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The coexistence of *dynamin 2* mutation and multiple mitochondrial DNA (mtDNA) deletions in the background of severe cardiomyopathy and centronuclear myopathy

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Abstract. *Dynamin2 (DNM2)* gene mutations may result in Charcot-Marie-Tooth disease and centronuclear myopathy. Here, we present a patient suffering from cardiomyopathy and centronuclear myopathy with repetitive discharges and mild axonal neuropathy due to *DNM2* mutation. Detailed cardiological and neurological examinations, electrophysiological tests, muscle biopsy, and molecular genetic analysis were performed. The patient developed left bundle branch block at age 40 and was fitted with a pacemaker at the age of 43. The patient has severe heart failure, ptosis, strabism, facial and proximal muscle weakness. Electrophysiological investigations found myopathy, complex repetitive discharges, and axonal neuropathy. Skeletal muscle biopsy detected centronuclear myopathy and cytochrome C oxidase (COX) negative fibers. Genetic analysis detected a pathogenic c.1105C>T (p.R369W) *DNM2* gene mutation and heteroplasmic multiple mitochondrial DNA (mtDNA) deletion. Our data broadens the phenotypic spectrum of *DNM2* mutations. The presence of the multiple mtDNA deletions may provide new aspects to understanding the pathogenesis of multisystemic symptoms in patients with *DNM2* mutations.

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Introduction

Dynamins are a family of GTPase proteins within the dynamin superfamily, which function as mechano-chemical scaffolding molecules and control trafficking from the trans golgi network [1, 2, 3]. *Dynamin 2 (DNM2)* is the most widely expressed of the three classical dynamins, which have roles in diverse cellular functions, including endocytosis and membrane trafficking [4, 5]. Mutations in the *DNM2* gene result in disrupted cellular organization [4], centronuclear myopathy (CNM), and/or Charcot-Marie-Tooth neuropathy (CMT2M) [6]. *DNM2*-associated CNM may be responsible for infantile onset early feeding- and respiratory difficulties, contractures, muscle hypertrophy, cardiomyopathy, and hematological abnormalities, which result in developmental delay (Table 1). *DNM2*-associated CMT is characterized by slow-onset progressive weakness and atrophy of the anterior and lateral muscles of the legs and intrinsic muscles of the hand with intermediate or axonal type neuropathy [7].

At present, 35 different *DNM2* mutations have been identified in more than 150 distinct clinical cases [8]. Alterations of the Pleckstrin homolog (PH) domain of *DNM2* were reported to be associated with severe neonatal onset, whereas milder phenotypes with later onset are often caused by mutations in the middle domain (MD) (Table 1) [8].

Dynamin molecules not only influence cellular organization but are also known to play a role in mitochondrial fusion and fission [9]. Through modulation of mitochondrial dynamics, *DNM2* mutations likely have an indirect effect on mitochondrial function. It is interesting to note that *DNM2* mutations frequently result in multisystemic symptoms (Table 1). In a few cases, *DNM2* mutations coexisted with mitochondrial dysfunction [10, 11, 12].

The present article reports the case of a 47-year-old woman affected by severe car-

Table 1. The previously published *DNM2* mutations and associated phenotypes based on the Leiden database and Pubmed literature search.

Dynamin domain	Amino acid substitution	Phenotype	Reference
Middle domain (MD)	R336Q	CNM, hypotonia, external ophthalmoplegia	[22]
	G358R	CMT	[7]
	E368K	CNM, neonatal hypotonia, delayed motor development, external ophthalmoplegia, ptosis, respiratory weakness, repetitive discharges	[13, 16, 22, 23]
	E368Q	CNM, CMT, mild cognitive impairment, learning difficulties, external ophthalmoplegia, ptosis	[24]
	R369Q	CNM, muscle hypertrophy	[13]
	R369W	CNM, muscle weakness	[13]
	V375G	CNM, facial weakness, ptosis, muscle wasting, pes cavus, epilepsy	[25]
	F379V	Lethal congenital contracture syndrome-5 (LCCS5)	[26]
	Y462C	CNM, neonatal hypotonia, external ophthalmoplegia	[21]
	R465W	CNM, external ophthalmoplegia, ptosis, facial weakness, arachnodactyly, biventicular dilated cardiomyopathy	[13, 16, 23]
Pleckstrin homolog (PH)	R522H	CMT, hypotonia, external ophthalmoplegia, repetitive discharges	[16]
	G537C	CMT	[27]
	E540K	CNM, strabism, external ophthalmoplegia, dysphagia, muscle wasting, severe pelvic muscle weakness	[25]
	D555_E558del	CMT	[28]
	D555Gfs*12	CMT	[28]
	K558E	CMT, cataracts, neutropenia	[7, 29]
	K558del	CMT, neutropenia	[7, 29]
	K559del	CMT, congenital cataracts, strabism, ptosis, external ophthalmoplegia	[30]
	E560K	CNM, external ophthalmoplegia, ptosis, facial weakness dysphagia,	[29]
	K562E	CMT	[7]
	K562del	CMT	[7]
	L570H	CMT	[27]
	D614N	CNM, ptosis, progressive muscle weakness	[31]
	A618D	CNM, hypotonia, external ophthalmoplegia, progressive muscle weakness, respiratory weakness,	[32]
	A618T	CNM, hypotonia, external ophthalmoplegia, IDDM, cryptorchidism	[16, 22]
	S619L	CNM, hypotonia, weak suckling, facial weakness, respiratory weakness, cryptorchidism	[18, 33]
	S619W	CNM	[33]
	L621P	CNM, cataract, hypotonia, respiratory and feeding difficulties	[16]
	624insA_G	Ptosis, facial weakness, pes cavus	[25]
GTPase effector domain (GED)	V625del	CNM, hypotonia, weak suckling	[33]
	P627H	CNM, decreased fetal movements, external ophthalmoplegia, respiratory weakness, arachnodactyly	[16]
	P627R	CNM, pes cavus	[25]
	E650K	CNM, ptosis, muscle weakness, mental retardation	[34]
Proline-rich domain (PRD)	T859_I860del	CMT	[7]

CMT = Charcot-Marie-Tooth disease, CNM = centronuclear myopathy, GED = GTPase effector domain, IDDM = insulin-dependent diabetes mellitus, LCCS5 = lethal congenital contracture syndrome-5.

diomyopathy, CNM with muscle relaxation difficulty, and mild axonal neuropathy, harboring both a *DNM2* mutation and multiple mitochondrial DNA (mtDNA) deletions.

Materials and methods

Detailed neurological, cardiological, psychiatric examinations, and laboratory investigations were performed. The patient's cardiac status was evaluated with ultrasonography (Philips iE33 ultrasound machine, Best, Netherlands) using transthoracic echo probe) and Holter (Rozinn Electronics, Glendale, NY, USA) electrocardiogram (ECG). Skeletal muscle biopsy was obtained for light- and electron microscopic examination using standard routine staining. Electromyography (EMG) and electroneurography (ENG) were performed using standard techniques (Dantec Keypoint, Skolunde, Denmark). Written, informed consent was obtained from the patient prior to molecular genetic testing. DNA was extracted from blood and muscle samples using a QIAamp DNA blood kit, according to the manufacturer's instructions (QIAgen, Hilden, Germany). The *dystrophia myotonica protein kinase (DMPK)* and *zinc finger protein 9 (ZNF9)* gene were investigated by Southern blot. The mitochondrial *polymerase gamma 1 (POLG1)* and *DNM2 (dynamin2)* genes were sequenced bidirectionally. Genetic sequence was compared with the human reference genome using NCBI's Blast® (National Center for Biotechnology, Informations, Bethesda, MD, USA) application. The mtDNA deletion was tested with long-range polymerase chain reaction (PCR) methodology.

Case report

Patient

The subject of this case report is a 47-year-old Hungarian female patient. She was referred to our center because of muscle stiffness and weakness. Her mother had severe cardiac failure, paresis of the external ocular muscles, generalized muscle stiffness, and limb girdle type muscle weakness and died at the age of 67 from a pulmonary embolism. The patient's siblings and children are asymptomatic.

Cardiological examinations and interventions

The patient's first clinical symptoms started at the age of 30, with shortness of breath upon exertion. At that time, hypertension was diagnosed and was accompanied by symptoms of heart failure, including effort dyspnea and fatigue. ECG showed left bundle branch block. Echocardiography revealed diffuse hypokinesis, impaired left ventricle systolic function, and ventricular dyssynchrony with significant intra- and inter-ventricular delay. The ejection fraction was decreased to 41% (normal: > 55%). Coronary catheterization did not show signs of coronary artery disease. At the age of 43, the patient became refractory to complex medical treatment, New York Heart Association (NYHA) class II – III heart failure developed, and, therefore, a biventricular pacemaker was implanted using coronary sinus stenting procedure for the left ventricular electrode. Since then, the patient has been in NYHA class I during regular follow-up visits in the past 4 years.

Neurological examinations and findings

The patient's perinatal period and early motor development was normal. Neurological symptoms started at the age of 32, with difficulty tiptoeing, stair climbing, and standing from sitting position. She suffered frequent ankle sprains, generalized muscle stiffness, and weakness of the hands. Her most recent complaints have been blurred vision in the morning and persisting horizontal diplopia. Neurological examination at the age of 45 revealed long, myopathic face, moderate bilateral ptosis, and ophthalmoparesis predominantly on the left side. Relaxation difficulty was observed in her hand muscles. Additionally, moderate atrophy of the small muscles of the hands and feet, hammer toes, and excavated arches was detected. Muscle weakness, mild and predominantly distal in the upper extremities, moderate and predominantly proximal type in the lower limbs, was evident. Deep tendon reflexes were decreased; pyramidal tract signs were not present. Pallhypesthesia was observed in both legs.

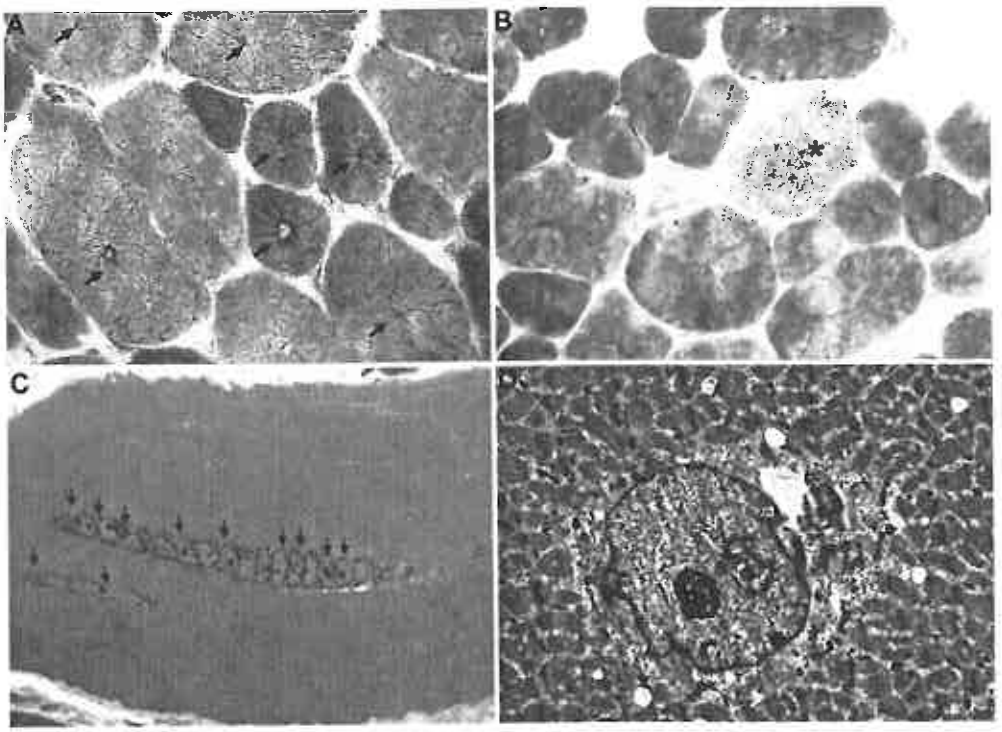
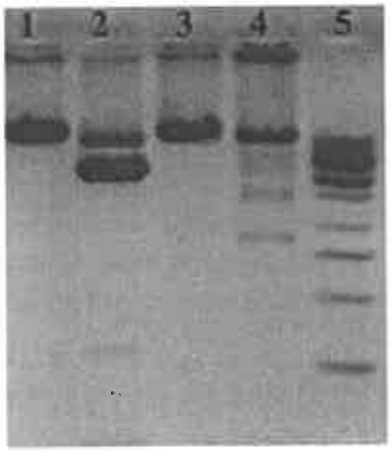


Figure 1. A: Centrally located nuclei in many muscle fibers with radiating sarcoplasmic strands. Arrows indicate central nuclei (NADH staining, 160×). B: COX negative fiber appearing as ragged blue fiber. Star indicates COX negative fiber (combined COX-SDH staining, 160×). C: Central core chain in muscle fibers on semi-thin section. Arrows indicate central nucleus (semi-thin resin sections, 420×). D: Central nucleus, surrounded by increased number of lipid and lipofuscin droplets and decreased number of mitochondria with rounded shape (electron microscopy, 8000×).



1. Wild type
2. 4977 bp „Common" deletion
3. Wild type
4. Patient sample – mtDNA multiple deletion
5. 1 kb DNA Ladder

Figure 2. Agarose gel electrophoresis of long PCR. In Lane 4, we show the multiple mtDNA deletion detected in the patient muscle biopsy.

Psychiatric and neuropsychological examinations and findings

Psychiatric assessment of the patient found mild depression, slightly increased interpersonal sensitivity (1.8/4), and paranoia (2.0/4) to be the most prevalent subscales. Somatic symptoms, memory and sleep problems were also marked. Neuropsychological examination detected mild cognitive dysfunction, which mainly affected the working and visual memory.

Neurophysiological examinations and findings

EMG of the tibial anterior muscle detected spontaneous activity characterized by fibrillation potentials, positive sharp waves, and complex repetitive discharges. The amplitudes were normal, while the duration of action potentials in the anterior tibial and lateral vastus muscles were below the normal range, indicating myopathic changes (anterior tibial amplitude: 377.2 ± 138.2 μV (normal value: 300 – 1,000 (μV), duration: 6.8 ± 0.7 ms (normal value: ≥ 13.8 ms ± 2 SD); lateral vastus muscle – amplitude: 592 ± 321 μV (normal value: 300 – 1000 μV), duration: 7.7 ± 1.5 ms (normal value: ≥ 14.1 ms ± 2 SD)). ENG revealed mild axonal neuropathy. The nerve conduction studies showed the following values: in the right median nerve motor: distal motor latency 2.5 ms (normal ≤ 4.5 ms), amplitude 5.9 mV (normal ≥ 5 mV), conduction velocity 56.6 m/s (normal ≥ 48 m/s); right ulnar nerve

motor: distal motor latency 2.3 ms (normal ≤ 4.5 ms), amplitude 4.4 mV (normal ≥ 5 mV), conduction velocity 50.0 m/s (normal ≥ 40 m/s); right peroneal nerve motor: distal motor latency 5.1 ms (normal ≤ 4.5 ms), amplitude 1.9 mV (normal ≥ 3 mV), conduction velocity 42.3 m/s (normal ≥ 40 m/s); right sural nerve motor: distal motor latency 2.5 ms (normal ≤ 4.5 ms), amplitude 4.4 mV (normal ≥ 5 mV), conduction velocity 56.8 m/s (normal ≥ 40 m/s).

Myopathological findings

Light microscopic investigation of the deltoid muscle detected moderate fiber caliber variation. A high proportion of the muscle fibers (> 70%) had central nuclei (Figure 1A and C) with perinuclear halo. In some fibers, snake coils were present. Three percent of the muscle fibers were cytochrome c oxidase (COX)-negative (6/200 fibers). These fibers appeared as ragged blue with modified succinic dehydrogenase (SDH) staining (Figure 1B). On resin section, some of the fibers contained a long row of central nuclei. In the neighborhood of the central nuclei increased numbers of lipid and lipofuscin droplets, intermyofibrillary moderately decreased numbers of mitochondria have been detected by electromicroscopy. Many mitochondria had rounded shape. Intramitochondrial paracristallin inclusions were not present (Figure 1D). In some fibers ,myofibrils were largely absent in the very center of the fiber, suggesting that a central nucleus is present in another level.

Routine clinical chemistry laboratory results

Decreased serum iron and folic acid levels were found, while triglyceride and LDL cholesterol levels were elevated. Creatinine kinase (CK) level was in the normal range. The resting serum lactate level and the lactate stress test were normal. Vitamin D3 level was also below normal.

Molecular genetic results

Genetic analysis was negative, both for type 1 (*DMPK* gene) and type 2 (*ZNF9* gene) myotonic dystrophy. In the muscle biopsy, multiple mitochondrial DNA (mtDNA) deletions were detected (Figure 2). The *DNM2* gene sequence analysis found 1 exonic nonsynonymous 1105C>T (p. R369W) mutation, 1 exonic synonym c.2139T>C (p.Ala713Ala) rs2229920 polymorphism, and 2 intronic variations (c.236-29C>G – rs3826803; c.1545+41C>T – rs2287029). Because mtDNA multiple deletions were found in the muscle tissue, *POLG1* gene analysis was performed. No pathogenic mutation was found in the *POLG1* gene. We detected 7 single nucleotide polymorphisms (SNPs): c.3708 G>T (p.Q1236H) – rs3087374, c.659+61 G>T – rs2283460, c.2071-22 T>C – rs2072267, c.2764+40InsGTAG, c.3105-36 A>G – rs2246900, c. 3105-11 T>C – rs2302084, and c.3483-19 T>G – rs2307438) in *POLG1* gene. The pathogenic *DNM2* R369W mutation was not present in the children of the patient. The patient's siblings did not agree to the genetic analysis, her mother had already passed away.

Discussion

This is the first report about a patient having severe cardiomyopathy, centronuclear myopathy, repetitive discharges, axonal neuropathy, and mild cognitive impairment due to *DNM2* gene mutation. The R369W mutation in the MD of *DNM2* has previously been described in a French family with autosomal dominant CNM [13].

The leading symptom in our patient was the severe cardiomyopathy. Up to now, only in a few cases have been reported of cardiomyopathy coexisting with CNM [14, 15, 16, 17]. However, in two of these studies, diagnosis was based on clinical and histopathological grounds, genetic analysis was not performed. The association of *DNM2* mutation and cardiomyopathy has been proven in only one case [15, 16]. However, in a zebrafish model, decreased DNM2 protein levels were observed in end-stage heart failure [18]. In this model, *DNM2* seemed to mediate heart failure by modulating Ca^{2+}-dependent apoptotic death of the cardiomyocyte [18]. Endogenous DNM2 protein levels have been demonstrated to decrease gradually, in a parallel fashion, with the progression of heart failure in different experimental animal models [18]. The cardiomyopathy was

only one component of the multisystemic involvement of *DNM2* mutation in our patient.

In the muscle biopsy of our patient, ragged blue and COX negative fibers were present, indicating, mitochondrial dysfunction additionally. In the muscle tissue, multiple mtDNA deletions were detected. The coexistence of *POLG1 gene* mutation, which is one of the most common causes of inherited mitochondrial disorders, was excluded with multiple deletions [19]. The association of DNM2 alterations and mitochondrial dysfunction has been described in a few cases [10, 11, 12]. The R369W mutation in our patient was associated with the presence of COX-deficient muscle fibers, a feature shared with a recent in vivo experimental mouse model [10]. Similar results were observed in HeLa cells following *DNM2* small interfering RNA (siRNA) knockdown. Expression of p.R369W *DNM2* in NIH3T3 cells led to a significant decrease in mitochondrial objects compared to controls, suggesting an important and emerging role for DNM2 in mtDNA maintenance and stability [10]. In a DNM2 mediated, CNM double knockout mouse model, reduced mitochondrial adenosine 5'triphosphate (ATP) levels were found, indicating mitochondrial abnormality [12]. The in vivo mouse model of Tinelli et al. [21] found some similar morphological changes in the skeletal muscle, such as increased lipid droplets and decreased density of mitochondria were found.

In summary, based on the literature and on our observations, *DNM2* gene mutations may result in a multisystemic disease. Previously, mutations of this gene have been shown to affect skeletal muscle, eyes, peripheral nervous system, and the hematopoietic system. Besides cardiomyopathy, our patient had centronuclear myopathy, muscle relaxation difficulty, mild axonal neuropathy, and mild cognitive impairment. The multisystemic phenotype we describe could be explained by an alternative, secondary mitochondrial dysfunction. However, the direct effect of the *DNM2* alteration is a strong candidate, given the evidence in the literature and does not preclude a compound effect of *DNM2* mutations with other causes of mitochondrial dysfunction.

In conclusion, our data broadens the phenotypic spectrum of *DNM2* mutations and suggests that CNM and CMT disease may only be a common part of an underlying multisystemic disease. Further studies are needed to clarify the complex interaction between DNM2 and mtDNA alterations.

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Conflict of interest

The authors declare that they have no conflict of interest.

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