A Novel Type of Hereditary Motor and Sensory Neuropathy Characterized by a Mild Phenotype

Peter De Jonghe, MD, PhD; Vincent Timmerman, PhD; Eva Nelis, PhD; Els De Vriendt; Ann Logren, MSc; Chantal Ceuterick, PhD; Jean-Jacques Martin, MD, PhD; Christine Van Broeckhoven, PhD, DrSc

Background: Three loci for autosomal dominant hereditary motor and sensory neuropathy type I (HMSN I) or Charcot-Marie-Tooth disease type 1 (CMT1) have been identified on chromosomes 17p11.2 (CMT1A), 1q21-q23 (CMT1B), and 10q21.1-q22.1 (designated here as CMT1D). The genes involved are peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), and the early growth response element 2 (EGR2), respectively. Probably a fourth locus (CMT1C) exists since some autosomal dominant HMSN I families have been excluded for linkage with the CMT1A and CMT1B loci. Four loci for autosomal dominant hereditary motor and sensory neuropathy type II (HMSN II) or Charcot-Marie-Tooth disease type 2 (CMT2) have been localized on chromosomes 1p35-p36 (CMT2A), 3q13-q22 (CMT2B), 7p14 (CMT2D), and 3p (HMSN-P).

Objective: To describe the clinical, electrophysiologic, and neuropathological features of a novel type of Charcot-Marie-Tooth disease.

Patients and Methods: We performed linkage studies with anonymous DNA markers flanking the known CMT1 and CMT2 loci. Patients and their relatives underwent clinical neurologic examination and electrophysiologic testing. In the proband, a sural nerve biopsy specimen was examined.

Results: Linkage studies excluded all known CMT1 and CMT2 loci. The clinical phenotype is mild and almost all affected individuals remain asymptomatic. Electrophysiologic and histopathological studies showed signs of a demyelinating neuropathy, but the phenotype is unusual for either autosomal dominant HMSN I or HMSN II.

Conclusion: Our findings indicate that the HMSN in this family represents a novel clinical and genetic entity.

Arch Neurol. 1999;56:1283-1288

The hereditary motor and sensory neuropathies (HMSNs) of the peripheral nervous system are characterized by progressive weakness and atrophy of distal muscles, areflexia, and distal sensory abnormalities. The HMSNs are classified in 7 types according to clinical phenotype, mode of inheritance, and neuropathological and electrophysiologic features.1 Hereditary motor and sensory neuropathy type I (HMSN I) or Charcot-Marie-Tooth disease type 1 (CMT1) is a demyelinating neuropathy with signs of extensive demyelination and remyelination and the formation of onion bulbs on nerve biopsy examination specimens. Motor and sensory nerve conduction velocities (NCVs) are severely slowed to less than 38 m/s for the motor median nerve. Hereditary motor and sensory neuropathy type II is an axonal neuropathy and shows normal or slightly reduced motor and sensory NCVs. Compound muscle action potentials have reduced amplitudes. Sensory nerve action potentials also have reduced amplitudes and are often not elicitable.

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Hereditary motor and sensory neuropathy type I is genetically heterogeneous and can be inherited as an autosomal dominant, autosomal recessive, or X-linked trait, while hereditary motor and sensory neuropathy type II (HMSN II) either shows an autosomal dominant or autosomal recessive mode of inheritance.1 A recessive X-linked CMT2 variant has also been described, but these patients do have additional clinical signs such as deafness and mental retardation.2 Molecular genetic studies3 have shown that the majority of autosomal dominant HMSN I families have mutations either in the peripheral myelin protein 22 gene (PMP22)4 localized on chromosome 17p11.2 (CMT1A).5-7

From the Laboratory of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Bohn-Bunge Foundation (BBS), University of Antwerp (UZA), Department of Biochemistry (Drs De Jonghe, Timmerman, Nelis, and Van Broeckhoven and Mss De Vriendt and Logren), the Department of Neurology, University Hospital Antwerp (UZA) (Drs De Jonghe and Martin), and Laboratory of Neuropathology, Bohn-Bunge Foundation (BBS) (Drs Ceuterick and Martin), Antwerpen, Belgium.
METHODS

FAMILY DATA

We studied a multigenerational family (CMT-54) in which an HMSN phenotype segregates as an autosomal dominant trait (Figure 1). The index patient (III.16), initially examined for vascular problems in the legs, showed signs of a peripheral neuropathy on clinical examination and NCV testing. Despite the negative family history of the disease, we examined the parents to eliminate the possibility of an inherited peripheral neuropathy. The asymptomatic father also showed slowed NCVs, and, consequently, we identified 11 other relatives with slowed NCVs.

ELECTROPHYSIOLOGIC TECHNIQUES

Motor NCVs in the median and peroneal nerves and sensory NCVs in the median and sural nerves were measured using surface electrodes and standard stimulation techniques.

NEUROPATHOLOGICAL TECHNIQUES

A biopsy of the superficial fibular nerve and the peroneus brevis muscle was performed on patient II.16 at the age of 54 years. The tissues were fixed in 4.5% phosphate-buffered glutaraldehyde, postfixed in 2% phosphate-buffered osmium tetroxide, and embedded in araldite. Semithin sections were stained with toluidine blue O. Quantitative studies on transverse semithin sections of the nerve were done using a Sony camera, projecting the picture on the 53 cm (21-in) screen of a Macintosh computer. A computing program, Image 1.31, from the National Institutes of Health, Bethesda, Md, was used to determine the density of the myelinated fibers per square millimeter. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a Philips CM 10 electron microscope at 60 kV.

MUTATION ANALYSIS AND LINKAGE STUDIES

Blood samples for genomic DNA extraction from the patients and their family members were obtained after informed consent. The absence of the CMT1A duplication in the patients was confirmed by Southern blot hybridization and short tandem repeat (STR) markers analysis. Single strand conformation polymorphism (SSCP) analysis was used to exclude the presence of mutations in PMP22, MPZ, Cx32, and EGR2.

Genotyping was performed with STR markers from chromosome 1p35–p36 ([D1S160 [MIT-MS48], D1S170 [MIT-COS37], D1S244 [AFM220yf4], and D1S288 [AFM196xb4]); chromosome 3 ([D3S179 [GATA8D02], D3S1290 [AFM198yb6m], D3S1744 [GATA3C02], D3S2465 [GGATA2G03], D3S2465 [GGAA16E09], and D3S2459 [GATA68D03]); 7p14 ([D7S1791 [ATA1B04], D7S516 [AFM224xg5], D7S2213 [GATA91C01], and D7S526 [FM248vc9]); and 8q23–q24 ([D8S128 [GATA21C12] and D8S1990 [GGAA23E06]]). Genomic DNA (0.15 pg) was amplified using oligonucleotide primers labeled with fluorophores (Applied Biosystems Inc, Foster City, Calif). Polymerase chain reaction was performed in a 25-µL reaction volume containing 1 µmol of each primer and 0.1–U Goldstar Taq DNA polymerase (Eurogentec, Seraing, Belgium) in a thermal cycler (Techne PHC-3; Techne Ltd, Cambridge, England). An aliquot of 1 µL of each amplified product was mixed with 2 µL of formamide and 0.5-µL fluorescent-labeled standard size Prism Genescan-350-ROX (PE Applied Biosystems, Foster, Calif) and denatured for 3 minutes at 95°C. The samples were loaded on 4% polyacrylamide sequencing gels and electrophoresed in the ABI Prism 377 DNA sequencer (PE Applied Biosystems). Finally, the data were collected and analyzed using ABI PRISM GENESCAN and GENOTYPER software (PE Applied Biosystems). The haplotypes were constructed according to the genetic maps of Dib and colleagues22 and the Cooperative Human Linkage Center (Web site: http://cgap.nci.nih.gov/CHLC). The 2-point linkage studies were performed with the FASTLINK computer package, using equal allele frequencies of the STR markers (1/n, n = number of alleles), a disease frequency of 1/10 000, and equal male and female recombination fractions. Individuals who had reduced NCVs were considered affected and those with normal NCVs were considered unaffected, implicating a fully penetrant phenotype.

RESULTS

CLINICAL FEATURES

The index patient (III.16) was healthy until the age of 41 years, when a diagnosis of thrombocytopenia obliterans or Buerger disease was made. An NCV study and electromyography, to eliminate the possibility of neuromuscular involvement due to vasculitis, showed uniformly slowed motor and sensory NCVs. Clinical neurologic examination

the myelin protein zero gene (MPZ) on chromosome 1q22–q23 (CMT1B),8,9 or the early growth response 2 gene (EGR2) on chromosome 10q21.1–q22.1.10 The most frequent DNA abnormality in CMT1A is a 1.5 million–base pair tandem duplication,11,12 containing the PMP22 gene. Initially, linkage studies have excluded a few autosomal HMSN I pedigrees from the CMT1A and CMT1B loci, implying that an additional locus (CMT1C) for autosomal dominant HMSN I has to exist.14,15 It cannot be excluded that the gene for CMT1C is actually the EGR2 gene since a mutation analysis of this gene has not yet been reported in these CMT1C families. The X-linked dominant form of HMSN I is caused by mutations in the Connexin 32 (GJB1) gene.16 Four genetic loci have been identified in HMSN II, ie, CMT2A at chromosome 1p35–p36,17 CMT2B at 3q13–q22,18,19 CMT2D at 7p14,20 and HMSN-P at 3p.21 The genes involved in CMT2, however, have not yet been identified.

We studied a large autosomal dominant HMSN family that was excluded for linkage to all known CMT1 and CMT2 loci. This family shows an unusual clinical, electrophysiologic, and neuropathological phenotype. In a previous article,22 we described in this family a mutation in the promoter of PMP22, which did not cosegregate with the disease. The clinical phenotype, electrophysiologic, and neuropathological features were not described in detail. The molecular genetic analysis was at that time limited to PMP22 and MPZ.
demonstrated bilateral pes cavus, slight weakness of the peroneal muscles, and distal areflexia in the lower limbs. Consequently, slowed NCVs were also observed in 12 relatives. However, all these individuals were asymptomatic and several of them frequently engaged in sports, such as tennis and soccer. Clinical neurologic examination results in all these persons were normal. They had no gait abnormalities and were able to walk on their heels and toes. They had no weakness or sensory signs and even the intrinsic muscles of the feet showed no atrophy. Several individuals had a Raynaud phenomenon with their fingers or toes turning white on exposure to cold. This trait also showed autosomal dominant transmission in this pedigree but did not cosegregate with the peripheral neuropathy.

ELECTROPHYSIOLOGIC CHARACTERISTICS

The motor and sensory NCVs of the median and ulnar nerves are given in Table 1. Nerve conduction velocities were uniformly slowed in all nerves tested except in patient IV.13. This young boy had normal sensory NCVs in both ulnar and median nerves. The motor NCVs in both ulnar nerves were normal in the forearms, but the F-wave latencies were severely slowed (38.2-43.7 and 38.7-42.2 milliseconds for the right and left ulnar nerve, respectively; normal <32 milliseconds), and the conduction over the elbow was severely reduced (40 and 34 m/s; normal ≥49 m/s); also both motor peroneal nerves were slowed (data not shown). All slowed NCVs fell within relatively narrow ranges: motor median nerve, 34 to 42 m/s; motor peroneal nerve, 27 to 36 m/s; sensory median nerve, 32 to 46 m/s; sensory ulnar nerve, 33 to 45 m/s; and sensory sural nerve, 28 to 35 m/s. The compound muscle action potentials (CMAPs) were normal in all nerves tested. Sensory nerve action potentials (SNAPs) were sometimes slightly reduced.

NEUROPATHOLOGICAL CHARACTERISTICS

Semithin cross sections revealed a severe loss of myelinated axons (6000 myelinated fibers per square millimeter instead of 11 281 per square millimeter for the superficial fibular nerve of healthy age-matched controls in our laboratory). The size of the remaining myelinated axons varied from 2 to 6 µm. Fibers with a diameter greater than 6 µm were considerably reduced. There was a loss of the bimodal distribution of myelinated fibers. Numerous fibers had a thin myelin sheath in comparison with the axon diameter (Figure 2). At higher magnifications, rare small onion bulbs and rare regenerative clusters were detected. By electron microscopy, few myelinated fibers were surrounded by sparse and incomplete Schwann cell processes, suggestive of early onion bulb formation. Occasional clusters of 2 to 3 regenerating axons and rarely, a denervated Büngner band were seen. No tomacula were found. There were no myelin debris and most unmyelinated axons were normal. Mild neurogenic changes were found in skeletal muscle.

MOLECULAR GENETICS

We previously excluded the 1.5–million base pair CMT1A duplication, which is the most common mutation in patients with CMT1.13 We also excluded linkage to the CMT1A locus on chromosome 17p11.2 by the identification of a C→G transversion in the noncoding exon 1A of PMP22, which did not cosegregate with the disease phenotype in family CMT-54.22 Also, linkage to the CMT1B locus on chromosome 1q22-q23 was excluded using an exonic polymorphism in the MPZ gene.22 In this study, we performed an SSCP analysis for the EGR2 gene located on chromosome 10q21.1-q22.1, but no altered fragments were found, indicating that EGR2 is not involved in the disease. We also performed a segregation

Figure 1. Pedigree of family CMT-54. Squares indicate males; circles, females; filled symbols, affected individuals; empty symbols, unaffected individuals; arrow, the proband; diagonal lines, deceased individuals; and asterisks, individuals clinically examined and sampled for DNA analysis.
The HMSN in family CMT-54 is inherited as an autosomal dominant trait. The clinical phenotype is extremely mild. The diagnosis in the proband was incidentally made during a clinical and electrophysiologic examination for vascular problems in the legs. The proband probably had no symptoms related to his peripheral neuropathy at that time. Subsequently, the diagnosis of HMSN was made in 12 relatives based on slowed NCVs. All these individuals were asymptomatic, including the 2 eldest persons (II.4 and II.7), who were 87 and 78 years old, respectively, at the time of clinical examination. Also mild clinical signs of a peripheral neuropathy were only found in the proband. All other affected but asymptomatic relatives were able to walk on their toes and heels. They had preserved tendon reflexes and all muscles, including the intrinsic foot muscles, were well developed.

A nerve biopsy specimen of the proband showed signs of demyelination and remyelination with the presence of small onion bulbs. The main morphologic characteristic of the nerve biopsy specimen was the loss of large myelinated axons without significant onion bulb formation. Thinning of the myelin sheaths as well as sparse and incomplete onion bulbs were suggestive of demyelination. There were no features comparable with those found in CMT1A, HNPP, or CMT1B. Limited evidence was found for axonal pathology with regeneration.

Motor and sensory NCVs were uniformly slowed within a rather narrow range for each nerve. Only individual IV.13 showed some borderline normal NCVs. Our patients carrying the 1.5-million base pair CMT1A duplication have motor NCVs of the median nerve ranging from 10 to 42 m/s (data not shown), and, therefore, the results obtained in family CMT-54 are still compatible with a diagnosis of HMSN I. There exists, however, a striking difference in the lower limits of motor and sensory NCVs obtained in patients with the CMT1A duplication and our CMT-54 family. Ninety-six percent of our patients with the CMT1A duplication have a motor median NCV between 10 and 33 m/s (data not shown), while none of the individuals in family CMT-54 had such slow NCVs (Table 1). The amplitudes of the CMAP were all normal and SNAPs with normal amplitudes could be elicited in most sensory nerves. This is in contrast with the findings in classical HMSN I and HMSN II, where the CMAP amplitudes are usually reduced and SNAPs are often not measurable. Also, muscle bulk of the intrinsic foot muscles, which are known to atrophy early in the course of the HMSNIs, was well preserved in patients of

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**Table 1. Electrophysiologic Features of Family CMT-54**

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<thead>
<tr>
<th>Patient</th>
<th>Median NCV</th>
<th>Sensory NCV</th>
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<td>Motor NCV</td>
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<tr>
<td></td>
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*Motor and sensory nerve conduction velocities (NCVs) are in meters per second. Normal NCVs for motor median and ulnar nerves are greater than or equal to 49 m/s and for sensory median and ulnar nerves are greater than or equal to 46 m/s. Amplitudes (Ampl) of the compound motor action potentials are in millivolts, with normal values greater than or equal to 6500 mV and Ampls of the sensory action potentials are in microvolts, with normal values at more than or equal to 10 µV. Abnormal values are given in bold and the nonbold values are the normal results.

†The patient numbers correspond to those listed in Figure 1.
CMT-54. The combination of slowed NCVs, preserved amplitudes, and absence of atrophy suggests that the pathological mechanism primarily affects the myelin sheaths and relatively spares the axons. However, a nerve biopsy specimen of the proband showed a severe loss of large myelinated fibers. The presence of clusters of regenerating fibers indicates that active regeneration is present and that this mechanism might balance the loss of large nerve fibers.

Genetic linkage studies with highly polymorphic DNA markers excluded linkage to the autosomal dominant HMSN 1 loci; the CMT1A locus at chromosome 17p11.229 and the CMT1B locus at 1q21-q23.29 Single strand conformation polymorphism and sequence analysis of the coding regions and promoter 1–exon 1A region of the PMP22 gene showed a polymorphism in the PMP22 promoter that did not cosegregate with the disease.22 No mutations were found in the coding region of the MPZ and EGR2 genes. Since NCVs in family CMT-54 are at the upper limit of HMSN 1 and sometimes exceed 38 m/s, we also examined linkage to the CMT2 loci. Conclusive negative logarithm of the odds scores were obtained with all 4 loci: CMT2A at 1p35-p36,17 CMT2B at 3q13-q22,18,19 CMT2D at 7p14,20 and HMSN-P at 3p.21

It has already been shown that an additional locus (CMT1C) for autosomal dominant HMSN 1 has to exist since some families were excluded for linkage to the CMT1A and CMT1B loci.14,15 However, mutations in the EGR2 gene still have to be excluded in these families. Only scarce clinical and electrophysiologic data have been reported on 2 non-CMT1A/non-CMT1B families. Patients in these 2 families have a typical Charcot-Marie-Tooth disease phenotype and the motor NCVs are severely slowed to a mean (SD) motor NCV of the median nerve of 15 (6.1) m/s and 22.9 (9.8) m/s, respectively.14,15 The clinical, electrophysiologic, and histopathological phenotype in our CMT-54 family looks different from the phenotype described in these non-CMT1A/non-CMT1B families, and we, therefore, conclude that the HMSN in family CMT-54 represents a novel clinical and genetic entity. A genomewide search will be performed to localize the gene defect in this novel type of HMSN.

Accepted for publication March 19, 1999.

This research was funded by a special research project of the University of Antwerp, Antwerpen, Belgium; the Fund for Scientific Research, Flanders, Brussel, Belgium; the Geneseskundige Stichting Koningin Elisabeth, Brussel; the Association Française contre les Myopathies, Paris, France; and the Muscular Dystrophy Association, Tucson, Ariz. Drs Timmerman and Neslis are research assistants of the Fund for Scientific Research. Dr Van Broeckhoven is the coordinator of the European Charcot-Marie-Tooth Consortium sponsored by European Union BIOMED2 grants CT961614 and CT960055.

The authors thank the patients and their relatives for their kind cooperation, Iris Smouts and Gisèle Smeyers for the genealogical studies, and Inge Bats for the histopathological figures.

Corresponding author: Peter De Jonghe, MD, PhD, Laboratory of Molecular Genetics, Department of Biochemistry, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium (e-mail: dejonghe@uia.ua.ac.be).

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