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Mutation Spectrum in the Large GTPase Dynamin 2, and Genotype–Phenotype Correlation in Autosomal Dominant Centronuclear Myopathy

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ABSTRACT: Centronuclear myopathy (CNM) is a genetically heterogeneous disorder associated with general skeletal muscle weakness, type I fiber predominance and atrophy, and abnormally centralized nuclei. Autosomal dominant CNM is due to mutations in the large GTPase dynamin 2 (*DNM2*), a mechanochemical enzyme regulating cytoskeleton and membrane trafficking in cells. To date, 40 families with CNM-related *DNM2* mutations have been described, and here we report 60 additional families encompassing a broad genotypic and phenotypic spectrum. In total, 18 different mutations are reported in 100 families and our cohort harbors nine known and four new mutations, including the first splice-site mutation. Genotype–phenotype correlation hypotheses are drawn from the published and new data, and allow an efficient screening strategy for molecular diagnosis. In addition to CNM, dissimilar *DNM2* mutations are associated with Charcot–Marie–Tooth (CMT) peripheral neuropathy (CMTD1B and CMT2M), suggesting a tissue-specific impact of the mutations. In this study, we discuss the possible clinical overlap of CNM and CMT, and the biological significance of the respective mutations based on the known functions of dynamin 2 and its protein structure. Defects in membrane trafficking due to *DNM2* mutations potentially represent a common pathological mechanism in CNM and CMT.

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KEY WORDS: centronuclear myopathy; congenital myopathy; Charcot–Marie–Tooth neuropathy; DNM2; ADCNM; CMTD1B; DI-CMTB; CMT2M; hereditary motor and sensory neuropathy type II; HMSNII; MTM1; myotubular myopathy; BIN1; RYR1; endocytosis

Background

Inherited neuromuscular disorders are mostly progressive diseases of the motor-unit-affecting components of the neuromuscular pathway, predominantly resulting in muscle weakness. Similar phenotypes can arise from mutations in different genes, and conversely mutations in a single gene can be associated with various clinical and histopathological phenotypes. Mutations in the large GTPase dynamin 2 (*DNM2*, 19p13; MIM# 602378), encoding *DNM2*, are associated with dominant Charcot–Marie–Tooth (CMT) peripheral neuropathies (MIM# 606482), as well as with autosomal dominant centronuclear myopathy (ADCNM; MIM# 160150) [Bitoun et al., 2005; Zuchner et al., 2005].

Centronuclear myopathies are characterized by muscle weakness associated with myofiber atrophy and abnormal nuclear centralization. In contrast to dystrophies, nuclei mispositioning in CNM appears not to be related to fiber regeneration. Three genetic forms have been described. The X-linked neonatal form (XLMTM; MIM# 310400) is associated with mutations in *MTM1* (MIM# 300415), encoding the 3-phosphoinositide phosphatase myotubularin, and involves life-threatening, generalized muscle weakness and respiratory insufficiency [Laporte et al., 1996]. An intermediate form with autosomal recessive inheritance (ARCNM; MIM# 255200) is related to mutations in the membrane-bending protein amphiphysin 2 (*BIN1*; MIM# 601248), with disease onset during infancy or early childhood [Nicot et al., 2007]. ADCNM, caused by mutations in *DNM2*, is usually clinically milder, and was reported with both neonatal and adult onset with a diffuse and slowly progressive muscle weakness, putatively accompanied by muscle hypertrophy [Bitoun et al., 2005]. Delayed motor milestones and facial weakness are common ADCNM features, and bilateral ptosis and ophthalmoparesis are seen in most patients. Biopsies typically show fiber size heterogeneity with type I fiber hypotrophy, radial arrangement of sarcoplasmic strands, and frequent centrally located nuclei [Romero, 2010; Toussaint et al., 2011]. CNM patients with *DNM2* mutations have a clinically wide spectrum ranging from severe neonatal to moderate adult onset characteristics [Bitoun et al., 2009a; Bitoun et al., 2007; Bitoun et al., 2009b; Bitoun et al., 2005; Echaniz-Laguna et al., 2007; Hanisch et al., 2011; Jeub et al., 2008; Jungbluth et al., 2010; Liewluck et al., 2010; Melberg et al., 2010; Schessl et al., 2007; Susman et al., 2010]. Computed tomography and magnetic resonance imaging (MRI) revealed main involvement of distal muscles [Fischer et al., 2006; Schessl et al., 2007].

In addition to the three established CNM genes, sequence variants in *RYR1* (MIM# 180901), coding for the ryanodine receptor calcium release channel, are associated with a variety of congenital myopathies, including cases with centralization of nuclei on muscle biopsy (MIM# 117000) [Bevilacqua et al., 2011; Wilmshurst et al., 2010]. An inactivating variant in hJUMPY/MTMR14 (MIM# 611089) has also been identified in a case of childhood onset CNM [Tosch et al., 2006].

CMT neuropathy, also called hereditary motor and sensory neuropathy, is the most common inherited neurological disorder, and it affects both motor and sensory nerves. It covers a heterogeneous group of genetically distinct disorders with related clinical appearance. Specific *DNM2* mutations are linked to dominant intermediate (CMTD1B; MIM# 606482) and axonal CMT neuropathy (CMT2M; MIM# 606482) with a classical mild to moderately severe phenotype, often including sensory loss, distal muscle weakness and atrophy, pes cavus, and decreased tendon reflexes. Neutropenia and cataracts have been reported in DNM2-related CMT and CNM [Fabrizi et al., 2007; Jungbluth et al., 2010; Liewluck et al., 2010; Zuchner et al., 2005].

Dynamin 2 is a multidomain protein 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 (PRD; Fig. 1A). To date,

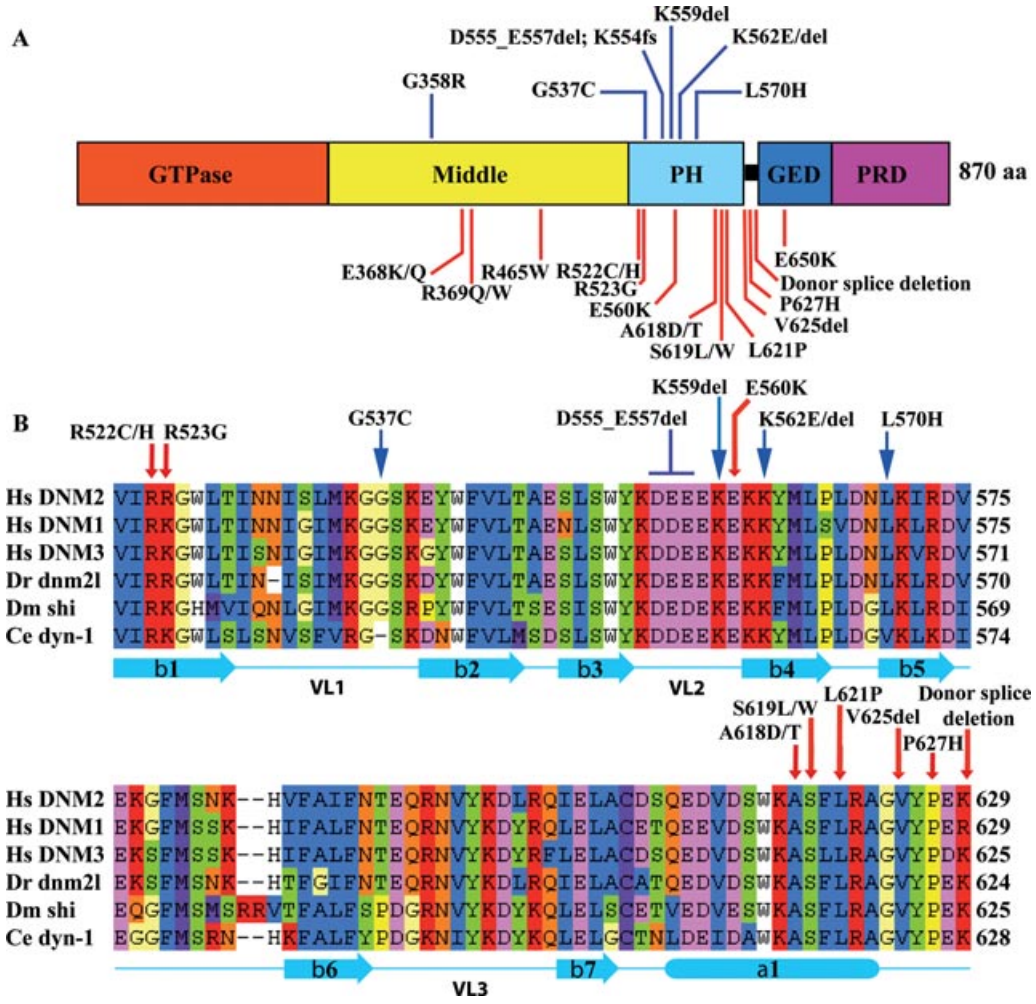

Figure 1. A: Dynamin 2 protein domains and mutations identified in CNM (red arrows) and CMT neuropathy (blue arrows). Dynamin 2 protein domains: GTPase domain, middle (MID), pleckstrin homology (PH) domain, GTPase effector domain (GED), and praline rich domain (PRD). B: Sequence comparison of the PH domain in dynamin 2 (DNM2), paralogs (DNM1 and DNM3), and orthologs. Hs, *Homo sapiens*; Dr, *Danio rerio*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*. Beta sheets (b1–b7), variable loops (VL1–VL3) and the alpha helix (a1) are indicated below.

mutations associated with either CNM or CMT have been found exclusively in the MID, GED, and PH domain. Dynamin 2 can bind membrane phospholipids through its PH domain; the GTPase is activated by the intrinsic GED and enhanced by homomultimerization at the membrane, and the PRD mediates protein–protein interactions [Chappie et al., 2009; Klein et al., 1998; Sever et al., 1999]. Dynamin 2 is a ubiquitously expressed GTPase involved in diverse cellular processes including endocytosis, cytokinesis, phagocytosis, and cell migration [Gold et al., 1999; Kruchten and McNiven, 2006; Praefcke and McMahon, 2004]. It acts as a mechanochemical enzyme involved in membrane fission, and is therefore a key player for endosome formation and membrane trafficking from the plasma membrane and the trans-Golgi network [Jones et al., 1998; Warnock et al., 1997]. Dynamin 1 was identified as a tubulin-binding protein regulating the stability of microtubules, and dynamin 2 was further demonstrated to be implicated in centrosome cohesion via gamma-tubulin binding, and in actin assembly, through direct and indirect interactions [Gu et al., 2010; Schafer et al., 2002; Shpetner and Vallee, 1989; Thompson et al., 2004].

To date, 15 different CNM-related *DNM2* mutations have been reported in 40 families [Bitoun et al., 2009a; Bitoun et al., 2007; Bitoun et al., 2009b; Bitoun et al., 2005; Echaniz-Laguna et al., 2007; Hanisch et al., 2011; Jeub et al., 2008; Jungbluth et al., 2010; Liewluck et al., 2010; Melberg et al., 2010; Schessl et al., 2007; Susman et al., 2010]. In this study, we describe a cohort of 60 novel ADCNM families harboring nine known and four new mutations, thereby expanding the mutation spectrum and defining regions more prone to mutations than others. Our results include the identification of two novel missense mutations in exon 14, one in exon 16, as well as a donor splice-site deletion of exon 16. We compared the position of CNM and CMT mutations in the light of recent structural models for dynamin protein domains. From the combined published and new results, we discuss genotype–phenotype correlations, as well as a possible expansion of the associated pathological signs, and we propose a sequencing hierarchy for hotspot containing exons according to the patient's clinical and histological data.

Mutations and Polymorphisms

Database

We have established a web-based, locus-specific database to list all identified variants for the *DNM2* gene. This database has been created with the universal mutation database software (UMD)

[Beroud et al., 2000], in compliance with the recommendations of the Human Genome Variation Society (www.umd.be/CSA/ and www.umd.be/CSB/). The database www.umd.be/DNM2/ is accessible online and will be updated with any newly reported variant from any team worldwide. A standardized form will be available online to submit any newly identified mutation to the curator.

Overview

To date, *DNM2* mutations have been reported in 40 CNM families: 26 with mutations in the MID, 11 in the PH domain, two in the PH–GED linker, and one in the N-terminus of the GED. In this study, we report the genetic and clinical characterization of 60 novel ADCNM families with *DNM2* mutations in the MID (36 families), in the PH domain (22 families), and in the PH–GED linker (2 families), including one family with a splice-site mutation leading to an in-frame insertion. Combining our results with the published data, the 100 families with *DNM2* mutation harbor five different mutations in the MID, nine mutations in the PH domain, four in the PH–GED linker, and one in the GED (Table 1, Supp. Table S1, and Fig. 1). The vast majority affect CpG dinucleotides. The N-terminal GTPase domain and the C-terminal PRD were never found to harbor disease-causing mutations in human patients. Apart from the novel exon 16 splice mutation and the deletion of Val625, all other mutations are missense changes affecting only 11 different positions in the 870 amino acid dynamin 2 protein. This strongly supports the idea that alterations of specific residues lead to ADCNM and that the mutations most probably do not involve loss-of-function, but rather gain-of-function, in agreement with the dominant inheritance. Alternatively, mutations may affect a specific subset of dynamin 2 functions. There is no overlap between ADCNM and CMT-related mutations.

De novo appearance of the mutation was confirmed in a large proportion of sporadic cases by Sanger sequencing of DNA samples of both parents. We also report in this study two patients from consanguineous families, who were first analyzed for ARCNM, but who were later found to carry de novo dominant *DNM2* mutations.

Mutations in the MID

Combining all available reports with our novel data, p.Arg465Trp is the most common mutation in the MID (27 families out of 100), followed by p.Glu368Lys (19 families), p.Arg369Trp (10 families), p.Arg369Gln (four families), and p.Glu368Gln (two families). In total, 62 families have been found with mutations in the MID, constituting the majority of patients (62%). Of note, all MID mutations are limited to two adjacent amino acids (Glu368 and Arg369) encoded on exon 8 and a single amino acid Arg465 encoded on exon 11.

Mutations in the PH Domain

Including this study, 33 ADCNM families with mutations in the PH domain have been reported. These mutations are more evenly distributed and less frequent than in the MID. The mutations include p.Arg522His (12 families), p.Ser619Leu (11 families), p.Ala618Thr (three families), and p.Glu560Lys (two families), and p.Ala618Asp, p.Ser619Trp, p.Leu621Pro, and the novel p.Arg522Cys and p.Arg523Gly mutations in one family each. It now becomes clear that similar to the MID, the PH domain contains mutation hotspots at Arg522/Arg523 and Ala618/Ser619 encoded by exons 14 and 16, respectively.

Mutations in the PH–GED Linker and in the GED

Two mutations in the linker domain between the PH domain and the GED were previously published: a single amino acid deletion (p.Val625del, one family) and a missense mutation (p.Pro627His, one family). Here we report the novel p.Pro627Arg (one family) and the first *DNM2* splice-site mutation associated with CNM (Fig. 2). The c.1885–1893+8del17 mutation was detected by direct Sanger sequencing of samples from a large family with seven affected members, and was found to segregate with the disease. The deletion removes the nine 3'-terminal nucleotides of exon 16, as well as the first eight intronic nucleotides including the exon 16 donor splice site. RNA isolation, reverse transcription polymerase chain reaction (RT-PCR), and cDNA sequence analysis confirmed the deletion, and revealed the presence of an aberrant transcript due to the use of a downstream cryptic in-frame donor splice site and the retention of 69 intronic nucleotides. The resulting predicted protein lacks three residues encoded by exon 16, but includes an in-frame insertion of 23 new amino acids in the linker region (Fig. 2C). The only previously detected mutation in the GED domain was pGlu650Lys (one family) [Bitoun et al., 2009b]. Mutations in the PH–GED linker and in the GED domain are rare and were found in 5% of all known *DNM2*-related CNM families.

Mutations in CMT

A single missense mutation leading to CMT peripheral neuropathy was found in the MID (p.Gly358Arg, Table 1) [Gallardo et al., 2008]. The other six mutations were identified in the PH domain and comprise three missense changes, two small deletions of a single amino acid, and the deletion of the donor splice site of exon 14, leading to multiple aberrant transcripts [Bitoun et al., 2008; Fabrizi et al., 2007; Zuchner et al., 2005]. All hitherto identified CMT mutations are unique.

Histology

Histological analyses of muscle biopsies from CNM patients typically reveal myofiber atrophy, centralized nuclei, and type I fiber predominance. Discriminatory features for ADCNM versus other CNM forms include prominent radial distribution of sarcoplasmic strands (sometimes referred to as "spokes of a wheel") and fiber size heterogeneity, displaying hypotrophic as well as hypertrophic muscle fibers. As a typical example, Figure 3 shows histopathological findings in deltoid muscle biopsies from patients belonging to the AHE2 family with five affected members harboring the common *DNM2* p.Arg369Trp mutation in the MID. Centrally located nuclei were found in most type I and some type II fibers, and were often surrounded by dense oxidative staining, suggestive of mitochondrial clustering. Periodic acid–Schiff staining reflects glycogen accumulation. All samples exhibited moderate fiber type I predominance and hypotrophy, as well as radial sarcoplasmic strands. Endomysial fibrosis and fatty infiltrations were also noted.

Clinical, Diagnostic, and Biological Significance

Phenotype Associated with Mutations in the MID

The p.Arg465Trp mutation is the most common *DNM2* mutation in ADCNM, and has been found in 27 families of different ethnic origin (Caucasian, Brazilian, Cuban, and Native American) presenting with marked interfamilial and intrafamilial variability

Table 1. *DNM2* Mutations in Centronuclear Myopathy and CMT Peripheral Neuropathy

Centronuclear myopathy							
Exon	Nucleotide change[a]	Predicted alteration	CpG	Protein domain	Number of families (total 100)	Age of onset	First description
8	c.1102G>C	p.Glu368Gln	Yes	MID	2	≤20	[Echaniz-Laguna et al., 2007]
8	c.1102G>A	p.Glu368Lys	Yes	MID	19	Neonatal to childhood	[Bitoun et al., 2005]
8	c.1106G>A	p.Arg369Gln	Yes	MID	4	Childhood to adulthood	[Bitoun et al., 2005]
8	c.1105C>T	p.Arg369Trp	Yes	MID	10	Childhood to adulthood	[Bitoun et al., 2005]
11	c.1393C>T	p.Arg465Trp	Yes	MID	27	Mainly childhood	[Bitoun et al., 2005]
14	c.1564C>T	p.Arg522Cys	Yes	PH	1	Adulthood	This study
14	c.1565G>A	p.Arg522His	Yes	PH	12	Mainly adulthood	[Susman et al., 2010]
14	c.1567A>G	p.Arg523Gly	No	PH	1		This study
15	c.1678G>A	p.Glu560Lys	No	PH	2	Childhood	[Bitoun et al., 2009b]
16	c.1853C>A	p.Ala618Asp	No	PH	1	Neonatal	[Melberg et al., 2010]
16	c.1852G>A	p.Ala618Thr	Yes	PH	3	Neonatal	[Bitoun et al., 2007]
16	c.1856C>T	p.Ser619Leu	Yes	PH	11	Neonatal	[Bitoun et al., 2007]
16	c.1856C>G	p.Ser619Trp	Yes	PH	1	Neonatal	[Bitoun et al., 2007]
16	c.1862T>C	p.Leu621Pro	No	PH	1	Neonatal	[Jungbluth et al., 2010]
16	c.1873_1875delGTC	p.Val625del	–	PH–GED	1	Neonatal	[Bitoun et al., 2007]
16	c.1880C>A	p.Pro627His	No	PH–GED	1	Neonatal	[Susman et al., 2010]
16	c.1880C>G	p.Pro627Arg	Yes	PH–GED	1	Childhood	This study
16	c.1885_1893+8del17 (AAGGACCAGgtgaggag)	Donor splice deletion	–	PH–GED	1	Childhood to adulthood	This study
17	c.1948G>A	p.Glu650Lys	No	GED	1	Childhood	[Bitoun et al., 2009a]

CMT neuropathy								
Family	Exon	Nucleotide change	Predicted alteration	CpG	Protein domain	Segregation	Number of patients	Reference
Only family	8	c.1072G>A	p.Gly358Arg	Yes	MID	Dominant	3	[Gallardo et al., 2008]
family A	14	c.1609G>T	p.Gly537Cys	Yes	PH	Dominant	4	[Fabrizi et al., 2007]
DUK1118	14	c.1664_1671+1delATGAGGAGg	p.Asp555_Glu557del;Lys554fs	–	PH	Dominant	21	[Zuchner et al., 2005]
Only patient	15	c.1675_1677delAAA	p.Lys559del	–	PH	Sporadic (de novo)	1	[Bitoun et al., 2008]
CMT310	15	c.1684A>G	p.Lys562Glu	No	PH	Dominant	22	[Zuchner et al., 2005]
CMT48	15	c.1684_1686delAAG	p.Lys562del	–	PH	Dominant	8	[Zuchner et al., 2005]
CMT-72	19	c.2576_2581delCCATTA	p.Thr859_Ile860del	–	PRD	Dominant	3	[Claeys et al., 2009]
Family B	15	c.1709T>A	p.Leu570His	No	PH	Dominant	3	[Fabrizi et al., 2007]

All patients are heterozygous for the nucleotide change.
[a]Mutations were numbered according to GenBank NM_001005360.2 and P50570.2. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon.
PH, pleckstrin homology; GED, GTPase effector domain; MID, middle domain.

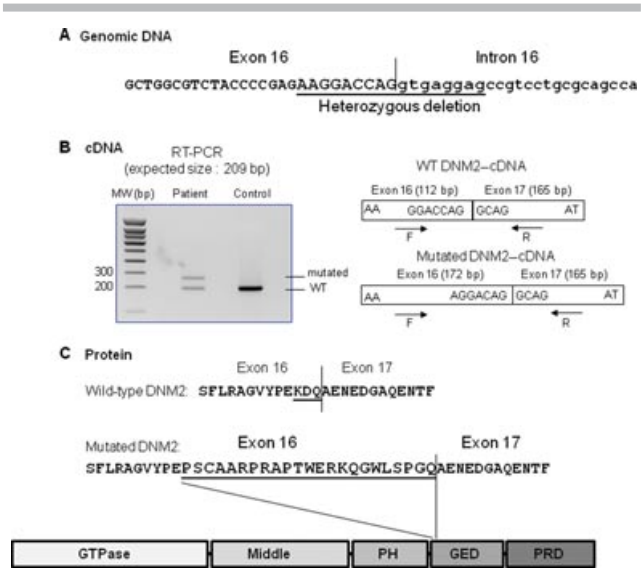

Figure 2. Impact of the novel exon 16 splice-site mutation. A: Genomic *DNM2* sequence at the exon–intron junction of exon 16. The heterozygous deletion of 17 nucleotides spanning the junction is underlined. B: Agarose gel electrophoresis of the patient and the control (left), and schematic representation of the strategy to verify abnormal splicing using exonic and intronic primers (right). A downstream in-frame cryptic splice site is used and induces an extended exon 16. C: Protein sequence of wildtype and mutated dynamin 2. The mutation induces the loss of three amino acids and the inclusion 23 supernumerary amino acids in the PH–GED linker domain.

(Supp. Table S2). This mutation was initially reported to be associated with adult onset [Bitoun et al., 2005]. In our cohort, we identified three cases with neonatal or early childhood onset (patients ALY5, BOS835-1, and BOS1059-1), while one patient developed first clinical CNM signs at the age of 56 years (patient AFW12). As an example of intrafamilial variability, in family E/393, the disease started in mid to late 40s for the father, and in early childhood for the affected daughter [Bitoun et al., 2005]. Likewise, ptosis, ophthalmoplegia/paresis, facial weakness, and motor development were variable within and across families. For some patients, hyperlordosis and ocular involvement were diagnosed. However, all patients had reduced or absent tendon reflexes, and walking difficulties were frequently noted. Furthermore, and contrasting with the variability described above, the four affected members of family ADT93 had similar age of onset and course of disease.

p.Glu368Lys is the second most frequent mutation among the ADCNM families. It was found in 19 unrelated families with a less variable and more severe phenotype compared with the p.Arg465Trp mutation. All patients with available detailed clinical description were reported with neonatal or infantile onset of muscle weakness, facial weakness, ptosis, reduced or absent tendon reflexes, and walking difficulties (Supp. Table S2), and muscle weakness was mostly diffuse. Hyperlordosis and dysarthria were seen in some, but not all patients. Myotonic discharges were noted for two patients. Patient DNM118 had an antenatal disease onset and presented with severe general muscle weakness, multiple skeletal deformities, cryptorchidism, inguinal hernia, fecal incontinence, dysphagia, and dysphonia. In two families, glutamic acid at position 368 was changed into glutamine: AIP45 (Supp. Table S2) and ABJ51 [Echaniz-Laguna et al., 2007]. In the first family, the only patient displayed neonatal disease onset, delayed speech and motor development, proximal and distal muscle involvement, ptosis and ophthalmoplegia/paresis, absent deep tendon reflexes, contractures, and cardiac involvement (ventricular septal defect). In the second family, all five affected members had distal muscle weakness, areflexia, and variable age of onset. Walking difficulties were not noted, and two displayed mild mental retardation and electromyography (EMG) findings consistent with a polyneuropathy. One of the five patients had additional ophthalmoplegia/paresis.

The p.Arg369Trp mutation was found in 10 families, and is associated with strong intrafamilial and interfamilial variability. Age of onset ranges from early childhood (AMR39) to the late 40s (ABZ97); muscle weakness can be proximal, distal, or diffuse, while facial weakness, ophthalmologic involvement, hyperlordosis, walking difficulties, and tendon reflexes are nonuniform. For patient ABZ97, the disease started at the age of 48 years, and he presented with diffuse muscle weakness together with facial weakness and ptosis. Disease onset for his son (AGJ49) was at the age of 15 years, and he had predominantly distal muscle weakness and no facial and ophthalmologic involvement. In four families, p.Arg369 was changed into Glutamine. In the patients described in this study (EH62 and EH64), the CNM phenotype was rather moderate with variable age of onset (5 of 24 years in our patients; adolescence/adulthood for the published cases); no eye movement defects were noted, and facial weakness was seen only for patient EH62.

Phenotype Associated with Mutations in the PH Domain and GED

The 13 patients (10 families) from our cohort with the exon 14 p.Arg522His mutation presented with a less severe phenotype compared with formerly published cases [Susman et al., 2010] with variable age of onset (infancy to the 50s) and walking abilities. Ptosis has been diagnosed in four patients (AFT16, ALY14, ALP71, and BOS1145-1), consistent with the previous results where eye movement defects were seen in one published family and not in the other. Family DNM62/63 shows intrafamilial variability with earlier disease onset (7 years) and no facial weakness for the father compared with his son (onset, 19 years; mild facial weakness). In addition, the now 55-year-old father (DNM62) has contractures, Achilles tendon retractions, scoliosis, dysphagia, and dysphonia. However, some of these clinical features might be age associated and not a feature of intrafamilial variability, as the son was 26 years old at the last medical exam. The only patient with the novel p.Arg522Cys mutation (AML18) presented with a similarly mild phenotype. Age of onset was between 40 and 50 years, and the patient had predominantly distal muscle weakness, ptosis, ophthalmoplegia/paresis, and facial weakness. He has a stepping gait and no cardiac or respiratory involvement. Likewise, the novel and unique p.Arg523Gly mutation is associated with a mild phenotype; the patient CHI4 presented with normal motor development, facial weakness, and weak tendon reflexes. Eye movement defects, and cardiac, respiratory, or mental impairments were not noted.

The p.Glu560Lys mutation was found once (EH58) in our cohort. Age of onset was 2 years, and at the age of 26 years, the patient had classical ADCNM symptoms such as diffuse muscle weakness, ptosis, ophthalmoplegia/paresis, and walking difficulties, with scoliosis and severe respiratory deficiency (vital capacity of 22%). The p.Glu560Lys mutation has been previously reported in an isolated case [Bitoun et al., 2009a] with disease onset at the age of 7 years, difficulty in walking, facial and muscle weakness, and ptosis at the age of 10 years.

Patients with p.Ser619Leu mutation with available clinical description were of different ethnic origin (Caucasian and African) and showed persistently severe CNM symptoms with neonatal

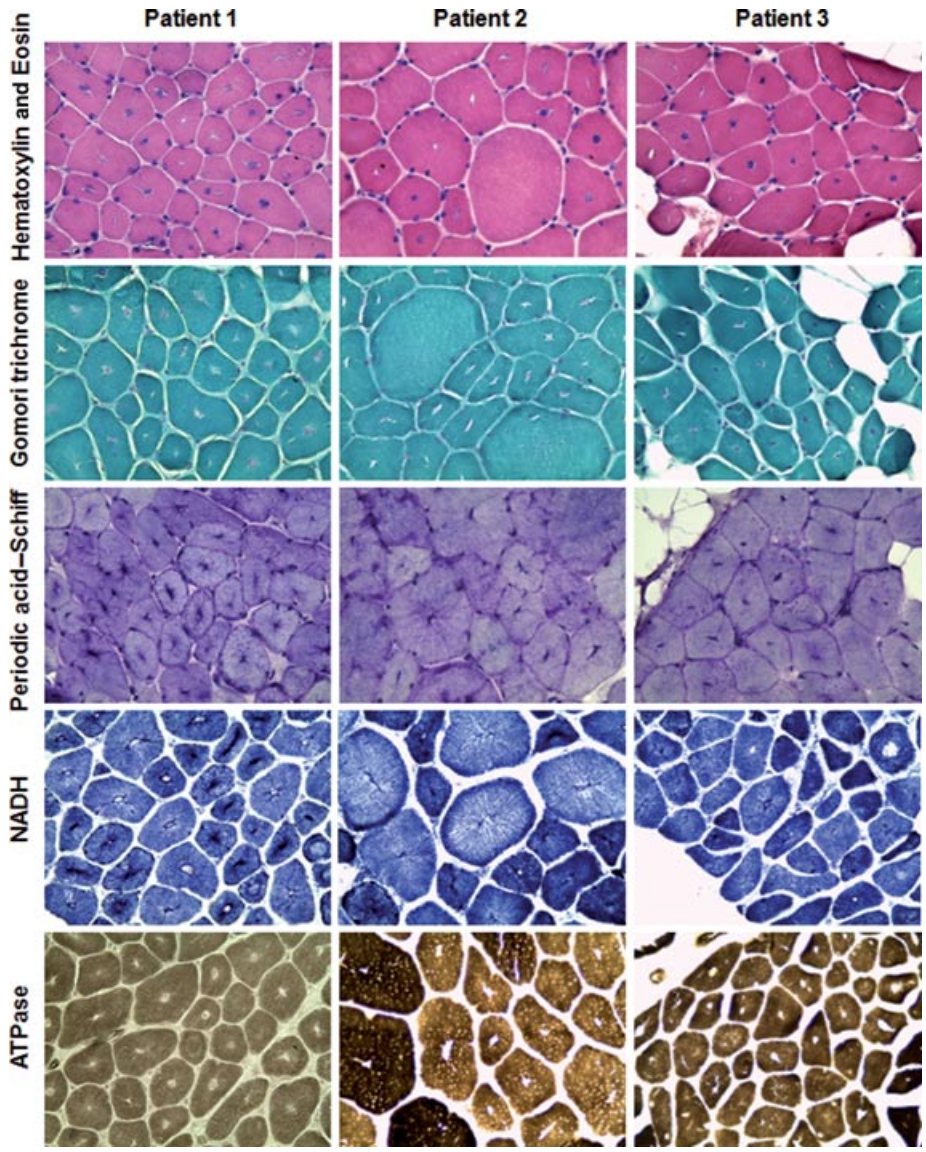

Figure 3. Histopathological and histochemical findings in deltoid muscle biopsies from three closely related patients with the *DNM2* p.Arg369Trp mutation (AHE2, AHE4, and AHE5). A: Hematoxylin and eosin staining demonstrated numerous centralized nuclei and fiber size heterogeneity. B: Gomori trichome staining revealed normal connective tissue and collagen. C: On periodic acid–Schiff and D: oxidative staining, a high number of fibers showed radial arrangements of sarcoplasmic strands. E: Fiber type I predominance was evident on ATPase staining (pH 9.4). All pictures have been made with a magnification of 40×.

disease onset. All presented with severe generalized hypotonia with low Apgar scores and ptosis. Most required ventilation; one had contractures, one cryptorchidism, and another required gastrostomy. All had delayed motor milestones, and only two were able to walk (although difficultly). Mental development was normal. Of note, the oldest p.Ser619Leu patient was 12 years old at the last medical examination; long-term clinical evaluation data are therefore not available. Our findings go along with previously published cases [Bitoun et al., 2007], indicating that neonatal ADCNM with severe hypotonia is most likely associated with the p.Ser619Leu mutation. In an isolated case, p.Ser619 was found to be changed to tryptophan, and the patient presented with similar clinical features at birth as the p.Ser619Leu patients, but reached independent ambulation at normal age [Bitoun et al., 2007]. Similarly, the three patients described with the p.Ala618Thr mutation [Bitoun et al., 2007; Susman et al., 2010; this study] had severe neonatal disease forms.

The only family with the novel p.Pro627Arg mutation (BOS1021-1) had childhood onset (3 and 7 years, respectively), but normal motor development. At the last medical examination, both patients presented with walking difficulties, reduced vital capacity, diffuse muscle weakness, and facial weakness. Eye movement defects were not noted. This is contrasting the only described patient with the p.Pro627His mutation [Susman et al., 2010]. The affected boy had ptosis from birth, required ventilation due to marked generalized muscle weakness, had delayed motor milestones, and lost ambulation during childhood.

DNM2 Splice-Site Mutation in CNM

An exon 16 donor splice mutation was found in seven affected members over two generations in a family from Sweden. A clinical description was available for four family members, and variable ages

of onset have been noted at 10, 15, 20, and 42 years, respectively. Muscle weakness was diffuse (two patients) or predominantly distal (two patients). Ptosis was seen in two patients and ophthalmoplegia/paresis in one; respiration was generally normal or slightly decreased (vital capacity 75–85%), and no cardiac involvement was noted. Histology showed classical CNM findings in all patients: prominent centralization of nuclei, predominance of type I fibers, hypotrophy, and radial sarcoplasmic strands.

Genotype–Phenotype Correlations

When the first reports implicating *DNM2* mutations in CNM or CMT neuropathy were published, it was proposed that mutations in the MID are associated with mild adult-onset CNM, while mutations in the PH domain are linked to either CMT or severe neonatal-onset CNM [Bitoun et al., 2007; Bitoun et al., 2005; Zuchner et al., 2005]. Together with recent reports and our present data on a total of 100 families with CNM-related *DNM2* mutations and clinical and histopathological descriptions, distinct mutation hotspots in exons 8, 11, 14, and 16 are apparent and allow the study of potential genotype–phenotype correlations, which might guide genetic counseling and molecular diagnostics (Supp. Table S2).

A "minimal" phenotype found in all patients with *DNM2* mutations is difficult to highlight, as phenotypic variability appears wider than previously reported, especially concerning the involved muscles, the severity of muscle weakness, and the age of onset. However, areflexia/hyporeflexia was seen in nearly all patients. The most consistent characteristics are the morphological hallmarks seen on biopsies including hypotrophic fibers with centralized nuclei and radial oxidative staining (Fig. 3). A vast majority of our patients did not have demonstrable cardiac involvement, respiratory insufficiency, or mental retardation. However, a limited intelligence quotient was noted in several cases; ECG revealed valvular irregularities and cardiac arrhythmia in four patients harboring the p.Glu368Lys mutation (patients CHI1, BOS183-1, BOS839-1, and BOS1132-1), one patient with the p.Arg465Trp mutation (BOS1061-1), one patient with the p.Arg522His mutation (ALP71), and one patient with the p.Ser619Leu mutation (ADT88). Furthermore, one patient with p.Glu368Gln mutation had a cardiac ventricular septal defect (AIP45), and one patient with p.Arg465Trp mutation had a bicuspid aortic valve (BOS601-1). As these are not uncommon findings in any population, the relationship of these abnormalities to the patients' primary skeletal myopathies must be considered uncertain. All patients with the p.Ser619Trp/Leu mutation, except two, had abnormal ventilation, and two patients had a vital lung capacity of ≤30% (patient DNM118 with the p.Glu368Lys mutation, and patient EH58 with the p.Glu560Lys mutation). As with other CNM forms, serum creatine kinase levels were normal or only slightly elevated in our cohort.

Taken together, CNM-related *DNM2* mutations can be grouped into three classes, from the most severe to the least severe phenotypes: p.Ala618Thr and p.Ser619Trp/Leu in the first group with severe clinical manifestations and early onset; p.Glu368Lys with intermediate severity in a second group; and p.Arg369Trp, p.Arg465Trp, p.Arg522His/Cys, and p.Arg523Gly mutations in the third group with generally milder phenotypes.

p.Ser619Leu is the most common mutation in the PH domain, and is invariably associated with a severe neonatal phenotype with general hypotonia and low Apgar score. Currently, none of the affected children were older than 12 years at the time of the last medical exam, precluding a long-term follow-up and clinical description of the phenotype. Nevertheless, all patients presented with delayed motor milestones, and only two were ambulant. Similar clinical findings were reported for the unique p.Ala618Thr and p.Ser619Trp mutations (found three times) [Bitoun et al., 2007; Susman et al., 2010; this study]. Importantly, and in contrast to the X-linked form with neonatal onset, where the muscle weakness remains severe, patients with the *DNM2* p.Ala618Thr or p.Ser619Trp/Leu mutation are strongly affected at birth, but improve by degrees over time before their overall health progressively deteriorates.

Another common mutation with largely neonatal onset is p.Glu368Lys. Patients present with severe muscle weakness at birth and other symptoms improve partially over the first years. Facial weakness and marked diffuse muscle weakness are, however, constant. In the medium term, this mutation is associated with a milder phenotype as compared with p.Ser619Trp and p.Ala618Thr.

The recurrent mutations p.Arg369Trp/Gln, p.Arg465Trp, and p.Arg522His are not associated with regular clinical features, and result in marked interfamilial and intrafamilial variability with both childhood, as well as adult onset cases. However, p.Arg522His seems to result in a particularly mild phenotype, as eye movement defects are absent for the majority and walking difficulties are generally less pronounced. Different PH domain mutations can, therefore, account for either severe or moderate phenotypes.

Diagnostic Relevance and Strategies

In contrast to X-linked CNM, where almost 450 *MTM1* mutations are evenly distributed over the coding and splice-relevant regions, the majority of *DNM2* mutations leading to ADCNM are clustered in distinct hotspot regions. Depending on the patient's phenotype and the age of onset, single *DNM2* exons should primarily be sequenced, allowing fast diagnosis, improved genetic counseling, and lower costs. As a general rule, predominant distal muscle involvement noted by MRI, or a large number of radial sarcoplasmic strands on oxidative staining should shift the focus to *DNM2*. However, for familial cases where the inheritance pattern can be clearly assessed, the three known genes (*MTM1* for X-linked CNM, *BIN1* for ARCNM, and *DNM2* for ADCNM) have to be considered with priority. Nevertheless, consanguinity does not exclude *DNM2* de novo mutations, as we have shown for two cases in this study. For sporadic male patients with neonatal onset and severe muscle weakness together with respiratory insufficiency, the first gene to be analyzed is *MTM1*. In patients with neonatal onset without respiratory distress, *DNM2* exons 8 (p.Glu368) and 16 (p.Ser619) should be sequenced. Exons 11, 14, 15, and 17 should be sequenced next, as these exons carry known mutations with variable age of onset and disease severity. For sporadic cases with childhood/adult onset, *DNM2* exons 11 (p.Arg465) and 14 (p.Arg522) have to be sequenced first. If no mutation is detected, all other coding exons should be analyzed. As several cases with myopathy associated with centralized nuclei have been recently linked to recessive *RYR1* mutations, this gene could be screened after the main CNM genes listed above, or with priority if cores were noted on biopsy.

Overlap with *DNM2*-related CMT Neuropathies

DNM2 mutations in the MID (one missense), the PH domain (three deletions, three missense), and the PRD (one in-frame deletion) have been associated with peripheral CMT neuropathy (Table 1) [Bitoun et al., 2008; Claeys et al., 2009; Fabrizi et al., 2007; Gallardo et al., 2008; Zuchner et al., 2005]. There is no *DNM2* mutation causing both CNM and CMT, although the same domains and even two adjacent amino acids are implicated in either disorder:

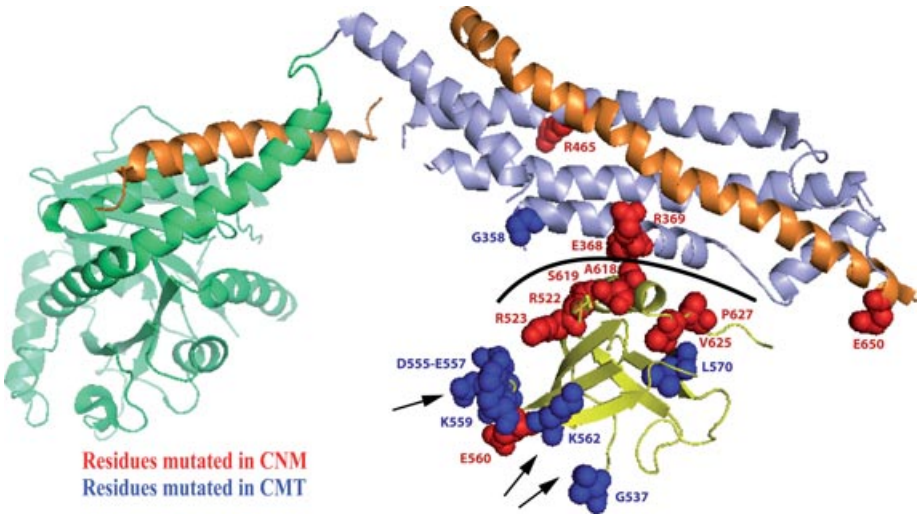

Figure 4. The coding *DNM2* mutations found in CNM and CMT patients are displayed on the crystal structure model of nucleotide-free human dynamin 1 [Faelber et al., 2011] using the PyMOL program. The GTPase domain (green), MID (light grey), PH domain (yellow), and GED (orange) domains are highlighted. The mutated amino acids are depicted in red (CNM) and blue (CMT). The binding interface between the PH domain and the MID is indicated by a black curved line, and the arrows show the main phosphoinositide binding sites.

pLys559del leads to CMT and p.Glu560Lys leads to CNM (Fig. 1) [Bitoun et al., 2009b; Bitoun et al., 2008]. There was no evidence for a skeletal myopathy on EMG or muscle biopsy for the p.Lys559del mutation, while both patients with the p.Glu560Lys mutation displayed typical CNM biopsies without neuropathic EMG. However, clinical overlap with CMT has been reported for some CNM patients presenting with a mild peripheral nerve involvement [Fischer et al., 2006], and EMG results for patients ABJ51, ABJ53 [Echaniz-Laguna et al., 2007], BOS183-1, BOS928-1, ABZ97, ACA46, ABZ99, and AFT 16 (Supp. Table S2) were suggestive of neuropathy. The patients with neuropathic signs were found to carry different *DNM2* mutations (p.Glu368Gln, p.Glu368Lys, p.Arg369Trp, p.Arg465Trp, pSer619Leu, and twice p.Arg522His, respectively). In contrast, primary myopathic features in CMT patients with *DNM2* mutations have never been reported, although there is a massive fatty atrophy of foot musculature seen by MRI in a single patient [Gallardo et al., 2008].

Biological Significance

It remains to be determined why specific *DNM2* mutations involve either skeletal muscle or peripheral nerve phenotypes. Recruitment of dynamin 2 to the plasma membrane leads to helical oligomerization around the vesicle neck, and endocytosis (fission of the vesicle) is mediated by a conformational change following guanosine triphosphate hydrolysis. Figure 4 illustrates the mutated residues on the three-dimensional structure of the crystallized human nucleotide-free dynamin [Faelber et al., 2011]. Most CNM mutations cluster in the interface between the MID and PH domain; the mutated residues 368 and 369 in the MID are in close proximity to the mutated residues 618, 619, 522, and 523 on the alpha helix of the PH domain. The impact of the MID mutations was assessed biochemically and turned out to enhance self-assembly, resulting in more resistant oligomer structures and increased GTPase activity compared with wild-type dynamin 2 [Wang et al., 2010]. Accordingly, the CNM mutations did not impair clathrin-mediated endocytosis in patient cells or after complementation in cells depleted of endogenous dynamin [Koutsopoulos et al., 2011; Liu et al., 2011b].

The CMT mutations are mainly located on the phosphoinositide-binding PH domain loops. The PH domain is essential for targeting dynamin 2 to the plasma membrane [Dong et al., 2000], and may also be critical for membrane fission. Functional investigations demonstrated that the CNM mutations in the C-terminal α-helix of the PH domain (p.Ala618Thr and p.Ser619Leu) do not affect phosphoinositide binding, but increase the basal GTPase activity of dynamin 2 [Kenniston and Lemmon, 2010], similar to the MID mutations. Conversely, the CMT mutation p.Lys562Glu abolishes phosphoinositide binding, but does not impact on the GTPase activity. Taken together, these observations suggest that most CNM mutations alter the autoinhibitory interface between the MID and the PH domain, supporting a gain-of-function mechanism, while most CMT mutations decrease lipid binding, suggesting a loss of function.

The *DNM2* knockout mouse is embryonic lethal [Ferguson et al., 2009], suggesting that the mutations found in humans do not completely abolish the dynamin 2 protein function, but rather involve subtle functional changes leading to progressive pathologies. The murine heterozygous knock-in model of the p.Arg465Trp mutation displayed muscle atrophy and structural disorganization [Durieux et al., 2010]. Centralized nuclei were rarely seen, contrasting histological findings upon adeno-associated virus (AAV)-mediated exogenous expression of either wild-type or p.Arg465Trp DNM2 constructs [Cowling et al., 2011], or transgenic overexpression of wild-type DNM2 [Liu et al., 2011a]. These mice develop key histological features of CNM, which clearly points to a dominant-negative effect and excludes, at least for the most common CNM-related *DNM2* mutation, haploinsufficiency as the underlying pathomechanism of ADCNM. As AAV p.Arg465Trp DNM2 was injected into adult wild-type murine tibialis interior [Cowling et al., 2011], and as transgene expression was driven by a muscle-specific promoter [Liu et al., 2011a], a primary involvement of nerves in CNM pathology is unlikely.

Future Prospects

The link between dynamin 2 and other proteins mutated in either CNM or CMT might be inferred from molecular studies. Myotubularin and amphiphysin 2, mutated in other CNM forms, are both implicated in membrane remodeling and trafficking [Nicot and Laporte, 2008]. Several proteins implicated in CMT neuropathies also regulate membrane trafficking or phosphoinositide metabolism (RAB7, FIG4, and myotubularin-related proteins 2 and 13). Defects in membrane trafficking may thus account for the common pathological mechanism connecting these neuromuscular diseases.

The origin of the tissue specific impact of different *DNM2* mutations is not understood. Future work will explore dynamin 2 tissue-specific isoforms and posttranslational modification, and search for potential tissue-specific interactors.

The identification of additional patients with *DNM2* mutations, and the screening of this gene for other diseases may clarify its physiological importance for human health. In addition, animal models targeting other CNM and CMT mutations are needed to decipher the pathological mechanisms and will be an asset to test rescue approaches.

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