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Myotonia in *DNM2*-related centronuclear myopathy

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Abstract Centronuclear myopathy (CNM) is a rare hereditary myopathy characterized by centrally located muscle fiber nuclei. Mutations in the dynamin 2 (*DNM2*) gene are estimated to account for about 50 % of CNM cases. Electromyographic recordings in CNM may show myopathic motor unit potentials without spontaneous activity at rest. Myotonic discharges, a distinctive electrical activity caused by membrane hyperexcitability, are characteristic of certain neuromuscular disorders. Such activity has been reported in only one CNM case without a known genetic cause. We sequenced the *DNM2* gene and the genes associated with myotonia (*CLCN1, SCN4A, DMPK* and *ZNF9*) in a sporadic adult patient with CNM and myotonic discharges. Sequencing the entire coding region and exon–intron boundaries revealed a heterozygous c.1106g-a substitution in exon 8, resulting in a R369Q change in the *DNM2*. Sequencing the *CLCN1, SCN4A, DMPK* and *ZNF9* genes ruled out mutations in these genes. This is the first report of *DNM2*-related CNM presenting with myotonia. The diagnosis of CNM should be considered in patients with myotonic discharges of an unknown cause.

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Introduction

Myotonia, a condition characterized by impaired relaxation of contracted skeletal muscles, can occur in certain neuromuscular disorders. It is a disorder of muscle membrane hyperexcitability that is caused by sarcolemmal ion channel dysfunction (Lehmann-Horn et al. 1995; Jurkat-Rott and Lehmann-Horn 2005; Lossin and George 2008). Hyperexcitable sarcolemma can result from specific mutations in the ion channel genes, particularly the sodium and chloride channels, or as a part of other specific muscle disorders, such as the myotonic dystrophies. Myotonic discharges, without clinical myotonia, have been reported in patients with myofibrillary myopathy (Hanisch et al. 2013). However, the presence of myotonia in association with myopathy, may serve as a valuable tool in the differential diagnosis of neuromuscular diseases.

The centronuclear myopathies (CNM) are inherited heterogeneous neuromuscular disorders, characterized by numerous centrally located nuclei in muscle fibers. X-linked myotubular myopathy is a severe disease with infantile onset, characterized by marked weakness and respiratory failure, and caused by mutations in the myotubularin gene (Laporte et al. 1996). Autosomal dominant and recessive forms exist with mutations in the dynamin 2 (*DNM2*) (Bitoun et al. 2005), amphiphysin 2 (*BIN1*) (Nicot et al. 2007), and the ryanodine receptor genes (*RYR1*) (Jungbluth et al. 2007).

In only a few case reports myotonic discharges were associated with centronuclear myopathy. Some of the

reported patients had cataract at young age. In none of them myotonic dystrophy was excluded by genetic studies, and no genetic studies for DNM2 mutation were available then (Hawkes and Absolon 1975, Gil-Peralta et al. 1978, Vallat et al. 1985, Zanoteli et al. 1998). Here, we describe the first patient with *DNM2*-related CNM with myotonic discharges.

Case report

A 27-year-old woman, born in Ukraine to non-consanguineous parents of Jewish origin, immigrated to Israel at age 24. Her past medical history is unremarkable, except for orthopedic surgery in her left knee in 2003. Her father, who died of a stroke at age 55, had difficulties walking and rising from a chair, which had never been investigated. Otherwise, no other family members had neurological illnesses.

The patient was referred to the Department of Neurology at Wolfson Medical Center in August 2011 for evaluation of muscle weakness in the lower extremities that had been progressing over one year. In mid 2010, she experienced severe pain in her left thigh shortly after strenuous exercise in a fitness center. After the pain subsided she noticed difficulties climbing stairs and rising from a sitting position, which slowly progressed with time. She denied muscle twitches or cramps. She noticed neither weakness in her upper extremities nor difficulties walking on flat surfaces. There were no sensory, visual or bulbar symptoms.

Neurologic examination revealed intact cranial nerves without ptosis or ophthalmoparesis, and normal strength of the neck muscles. In the upper extremities, proximal and distal muscle weakness was symmetric, 4/5 (Medical Research Council Scale), of the deltoid, infraspinatus, pectoralis, finger extensors and interossei. In the lower extremities, iliopsoas, glutei, ankle and toe extensors were all 4/5. The patient could not step on her toes and heels, and had to use her arms to get up from a squatting position. Deep tendon reflexes were normal, with flexor plantar response. Neither spontaneous or percussion myotonia nor muscle hypertrophy were observed. Sensation was normal to all modalities. Physical examination was normal, there was no skin rash.

Serum creatine kinase (CK) was initially 11,000 IU/L (normal values < 200), however, further values on follow-up were 800–1,200 IU/L. Motor and sensory nerve conduction studies were normal. Electromyogram (EMG) showed numerous myotonic discharges, without fibrillations or positive sharp waves, mainly in the proximal muscles of the upper and lower extremities. The myotonic discharges were evoked by inserting or moving the needle electrode, they waxed and waned with variation of amplitude and frequency (Fig. 1). Motor unit potentials (MUPs) were of short duration and low amplitude, particularly in the quadriceps muscles, with early and nearly full recruitment. These findings were consistent with the diagnosis of myopathy with myotonia. Therefore, genetic testing for myotonic dystrophy types I (DM1) and II (DM2) were performed. The results showed normal repeat lengths in the *DMPK* and *ZNF9* genes, ruling out these diseases. A repeat EMG study several months later showed similar abundant myotonic discharges in the upper and lower limb muscles.

Materials and methods

Muscle biopsy

Part of the muscle was fixed with formalin and embedded in paraffin. Another sample was frozen in isopentane chilled in liquid nitrogen. Seven micrometer thick transverse sections were stained with haematoxylin and eosin, modified Gomori trichrome, PAS, Sudan black B, NADH-tetrazolium reductase, succinate dehydrogenase and cytochrome oxidase, ATPase at pH 9.4 and after preincubation at pH 4.3 and 4.6. The immunocytological reactions

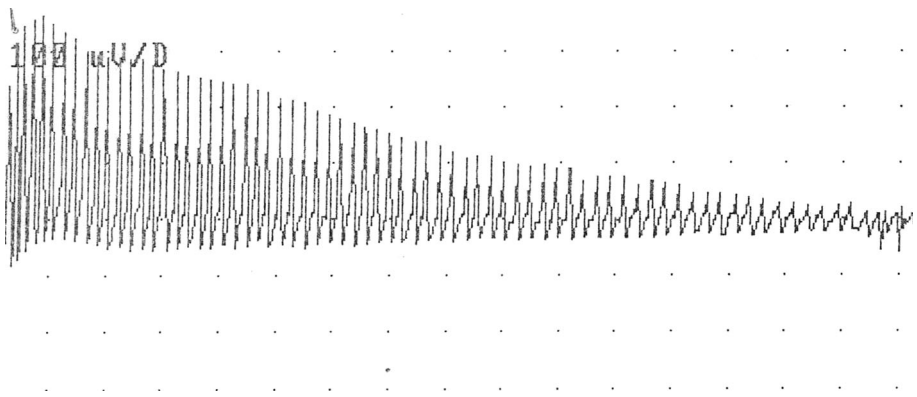

Fig. 1 Myotonic discharges recorded in the right vastus lateralis muscle

included dystrophin 1–3, sarcoglycans, merosin, caveolin 3, dysferlin and collagen VI.

Molecular analysis

Genomic DNA was extracted from peripheral blood by the Puregene kit (Gentra, Minneapolis, USA), according to the manufacturer's instructions. Genomic DNA was used as a template for PCR amplification of each of the 21 exons and exon–intron boundaries of the DNM2 gene. The reaction was performed in a 25 µl volume containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ 250 µM dNTPs, 1 µM of each primer, 100 ng of genomic DNA and 1.25U of AmpliTaq Gold DNA polymerase (Perkin Elmer Applied Biosystem). An initial denaturation step of 10 min at 95 °C was carried out to activate the polymerase, followed by 35 cycles of 94 °C 15 s, 55 or 60 °C 45 s, 72 °C and 45 s and a final elongation of 10 min at 72 °C. Predicted amplicon sizes were confirmed by agarose gel electrophoresis. The amplified PCR products were purified with the ExoSapIT kit (Amersham Pharmacia biotech, Amersham, Buckinghanshire, UK), according to the manufacturer's instructions, and sequenced with fluorescently labeled dedeoxynucleotide terminators and an Applied Biosystem 3730A automated sequencer. Sequences were compared with the DNM2 transcript (ENST00000389253), using the DNA variant analysis Surveyor software, version 3.3 (http://www.softwaregenetics.com). Polymerase chain reaction (PCR) and direct PCR sequencing of the 23 *CLCN1* and the 24 *SCN4A* exons were performed as described previously (Lehmann-Horn et al. 1995). To test for an exon-wide deletion or duplication on one allele that would not be identified by the method described above, multiplex ligation-dependent probe amplification (MLPA) analysis was performed. A commercially available kit (SALSA MLPA kit P350-A1 CLCN1) was used according to the manufacturer's guidelines (MRC-Holland, Amsterdam).

Results

Morphological findings

Muscle biopsy from the quadriceps muscle demonstrated variability in fiber size with uneven distribution. The majority of muscle fibers were small, with only a minor proportion of large fibers. Central nuclei were observed in all muscle fibers. With the oxidative enzymes and PAS there was enhanced rim staining around the nuclei with radial organization of sarcoplasmic strands. The normal intermyofibrillary network was lost. With the ATPase stain almost all fibers were of type 1, with a central zone of enzymatic inactivity (Fig. 2).

DNM2 mutation

Sequencing the entire coding region and exon–intron boundaries of *DNM2* revealed a heterozygous c.1106g-a substitution in exon 8, resulting in R369Q change in the gene product (Fig. 3). This mutation appears as pathologic according to prediction software (polyphen2; score-0.8), mutation taster (score-0.999). The substituted amino acid is located in the middle domain (MID) of the dynamin protein. This mutation has been described in CNM cases (Bitoun et al. 2005; Böhm et al. 2012). *CLCN1* and *SCN4A* mutations were excluded.

Discussion

This is the first study to describe the presence of myotonia in a case of CNM due to a mutation in the dynamin 2 gene. The R369Q mutation (p.Arg369 is changed into glutamine) has been documented in four families (Bitoun et al. 2005; Böhm et al. 2012). The severity of the CNM phenotype was moderate. The age of onset ranged from childhood to adulthood, and no prominent eye movement deficits were reported. In all cases, the R369Q mutation was heterozygous, thus conferring dominant trait of inheritance. Our patient's father had an undiagnosed proximal weakness. We suspect that he had the same disease, which is congruent with autosomal dominant trait inheritance.

Clinical myotonia could not be elicited in our patient, however, an electromyogram showed abundant typical myotonic discharges with considerable variation in amplitude and frequency (waxing and waning) (Fig. 1). Myotonic discharges in the absence of clinical myotonia have been reported in several muscle disorders such as Pompe disease, drug-induced myopathy and myofibrillary myopathy (Hanisch et al. 2013).

Dynamin 2 is a ubiquitously expressed large GTPase protein that plays an important role in multiple cellular pathways. It is composed of an N-terminal GTPase domain, a middle domain (MID), a pleckstrin homology (PH) domain, a GTPase effector domain (GED), and a C-terminal proline-arginine rich domain. Dynamin 2 is involved in membrane modulation, particularly membrane fission and fusion: it can bind to membrane phospholipids and exhibit GTP hydrolysis. It acts as a key player in endocytosis, phagocytosis, endosome formation, cell migration, membrane trafficking from the plasma membrane to the *trans*-Golgi membrane, and other membrane processes (Klein et al. 1998; Gold et al. 1999; Praefcke and

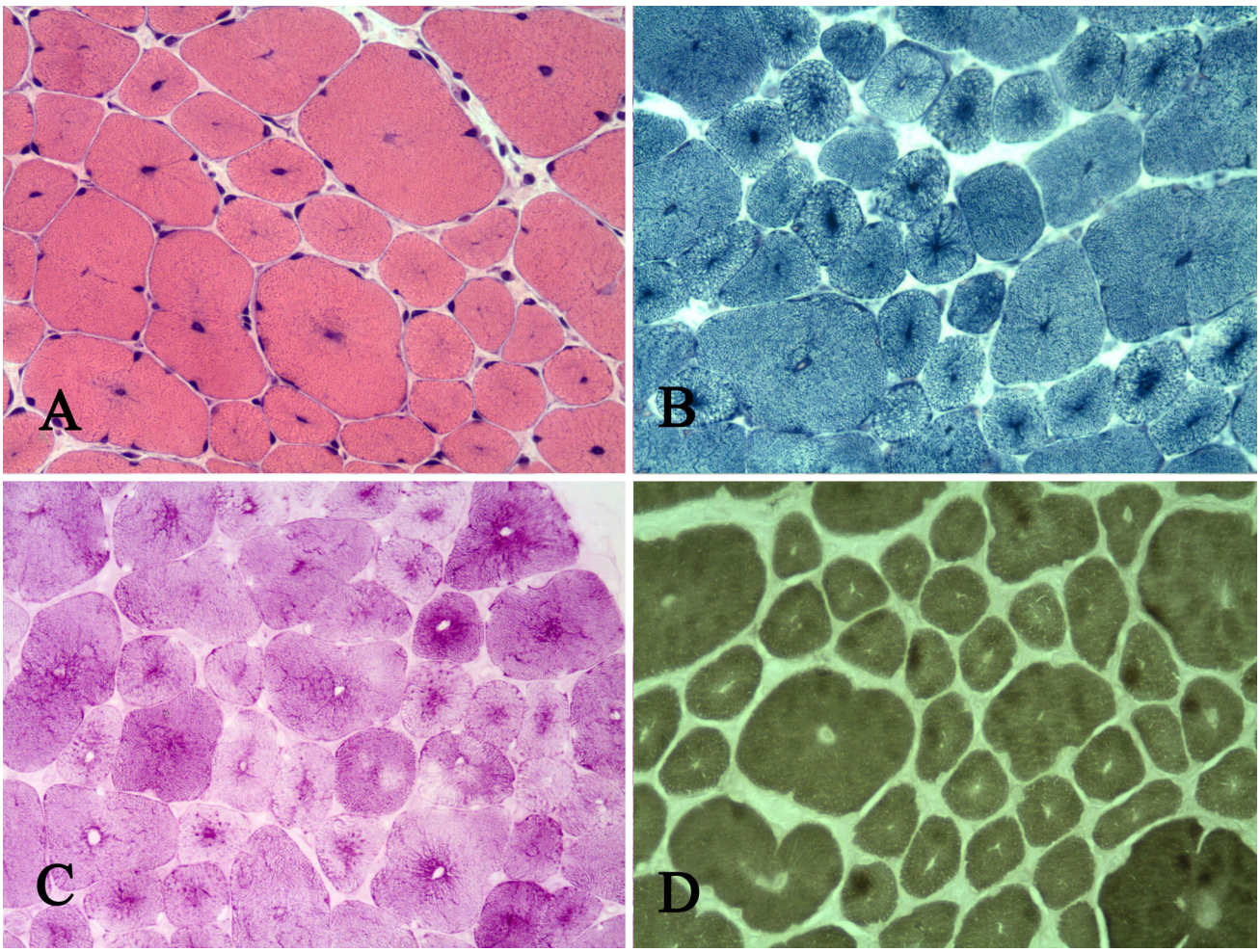

Fig. 2 Transverse sections of muscle biopsy. **a** Central nuclei are observed in every muscle fiber. H&E. **b–c** Abnormal internal architecture with central rim of enhanced staining and radial strands. NADH-TR (**b**) and PAS (**c**). **d** All fibers are type 1. In every fiber there is a central area devoid of enzymatic activity. ATPase at pH 9.4

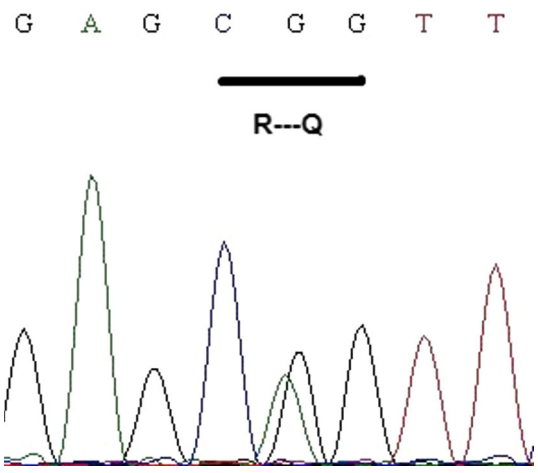

Fig. 3 Sequence electropherogram demonstrating the R369Q change in the DNM2 gene

McMahon 2004; Krutchten and McNiven 2006; Chappie et al. 2009; Catteruccia et al. 2013).

Mutations in *DNM2* are rare, and have been found to cause two autosomal dominant disorders: centronuclear myopathy (CNM) and Charcot–Marie–Tooth (CMT) neuropathy (Durieux et al. 2010; Romero 2010; Tanabe and Takei 2012; Catteruccia et al. 2013). It is yet unclear why certain mutations cause peripheral nerve disease while others cause muscle disease. *DNM2*-related CMT (CMT2 M) is generally of mild to moderate severity, with intermediate or axonal neuropathy. Typically, the sensory and motor fibers are involved, resulting in distal muscle weakness and atrophy, decreased tendon reflexes, pes cavus and distal sensory loss (Durieux et al. 2010; Romero 2010). *DNM2* mutations are estimated to account for around 50 % of CNM. Generally, *DNM2*-related CNM is clinically milder than severe early onset X-linked

myotubularin and autosomal recessive *BIN1* associated CNM. The clinical spectrum of *DNM2*–CNM is broad, ranging from severe neonatal congenital myopathy to mild late onset phenotype (Catteruccia et al. 2013; Kierdaszuk et al. 2013). The majority of patients exhibit facial weakness, ophthalmoparesis and bilateral ptosis, with variable degrees of distal muscle weakness and atrophy. As mentioned, our patient did not exhibit facial or eye movement weakness, which is similar to reported symptoms of R369Q patients. The histological characteristics of ADCNM consist of centralized nuclei, myofiber atrophy, type I fiber predominance and radial arrangement of sarcoplasmic strands (also referred to as "spokes of a wheel").

In conclusion, DNM2-related centronuclear myopathy should be considered in the differential diagnosis of patients with myotonia.

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