 1 patient who died at the age of 16 years (Patient 1-1), although no detailed information on the cause of death was available. Neither cardiac nor respiratory failure occurred in any patient, except Patient 3-2 who underwent tracheotomy for dyspnea secondary to severe pneumonia. All the 3 patients who were above 50 years of age are still ambulant. With an exception of Patient 3-2,

Table 1
Clinicopathological features of DNM2-CNM.

			1	2	3					4
	Family									
Demographic data	Patient number		1-1	2-1	3-2	3-6	3-7	3-10	3-11	4-1
	Mutation		c.1102G>A (p.E368K)	c.1393C>T (p.R465W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)
	Age/sex		16/M	42/M	55/M	50/M	47/F	22/F	19/F	55/M
Clinical features	Ability to walk		NR	Ambulatory	With cane	With cane	Ambulatory	Ambulatory	Ambulatory	Ambulatory
	Opthalmoplegia		NR	–	–	–	–	–	–	–
	Ptosis		NR	+	+	–	–	–	–	–
	MMT upper extremities	Proximal	NR	5	5	5	4	4	4	4
		Distal	NR	5	5	2	3	2	2	3
	MMT lower extremities	Proximal	NR	4	5	4	3	3	4	4
		Distal	NR	4	5	2	2	2	2	3
	Deep tendon reflexes		NR	–	NR	N	↓	–	NR	↓
	Joint contractures		NR	Elbow, wrist, ankle	Ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Ankle
	Muscle atrophy	Leg	NR	+	+	+	+	+	+	+
		Paraspinal	NR	NR	NR	+	+	+	+	+
		Plantar	NR	+	+	+	+	+	+	+
	Cardiovascular		NR	N	N	NR	NR	N	N	N
	Respiratory		NR	NR	Tracheotomy	Normal vital capacity	Normal vital capacity	Normal vital capacity	Normal vital capacity	NR
	Electromyography		NR	M	M	M	M	M	NT	M
	Nerve conduction studies		NR	*	N	N	N	N	N	NR
	Serum CK		NR	N	N	N	N	NR	N	N
	Muscle CT	Calf	NR	+	+	+	NR	NR	–	+
		Thigh	NR	2+	2+	2+	NR	NR	+	2+
Findings on muscle biopsy	% of centrally nucleated fibers		65	68	55	60				70
	Radial distribution of myofibrils		+	+	NT	+				+
	Type 1 predominance (%)		80	79	NT	88				90
	Type 1 atrophy		+	+	NT	+				+
	Type 2B deficiency		+	+	NT	+				+
	Type 2C fibers (%)		2	1	NT	2				5

Abbreviations: MMT, manual muscle testing; +, present; –, absent; N, normal; NR, no record; NT, not tested; ↓, decreased; EMG, electromyography; M, myogenic changes; and NCS, nerve conduction study. The CT scores are as follows: 1+: decreased signal density and 2+: decreased signal density with severe muscle atrophy.
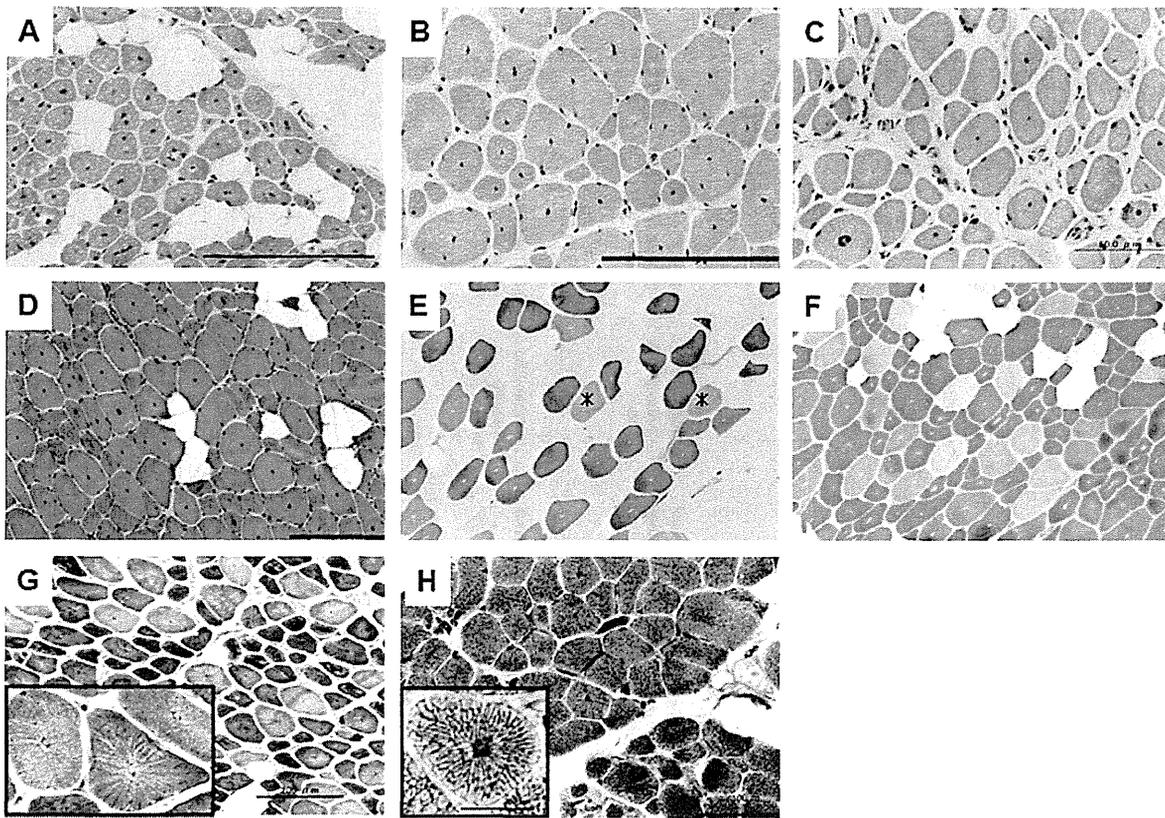
Fig. 1. Biopsy findings of DNM2-CNM (A-G) and non-DNM2-CNM (H). Hematoxylin and eosin stain of muscle sections from Patients 1-1 (A), 2-1 (B), 3-2 (C), and 3-6 (D). Numerous centronuclear fibers (up to 55%) and interstitial fibrosis were observed. Histochemical findings in muscle sections from Patient 3-6 (E–G). Type 1 predominance and hypotrophy (E, ATPase staining pH 10.6), a few type 2C fibers (F, ATPase pH 4.6), and radial distributions (G, NADH-TR) were observed. Radial distributions were also observed in some non-DNM2-CNM muscles (H, NADH-RT).

neither ptosis nor ophthalmoplegia was observed in the affected family members.

Clinical features of the CNM patients without DNM2 mutations (non-DNM2-CNM) along with the number of patients are given below: proximal weakness (2/18), floppy infant (8/18), scoliosis (1/18), mental retardation (1/18), dysphagia (1/18), myalgia (1/18), and high-arched palate (8/10). Furthermore, only 1 of 10 patients with non-DNM2-CNM showed joint contracture. The clinical features of non-DNM2-CNM varied more widely than those of DNM2-CNM.

3.3. Summary of the pathological features

In all patients with DNM2-CNM, the pathological findings were rather similar: (1) radial distribution of myofibrils in scattered fibers, (2) type 1 fiber atrophy, (3) type 1 fiber predominance, and (4) a small number of type 2C fibers (Table 1, Fig. 1). In addition, the frequency with which muscle fibers with centrally placed nuclei were observed in DNM2-CNM patients was $63.1 \pm 6.1\%$ (mean ± SD), range, 55–70%, which is much lower than that observed in non-DNM2-CNM patients (24.7 ± 13.2%, range, 8–50%).

In contrast, none of the non-DNM2-CNM patients had all the 4 abovementioned pathological features. Definite radial distribution of myofibrils was seen only in 2 of 18 cases. In 4 of 18 cases, equivocal radial distribution was observed. Among the 18 cases, type 1 fiber atrophy was noted in 12 patients; type 1 fiber predominance, in 16 patients; and type 2C fibers, in 12 patients. In addition, type 2 fiber atrophy was seen in 2 of 18 patients.

4. Discussion

This is the first report to document DNM2 mutations in CNM patients in Japan with a low frequency, similar to the cases found in Europe, the United States, Central Africa, and Argentina [7,10,11]. All affected family members had distal muscle atrophy, finger and ankle contractures, and pes cavus caused by plantar muscle atrophy in their childhood (Table 1, Fig. 3A–D). Atrophy and fatty changes in the gastrocnemius muscle were the earliest signs observed on CT and were noted in the second decade of their lives (Fig. 3E). Thigh flexor, gluteus maximus, and paraspinal muscles were involved in the later stages (Fig. 3F).

The clinical and pathological features of DNM2-CNM were rather homogeneous in our series, as in previous reports [6,10,16]. This can be helpful in establishing a working diagnosis in CNM patients. The phenotypes of the mutations identified here (E368K, R465W) were almost identical to those identified in previous cases [7], although only 1 patient with E368K and 1 with R369W showed ptosis and ophthalmoplegia. In addition, the early death of Patient 1-1 and the respiratory failure of Patient 3-2 are unusual occurrences for DNM2-CNM, although we could not obtain detailed information.

A high occurrence of ptosis (9/10 [10], 7/11 [18]) and ophthalmoplegia (2/10 [10], 5/11 [18]) among DNM2-CNM patients is observed in other countries [10], while in our series, ptosis was much more rare (2/8), and ophthalmoplegia was not seen in our
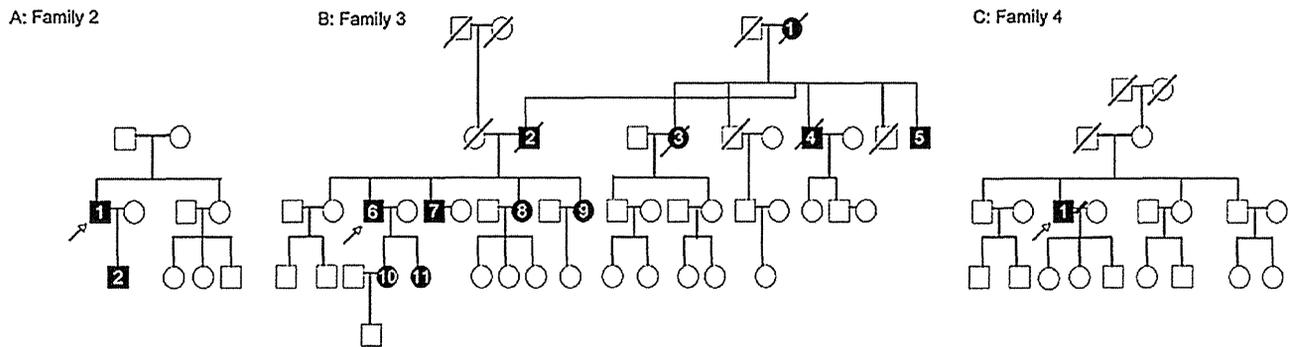
Fig. 2. The DNM2-CNM family tree. Families 2 and 3 had obvious autosomal dominant inheritance. On the contrary, Patient 4-1 had a sporadic onset. His parents and children did not show any symptoms.

cohort. In previous studies, most patients who did not have ptosis and ophthalmoplegia were from a p.R522H family, and most of them were infants [17]. The Japanese patients in our study, including those with the p.R465W mutation that causes DNM2-CNM with ptosis and ophthalmoplegia, as shown in a European study, did not have ophthalmoplegia, and only 2 (p.R465W and R369W) patients exhibited ptosis. Ethnic background may be a contributing factor to the occurrence of ptosis because there are some anatomical differences between the eyelids

of Asian and European populations: Asians have shallower eyelids than Europeans [18,19]. Since the severity of ptosis is correlated to the severity of myopathy, ptosis caused by mutations in the middle domain in DNM2 in DNM2-CNM patients could be mild enough not to be recognized in the eyelids of Asians.

On the other hand, among non-DNM2-CNM patients, ptosis or ophthalmoplegia was also seen in 4 of 18 cases, suggesting that ocular symptoms may not be a specific indicator of DNM2 mutations
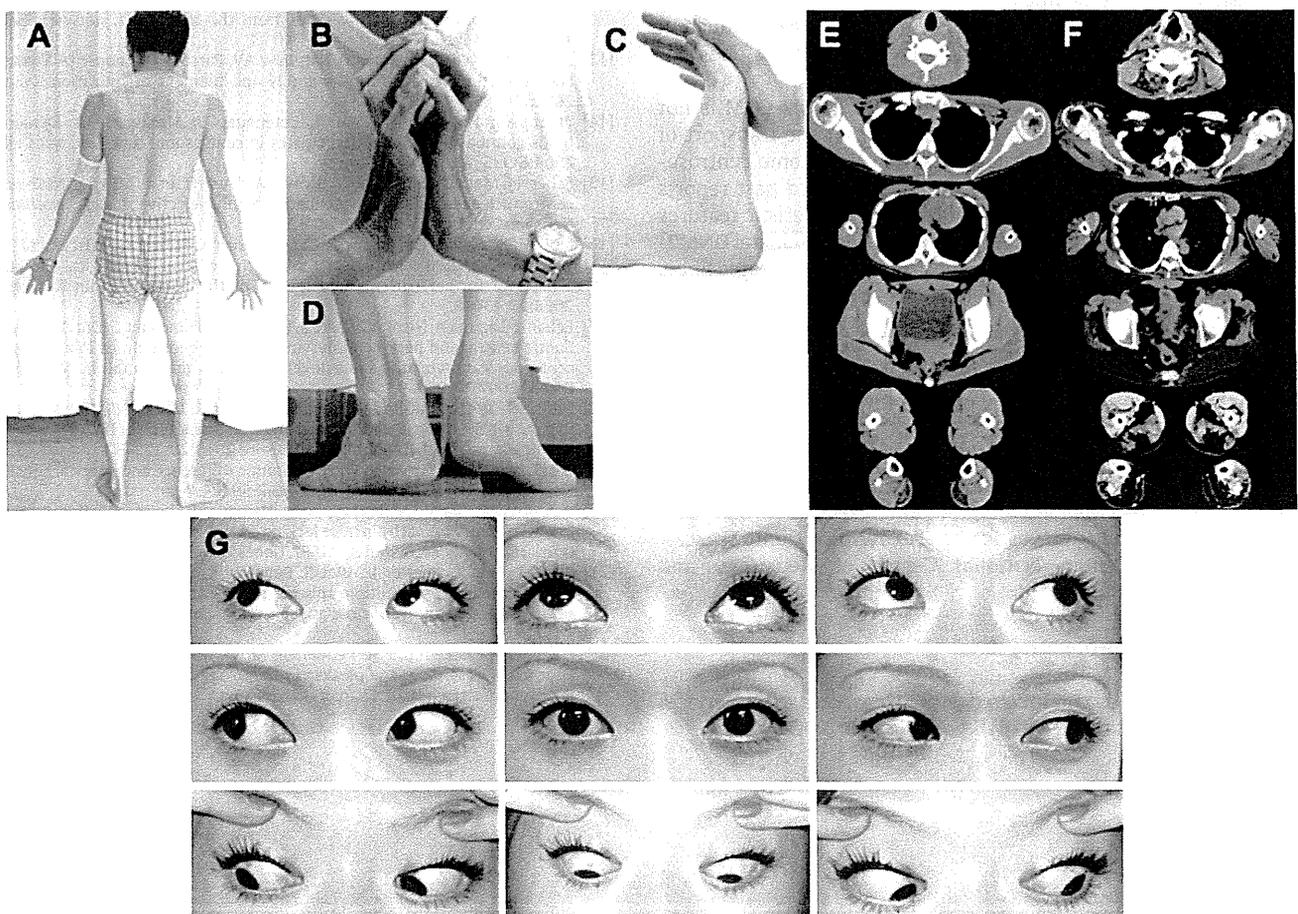
Fig. 3. Photograph of Patient 3-6: distal muscular atrophy (A), joint contracture of fingers (B), and ankle contracture (C); patient could not put his heels on the floor because of the ankle contracture (D). Muscle CT of Patient 3-6 (F) and Patient 3-11 (E) depicting lower-leg muscle atrophy of the posterior compartment (gluteus maximus, hamstrings, gastrocnemius, and soleus), thigh abductor, and paraspinal muscles. Note the early involvement of the biceps femoris, gastrocnemius, and soleus in Patient 3-11 when she was 19 years old. Ophthalmoplegia and ptosis are not observed in most patients (G: Patient 3-11).

in CNM patients, at least in Japanese patients, further highlighting the importance of the frequency of ocular involvement as a genetic factor.

Other symptoms of the central nervous system and peripheral neuropathy were also observed in our cohort and were reported as subtle complications of DNM2-CNM [17]. Among the non-DNM2-CNM patients, other causative genes for CNM were considered. *MTM1* mutations are implicated in CNM, but in our cohort, *MTM1* mutations were excluded. Compound heterozygous mutations in *hJUMPY*, a gene that encodes a phosphoinositide phosphatase, were reported as a cause of sporadic CNM [8]. Additionally, *BIN1* is a newly identified causative gene for autosomal recessive CNM [9]; patients with autosomal recessive CNM show typical CNM muscle pathology and proximal-dominant muscle weakness, which is more severe than observed in DNM2-CNM patients. The pathology of CNM with *BIN1* mutations does not have a radial distribution, which is thought to be a hallmark of DNM2-CNM [9]. Notably, some of our non-DNM2-CNM patients showed a radial distribution. However, none of our non-DNM2-CNM patients had a family history that would indicate autosomal recessive inheritance and merit further mutational analysis of the *hJUMPY* and *BIN1* genes. Other candidate genes include *Srpk3* [20] and *PTPLA* [21], which were thus far implicated as causative genes of CNM in mice and dogs.

DNM2 mutations were also identified in AD Charcot–Marie–Tooth disease (CMT) [22]; in fact, some DNM2-CNM patients also show very mild reductions in nerve conduction velocities in the lower legs [10] or pathological changes in both myelinated and unmyelinated nerve fibers [18]. Nevertheless, none of our patients showed any abnormality in nerve conduction studies, suggesting that peripheral nerve involvement does not occur frequently in DNM2-CNM patients.

Although the precise pathomechanism of DNM2-CNM is not known, mutations are thought to hinder either the transport of DNM2 to the centrosome or its interaction with some centrosomal component [7]. Interestingly, in our study and past reports of *DNM2* mutations in the middle domain, characteristic features were observed in muscle pathology, as opposed to neonatal DNM2-CNM with PH domain mutations in which centrally nucleated fibers and radial distribution of myofibers are less prominent [11].

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.clineuro.2011.10.040.

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ELSEVIER

Respiratory dysfunction in patients severely affected by GNE myopathy (distal myopathy with rimmed vacuoles)

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Abstract

GNE myopathy is a rare and mildly progressive autosomal recessive myopathy caused by *GNE* mutations. Respiratory dysfunction has not been reported in GNE myopathy patients. In this study, we retrospectively reviewed the respiratory function of 39 severely affected GNE myopathy patients (13 men, 26 women) from medical records, and compared these parameters with various other patient characteristics (e.g., *GNE* mutations, age at onset, creatine kinase levels, and being wheelchair-bound) for correlations. The mean % forced vital capacity [FVC] was 92 (26) (range, 16–128). In 12/39 (31%) patients, %FVC was <80%. Of these 12 patients, 11 (92%) were entirely wheelchair-dependent. These patients exhibited significantly earlier onset (20 [4] vs. 30 [8] years, $p < 0.001$) and lower creatine kinase levels (56 [71] vs. 279 [185] IU/L) than patients with normal respiratory function. Two patients exhibited severe respiratory failure and required non-invasive positive pressure ventilation. Patients with a homozygous mutation in the *N*-acetylmannosamine kinase domain exhibited lower %FVC, while only one compound heterozygous patient with separate mutations in the uridinediphosphate-*N*-acetylglucosamine 2-epimerase and the *N*-acetylmannosamine kinase domains had respiratory dysfunction. Our results collectively suggest that GNE myopathy can cause severe respiratory failure. Respiratory dysfunction should be carefully monitored in patients with advanced GNE myopathy characterized by early onset and homozygous homozygous mutations in the *N*-acetylmannosamine kinase domain.
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Keywords: GNE myopathy; Distal myopathy with rimmed vacuoles (DMRV); Hereditary inclusion body myopathy; Respiratory dysfunction; Uridinediphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase domain; *N*-acetylmannosamine kinase domain

1. Introduction

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy, or hereditary inclusion body myopathy (hIBM), is an early adult-onset, slowly progressive myopathy that preferentially affects the tibialis anterior muscle but relatively spares the quadriceps femoris muscles [1,2]. Respiratory dysfunction has not been reported in GNE myopathy [3]. Nonaka

et al. reported that respiratory muscles were rarely involved even in bed-ridden patients, but no data were presented [1]. However, we had noticed that a few patients with GNE myopathy exhibited mild but progressive respiratory loss, with some experiencing recurrent pneumonia due to reduced airway clearance. Recent recommendations suggest training patients with neuromuscular disease with respiratory dysfunction using the air stacking technique to increase their thorax capacity and assisted cough peak flow (CPF) from an early stage to maintain lung compliance and chest mobility, and to clean the airways [4]. If respiratory dysfunction is not rare in patients with GNE

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myopathy, then, physicians should punctually monitor their respiratory function with pulmonary function tests to look for early signs of respiratory dysfunction, perform respiratory training, coup with airway infection using a mechanical in-exsufflator (MI-E), and induce mechanical ventilation if required, as they do for patients with neuromuscular disease who exhibit respiratory failure.

The aim of this study is to evaluate past and present clinical respiratory function test parameters of GNE myopathy patients, and analyze factors that correlate with disease severity.

2. Patients and methods

2.1. Study population

Medical records of all genetically confirmed GNE myopathy patients who underwent pulmonary function tests at the National Center Hospital, National Center of Neurology and Psychiatry, were retrospectively reviewed. We collected data on genetic diagnosis, respiratory function (% vital capacity [%VC], % force vital capacity [FVC], cough peak flow [CPF]), creatine kinase (CK), chest X-ray and/or CT scan and body mass index (BMI) for analysis.

2.2. Data handling and analysis

Data were summarized using descriptive statistics, and each variable was compared against age, sex, respiratory dysfunction (whether their %FVC was up to or over 80%), and domain mutation (i.e., within the UDP-GlcNAc 2-epimerase domain: ED or N-acetylmannosamine kinase domain: KD). The t-test was used to compare the means of each group. Data for the two study populations were calculated using chi-square contingency table analysis. Multivariate regression analysis was performed with %FVC as the dependent variable. Explanatory variables included age at disease onset, CK and BMI. We found that the variables age, duration from onset to present, age upon wheelchair use, age at loss of ambulation, were highly correlated (over 0.5) with age at disease onset. As such, we eliminated these three due to multicolinearity in the multivariate regression analysis. When past %FVC data were available, the present data were compared with serial changes in respiratory function during the preceding 5–7 years, and changes in %FVC over time were determined by calculating the difference between past and present data. All analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

3. Results

3.1. General characteristics

A total of 39 Japanese patients (13 men, 26 women) were recruited. The mean age at the time of data collection was 43.1 (11.3) years (mean [standard deviation, SD]) (Table 1).

The mean age at first appearance of symptoms was 26.8 (9.0) years (range, 15–58 years; median, 25 years). Present age, age at disease onset, age at wheelchair use, and present ambulation status were not significantly different between men and women; 20.5% (8/39) had symptom onset before age 20. Of the 39 patients, 51.3% (20/39) could walk but needed assistance, and 69.2% (27/39) were wheelchair-bound (8/27 and 19/27 were partially and totally wheelchair-bound, respectively). Age at first use of a wheelchair was 33.3 (10.8) years (range, 18–59 years; median, 31.5 years) and that for loss of ambulation was 36.9 (11.9) years (Table 1).

3.2. GNE mutations

Of the 39 patients, 30.7% (12/39) carried homozygous mutations, while 69.2% (27/39) harbored compound heterozygous mutations (Supplementary Table 1). Among the homozygous patients, 66.7% (8/12) harbored the p.V572L mutation. Among the compound heterozygous patients, 25.9% (7/27) exhibited the p.D176V/p.V572L genotype, while the other patients each had a different mutation. With respect to the location of the mutation (i.e., protein domain), 28.2% (11/39) homozygous patients carried mutations only in ED (ED/ED), 46.2% patients (18/39) were compound heterozygotes with 1 mutation each in the ED and KD (ED/KD), and 25.6% patients (10/39) had a mutation in the KD of both genes (KD/KD) (Table 2). The allelic frequencies of p.V572L, p.D165V, p.C13S, and p.R129Q were 35.9% (28/78), 28.2% (22/78), 11.5% (9/78), and 2.6% (2/78), respectively, while all other mutations had only 1 allele each (Supplementary Table 1).

3.3. Respiratory function

None of the patients had lung and/or thoracic diseases that could affect their respiratory function in chest X-ray and/or chest computed tomography. The %VC and %FVC in patients with GNE myopathy were 91.9 (26.9) (range, 18.2–126.3; median, 100.3) and 92.0 (25.8) (range, 16.4–128.5; median, 100.5; Table 1), respectively.

3.4. Patients with respiratory dysfunction

In 30.7% of patients (12/39), %FVC was <80. Of these 12 patients, 91.6% (11/12) were wheelchair-dependent and 83.3% (10/12) had already lost ambulation. Their onset was significantly earlier (19.3 [4.4] vs. 30.3 [8.4], $p < 0.001$) and mean CK level was significantly lower (55.8 [71.6] vs. 279.0 [184.7], $p = 0.004$) than those of patients with normal respiratory function. Four patients exhibited advanced respiratory dysfunction (%FVC < 50% and cough peak flow [CPF] < 160 L/min) (Table 2). All 4 patients had experienced recurrent pneumonia, and 2 patients required nocturnal NPPV. They were all early onset (before 20 years old) and non-ambu-

Table 1
Patient characteristics by respiratory function.

n	Total 39	%FVC ⩾ 80% 27	%FVC < 80% 12	p
Age (years)	43.0 ± 11.3	44.3 ± 11.7	39.9 ± 10.3	0.267
Age at onset (years)	26.8 ± 9.0	30.2 ± 8.4	19.2 ± 4.4	<0.001
GNE/GNE	10 (25.6%)	7 (70.0%)	3 (30.0%)	0.640
GNE/MNK	18 (46.2%)	16 (88.9%)	2 (11.1%)	0.018
MNK/MNK	11 (28.2%)	4 (36.4%)	7 (63.6%)	0.009
Duration from onset of disease to present	16.2 ± 8.4	14.1 ± 7.8	20.8 ± 8.2	0.021
Wheelchair use (%)	27 (69.2%)	16 (59.3%)	11 (40.7%)	0.141
Wheelchair use since (years)	33.3 ± 10.8	37.9 ± 11.3	26.6 ± 5.1	0.002
Lost ambulation	19 (48.7%)	8 (42.1%)	11 (57.9%)	0.014
Age at lost ambulation (years)	36.9 ± 11.9	41.2 ± 11.7	28.2 ± 6.4	0.018
CK (IU/L)	201.3 ± 187.5	279.0 ± 184.7	55.8 ± 71.6	0.004
BMI	21.1 ± 4.2	20.8 ± 3.2	21.9 ± 5.8	0.457
FVC (%)	91.9 ± 26.9	106.9 ± 12.5	58.2 ± 18.7	<0.001
VC (%)	92.0 ± 25.8	106.4 ± 11.6	59.5 ± 17.6	<0.001
CPF (L/min)	334.2 ± 139.5	378.0 ± 105.7	250.2 ± 161.5	0.008

Most patients with reduced respiratory function had already lost ambulation and were entirely wheelchair-dependent. Their onset was significantly earlier and CK levels significantly lower than those of patients with normal respiratory function. FVC: forced vital capacity, VC: vital capacity, CPF: cough peak flow, BMI: body mass index, CK: creatine kinase.

Table 2
Patients with FVC < 50% and CPF < 160 L/min.

Case	Age	Sex	Mutation	Mutant domain	Ambulation status	Disease onset	Disease duration	Age at lost ambulation	%VC	%FVC	CPF (L/min)	Reccurent pneumonea	NPPV	CK (IU/L)	BMI
1	51	Man	p.C13S homozygote	ED/ED	Non-ambulant	17	34	25	18.2	16.4	48.0	Yes	Nocturnal	13	18.6
2	42	Woman	p.V572L homozygote	KD/KD	Non-ambulant	16	26	23	37.6	34.4	141.6	Yes	Nocturnal	13	22.2
3	45	Woman	p.V572L homozygote	KD/KD	Non-ambulant	17	28	31	49.0	48.3	147.6	Yes	No	8	31.6
4	37	Woman	p.V572L homozygote	KD/KD	Non-ambulant	16	21	24	53.7	48.6	118.8	Yes	No	No data	20.4

Table 3
Multivariate regression analysis of predictive factors for respiratory dysfunction.

	Regression coefficient	p	Lower limit of 95% confidence interval	Upper limit of 95% CI
Age at onset	0.949	0.042	0.038	1.86
CK	0.068	0.008	0.02	0.115
BMI	−1.8	0.09	−3.811	0.302

Multivariate linear regression analysis was performed to evaluate the relationship between %FVC and other clinical parameters. Age at onset and CK were significantly correlated with %FVC.

lant. The majority (7/12) of patients had KD/KD mutations, whereas significantly fewer patients with respiratory dysfunction had ED/KD mutations.

In order to identify predictive factors for respiratory dysfunction in GNE myopathy, we performed multivariate analysis to determine the relationship with %FVC. This revealed age at onset (p = 0.042) and CK (p = 0.008) as significantly correlated to %FVC (Table 3, Fig. 1).

Past (5–7 years ago) data were available for 9 patients. The %FVC decrements in 5 patients with respiratory dys-

function were significantly greater than those of patients without dysfunction (20.9 [6.0] vs. 0.8 [9.7], p = 0.004; Supplementary Table 2).

4. Discussion

To our knowledge, we are the first to report respiratory dysfunction in GNE myopathy. Our study demonstrates that (1) certain GNE myopathy patients in Japan exhibit respiratory dysfunction, and (2) early onset and lower CK levels resulting from severe muscle atrophy and weakness, and KD/KD mutations can be risk factors for respiratory dysfunction.

Malicdan et al. reported that pathological changes in the diaphragms of the GNE (−/−) hGNED176V-Tg model mice were variable and ranged from almost normal to the presence of marked fibrosis and rimmed vacuoles. On the other hand, the gastrocnemius muscles of all mice exhibited myopathic features [5]. The features in these mice correspond to individual differences observed in the patients of our study. The fact that not all cases in our study exhibited respiratory dysfunction as observed in the GNE (−/−)
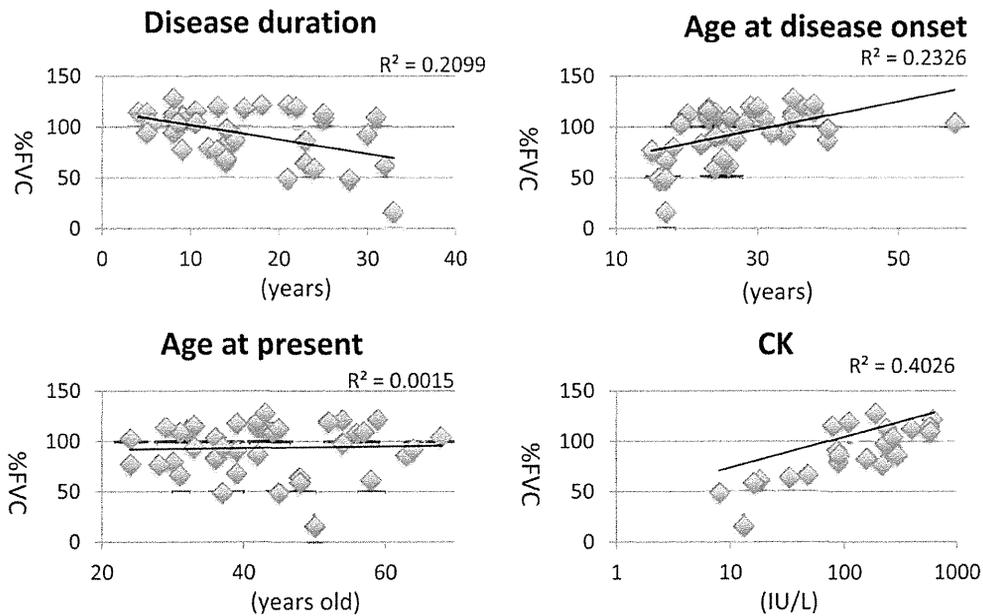
Fig. 1. Scatterplots of %FVC as functions of age, age at disease onset, disease duration, and creatine kinase (CK) level. Age at disease onset, disease duration, and CK level were correlated with %FVC.

hGNED176V-Tg mice indicates that severe respiratory muscle involvement is not a constant feature of GNE myopathy. Yet, since about 30% of patients had decreased %FVC and severe respiratory dysfunction was overlooked by neurologists or physicians, clinicians should be made more aware of the possibility of respiratory dysfunction, particularly in patients with advanced GNE myopathy. If %VC decreases to 70%, patients should be taught air stacking as with other neuromuscular disorders [4,6]. CPF should be routinely measured in patients with GNE myopathy, given that its decrement was associated with recurrent pneumonia in our study. Early induction of assisted CPF and/or MI-E is required if patients with reduced CPF have an airway infection. Serial data suggest that %FVC decreased from the normal range to %FVC < 80, indicating that continuous monitoring is required even in patients with normal respiratory function. Moreover, respiratory function parameters may provide quantitatively useful data for clinical trials, particularly those directed to non-ambulant patients.

All 4 patients with severe respiratory dysfunction exhibited early onset, homozygous mutations, and advanced muscle weakness. However, not all early onset, homozygous, or non-ambulant patients exhibited severe respiratory dysfunction. Although the underlying reasons are unclear, we also found that ED/KD mutations were less associated with decreased respiratory function, while many patients with KD/KD mutations showed respiratory dysfunction. A large scale, cross-sectional study could better identify key factors responsible for respiratory dysfunction and genotype-phenotype correlations.

We are aware that the recruitment of patients from NCNP, highly specialized for muscle disease, is a potential source of selection bias, because they may be particularly more severely affected than the general patient population. Therefore, our study may not correctly reflect the general patient population. Investigations of small populations may underestimate the statistical significance as well. However, our previous GNE myopathy questionnaire study revealed a similar correlation between genotypes and phenotypes [7]. We are currently in the process of establishing a Japanese national GNE myopathy patient registry called Registration of Muscular Dystrophy (REMUDY, http://www.remudy.jp) to perform a broader epidemic investigation of associated conditions, including respiratory dysfunction. To clarify the relationship between respiratory dysfunction and other clinical/laboratory factors, we have initiated a prospective observational study on GNE myopathy.

Three of 4 patients with severe respiratory dysfunction had homozygous p.V572L mutations. Given the frequency of the p.V572L mutation in the Japanese population, it will be interesting to determine whether non-Japanese individuals harboring this mutation also exhibit respiratory dysfunction.

In conclusion, advanced GNE myopathy patients are at risk for respiratory dysfunction. The KD/KD genotype, early onset, loss of ambulation/wheelchair use, and low CK level resulted in advanced muscle atrophy may be associated with respiratory dysfunction.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2012.09.007.

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