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Sporadic Centronuclear Myopathy with Muscle Pseudohypertrophy, Neutropenia, and Necklace Fibers due to a *DNM2* mutation

Teerin Liewluck^{a,*}, Tracy L. Lovell^b, Anna V. Bite^a, and Andrew G. Engel^a

^a Department of Neurology and Muscle Research Laboratory, Mayo Clinic College of Medicine, Rochester, MN

^b Northeast Georgia Diagnostic Clinic, Gainesville, GA

Abstract

Dynamin 2 gene (*DNM2*) mutations result in an autosomal dominant centronuclear myopathy (CNM) and a Charcot-Marie-Tooth (CMT) neuropathy. *DNM2*-CMT but not *DNM2*-CNM patients were noted to have neutropenia. We here report a man with paravertebral muscles hypertrophy and mild neutropenia. His muscle biopsy was typical for CNM with additional “necklace” fibers. Sequencing of *DNM2* revealed a known heterozygous c.1269C>T (p.Arg369Trp) mutation. Necklace fibers were considered as a pathological hallmark of late onset X-linked CNM due to mutations in *MTM1* but have not been observed in *DNM2*-CNM. The findings broaden the features of *DNM2*-myopathy.

Keywords

Centronuclear myopathy; *DNM2*; dynamin 2; muscle hypertrophy; muscle pseudohypertrophy, necklace fibers; neutropenia

1. Introduction

Centronuclear myopathies (CNM) are genetically heterogeneous congenital myopathies with the common pathological feature of numerous myofibers containing centrally-placed nuclei without significant muscle fiber necrosis or regeneration [1]. Mutations in the myotubularin (*MTM1*) [2], the amphiphysin 2 (*BIN1*) [3], and the dynamin 2 (*DNM2*) [4] genes cause X-linked, autosomal recessive, and autosomal dominant forms of CNM, respectively. A mutation in the skeletal muscle ryanodine receptor (*RYR1*) was reported in one CNM patient [5]. Interestingly, myotubularin, amphiphysin 2, and dynamin 2 are essential for clathrin-mediated endocytosis and their defects cause similar morphologic changes [6,7]. However, the necklace fibers were considered a specific marker for late-onset *MTM1*-related CNM [8].

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* Corresponding author. Address: Department of Neurology and Muscle Research Laboratory, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN, 55905, USA. Tel.: +1 507 284 5102; fax: +1 507 284 5831; liewluck.teerin@mayo.edu.

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Dynamin 2, a ubiquitously expressed 100-kDa GTPase, plays a role in vesicle endocytosis and centrosome organization [4]. It is composed of 5 domains including a N-terminal GTPase domain, a middle domain, a pleckstrin homology domain, a GTPase effector domain, and a C-terminal proline-rich domain [4]. *DNM2* mutations give rise to autosomal dominant CNM and Charcot-Marie-Tooth (CMT) neuropathy with intermediate or normal nerve conduction velocities [4,9,10]. To date, all *DNM2* mutations causing myopathy occurred in the middle domain, the C-terminal of the pleckstrin homology domain, or the GTPase effector domain [11]. Patients carrying mutations in the C-terminal of the pleckstrin homology domain have an unusual perinatal onset [12]. In contrast, most mutations in *DNM2*-CMT occur in the N-terminal of the pleckstrin homology domain with only single mutations in the middle and the proline-rich domains [9].

Both *DNM2*-myopathy and *DNM2*-CMT patients have distal limb muscle weakness. Involvement of ocular and eyelid elevator muscles has been noted in *DNM2*-CNM and less often in *DNM2*-CMT [9,13]. A minority of *DNM2*-myopathy patients has a mild axonal neuropathy, but none of *DNM2*-CMT patients have a myopathy by electrophysiologic and pathologic criteria [13-15]. *DNM2*-CMT patients may also have neutropenia and cataracts, but no *DNM2*-CNM patients have neutropenia and only one *DNM2*-CNM patient had cataracts [9,16].

We report a patient with paravertebral muscle pseudohypertrophy, mild neutropenia, and necklace fibers due to a known heterozygous mutation affecting the middle domain of dynamin 2.

2. Case report

2.1 Patient

A 21-year-old man presented at the emergency room with acute abdominal pain, nausea, and mild epigastric tenderness. He had mild neutropenia with an absolute neutrophil count of 1900 cells/ μ l (normal 2200-4800 cells/ μ l). Other routine blood tests as well as serum amylase and lipase levels were normal. CT scan of the abdomen showed no intra-abdominal abnormality but revealed an increase in size and fatty replacement of the thoracolumbar paravertebral and anterior abdominal muscles (Fig.1A). The abdominal pain resolved after administration of analgesic and antiemetic agents.

Retrospectively, the patient was born at full term with no perinatal complications. His motor development was normal. He kept up with his peers in physical activities during his childhood, and has noted no muscle weakness or sensory symptoms. No other family members were similarly affected. There was no spinal deformity and spine mobility was unrestricted. Except for hypertrophy of the paraspinal muscle (Fig.1B), his examination was normal. The serum CK level was 364 U/L (normal 24-195 U/L). The blood thyrotrophin level was normal. The mild neutropenia had been noted three years ago; there was no history of severe or recurrent infections. No electrodiagnostic studies were performed.

2.2 Muscle biopsy

A biopsy of the rectus abdominis muscle showed the muscle fiber diameters to vary from 30 to 90 μ m, with a moderate to marked increase in perimysial fatty and a mild increase in endomysial fibrous connective tissue. Ninety-four percents of the muscle fibers harbored one or more centrally-placed nuclei (Fig.1C). A single necrotic fiber was observed. Regenerating fibers were absent. In oxidative enzyme-reacted sections, myofibers with central nuclei showed a radial arrangement of the myofibrils, and scattered fibers displayed peripheral accentuation of enzyme activity in a necklace pattern (Fig.1D). Each necklace fiber harbored a single central nucleus. Muscle fibers in PAS and myophosphorylase-reacted

sections also showed a necklace-like accentuation. Twelve percents of myofibers in myophosphorylase-reacted section and eight percents of myofibers in NADH dehydrogenase-reacted section were necklace fibers. As adjudged by ATPase-reacted sections, type 1 fibers were more abundant and had a smaller mean diameter than type 2 fibers. Sarcoplasmic/Endoplasmic reticulum calcium ATPase 2(NCL-SERCA2, 1:200, Novocastra), dihydropyridine receptor- α 1 subunit (DHPR α 1s, 1:10, Hybridoma), calveolin 3 (26/Caveolin 3, 1:800, BD Biosciences), desmin (DE-U-10, 1:80, Sigma) and α B-crystallin(1B6.1-3G4, Stressgen, 1:1000) were immunolocalized with monoclonal antibodies. The necklaces were positive for SERCA2 (Fig.1D inlet), DHPR α 1s, desmin and α B-crystallin antibodies, but not for caveolin 3 antibody.

2.3 Genetic analysis

Because necklace fibers were reported as a histologic marker for late-onset *MTM1*-CNM, we first analyzed the *MTM1* but found no mutations. Next, we sequenced all exons and flanking untranslated regions of *DNM2* and identified a previously reported heterozygous c. 1269C>T substitution in exon 8 predicting an arginine to tryptophan substitution at position 369 (p.Arg369Trp) (nucleotide positions are based on *DNM2* reference sequence NM_001005360) [6] (Fig.1E). No parental DNA was available for analysis.

3. Discussion

Muscle hypertrophy/pseudohypertrophy occurs in various conditions including overuse, neuromuscular hyperexcitability, muscular dystrophies, lipodystrophies, amyloidosis, hypothyroidism, acromegaly, myostatinopathy, parasitic infestations (cysticercosis, schistosomiasis, and trichinosis) and rarely in neurogenic disorders [17]. The diagnosis of centronuclear myopathy in our patient was supported by the high frequency of fibers harboring central nuclei and a radial arrangement of the myofibrils in some fibers. The increase of fibrous and fatty connective tissue likely explains the increased size of the truncal muscles. Increase in muscle size was previously noted in two autosomal dominant CNM families and in a sporadic adult-onset CNM patient before any CNM disease genes were identified [18-20].

The necklace fibers in our patient with *DNM2*-CNM are of a special interest. Bevilacqua and colleagues recently reported these fibers as a histologic marker of adult patients with childhood-onset *MTM1*-CNM, but they did not see these fibers in 40 patients with *DNM2*-CNM or in one patient with *BINI*-CNM [8]. Interestingly, the necklace fibers in our patient differ only slightly from those in *MTM1*-CNM in that in our patient they do not have a smaller mean diameter than non-necklace fibers, [8]. In *MTM1*-CNM the myonuclei appear adjacent to the necklace in a bead-like pattern, but in our *DNM2*-CNM patient the nuclei remain central [8]. In our patient, like other *DNM2*-CNM patients, muscle biopsy showed a radial arrangement of the sarcoplasmic strands, but this was not noted in *MTM1*-CNM patients with necklace fibers [8]. In future studies, these subtle differences may point to *DNM2* rather than *MTM1* as the disease gene.

DNM2-CNM has several unique clinical presentations that set it apart from *MTM1*-CNM and *BINI*-CNM (Table 1). Most *DNM2*-CNM patients have predominantly distal weakness [1,6]. Exercise-induced myalgia as an initial presentation is not unusual [15]. Generalized muscle hypertrophy was previously reported in 7 patients in a single French-Guyana family carrying p.Arg369Gln *DNM2* mutation, but some CNM patients with the same mutation do not have muscle hypertrophy [4,21]. All patients had ptosis and some patients had weakness of the distal arm muscles [21]. Three patients had rigid spine [21]. In our patient, only the paraspinal muscles were enlarged and ptosis and limb weakness were absent.

The only other myopathy known to be associated with isolated neutropenia is Barth syndrome (3-methylglutaconic aciduria type 2) [22]. In *DNM2*-CMT but not in *DNM2*-myopathy, neutropenia and cataracts are associated with the p.Lys558Glu, p.Lys558del or p.Lys559del mutations [9,13]. The absolute neutrophil count is very variable, ranging from < 500 to as high as 2000 cells/ μ l and not constantly present [9]. Unlike in Barth syndrome, CMT patients with neutropenia have no severe or recurrent infections [9,11], but a few patients have mild anemia and thrombocytopenia [9]. The present patient is the first *DNM2*-myopathy patient with neutropenia. The mechanism of the neutropenia is unclear. Zuchner et al. sequenced the neutrophil elastase gene (*ELA2*), a causative gene of cyclic neutropenia which is in the close proximity to *DNM2*, and found no mutations [23]. Another possible explanation is that normal dynamin 2 is required for the endocytosis of granulocyte colony-stimulating factor (GCSF) receptor (GCSFR), which is a major regulator of neutrophil proliferation, maturation and survival [10,24]. Defective internalization of GCSF-GCSFR complex due to a mutation in *GCSFR* itself is known to cause severe congenital neutropenia [25].

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References

1. Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. *Orphanet J Rare Dis* 2008;3:26. [PubMed: 18817572]
2. Laporte J, Hu LJ, Kretz C, et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* 1996;13:175–182. [PubMed: 8640223]
3. Nicot AS, Toussaint A, Tosch V, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet* 2007;39:1134–1139. [PubMed: 17676042]
4. Bitoun M, Maugenre S, Jeannet PY, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet* 2005;37:1207–1209. [PubMed: 16227997]
5. Jungbluth H, Zhou H, Sewry CA, et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul Disord* 2007;17:338–345. [PubMed: 17376685]
6. Jungbluth H, Wallgren-Pettersson C, Laporte JF. Centronuclear (Myotubular) Myopathy Consortium. 164th ENMC International workshop: 6th workshop on centronuclear (myotubular) myopathies, 16–18th January 2009, Naarden, The Netherlands. *Neuromuscul Disord* 2009;19:721–729. [PubMed: 19683444]
7. Dowling JJ, Gibbs EM, Feldman EL. Membrane traffic and muscle: lessons from human disease. *Traffic* 2008;9:1035–1043. [PubMed: 18266915]
8. Bevilacqua JA, Bitoun M, Biancalana V, et al. “Necklace” fibers, a new histological marker of late-onset MTM1-related centronuclear myopathy. *Acta Neuropathol* 2009;117:283–291. [PubMed: 19084976]
9. Claeys KG, Züchner S, Kennerson M, et al. Phenotypic spectrum of dynamin 2 mutations in Charcot-Marie-Tooth neuropathy. *Brain* 2009;132:1741–1752. [PubMed: 19502294]
10. Fabrizi GM, Ferrarini M, Cavallaro T, et al. Two novel mutations in dynamin-2 cause axonal Charcot-Marie-Tooth disease. *Neurology* 2007;69:291–295. [PubMed: 17636067]
11. Bitoun M, Durieux AC, Prudhon B, et al. Dynamin 2 mutations associated with human diseases impair clathrin-mediated receptor endocytosis. *Hum Mutat* 2009;30:1419–1427. [PubMed: 19623537]

12. Bitoun M, Bevilacqua JA, Prudhon B, et al. Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. *Ann Neurol* 2007;62:666–670. [PubMed: 17932957]
13. Bitoun M, Stojkovic T, Prudhon B, et al. A novel mutation in the dynamin 2 gene in a Charcot-Marie-Tooth type 2 patient: clinical and pathological findings. *Neuromuscul Disord* 2008;18:334–338. [PubMed: 18394888]
14. Echaniz-Laguna A, Nicot AS, Carré S, et al. Subtle central and peripheral nervous system abnormalities in a family with centronuclear myopathy and a novel dynamin 2 gene mutation. *Neuromuscul Disord* 2007;17:955–959. [PubMed: 17825552]
15. Fischer D, Herasse M, Bitoun M, et al. Characterization of the muscle involvement in dynamin 2-related centronuclear myopathy. *Brain* 2006;129:1463–1469. [PubMed: 16585051]
16. Jungbluth H, Cullup T, Lillis S, et al. Centronuclear myopathy with cataracts due to a novel dynamin 2 (*DNM2*) mutation. *Neuromuscul Disord* 2010;20:49–52. [PubMed: 19932620]
17. Bertorini, TE. *Neuromuscular case studies*. Butterworth Heinemann; Philadelphia: 2008. p. 601–608.
18. Lovaste MG, Aldovini D, Ferrari G. Centronuclear myopathy with unusual clinical picture. *Eur Neurol* 1987;26:153–160. [PubMed: 3569369]
19. Matsushita S, Ikeda S, Hanyu N, Yamamoto K, Yanagisawa N. Muscular pseudohypertrophy in an adult case with centronuclear myopathy. *Eur Neurol* 1991;31:172–174. [PubMed: 2044633]
20. Ferrer X, Vital C, Coquet M, et al. Autosomal dominant centronuclear myopathy. *Rev Neurol (Paris)* 1992;148:622–630. [PubMed: 1295057]
21. Jeannet PY, Bassez G, Eymard B, et al. Clinical and histologic findings in autosomal centronuclear myopathy. *Neurology* 2004;62:1484–1490. [PubMed: 15136669]
22. Schlame M, Ren M. Barth syndrome, a human disorder of cardiolipin metabolism. *FEBS Lett* 2006;580:5450–5455. [PubMed: 16973164]
23. Züchner S, Noureddine M, Kennerson M, et al. Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat Genet* 2005;37:289–294. [PubMed: 15731758]
24. Touw IP, van de Geijn GJ. Granulocyte colony-stimulating factor and its receptor in normal myeloid cell development, leukemia and related blood cell disorders. *Front Biosci* 2007;12:800–815. [PubMed: 17127321]
25. Boxer LA, Newburger PE. A molecular classification of congenital neutropenia syndromes. *Pediatr Blood Cancer* 2007;49:609–614. [PubMed: 17584878]

Fig.1.

(A) Abdominal CT scan reveals fatty infiltration and enlargement of paraspinal (arrow) and anterior abdominal muscles (arrowhead). (B) The patient with bilateral, paraspinal muscle (asterisk) hypertrophy. (C) Hematoxylin and eosin section displays numerous myofibers harboring centrally-placed nuclei. Arrow points to a single necrotic fiber replaced by macrophages. There is a moderate to marked increase in perimysial fatty (asterisk) and a mild increase in endomysial fibrous connective tissue. (D) In NADH dehydrogenase-reacted section, some fibers show radial arrangement of the myofibrils and harbor central nuclei (arrow). Scattered myofibers with centrally-located nuclei displays peripheral accentuation of enzyme reactivity in a necklace-like pattern (arrowhead). The necklaces were positive for Sarcoplasmic/Endoplasmic reticulum calcium ATPase 2 (SERCA2) (inlet). (E) Chromatogram shows a heterozygous c.1269C>T substitution in *DNM2* exon 8 causing p.Arg369Trp. Bar: 100 μ m in C and 50 μ m in D.

Table 1

Comparison of mutation-identified centronuclear myopathies

Characteristics	DNM2-related disorders		MTM1-CNM [2,6,8]	BIN1-CNM [3,6]
	CNM [4,6,12,15,16, present study]	CMT [9,13,23]		
Mode of inheritance	Autosomal dominant	Autosomal dominant	X-linked recessive	Autosomal recessive
Onset	Adolescent to adult onset except pleckstrin homology domain mutations cause neonatal onset	Childhood to adolescent onset	Neonatal to infantile onset; manifesting carriers develop symptoms later in life	Neonatal to childhood onset
Limb weakness	Distal or proximal predominant	Distal predominant	Proximal predominant	Proximal predominant
Ptois or ophthalmoparesis	++++	+	++++	++++
Neuropathy	+	++++	–	–
Muscle hypertrophy	++	–	–	–
Cataract	+	++	–	–
Neutropenia	+	++	–	–
Radial arrangement of myofibrils	++++	–	++	–
Necklace fibers	+	–	++ in late-onset patients	–

CMT, Charcot-Marie-Tooth neuropathy; CNM, centronuclear myopathy; frequency of each feature ranging from common (++++), to absent (–)