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# Two novel mutations in dynamin-2 cause axonal Charcot–Marie–Tooth disease

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**ABSTRACT Background:** Recently, mutations affecting different domains of dynamin-2 (*DNM2*) were associated alternatively with autosomal dominant centronuclear myopathy or dominant intermediate (demyelinating and axonal) Charcot–Marie–Tooth disease (CMT) type B. **Objective:** To assess the etiologic role of *DNM2* in CMT. **Methods:** We performed a mutational screening of *DNM2* exons 13 through 16 encoding the pleckstrin homology domain in a large series of CMT patients with a broad range of nerve conduction velocities and without mutations in more common genes. **Results:** We identified two novel *DNM2* mutations that cosegregated with purely axonal CMT in two pedigrees without clinical evidence of primary myopathy. **Conclusion:** Patients with axonal Charcot–Marie–Tooth disease type 2 neuropathy without mutations in more common genes should undergo investigation for *DNM2* pleckstrin homology. **NEUROLOGY 2007;69:291–295**

Autosomal dominant Charcot–Marie–Tooth disease (CMT) has numerous molecular actors. Four genes are associated with demyelinating CMT type 1 (CMT1), and at least seven genes are associated with axonal CMT type 2 (CMT2).

The rarer dominant intermediate CMT (DI-CMT) is also heterogeneous.<sup>1</sup> DI-CMT grouped those kindreds that did not fit in with CMT1 or CMT2 because motor nerve conduction velocities (MNCVs) in the median nerve in multiple affected members broadly spanned the CMT1–CMT2 watershed value of 38 m/s.<sup>2</sup> Accordingly, nerve biopsies were consistent with a mixed demyelinating and axonal neuropathy.<sup>2,3</sup> DI-CMTA was mapped to chromosome 10q24.1–q25.1,<sup>4</sup> DI-CMTB was associated with the dynamin-2 gene (*DNM2*) in three singleton kindreds,<sup>5–8</sup> and DI-CMTC was associated with the tyrosyl-transfer RNA synthetase gene (*YARS*) in two kindreds and a sporadic patient.<sup>9</sup> The roles of *DNM2* and *YARS* need to be confirmed.

*DNM2* is a ubiquitously expressed protein associated with microtubules and implicated in endocytosis and cell motility. *DNM2* has a tripartite guanosine triphosphatase (GTPase) domain, a middle region, a pleckstrin homology (PH) domain that mediates membrane association, a GTPase effector domain, and a proline-rich domain.<sup>8</sup> Whereas mutations in the PH domain cosegregated with DI-CMT1B,<sup>8</sup> mutations in the middle domain caused autosomal dominant centronuclear myopathy (CNM),<sup>10</sup> sometimes associated with electrophysiologic signs of axonal neuropathy.<sup>11</sup>

By testing a large series of unrelated CMT patients with a broad range of MNCVs, we identified two novel *DNM2* PH mutations associated with axonal CMT.

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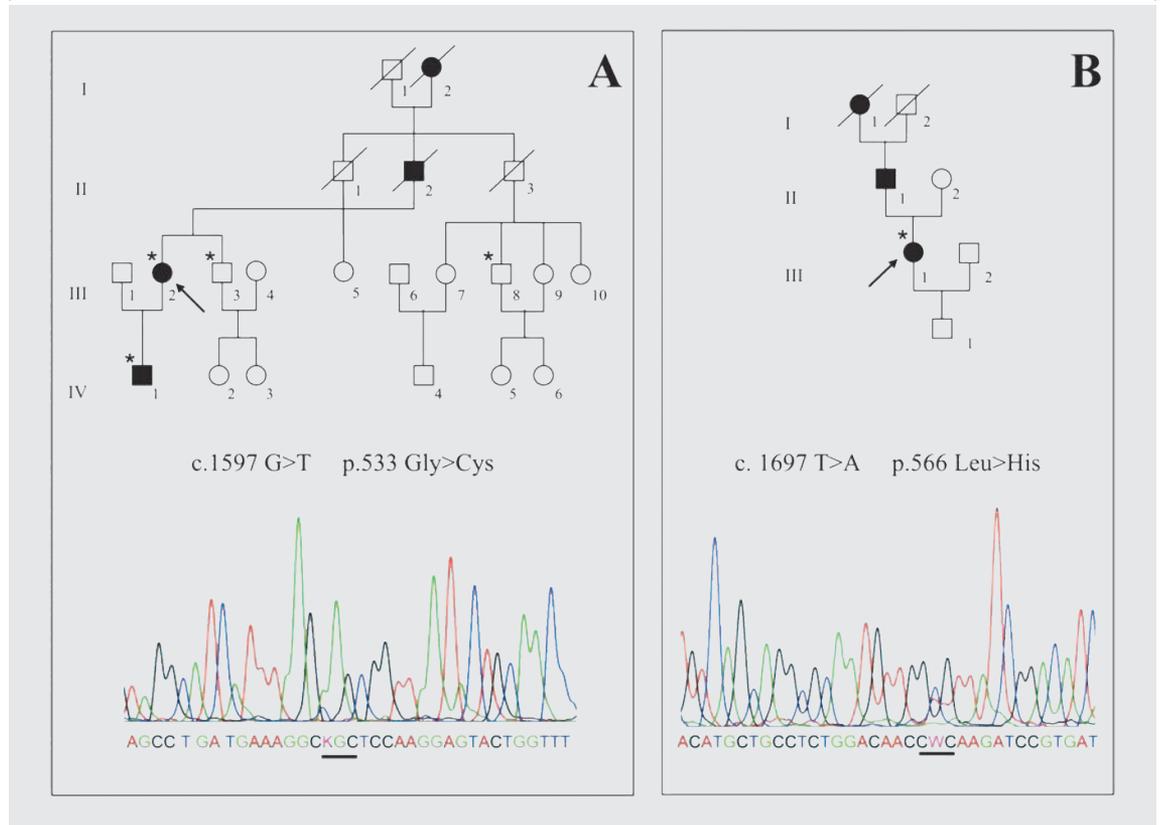
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(A) Pedigree A (top) harboring the Gly533Cys mutation (bottom). (B) Pedigree B (top) harboring the Leu566His mutation (bottom). Pedigree symbols: square = male; circle = female; solid black symbol = affected individual; arrow = proband; asterisk = individual analyzed molecularly, clinically, and electrophysiologically. In the automated nucleotide sequences, the mutated codons are underlined. K = G to T transversion; W = T to A transversion.

**Figure 1** Pedigrees and corresponding *DNM2* mutations



**METHODS Patients.** *DNM2* was analyzed in 210 unrelated CMT patients without mutations in the more common genes. The series included 133 males and 77 females; 53 patients had dominant inheritance, whereas 157 patients had an unclear or negative family history. Median MNCV was >38 m/s in 143 patients and <38 m/s in 67.

Nerve conduction studies and needle EMG were performed according to standard methods. Archive sural nerve biopsies, processed for histologic, ultrastructural, and teased-fiber analysis,<sup>12</sup> were available for the 32-year-old proband of Pedigree A and the 40-year-old proband of Pedigree B. MRI of thigh and calf muscles from patients in Pedigree A was performed on the transversal plane by a 1.5-T Siemens magnet scanner with spin echo T1-weighted and short TI inversion recovery T2-weighted sequences.

**Mutational analysis.** Blood genomic DNA was extracted from informed and consenting patients and controls. Mutations in the genes coding peripheral myelin protein 22 (*PMP22*), myelin protein zero (*MPZ/P0*), lipopolysaccharide-induced tumor necrosis factor alpha (*LITAF*), early growth response 2 (*EGR2*), mitofusin 2 (*MFN2*), neurofilament light chain (*NEFL*), and connexin 32 (*Cx32*; also known as gap-junction protein beta 1 [*GJB1*]), were excluded as described.<sup>12</sup> The PH-coding exons 13 through 16 and related exon-intron boundaries were analyzed by denaturing high-performance liquid chromatography (DHPLC) using a WAVE System 1500 (Transgenomic) (table E-1 on the *Neurology* Web site at www.neurology.org). PCR fragments containing DHPLC alterations were analyzed by a CEQ 8800 capillary sequencer using the GenomeLab Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter). Nucleotide changes were numbered start-

ing from the A of the ATG initiation codon of the GeneBank complementary DNA clone NM\_004945.

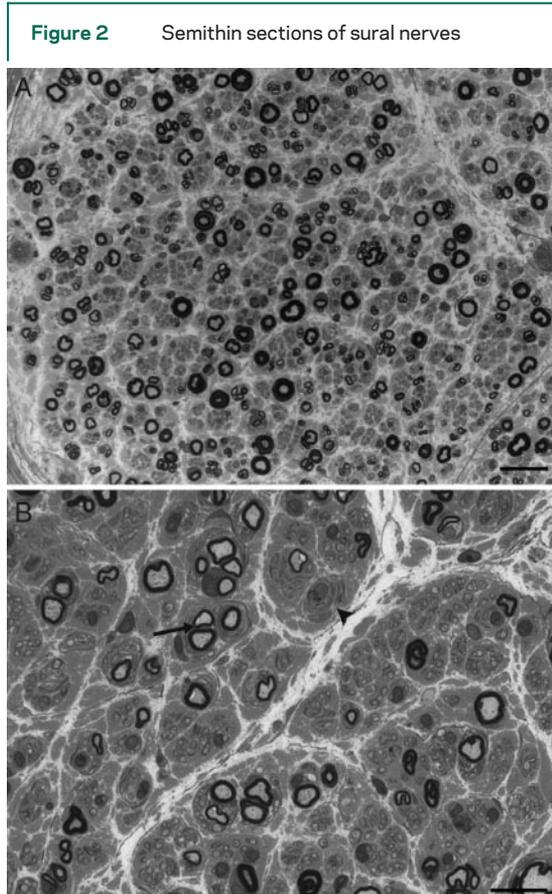
Cosegregation of the identified mutations with CMT was investigated by mutational analysis of available affected and unaffected pedigree members and of 100 healthy, white, unrelated individuals.

**RESULTS *DNM2* mutations.** A novel c.1597 G>T transversion leading to a glycine-to-cysteine mutation at codon 533 (Gly533Cys) cosegregated with CMT in Pedigree A. A novel c.1697 T>A transversion leading to a leucine-to-histidine mutation at codon 566 (Leu566His) was present in the proband of Pedigree B (figure 1).

Both mutations introduced nonconservative changes: glycine is nonpolar neutral, whereas cysteine is polar; leucine is nonpolar, whereas histidine is charged and polar. Furthermore, both mutations affected residues highly conserved in various ortholog and paralog proteins (figure E-1). Finally, they were absent in 100 control individuals.

**Clinical findings. Pedigree A.** Proband III-2 is a 50-year-old woman evaluated at age 32 years because of painful paresthesias and exercise-related leg weakness manifested during pregnancy. In childhood, she had recurrent ankle sprains. Examination disclosed bilateral pes cavus; mildly ataxic gait, dif-

(A) Pedigree A's proband (bar = 30  $\mu\text{m}$ ). (B) Pedigree B's proband (bar = 15  $\mu\text{m}$ ). Lower magnification discloses numerous regenerating clusters. Higher magnification shows a denervated onion bulb (arrowhead) and a simple onion bulb encircling a regenerating cluster (arrow).



difficult on heels; weakness of foot dorsiflexion (Medical Research Council [MRC] scale of strength = 3) and plantar flexion (MRC = 4); mild wasting of intrinsic hand muscles without significant weakness; stocking reduction of vibration sense; and absent ankle jerks. Neurologic examinations at ages 42 and 50 years disclosed no significant evolution and preservation of the upper limbs. Blood tests, including creatine kinase (CK) levels and neutrophil count, were normal. The 19-year-old son of the proband, IV-1, had recurrent ankle sprains in childhood and since adolescence has experienced painful

paresthesias exacerbated by prolonged tractions and exercise-related leg weakness. Examination disclosed bilateral pes cavus with clawed toes, retraction of Achilles tendons, and wasting of peroneal muscles prevalent on the right side; stepping gait with sensory ataxia; weakness of toes, foot dorsiflexion (MRC = 2, right; 3, left), and plantar flexion (MRC = 4); stocking reduction of vibration sense; absent ankle jerks and reduced knee jerks; and preservation of intrinsic hand muscles. Blood CK values and neutrophil count were normal.

**Pedigree B.** The 45-year-old female proband had bilateral plantar paresthesias; since childhood, she had been wearing arch supports. At age 40 years, examination disclosed bilateral pes cavus with clawed toes and retraction of the left Achilles tendon; gait impossible on heels and difficult on toes; prominent wasting of the left calf muscles, involving both the anterior and posterior compartments; weakness of toes and foot dorsiflexion (MRC = 3, left; 4, right) and of left plantar flexion (MRC = 4); mild wasting of the intrinsic hand muscles; and stocking decrease in vibration sense bilaterally. Tendon reflexes were lost at the ankles and brisk at the knees, but preserved in the upper limbs. Blood CK values and neutrophil count were normal.

**Neurophysiologic findings.** Nerve conduction studies are reported in the table. In the examined patients, EMG disclosed diffuse changes of denervation with reduced interference pattern on maximum voluntary contraction, increased mean amplitude of motor unit potentials and fibrillation potentials.

**Muscle MRI findings.** In the examined patients III-2 and IV-1 of Pedigree A, signal abnormalities were localized in the calf muscles and spared the thigh muscles (figure E-2).

**Nerve biopsy findings.** The two probands examined had a similar pattern of pathologic changes. Fiber

Table	Results of electroneurography																
	Median nerve				Ulnar nerve				Peroneal nerve				Tibial nerve	Median nerve		Sural nerve	
	MNCV	cMAP	DL	F-wave	MNCV	cMAP	DL	F-wave	MNCV	cMAP	DL	F-wave	F-wave	SNCV	SNAP	SNCV	SNAP
<b>Normal</b>	50-60	>6	<3.5-4	<29	52-62	>10	<2.6-6.2	<29	44-52	>6	<4.5-5.5	<55	<52	>55	>8	44-50	>8
<b>Pedigree 1</b>																	
III-2/F/32 y	57.4	18	3.7	27.6	47.4	18	4	29	30	1	6.5	62	70	56	38	NE	NE
III-2/F/42 y	50	11	3.9	29.7	NP	NP	NP	NP	29.5	1	8.3	NE	NP	52	29	NE	NE
IV-1/M/19 y	42.9	14.9	4.2	38	46.6	7.4	3.7	30	NE	NE	NE	NE	NE	58	22.2	NE	NE
<b>Pedigree 2</b>																	
III-1/F/40 y	56	8	3.9	26	49.1	5.5	3.3	28.2	42	3.2	4.9	NE	NE	60	26	NE	NE

For each nerve, data from the worse side are shown.

MNCV = motor nerve conduction velocity (m/s); cMAP = compound motor action potential (mV); DL = distal motor latency (ms); F-wave = F-wave latency (ms); SNCV = sensory nerve conduction velocity (m/s); SNAP = sensory nerve action potential ( $\mu\text{V}$ ); F = female; M = male; NE = not evoked; NP = not performed.

densities were reduced (Pedigree A: 5,149/mm<sup>2</sup>; Pedigree B: 3,703/mm<sup>2</sup>; range in four age-matched controls = 6,455 to 6,893/mm<sup>2</sup> of the fascicular area); histograms revealed a unimodal, skewed-to-the-left distribution with loss of large-diameter fibers (percentages of fibers with diameter above 6 μm = 22% in Pedigree A and 19% in Pedigree B). There were numerous clusters of regenerating fibers (figure 2A) and rare onion bulb structures either denervated or encircling fibers (figure 2B). Teased-fiber analysis disclosed absence of paranodal or segmental demyelination in both patients; 50% of fibers in Pedigree A's proband and 60% of fibers in Pedigree B's proband had homogeneously shortened internodes. Electron microscopy confirmed the histologic findings and revealed numerous collagen pockets indicating loss of small unmyelinated fibers; myelin uncompactation was detectable only in few regenerating fibers (figure E-3).

**DISCUSSION** The characterization of two novel mutations of *DNM2* corroborated the etiologic role of the gene in hereditary neuropathies and added CMT2 to the phenotypical spectrum.

The neuropathy reported here had mild-to-moderate impairment with scarce evolution in middle age, left the upper limbs relatively preserved, and could manifest an asymmetric involvement of calves affecting muscles of both anterior and posterior compartments. The syndrome diverged from the DI-CMTB described in the Australian and North American kindreds that manifested severe involvement of distal and proximal limb segments with progression to wheelchair after two to three decades.<sup>1</sup> None of our patients had the neutropenia that cosegregated with DI-CMT in the Australian and Belgian pedigrees.<sup>8</sup>

Nerve conduction changes mainly involved the lower limbs and were consistent with CMT2, indicating axonopathy. Minor demyelinating features such as slightly increased distal motor and F-wave latencies and slowed MCNV in the upper limbs were present only in Patient IV-1 in Pedigree A. In that patient, the median MNCV was nevertheless above the cutoff value of 38 m/s in the range of CMT2. Because of the small number of patients examined, we cannot exclude that the mutations reported may occur in pedigrees with intermediate nerve conduction velocity alterations. Nerve biopsies were also consistent with CMT2 disclosing an axonopathy unaccompanied by detectable demyelination on semithin and ultrathin sections or teased fibers. The histologic picture overlapped that of axonal CMT related to *P0* (CMT2I) and *MFN2* (CMT2A),<sup>13,14</sup> and diverged from that of DI-CMTB,

concisely described as resembling DI-CMTA.<sup>5</sup> DI-CMTA was characterized by the coexistence of axonal degeneration with demyelinating features such as segmental demyelination, onion bulb formations in cytoplasmic Schwann cell processes, and uncompactation of the myelin sheath.<sup>3,15</sup>

Of the 210 patients screened for the PH-related nucleotide sequences, only two index patients had *DNM2* mutations. Although no conclusions can be drawn without systematic studies of the entire gene, such a low rate suggests that *DNM2* defects are a rare cause of CMT. So far, we propose that *DNM2* should be analyzed not only in the rare pedigrees that fit the criteria for DI-CMT but also in pedigrees or patients with CMT2. CMT2 is a puzzling genetic entity: most genes were discovered but many cases remain molecularly unsolved; *DNM2* could be analyzed in those patients who have no mutations in more common genes such as *MFN2* and *NEFL*.<sup>16</sup>

In CNM, a congenital myopathy related to the middle domain of *DNM2* with onset in childhood or adolescence, approximately half of the patients had electrophysiologic signs of mild axonal peripheral nerve involvement.<sup>11</sup> We did not find the reverse condition: none of the patients had features of CNM such as weakness of the extraocular and facial muscles or involvement of proximal limb muscles, and none had myopathic changes on EMG or muscle MRI. Hence, although muscle biopsy would be necessary to provide definitive evidence, we suggest that mutations in *DNM2* PH do not cause an associated myopathy.

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