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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.

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,