INVITED EDITORIAL
Axonal Charcot-Marie-Tooth Disease and the Neurofilament Light Gene (*NF-L*)

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Charcot-Marie-Tooth disease (CMT) is the most common inherited disorder of the human peripheral nerve, and, at a frequency of ~1/2,500, it represents one of the most common disease traits. It can be inherited as an autosomal dominant, autosomal recessive, or X-linked trait. In addition, many sporadic cases occur and have often been shown to result from new dominant mutations. The disease has generally been divided into two major types, on the basis of results of electrophysiologic studies. The type 1 form, CMT1, is accompanied by decreased motor nerve conduction velocities and primarily affects the myelin. The type 2 form, CMT2, manifests normal or slightly reduced motor nerve conduction velocities with decreased amplitudes and primarily affects the axon.

In the past several years, much has been learned about CMT1, with >12 linked loci delineated and 6 genes identified (reviewed in Lupski [1999] and Warner et al. [1999]) (table 1). These genes encode proteins with various functions that are important for myelin formation, structure, and integrity. However, from a genetic standpoint, precious little has been learned about CMT2, because, to date, no single gene has been identified, despite reports of the existence of four different CMT2-linked loci (table 2). In this issue of the *Journal,* Mersiyanova et al. (2000) provide substantive evidence that they have identified the first CMT2 gene. The gene *NF-L* encodes neurofilament light protein, which is one of three major neurofilament protein constituents. Neurofilaments are important for the structure and function of axons and may be necessary for axonal transport, regeneration, and longevity. Although Mersiyanova et al. report a single mutation in a large Russian family segregating autosomal dominant CMT2, they nevertheless provide important insights into peripheral nerve neurobiology and point to other potential CMT2 genes.

Mersiyanova et al. (2000 [in this issue]) studied a large six-generation family from Mordovia, Russia, who segregated clinical and nerve conduction velocity findings consistent with CMT2. After exclusion of the four previously linked loci, the results of a genome-wide search revealed linkage to markers mapping at 8p21. Historical recombinants further narrowed the locus to a 16-cM interval that contained two excellent positional candidate genes: *NF-L,* which encodes the neurofilament light proteins, and *NF-M,* which encodes the neurofilament medium proteins. The entire coding regions were screened for both genes. An A→C transversion mutation predicted to result in a Gln333Pro missense mutation was identified in the first exon of *NF-L*. The mutation cosegregated with the disease and was not found in 180 normal chromosomes used as controls. This highly conserved amino acid position is surrounded by the protein rod domain, which is responsible for neurofilament assembly (Carpenter and Ip 1996). Gln333 is located in the coil 2B domain, which is the last and the largest of four coil domains that form the rod region. A

Table 1

Genes in Which Mutation or Altered Dosage May Cause CMT

Gene	Function
CMT1:	
PMP22	Myelin structure/growth arrest?
Cx32	Gap-junction formation
MPZ	Myelin structural protein, homophilic adhesion
EGR2	Transcription factor
MTMR2[a]	Protein tyrosine phosphatase/ dual-specificity phosphatase
NDRG1[b]	Growth arrest/cell differentiation
CMT2:	
NF-L	Neurofilament organization and regulation

[a] See Bolino et al. (2000).

[b] Kalaydjieva et al. (2000 [in this issue]) recently described CMT1 genes primarily associated with autosomal recessive disease.

Received May 18, 2000; accepted for publication May 19, 2000; electronically published June 7, 2000.

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This article represents the opinions of the authors and has not been peer reviewed.

0002-9297/2000/6701-0004$02.00

Table 2

CMT2-Linked Loci

Locus	Genome Position	Reference
CMT2A	1p35-36	Ben Othmane et al. (1993) Timmerman et al. (1996)
CMT2B	3q13-q22	Kwon et al. (1995) De Jonghe et al. (1997) Ionasescu et al. (1996)
CMT2D	7p14	Ionasescu et al. (1996)
HMSNP	3q13.1	Takashima et al. (1997, 1999)

Leu394Pro mutation in the same coil domain in mice resulted in a severe peripheral neuropathy phenotype (Lee et al. 1994; Cleveland et al. 1996), whereas *NF-L* null mice did not have a CMT-like phenotype suggesting a dominant gain-of-function mechanism.

The neuronal cytoskeleton is composed of three interconnected filaments: the actin microfilaments, microtubules, and intermediate filaments (IFs). Neurofilaments (diameter 10 nm) are the major type of IFs in adult neurons. In the higher eukaryotes, there are three neurofilament proteins: light (NF-L), medium (NF-M), and heavy (NF-H) neurofilament proteins. These three neurofilament proteins share, among themselves and with other members of the IF family, a central coiled domain that is involved in the assembly of 10-nm filaments (Julien 1999). The carboxy terminals of the NF-H and NF-M subunits form side-arm projections at the periphery of the neurofilaments. It is believed that phosphorylation of their KSP (Lys-Ser-Pro) repeats, particularly in NF-H, increases their negative charge and thereby causes increased neurofilament spacing (Julien 1999). KSP domains are substrates for the mitogen-activated protein kinase family, which includes stress-activated protein kinase 1 (Giasson and Mushynski 1996) and extracellular signal-regulated kinases 1 and 2 (Veeranna et al. 1998). Interestingly, an elevation of neurofilament phosphorylation has been reported in the sensory neurons of rats with diabetic neuropathy (Fernyhough et al. 1999). NF-L seems to play the most important role in neurofilament assembly, since it is the only neurofilament protein capable of organizing filaments by itself (Geisler and Weber 1981; Carpenter and Ip 1996), and it plays a part in regulation of the expression of other neurofilament proteins.

It is of note that Mersiyanova et al. (2000 [in this issue]) allude to a potential final common pathway for the pathologic process in CMT: a chain of events resulting in axonal loss and muscle degeneration. Even in CMT1, the demyelination is not the direct cause of muscle atrophy and weakness but, instead, appears to act by initiating axonal loss (Scherer 1999). Several lines of evidence illustrate this process of axonal loss. First, altered neurofilament phosphorylation and β-tubulin isotypes have been observed in CMT1 (Watson et al. 1994). Additionally, *Trembler* mice, which harbor a *PMP22* missense mutation, undergo local axonal demyelination, which results in decreased neurofilament phosphorylation and slow axonal transport and reduced axonal diameter, yet the myelinated regions of the same axon have normal parameters (de Waegh et al. 1992). The results of nerve xenograft studies using sural nerve from patients with a *PMP22* duplication or deletion show distal axonal loss (Sahenk et al. 1999), whereas the results of longitudinal nerve conduction velocity studies in patients with duplication of *PMP22* also support an axonal role in pathologic progression (Killian et al. 1996; Garcia et al. 1998). Finally, when mutated in mice, another myelin gene, *MAG,* which encodes myelin-associated glycoprotein, results in axon degeneration that correlates with a decrease in neurofilament phosphorylation and reduced axonal caliber (Yin et al. 1998). Thus, mutations in myelin proteins can act as signals that initiate a similar pathological process in the axons. These findings again emphasize the importance of axon-glia interactions.

As aptly pointed out by Mersiyanova et al. (2000 [in this issue]), neurofilament proteins are involved in the pathogenesis of several other neurological disorders, including giant axonal neuropathy, amyotrophic lateral sclerosis (ALS), and Parkinson and Alzheimer diseases. The finding of an *NF-L* mutation associated with CMT2 supports a role for neurofilament proteins in this disease and also delineates a potential final common pathway for different forms of CMT1. Furthermore, since neurofilament proteins apparently are key targets in axonal pathology, their corresponding genes—as well as genes for proteins that interact with or regulate neurofilaments—should be considered as candidate genes for other CMT loci.

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