MITOCHONDRIAL ALTERATIONS IN DYNAMIN 2-RELATED CENTRONUCLEAR MYOPATHY

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Centronuclear myopathy (CNM) is clinically characterized by diffuse involvement of skeletal muscles, with onset mainly in early childhood and a slowly progressive course. The basic histological abnormalities in muscle biopsies are a high percentage of centrally located nuclei of muscle fibers, radial arrangement of sarcoplasmic strands around the central nuclei, and predominance and hypotrophy of type 1 fibers[1-3]. Three forms of the disease have been recognized that differ in age of onset and severity of the symptoms: The severe X-linked myotubular myopathy, caused by mutations in the *MTM1* gene; the childhood onset form; and the juvenile/adult onset form that fully manifests during the third decade of life. In the juvenile/adult onset form, most of the families have an autosomal dominant inheritance, and patients present with a slowly progressive disease, but with mild clinical manifestations[2]. This form has been associated with mutations in the *dynamin 2* gene (*DNM2*)[4]. More recently, Nicot et al.[5] described in three CNM families with autosomal recessive inheritance mutations in *amphiphysin 2* gene, which disrupt the interaction with dynamin 2 protein.

We have now identified an adult-CNM patient with mutation in *DNM2* and overt mitochondrial changes in muscle biopsy.

CASE

A 50-year-old male, from a large Brazilian family, presented since the age of 30 with slowly progressive muscle weakness associated with impaired capacity to stand up, run and climb the stairs (Fig 1, III-6). Clinical examination confirmed weakness predominantly in the distal part of the limbs (Table). The Gowers' signal was present. He could not walk on his heel. Atrophy of the limbs was evident, especially in the distal parts, as well as tendon hyporeflexia with normal muscular tonus. There was no noticeable weakness of facial or ocular movement; and ptosis was absent. The patient's most consistent complaint was easy fatigue even during regular daily activity.

Other four affected members of his family were also examined (Fig 1) (Table). These patients had similar complaints of fatigue, muscle pain and cramps. All patients presented a slowly progressive disease, and could not walk on their heels. All patients had normal ocular movements. Normal muscular tonus with hyporeflexia was observed in all affected patients. Patients II-6, III-5, and IV-2 presented a clear distal atrophy of the limbs, especially in the lower legs. Neonatal manifestations such as mild hypotonia, suction and cry weakness were described only for the patient III-5; however the motor development was normal.

Deltoid muscle biopsy from patient III-6, performed at the age of 50, showed increased number of central nuclei (more than

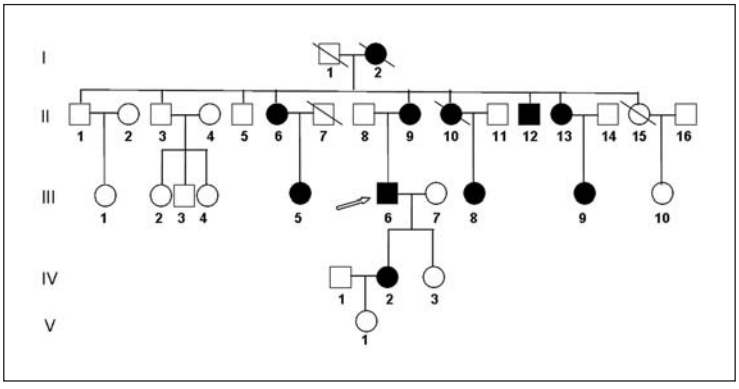

Fig 1. Pedigree of the Family. Black symbols represent affected members, either by clinical history, physical exam or molecular analysis.

ALTERAÇÕES MITOCONDRIAIS NA MIOPATIA CENTRONUCLEAR RELACIONADAS A DINAMINA 2

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Table. Clinical data from 5 patients with DNM2-CNM from the same family.

Case	Sex/ age (y)	Onset (y)	First symptoms	Complains	Facial muscles	Proximal movements (MRC)	Distal movements (MRC)	Cervical movements (MRC)	Osteoskeletal changes
II-6	F/64	40	Fatigability and weakness for walking	Muscle pain, fatigue, cramps	Mild dysphagia	Hip flexion (4+), leg extension (4+), leg adduction (4)	Ankle dorsiflexion and eversion (4-), ankle flexion and inversion (4+)	Flexion (4)	Pes cavus equinovarus, wrist and Achilles retraction
III-5	F/35	?	Walk weakness. Neonatal hypotonia, weak suction and cry.	Muscle pain, fatigue, cramps	Ptosis since child	Hip flexion (4+), leg extension (4+)	Ankle dorsiflexion and eversion (4), ankle flexion and inversion (4+)	nl	no
III-6	M/50	30	Weakness	Fatigue	Masticatory muscles atrophy	Arm abduction (4) and extension (4), leg extension-flexion (4) and adduction (4)	Ankle dorsiflexion (4-), ankle eversion (4-), foot plantar flexion (4) and wrist extension-flexion (4)	Flexion and extension (4)	no
III-8	F/32	?	?	Fatigue, cramps	High-arched palate	Hip flexion (4+), arm extension (4+)	Ankle dorsiflexion (4)	Flexion (4)	Achilles retraction
IV-2	F/20	?	?	no	nl	Hip flexion (4+), arm extension (4+)	Ankle dorsiflexion (4)	nl	no

Age refers to the time of examination; MRC, Medical Research Council scale; +, present; –, absent; y, years; nl, normal; F, female; M, male; DNM2-CNM, dynamin 2-related centronuclear myopathy.

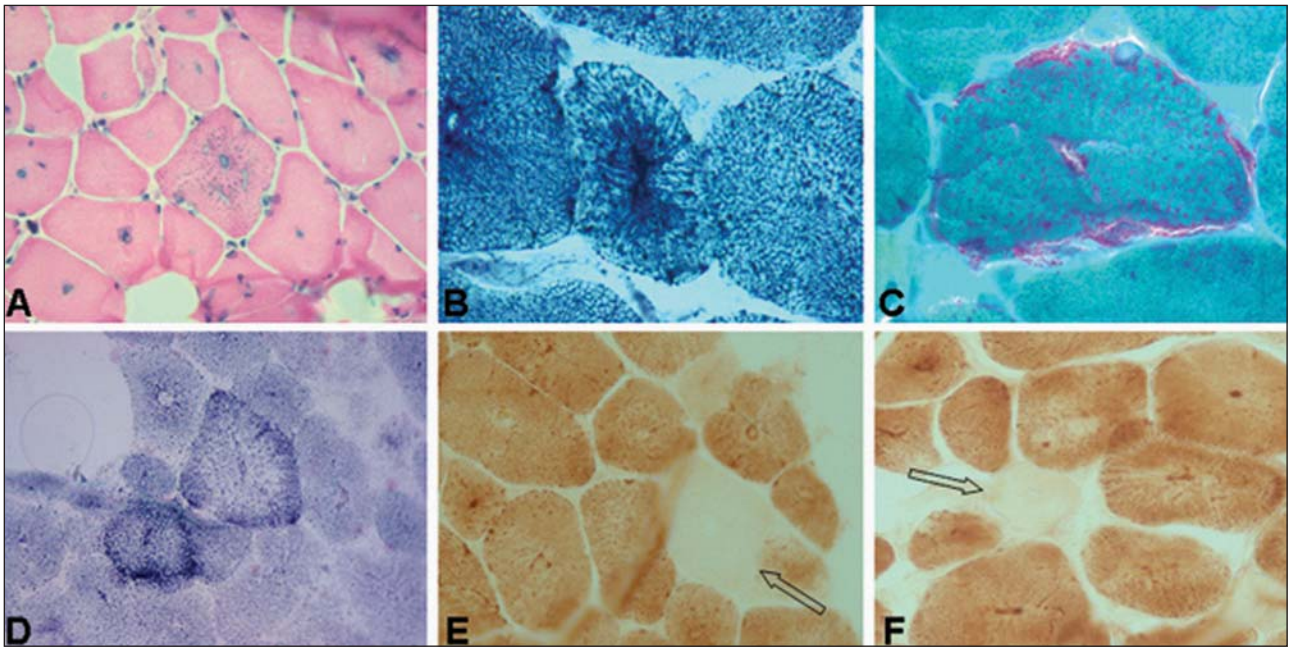

Fig 2. Muscle biopsy analysis from CNM patient with DNM2 mutation (III-6). Characteristic histological findings of CNM and mitochondrial alterations. (A) Increased number of centralized nuclei in hematoxylin & eosin staining, and (B) spoke-like appearance of the fibers seen on NADH-tr staining. (C) Typical ragged-red fiber in Gomori's trichrome staining, and (D) increased subsarcolemmal enzyme activity seen on SDH staining. (E) and (F) Presence of COX negative fibers (arrows).

80% of the fibers), intense variation in fiber size, moderate increase of the endomysial and perimysial connective tissue and intense perifascicular fat infiltration (Fig 2A). The histochemical study revealed predominance and hypotrophy of type 1 fibers. Spoke-like appearance was observed in most of the fibers (Fig 2B). About 4% of the fibers showed a ragged-red aspect (Fig 2C). Additionally, there were fibers with increased SDH activity (Fig 2D) and reduced or absent COX activity (about 5%) (Fig 2E–F). Inflammation and necrosis were not observed. The muscle biopsy from another family member (III-5), performed at the age of 35, showed typical characteristics of CNM, but no ragged-red fibers, SDH positive fibers or COX deficient fibers.

The exons and intron-exon boundaries from the middle domain of the *DNM2* gene were sequenced, and a heterozygous missense mutation in exon 11 (1393C–T), which results in the amino acid substitution R465W, was identified in patients II-6, III-5, III-6, III-8 and IV-2.

The muscle biopsies and the DNA from the patients were obtained with informed consent following protocols approved by the ethical committee from the Institution.

DISCUSSION

We report here a case of adult onset CNM with mutation in the *DNM2* gene and additional histological findings typically seen in mitochondrial diseases.

Only a few reports are present in the literature describing mitochondrial changes in CNM patients. In one patient, crystalline inclusions were found in mitochondria adjacent to the centralized nuclei[6]. In another case, mitochondrial abnormalities, including reduction of the number of central and subsarcolemmal mitochondria, reduction of cristae, and mitochondrial remnants within lysosomes, were observed[7], and Naumann et al.[8] described two typical adult CNM patients with ragged-red fibers and fibers with absence of COX staining in the muscle biopsy. Although no molecular analyses were done on any of these patients, their histological alterations were similar to those described in CNM patients carrying mutations in the *DNM2* gene, i.e. fiber type variability, type 1 atrophy and spoke-like appearance[4]. Furthermore, these patients also had a late onset form of the disease. Based on our findings, we believe that also those early cases might have carried mutations in the *DNM2* gene, indicating that the combined occurrence of CNM with *DNM2* mutations and mitochondrial changes could be more frequent than recognized until now. More recently, ragged red fibers were identified in the muscle biopsy from a patient with DNM2-related CNM (DNM2-CNM)[9].

We could speculate that altered DNM2 protein makes the skeletal muscle of CNM patients more prone to develop a mitochondrial dysfunction as they age. This could explain the lack of mitochondrial changes in another affected member of the same family, who was biopsied at the age of 35. In addition, we cannot exclude interfamilial variability, which is commonly observed in muscle diseases.

Bitoun et al.[4] demonstrated that in DNM2-CNM the DNM2 protein presents an aberrant centrosomal localization *in vitro*, indicating that *DNM2* mutations might cause CNM by interfering with centrosome function. Dynamins are large GTPases involved in membrane trafficking that act as mechanochemical scaffolding molecules that can hydrolyze GTP to alter biological membranes[10]. Among the dynamin family, the 100-kDa GTPase DNM2 is known to be involved in endocytosis and membrane trafficking[11], actin reorganization[12], and centrosome cohesion[13]. Furthermore, dynamin-related GTPase has been suggested to be involved in the processes of mitochondrial fission and fusion[14,15], both of which are known to play a key role in cell division and differentiation. Considering the broad range of cellular mechanisms in which DNM2 is involved, it is conceivable that an altered DNM2 protein could also interfere with the correct formation and/or function of the mitochondria in affected patients, especially during the aging. We conclude that muscle biopsy from DNM2-CNM patients should be investigated for associated mitochondrial changes.

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