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Subtle central and peripheral nervous system abnormalities in a family with centronuclear myopathy and a novel *dynamin 2* gene mutation

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Abstract

Mutations in dynamin 2 (DNM2), an ubiquitously-expressed large GTPase, cause autosomal dominant centronuclear myopathy (DNM2-CNM) and AD Charcot-Marie-Tooth disease type 2B (DNM2-CMT2B). We report a series of 5 patients from the same family who all presented with dominant centronuclear myopathy, mild cognitive impairment, mild axonal peripheral nerve involvement, and the novel E368Q mutation in the DNM2 gene. This study suggests that the phenotypes of dynamin 2 related centronuclear myopathy and Charcot-Marie-Tooth disease overlap and that DNM2 mutations may alter cerebral function. This report extends the clinical knowledge of DNM2-centronuclear myopathy and shows that the role of DNM2 mutations in the central nervous system should be further studied.

Keywords: Centronuclear myopathy; Dynamin 2; Mental retardation; DNM2-DI-CMTB

1. Introduction

X-linked myotubular myopathy (XLMTM) and autosomal centronuclear myopathies are characterized morphologically by chains of centrally located nuclei in a large proportion of muscle fibers [1,2]. While XLMTM is a very severe congenital disease resulting from mutations in myotubularin, a phosphoinositides phosphatase, centronuclear myopathies are genetically heterogeneous congenital myopathies that usually display recessive or dominant inheritance and generally present with childhood or adolescent onset [1,2]. Missense mutations affecting the Middle domain of the dynamin 2 (*DNM2*) protein, a large GTPase implicated in membrane and cytoskeletal remodeling, have recently been demonstrated to cause the slowly pro-

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gressive autosomal dominant centronuclear myopathy (DNM2-centronuclear myopathy) [3]. Moreover, DNM2 mutations restricted to the Pleckstrin Homology (PH) domain of the *DNM2* gene have also been recently identified in patients with autosomal dominant intermediate Charcot-Marie-Tooth disease (DNM2-DI-CMTB), an hereditary motor and sensory axonal neuropathy [4]. More recently, a *de novo* mutation in the skeletal muscle ryanodine receptor (RYR1) gene has been demonstrated in 1 patient with centronuclear myopathy [5].

We describe a family that presented with autosomal dominant centronuclear myopathy, mild axonal polyneuropathy, cognitive impairment, and a novel mutation in the Middle domain of the *DNM2* gene.

2. Patients and methods

We studied 5 patients (4 females, 1 male) with autosomal dominant centronuclear myopathy from 1 pedigree originating from France (Fig. 1A). All patients underwent standardized clinical examinations and biological tests, including serum creatine kinase (CK) levels. Karyotype was obtained in 3 patients.

Neurophysiological studies were performed in all patients and included nerve conduction study in the lower limbs and electromyography (EMG) using a concentric needle electrode in at least 3 muscles. Electrophysiological studies were performed using conventional equipment and standard methods, and skin temperature was maintained in the 32–34 °C range. The nerve conduction study was performed on: [1] peroneal nerve with motor conduction velocity (MCV), amplitude of compound muscle action potential (CMAP), and distal latency (DL); [2] sural nerve with sensory conduction velocity (SCV) and amplitude of sensory action potential (SAP). The SCV of sural nerve (antidromic) was measured in the distal third between calf and lateral malleolus. The amplitude of a sensory nerve action potential was measured peak to peak. Motor response amplitudes were measured from baseline to peak of the negative component and areas were measured by the computer program in the EMG machine.

Muscle biopsy was performed in all 5 patients and in one unaffected family member (patient II-3) and was processed with standard methods for histology, histochemistry, and electron microscopy. Brain magnetic resonance imaging (MRI) was performed in 2 patients. Neuropsychological testing was performed in 3 patients and one unaffected sibling. Electrocardiogram (EKG) and echocardiogram were performed in all patients.

Genomic DNA was extracted from the peripheral blood of 2 patients, and DNM2 sequencing was performed according to previously described methods [3]. The entire coding region of DNM2 was screened and both strands of the DNA sample were sequenced. Fragile X syndrome was tested by PCR amplification of the FRAXA 5′-region. Lymphoblastoid cell lines were established at Genethon (Evry, France) and proteins extracted with TGEK buffer (50 mM Tris, pH 7.8, 10% glycerol, 1 mM EDTA, 50 mM KCl).

3. Results

The index case was patient II-1, who was diagnosed with centronuclear myopathy at age 14. The mean age at onset in this family was 14 years (10–20 years). Whether the patients had normal motor milestones and language development is unknown. However, patients II-4 and II-5 required adapted schooling due to learning difficulties and all 5 patients are said to have presented with "mild" mental retardation since childhood. The first neurological symptoms were distal upper and lower limbs mild atrophy and weakness often associated with subtle bilateral ptosis and ophtalmoparesis (Fig. 1B). All patients had abolished deep tendon reflexes in all limbs, Achilles tendon contractures, and hyperlordosis (Fig. 1B). They all progressively developed mild proximal upper and lower limbs atrophy and weakness. However, disease severity remained globally mild and all patients were still able to walk without assistance with a mean disease duration of 33 years (17–51 years).

Serum CK levels were normal in all patients. Karyotype was normal in patients II-1, II-4, and II-5. All patients displayed a reduction of 10–30% of the CMAP amplitude of the peroneal nerve and a very mildly diminished SAP amplitude of the sural nerve (90% of the normal value), whereas SCV, DL, and MCV were normal. EMG examination showed in all patients increased polyphasic and low-amplitude motor unit potentials in distal and proximal muscles in the upper and lower limbs, indicative of myopathic changes. All patients also demonstrated mild pathological spontaneous activity (fibrillation potentials) in distal muscles in the lower limbs, indicative of neuropathic changes. Pathological spontaneous activity was observed in the *flexor hallucis brevis*, *tibialis anterior* and *gastrocnemius* (medial head) muscles in patients I-1 and II-1, and in the *flexor hallucis brevis* and *tibialis anterior* muscles in patients II-2, II-4, and II-5. EKG and echocardiogram were normal in all patients. Muscle biopsies taken from the *tibialis anterior* were performed in all patients and displayed an increased number of central nuclei, a predominance with an atrophy of type I fibers and a radial distribution of sarcoplasmic strands, highly characteristic for centronuclear myopathy (Fig. 1C–E). Neuropsychological testing showed a total IQ of 80, 82, and 83 in patients II-1, II-4, and II-5 respectively (average age-matched IQ: 100; standard deviation: 15). Brain MRI was normal in patients I-1 and II-5, and there was no abnormal CGG expansion in the FRAXA locus. Molecular analysis found the novel heterozygous E368Q mutation in the Middle domain of the *DNM2* gene in patients I-1 and II-5, affecting a highly conserved residue (Fig. 2A–C). Protein level appeared normal in cultured lymphoblasts (Fig. 2D). The E368Q mutation was not found in 154 control DNAs that were amplified for exon 8 of dynamin