

Clinical and Electrophysiological Features in Charcot-Marie-Tooth Disease With Mutations in the *NEFL* Gene

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Background: To date, 13 different neurofilament light-chain polypeptide gene (*NEFL*) mutations have been identified in 55 patients with Charcot-Marie-Tooth disease (CMT) from 16 families. *NEFL* mutations were found to be associated with axonal and demyelinating variants of CMT.

Objectives: To describe the clinical features of 11 patients with CMT and *NEFL* mutations and to explore possible genotype-phenotype correlations.

Design: Standardized neuromuscular and nerve conduction studies were performed, and the coding regions of the peripheral myelin protein 22 (*PMP22*), myelin protein zero (*MPZ*), gap junction β -1 protein (*GJB1*), and *NEFL* genes were analyzed by direct DNA sequencing.

Setting: Two university hospitals in Austria (referral centers for neuromuscular disorders).

Patients: Eleven patients with CMT and *NEFL* mutations.

Main Outcome Measure: We genotyped *NEFL* in all of the patients and healthy relatives and correlated the genotype with the phenotype.

Results: A novel *NEFL* mutation (p.L93P) was detected in 1 family with 4 affected individuals exhibiting a severe CMT phenotype. Nerve conduction velocities were intermediately slowed to a range of 35 to 39 m/s. In a second family and in a sporadic patient, a p.P8R mutation was identified with intermediate and severe nerve conduction slowing.

Conclusion: The results argue against an obvious genotype-phenotype correlation regarding disease onset, degree of muscle weakness, and nerve conduction slowing caused by *NEFL* mutations.

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CHARCOT-MARIE-TOOTH disease (CMT) (Online Mendelian Inheritance in Man #118220), also called hereditary motor and sensory neuropathy, has been subdivided into 2 main groups based on the underlying pathological findings.¹⁻⁵ The demyelinating variant, CMT1, is electrophysiologically characterized by reduced motor nerve conduction velocities (NCVs) of the median nerve (NCV < 38 m/s) as well as segmental demyelination, segmental remyelination, and hyperplasia of Schwann cells

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causing onion bulb formations on histopathological examination. In the axonal variant, CMT2, NCVs are almost normal, but amplitudes of compound motor evoked potentials are reduced and nerve pathological findings show axonal loss and regenerative sprouting. The additional clas-

sification of intermediate CMT has been suggested in cases with motor NCVs between 25 and 45 m/s.^{6,7}

Charcot-Marie-Tooth disease is genetically very heterogeneous, and mutations in 36 genes have been described so far (Inherited Peripheral Neuropathies Mutation Database, <http://www.molgen.ua.ac.be/CMTMutations/>). A large study of 323 patients with CMT by Jordanova et al⁸

**See also pages 935,
959, and 974**

found neurofilament light-chain polypeptide gene (*NEFL*) mutations in 2% of the cases. Thirteen disease-causing *NEFL* mutations and clinical features of 55 patients have been described, providing a limited set of data for genotype-phenotype analysis.⁸⁻¹⁷

Here we describe the results of clinical, electrophysiological, and genetic studies in 2 Austrian families and 1 single patient exhibiting a severe clinical CMT phenotype and a novel *NEFL* mutation.

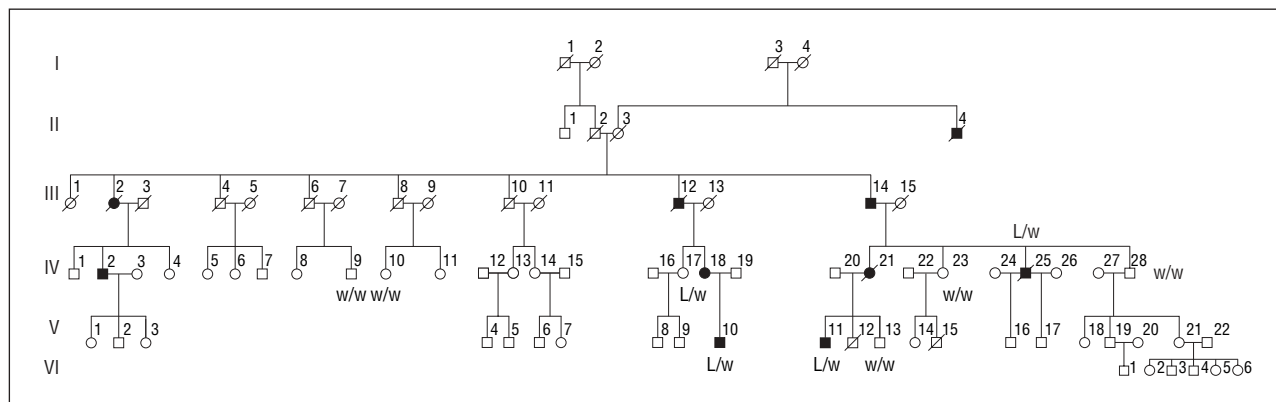


Figure 1. Pedigree of family 1. Circles indicate females; squares, males; filled symbols, affected individuals; open symbols, unaffected individuals; slash, deceased; L/w, heterozygous p.L93P mutation; and w/w, absence of mutation (w indicates wild-type allele).

METHODS

PATIENTS

Two Austrian families (family 1 and family 2) and 1 sporadic patient with classical CMT were studied. Neurological examinations and standard nerve conduction studies were performed in 9 individuals (4 affected and 5 at risk) of family 1, in 10 individuals (6 affected and 4 at risk) of family 2, and in the sporadic patient. Written informed consent was obtained from all of the participants according to the Declaration of Helsinki, and the study was approved by the local ethics committee.

MUTATION ANALYSIS

Participants' DNA was extracted from peripheral blood samples using an automated extractor according to the manufacturer's protocols (GenoM 48; Qiagen, Vienna, Austria). Mutations in the most common genes (peripheral myelin protein 22 gene [PMP22] duplication and deletion, PMP22 point mutations, and mutations in myelin protein zero [MPZ] and gap junction β -1 protein [GJB1] genes) were excluded first. Intronic primers were designed to flank each of the 4 NEFL (GenBank accession number NM_006158) exons encoding 542 amino acids. Each polymerase chain reaction fragment was bidirectionally sequenced in each index case (ABI 3100 DNA sequencer with BigDye terminator mix and SeqScape Software version 2.0; Applied Biosystems, Vienna, Austria). Eight further individuals from family 1 and 9 individuals from family 2 were investigated for the presence of the respective sequence change identified in the index case. Sequencing of these 2 positions was performed in 200 normal chromosomes derived from a reference set of ethnically matched individuals.

RESULTS

CLINICAL FEATURES

In family 1, 56 individuals were identified (**Figure 1**). In all of the affected individuals, disease onset was during the second decade of life related to plantar extensor weakness and foot deformity, ie, pes cavus, that resulted in ankle arthrodesis in 2 patients at ages 14 (V:10) and 17 (IV:18) years. Distal lower limb atrophy and weakness progressed over years and involved distal upper limbs at older ages. Two patients became wheel-

chair bound. Sensory loss predominantly affected the lower limbs, and large fiber function and tendon reflexes were absent in the lower limbs (**Table 1**).

In family 2, disease onset was before age 15 years in all of the patients except for 1 in whom gait problems were not reported until age 25 years. Symptoms were slowly progressive, resulting in a severe and disabling CMT phenotype. Interestingly, the oldest patient was initially diagnosed with spinocerebellar ataxia, as she presented with markedly dysarthric speech, nystagmus, and ataxia (**Table 1**).

A severe CMT phenotype without signs of ataxia was observed in the single patient who had no reported family history (**Table 1**).

ELECTROPHYSIOLOGICAL RESULTS

Electrophysiological studies (**Table 1**) of families 1 and 2 demonstrated intermediate motor NCVs and absent median and sural nerve sensory action potentials. Compound motor action potentials were reduced to variable degrees. All of the clinically unaffected individuals who were tested had normal nerve conduction study results. The sporadic patient (patient 33) had severely reduced NCVs and compound motor action potentials.

MOLECULAR RESULTS

The index patient of family 1 (IV:25) showed a heterozygous single nucleotide exchange (c.278T>C) resulting in a leucine-to-proline amino acid change at codon 93 (p.L93P) in the coil 1a domain of the NEFL protein. The leucine-93 residue is highly conserved (**Figure 2**). No other nucleotide changes were detected. All of the affected family members but none of the 5 unaffected family members carried the mutation (**Figure 1**), which was also absent in 100 controls.

In the affected patients of family 2 as well as in the sporadic patient, we found a heterozygous C-to-G nucleotide change in the first coding exon of NEFL (c.23C>G) resulting in a proline-to-arginine substitution at codon 8 (p.P8R) located in the head domain of the NEFL protein. The p.P8R change was absent in clinically unaffected individuals of family 2. It was absent in the unaffected

Table 1. Clinical and Electrophysiological Features of Patients With Charcot-Marie-Tooth Disease Investigated in This Study

Patient	Age at Study Time, y	Age at Onset, y	Initial Symptoms	Weakness in LL/UL ^a	Atrophy in LL/UL ^b	Vibration ^c		Pain or Temperature in LL/UL	Reflexes in LL/UL	Pes Cavus	Additional Disorders	NCV, m/s (CMAP, mV) for Median/Ulnar
						LL	UL					
Family 1												
IV:25	63 ^d	~ 15	Gait problems	+++ / +	+++ / +	0/8	6/8	R/N	A/A	Yes	Hypertension	38 (2.9)/40 (3.3)
V:11	55	~ 15	Clumsiness during sport	+++ / -	+++ / -	0/8	7/8	R/N	A/D	Yes	Psoriasis	35 (2.0)/36 (4.1)
IV:18	70	~ 14	Gait problems	+++ / ++	+++ / ++	0/8	7/8	N/N	A/D	Yes	Diabetes, hypertension, coronary heart disease	36 (1.2)/29 (1.5)
V:10	46	~ 8	Gait problems	++ / -	+ / -	0/8	8/8	N/N	A/A	Yes		38 (5.0)/40 (2.4)
Family 2												
Patient 1	49	12	Unsteady gait	+++ / ++	+++ / ++	ND	ND	ND/ND	A/A	Yes	Hyperlordosis	ND
Patient 2	77	15	Gait problems	+++ / ++	+++ / ++	ND	ND	R/R	A/A	Yes	Cerebellar signs	39 (0.2)/ND
Patient 3	71	12	Unsteady gait	+++ / ++	+++ / ++	ND	ND	ND/ND	A/A	Yes	Kyphoscoliosis	ND
Patient 4	61	25	Gait problems	+++ / ++	+++ / ++	ND	ND	R/R	A/A	Yes	Fasciculations	ND
Patient 5	51	13	Unsteady gait	+++ / ++	+++ / ++	ND	ND	R/R	A/A	Yes	Ataxia, tremor	36 (1.9)/ND
Patient 6	50	ND	Unsteady gait	+++ / ++	+++ / ++	ND	ND	R/R	A/A	ND	Schizophrenia, ataxia	ND
Patient 33	29	8	Foot deformity	+++ / ++	+++ / ++	ND	ND	R/R	A/A	Yes		23 (0.5)/ND

Abbreviations: A, absent; CMAP, compound motor action potential; D, diminished; LL, lower limbs; N, normal; NCV, nerve conduction velocity; ND, no data; R, reduced; UL, upper limbs.

^aFor weakness in LL, + indicates ankle dorsiflexion less than grade 4 Medical Research Council (MRC); ++, ankle dorsiflexion less than grade 4 MRC and proximal weakness; and +++, ankle dorsiflexion less than grade 4 MRC and wheelchair bound. For weakness in UL, - indicates no weakness; +, intrinsic hand muscle weakness grade 4 MRC; and ++, intrinsic hand muscle weakness less than grade 4 MRC.

^bFor atrophy, - indicates no atrophy; +, mild atrophy; and ++, pronounced atrophy.

^cVibration in the LL was measured at the metacarpophalangeal joint of the hallux; vibration in the UL was measured at the distal interphalangeal joint of the index finger.

^dPatient died in 2004.

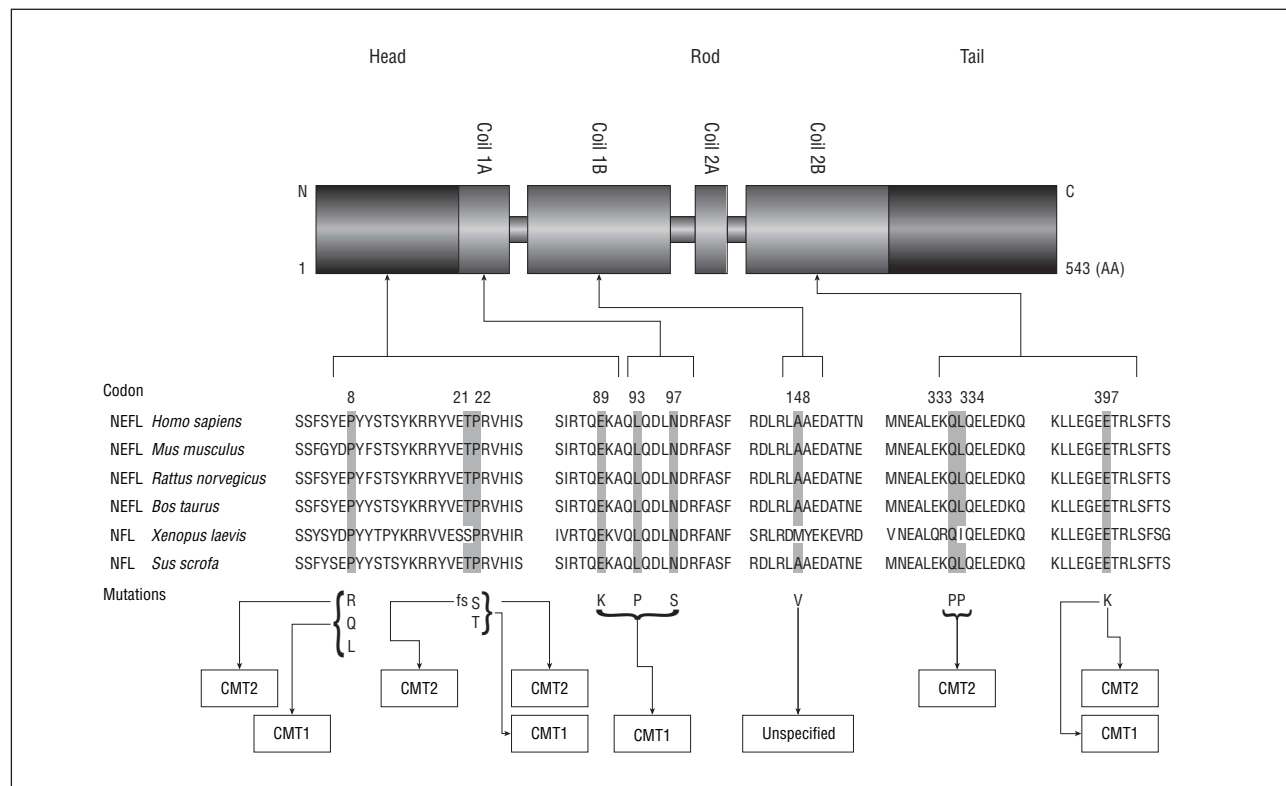


Figure 2. Compilation, sequence homology, and localization of NEFL mutations with respect to protein domains. Mutation sites are indicated in gray. Amino acid changes and associated phenotypes (according to the original publications) are indicated below the mutation sites. CMT1 indicates Charcot-Marie-Tooth disease, demyelinating variant; CMT2, Charcot-Marie-Tooth disease, axonal variant; N, N terminus; C, C terminus; AA, amino acid.

Table 2. Clinical and Electrophysiological Features of Patients With Charcot-Marie-Tooth Disease With Mutations in the *NEFL* Gene Described in the Literature

Domain	Mutation	Source	Patients, No.	Families/ Sporadic Patients, No.	Age at Onset, y	Muscle Weakness ^a		Atrophy ^b		Sensory Loss ^c	Reflexes	Pes Cavus	NCV, Median, m/s (CMAP, mV)	CMT Variant
						LL	UL	LL	UL					
Head	p.P8R	Jordanova et al ⁸ (2003), De Jonghe et al ¹⁰ (2001), present study	20	3/2	7-25	+ to +++	ND to +	+	+	+	N, D, A	Yes	23-39 (0.5-4.9)	CMT1, CMT2
Coil 1a	p.P8Q	Jordanova et al ⁸ (2003)	1	1/0	< 5	+++	+	+	+	+	D	Yes	21 (1.0)	CMT1
	p.P8L	Jordanova et al ⁸ (2003)	1	0/1	< 2	++	++	+	+	-	A	Yes	13-15 (2.0)	CMT1
	p.T21fs	Leung et al, ¹⁷ (2006)	1	0/1	71	+++	ND	+	ND	+	A	Yes	ND	CMT2
	p.P22T	Yoshihara et al ¹³ (2002)	2	1/0	18-24	+++ to ++++	ND	+	ND	+	ND	ND	29.3-35.7 (0.01-0.74)	CMT1
	p.P22S	Georgiou et al ¹⁴ (2002), Fabrizi et al ¹⁵ (2004)	12	2/0	< 10 to 36	+ to ++	+ to ++	+	+	+	D, A	Yes	21-54 (0.8-7.4)	CMT1, CMT2
	p.E89K	Jordanova et al ⁸ (2003)	1	0/1	< 2	+	+	ND	ND	+	A	Yes	27 (3.9)	CMT1
	p.L93P	Present study	4	1/0	~ 8 to ~ 15	++ to +++	- to ++	++	+	ND	A	Yes, no	35-38 (1.2-5.0)	CMT2
Coil 1b	p.N97S	Jordanova et al ⁸ (2003), Yoshihara et al ¹³ (2002)	2	0/2	< 1 to 15	+ to +++	+ to ++	+	+	ND to +	A	Yes	22-26 (0.52-4.7)	CMT1
	p.A148V	Yoshihara et al ¹³ (2002)	1	0/1	33	+	+	+	+	+	ND	ND	ND	Unspecified
Coil 2b	p.Q333P	Mersiyanova et al ⁹ (2000)	12	1/0	10-20	ND	ND	+	ND	ND	ND	ND	ND	CMT2
	p.L334P	Choi et al ¹¹ (2004)	1	0/1	ND	ND	ND	ND	ND	ND	ND	ND	ND	CMT2
	p.E397K	Choi et al ¹¹ (2004), Züchner et al ¹² (2004)	8	2/0	4-46	+ to ++	-	ND	ND	+	N, D	Yes, no	57-63 (10-14.8)	CMT1, CMT2

Abbreviations: A, absent; CMAP, compound motor action potential; CMT1, Charcot-Marie-Tooth disease, demyelinating variant; CMT2, Charcot-Marie-Tooth disease, axonal variant; D, diminished; LL, lower limbs; N, normal; NCV, nerve conduction velocity; ND, no data; UL, upper limbs.

^aFor weakness in LL, + indicates ankle dorsiflexion less than grade 4 Medical Research Council (MRC); ++, ankle dorsiflexion less than grade 4 MRC and proximal weakness; and +++, ankle dorsiflexion less than grade 4 MRC and wheelchair bound. For weakness in UL, - indicates no weakness; +, intrinsic hand muscle weakness grade 4 MRC; and ++, intrinsic hand muscle weakness less than grade 4 MRC.

^bFor atrophy, - indicates absent; +, present.

^cFor sensory loss, - indicates no; +, yes.

parents of the sporadic case, suggesting that this mutation arose de novo (Figure 2 and Table 1).

COMMENT

To date, 13 different *NEFL* mutations have been identified in 55 patients with CMT from 16 families. We describe phenotypic features of 11 further patients with CMT from 3 families with *NEFL* mutations, and we also describe a novel *NEFL* mutation, p.L93P. Nerve conduction studies suggested that this mutation resulted in an axonopathy with secondary demyelination in all of the affected individuals, which was also seen in family 2 associated with a p.P8R mutation. Indeed, neuropathological studies in 2 cases demonstrated predominantly axonal atrophy in intermediate CMT, but axonal swelling, paranodal abnormalities, and onion bulb formations have also been observed.^{12,15}

So far, 22 patients with a mutation involving the proline residue at codon 8^{8,10} have been described and at least 5 different mutational events can be inferred, suggesting that codon 8 is a hot spot for *NEFL* mutations. Of 13

patients with this mutation, 1 was classified as having intermediate CMT and the others were classified as having CMT1, with NCVs ranging from 13 to 39 m/s. However, the classification based on nerve conduction studies alone becomes difficult in cases where the compound motor action potential amplitude is reduced, as in our single patient. Despite this, phenotypic variability can be seen with codon 8 mutations not only with NCVs but also with disease onset, which varied between ages 2 and 25 years.^{8,10}

The phenotypic variability found for codon 8 mutations can also be observed for mutations at codon 22 in the head domain of the *NEFL* protein¹³⁻¹⁵ or at codon 397 in the coil 2b domain^{11,12} (Figure 2).

This indicates that no simple genotype-phenotype correlation exists and suggests the existence of modifiers in *NEFL*-related CMT. However, it appears that mutations localizing to the *NEFL* protein head domain can cause more severe motor and sensory NCV slowing than mutations in the coil 2B domain (Figure 2 and **Table 2**),^{8,12} while the intrafamilial phenotype appears to “run true.”

In addition, the phenotypic variability of the *NEFL* mutation seems to also include possible central nervous sys-

tem involvement, as 1 patient of family 2 showed signs of cerebellar dysfunction and was initially diagnosed with spinocerebellar ataxia. Of previously described patients with *NEFL* mutations, 1 was initially diagnosed with Friedreich ataxia¹² and another had a complex phenotype with nystagmus and global developmental delay.⁸ Although the pathophysiological abnormalities underlying central nervous system dysfunction remain unclear, their occurrence is suggested by the widespread presence of neurofilament light-chain polypeptide in the central nervous system. However, concomitant mutations in *NEFL* and other genes cannot be excluded.¹⁸

ADDENDUM

Since the manuscript was accepted for publication, 3 additional mutations in the *NEFL* gene have been reported, highlighting the phenotypic variability in CMT with *NEFL* mutations.^{19,20} The *NEFL* reference sequence has recently been changed; therefore, the new mutation reported in this article is now described as c. 281T>C, p.L94P.

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