

Report

Dominant Intermediate Charcot-Marie-Tooth Type C Maps to Chromosome 1p34-p35

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Dominant intermediate Charcot-Marie-Tooth (DI-CMT) neuropathy is a genetic and phenotypic variant of classical CMT, characterized by intermediate nerve conduction velocities and histological evidence of both axonal and demyelinating features. We report two unrelated families with intermediate CMT linked to a novel locus on chromosome 1p34-p35 (DI-CMTC). The combined haplotype analysis in both families localized the DI-CMTC gene within a 6.3-cM linkage interval flanked by markers D1S2787 and D1S2830. The functional and positional candidate genes, Syndecan 3 (*SDC3*), and lysosomal-associated multispanning membrane protein 5 (*LAPTM5*) were excluded for pathogenic mutations.

Charcot-Marie-Tooth disease (CMT [MIM 118300]) is a clinically heterogeneous hereditary peripheral neuropathy characterized by progressive weakness and atrophy of the distal limb muscles, sensory abnormalities, and absent deep-tendon reflexes (Dyck et al. 1993). Most frequently, CMT is transmitted as a dominant trait. Since CMT affects ~1 in 2,500 individuals, it represents one of the most common inherited neuromuscular disorders (Skre 1974). On the basis of electrophysiological and histopathological criteria, CMT neuropathy is divided into two major clinical entities. CMT type 1 (CMT1), the demyelinating form, is characterized by median motor nerve-conduction velocities (NCVs) <38m/s. CMT type 2 (CMT2), the axonal form, is characterized by normal or slightly reduced motor NCVs (Harding and Thomas 1980). However, in some families with CMT, patients have median motor NCVs

with a range of 25–45m/s and are therefore difficult to classify using traditional electrophysiological criteria. One proposal has been to designate this type of CMT “intermediate CMT” (Davis et al. 1978).

CMT shows extensive genetic heterogeneity. Disease-causing mutations have been reported in different genes with a wide range of biological functions (Inherited Peripheral Neuropathies Mutation Database). The majority of patients with CMT1 have a 1.5-Mb tandem duplication on chromosome 17p11.2-p12 (CMT1A [MIM 118220]) (Lupski et al. 1991; Raeymaekers et al. 1991) that harbors the peripheral myelin protein 22 gene (*PMP22* [MIM 601097]) (Matsunami et al. 1992; Patel et al. 1992; Timmerman et al. 1992; Valentijn et al. 1992*b*). Furthermore, mutations in the following genes have been found to cause various types of dominant CMT1 and CMT2: *PMP22* in CMT1A (Valentijn et al. 1992*a*), myelin protein zero (*MPZ/P0* [MIM 159440]) in CMT1B (MIM 118200) (Hayasaka et al. 1993), lipopolysaccharide-induced tumor necrosis factor- α (*LI-TAF/SIPMLE* [MIM 603795]) in CMT1C (MIM 601098) (Street et al. 2003), early-growth-response element 2 (*EGR2* [MIM 129010]) in CMT1D (MIM 607678) (Warner et al. 1998), connexin 32 (*Cx32/GJB1* [MIM 304040]) in CMTX1 (MIM 302800) (Bergoffen

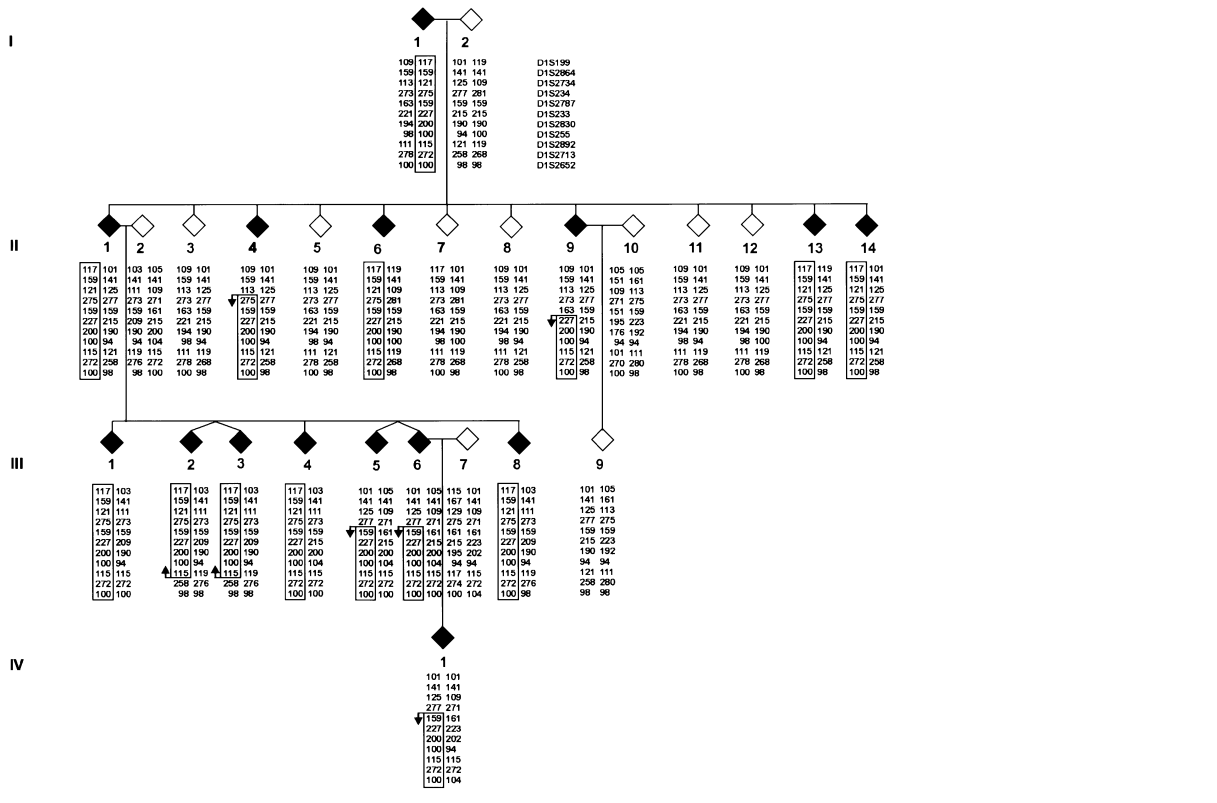
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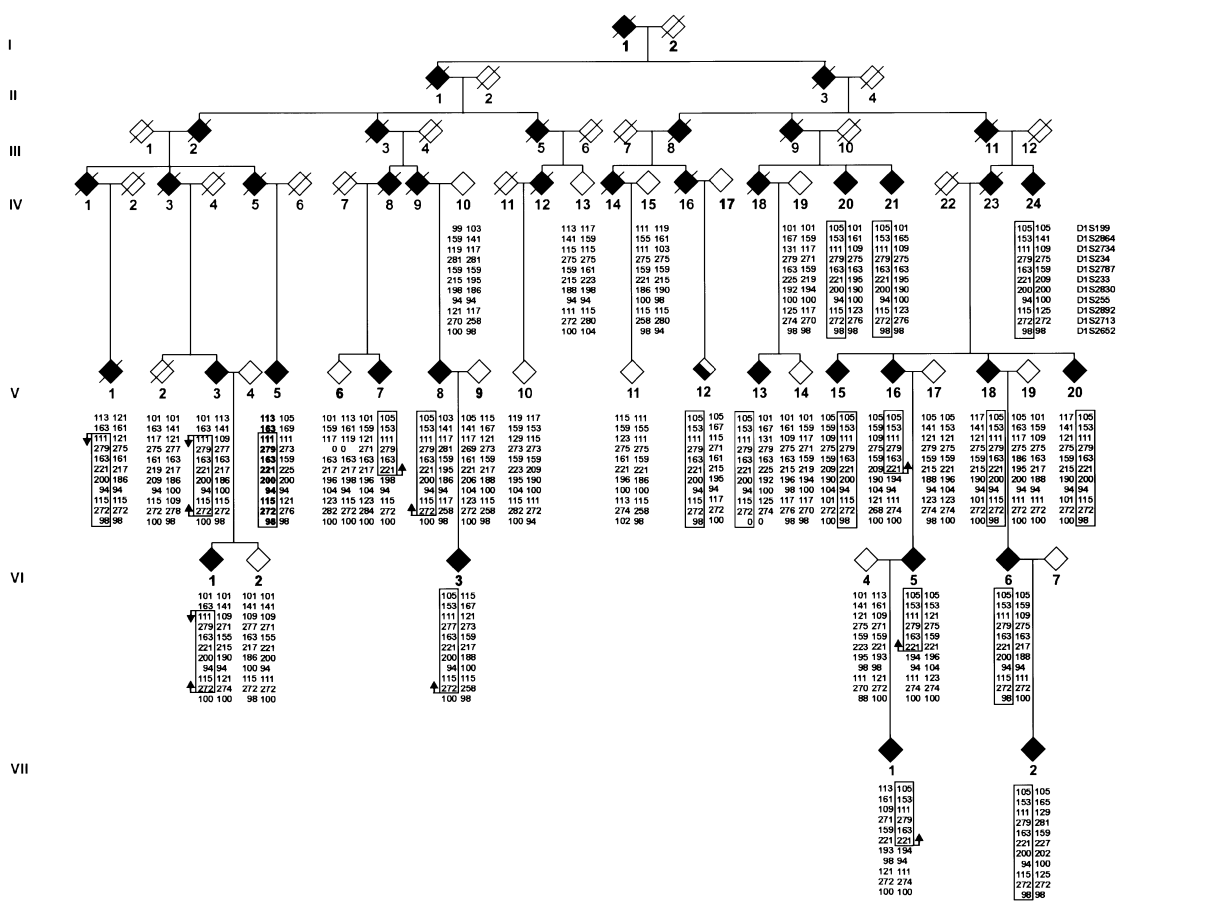
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A



B



et al. 1993), kinesin family member 1B (*KIF1B* [MIM 605995]) in CMT2A (MIM 118210) (Zhao et al. 2001), RAS-associated protein RAB7 (*RAB7* [MIM 602298]) in CMT2B (MIM 600882) (Verhoeven et al. 2003), glycyl tRNA synthetase (*GARS* [MIM 600287]) (Antonellis et al. 2003) in CMT2D (MIM 601472) (Ionasescu et al. 1996), and neurofilament light chain (*NF-L* [MIM 162280]) in CMT2E (MIM 607684) (Mersiyanova et al. 2000). So far, the genes involved in CMT2C (MIM 606071) on 12q23-q24 (Klein et al. 2003) and CMT2F (MIM 606595) on 7q11-q21 (Ismailov et al. 2001) remain to be found.

Recently, linkage was reported in two families with intermediate CMT. The first locus for dominant intermediate CMT (DI-CMT) was mapped to 10q24.1-q25.1 (DI-CMTA [MIM 606483]) in an Italian family (Verhoeven et al. 2001), and the second locus was mapped to 19p12-p13.2 (DI-CMTB [MIM 606482]) in an Australian pedigree (Kennerson et al. 2001). To date, no gene for DI-CMT has been identified. Here, we report a novel locus for DI-CMTC, mapping to chromosome 1p34-p35 in two unrelated large pedigrees from Bulgaria and the United States.

The American family (CMT-160) contained affected members in four generations (fig. 1A). Its founders originated from northeast Germany and were of German and Polish origin. The age at onset in the 15 patients was mainly in the 1st and 2nd decades. The most common initial complaints were distal leg and arm weakness and numbness. Although motor symptoms predominated, sensory signs were also prominent. Men and women were similarly affected. The motor median NCVs had a range of 30–40 m/s. Sural nerve biopsies from three generations demonstrated clusters of regenerating fibers and age-dependent reduction in fiber density and myelin thickness but no onion bulbs.

The Bulgarian family (CMT-176) contained affected members with a motor and sensory neuropathy in seven generations (fig. 1B). In all 19 patients, motor symptoms and lower limb involvement predominated. Disease onset (weakness of the feet, gait disturbance, and foot deformities) varied with an age range of 7–59 years. The clinical features showed some gender differences, with a relatively milder involvement in women. Median motor

NCVs had a range between 33 m/s and normal. We could trace the family history to 1850, when two affected siblings (II.1 and II.3) founded the two large branches of the pedigree. Some of the pedigree members (descendants from individual II.3) live in and around the town of Lom, about which a novel type of recessive inherited peripheral neuropathy was reported in Bulgarian Gypsies (HMSN-Lom [MIM 601455]) (Kalaydjieva et al. 1996). However, the members of family CMT-176 are of Bulgarian origin and have a genetically and clinically distinct CMT phenotype. The remaining part of the family lived in several towns in northwest Bulgaria.

For molecular genetic studies, genomic DNA of members of families CMT-160 and CMT-176 was extracted from peripheral blood samples by use of standard procedures. Informed consent was obtained, according to the Declaration of Helsinki and protocols approved by the institutional review boards of Sofia Medical University and St. Louis University. Initially, we excluded the CMT1A duplication/HNPP deletion and disease-causing mutations in genes that are implicated in the most common types of CMT; that is, *PMP22*, *MPZ*, *Cx32*, and *EGR2*. In addition, we excluded linkage to the CMT1A, CMT1B, CMT2D, CMT2E, DI-CMTA, and DI-CMTB loci by STR analysis (data not shown). Since simulation linkage studies with SLINK (Ott 1989; Weeks et al. 1990) indicated that families CMT-160 and CMT-176 had enough power to demonstrate significant linkage ($Z > 3.0$ at $\theta = 0.0$), we performed a genomewide search using the 382 STR markers of the ABI Prism Linkage Mapping Set, v2.5 (Applied Biosystems), spaced at average intervals of 10 cM. In both families, we detected linkage with marker D1S255 on chromosome 1p35. Additional STR markers were selected from the ABI Prism linkage mapping set HD-5 and the Marshfield chromosome 1 sex-averaged linkage map (Marshfield Center for Medical Genetics) and were genotyped (fig. 2). Several markers gave significant positive two-point LOD scores (i.e., $Z > 3.0$ at $\theta = 0.0$) in both pedigrees (table 1). The maximal cumulative LOD score ($Z_{\max} = 12.20$ at $\theta = 0.0$) was obtained at D1S233. The haplotypes were constructed with the STR alleles, according to the marker order reported by the Marshfield chromosome 1 sex-averaged linkage map and the NCBI Map Viewer (build 33). In family

Figure 1 Haplotype analysis in pedigrees CMT-160 (A) and CMT-176 (B). Pedigree structures and sex of patients were disguised to preserve anonymity. For reasons of confidentiality, we did not reveal the genotypes of the asymptomatic individuals in the youngest generations. These persons were also excluded from the linkage calculations. Patient CMT-176.V.12 was considered to have unknown disease status, since no NCVs were available. Patient CMT-176.VI.3 shared the disease-associated haplotype of family CMT-176 but had a 2-bp difference in the allele size of marker D1S234, probably owing to *Taq* DNA polymerase slippage during PCR. Markers were PCR amplified on a PTC-220 DNA Engine DYAD (MJ Research) and were pooled using a Beckman Biomek workstation. Fragment analysis was performed by capillary electrophoresis on an ABI PRISM 3700 DNA analyzer and was processed with GeneScan Analysis, version 3.5, and Genotyper, version 3.7, software (Applied Biosystems). Alleles were sized according to the CEPH control DNA (1347.02) and are shown in base pairs. STR markers are ordered from telomere (top) to centromere (bottom). Unblackened diamond = unaffected; blackened diamond = affected; half-blackened diamond = unknown status; slashed diamond = deceased; arrow = recombination event; box = disease-associated haplotype; 0 = failed genotype.

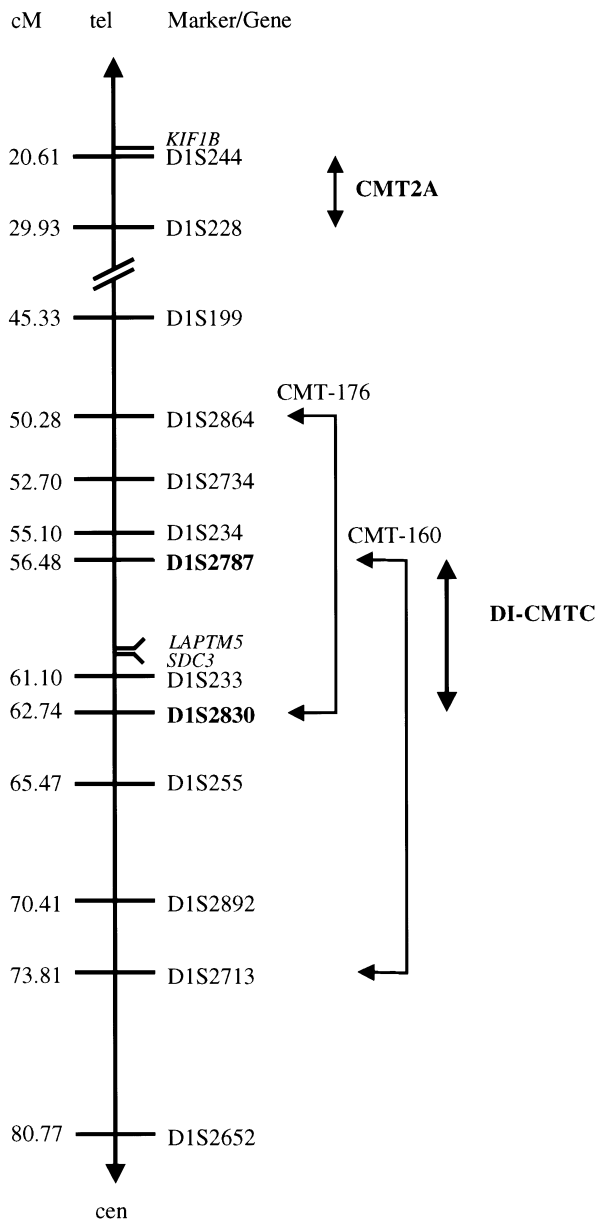


Figure 2 Sex-averaged linkage map of chromosome 1p34-p36 markers used in the present study. The genetic position of each marker (in cM) is obtained from the Marshfield chromosome 1 sex-averaged linkage map. Markers defining the DI-CMTC region are shown in boldface type. Arrows delineate the CMT2A locus, as defined by Ben Othmane et al. (1993) and Saito et al. (1997), and the DI-CMTC locus, as defined in the present study by the genetic analysis of families CMT-160 and CMT-176. *SDC3* and *LAPTM5* are positional candidate genes for DI-CMTC. *KIF1B* is the gene mutated in CMT2A (Zhao et al. 2001).

CMT-160, we found a disease-associated haplotype (227-200-100-115) with four STR markers: D1S233-D1S2830-D1S255-D1S2892 (fig. 1A). Individual CMT-160.II.9 inherited a recombinant chromosome between

markers D1S2787 and D1S233, indicating that D1S2787 is the distal flanking marker. In family CMT-176, several recombination events were found that limit the disease-associated haplotype (111-279-163-221) to four markers: D1S2734-D1S234-D1S2787-D1S233. Patients CMT-176.V.16 and CMT-176.VI.5 transmitted a chromosome recombining between markers D1S233 and D1S2830 and therefore defining D1S2830 as the proximal flanking marker. The combined analysis of key recombinants in both families showed a minimal genetic region of 6.3 cM, flanked distally by D1S2787 and proximally by D1S2830 (fig. 2). The two families do not share a common disease-associated haplotype, which indicates that the mutations underlying DI-CMTC arose independently in the families.

The DI-CMTC region on chromosome 1p34-p35 is covered by five sequenced NT contigs (NT_037485, NT_077385, NT_004538, NT_077923, and NT_004511) and contains >100 genes, according to build 33 of the NCBI MapViewer. As the positional and functional candidate gene, we first screened Syndecan 3 (*SDC3* [GenBank accession number NM_014654]), which codes for a heparan sulfate proteoglycan and is expressed during early postnatal development in the nervous tissues (Carey 1996). The *SDC3* coding region and its flanking intronic nucleotide sequences were sequenced on the genomic DNA level in four affected individuals from both families with DI-CMTC and in two normal control individuals, but no disease-associated mutations were detected (table A [online only]). However we found three coding SNPs (NCBI SNP cluster rs2491132, rs4949184, and rs1539360). Since several patients in both families were heterozygous for the SNP alleles, we do not expect genomic deletions in the *SDC3* gene. In addition, sequencing of cDNA (SuperScript first strand synthesis system for RT-PCR [Invitrogen]), obtained from transformed lymphoblast cell lines of patient CMT-176.VI.3, revealed no alternative spliced variants (table A [online only]). Furthermore, we sequenced on the genomic DNA level the Lysosomal-associated multispinning membrane protein 5 (*LAPTM5* [GenBank accession number NM_006762]) (Adra et al. 1996) gene—a positional candidate in the DI-CMTC region (table A [online only]). We observed one coding SNP and one noncoding SNP in the gene (NCBI SNP cluster rs1050663 and rs3795438); however, we found no sequence variations that cosegregate with the phenotype in both families.

The DI-CMTC locus is the second locus for CMT on the short arm of chromosome 1. It is interesting that the CMT2A locus maps on 1p35-p36 (Ben Othmane et al. 1993) and that a missense mutation in the *KIF1B* gene was found in a Japanese family with CMT2A (Zhao et al. 2001) (fig. 2). Two lines of evidence indicate that there are separate loci for DI-CMTC and CMT2A that do not overlap. First, CMT2A maps 26 cM distally to D1S2787, the flanking marker of the DI-CMTC locus.

Table 1

Two-Point LOD Scores between the Dominant Intermediate CMT Type C Locus and STR Markers on Chromosome 1p34-p36

MARKER AND FAMILY	LOD SCORE AT $\theta =$							
	.0	.001	.01	.05	.10	.20	.30	.40
D1S199:								
CMT-160	-12.30	-8.26	-4.07	-1.06	.04	.76	.77	.43
CMT-176	-5.52	.17	2.07	3.02	3.05	2.44	1.55	.68
D1S2864:								
CMT-160	-4.09	-3.68	-1.91	-.60	-.12	.22	.28	.20
CMT-176	.66	3.04	3.94	4.18	3.86	2.84	1.68	.66
D1S2734:								
CMT-160	-10.30	-6.31	-2.37	.13	.96	1.35	1.13	.58
CMT-176	6.58	6.57	6.45	5.89	5.18	3.73	2.27	.95
D1S234:								
CMT-160	-10.30	-3.31	-.38	1.41	1.91	1.95	1.50	.75
CMT-176	6.05	6.04	5.90	5.30	4.54	3.00	1.56	.50
D1S2787:								
CMT-160	2.21	2.21	2.17	2.00	1.78	1.31	.80	.30
CMT-176	4.26	4.25	4.16	3.77	3.26	2.24	1.29	.51
D1S2781:								
CMT-160	4.18	4.18	4.12	3.85	3.50	2.74	1.87	.88
CMT-176	5.96	5.95	5.87	5.44	4.86	3.60	2.28	1.03
D1S233:								
CMT-160	5.39	5.38	5.31	4.97	4.52	3.55	2.46	1.19
CMT-176	6.81	6.80	6.69	6.16	5.46	3.98	2.47	1.07
D1S2830:								
CMT-160	3.88	3.88	3.82	3.56	3.2	2.50	1.69	.77
CMT-176	-4.45	1.07	2.98	3.94	3.94	3.20	2.12	.99
D1S255:								
CMT-160	4.89	4.89	4.82	4.51	4.10	3.21	2.21	1.06
CMT-176	3.37	3.36	3.31	3.04	2.66	1.86	1.10	.47
D1S2892:								
CMT-160	5.39	5.38	5.31	4.97	4.52	3.55	2.46	1.19
CMT-176	.26	2.77	3.70	4.05	3.86	3.05	2.03	.97
D1S2713:								
CMT-160	-.60	-.31	1.32	2.41	2.61	2.35	1.72	.84
CMT-176	-.89	1.78	1.34	1.99	2.08	1.73	1.15	.54
D1S2652:								
CMT-160	-4.09	-3.84	-2.20	-.88	-.37	.02	.14	.12
CMT-176	-6.56	-5.24	-3.24	-1.32	-.63	-.15	-.04	-.02

NOTE.—Two-point linkage was performed using LINKAGE 5.1 and FASTLINK programs (Lathrop and Lalouel 1984; Cottingham et al. 1993). Six different penetrance classes were assigned, dependent on the age at onset of the disease. (For family CMT-160, age groups/mean penetrance are 0–5 years/0.07, 6–10 years/0.31, 11–15 years/0.69, 16–20 years/0.92, 21–30 years/0.98, and 31–80 years/0.99; for family CMT-176, age groups/mean penetrance are 0–10 years/0.09, 11–20 years/0.28, 21–30 years/0.56, 31–40 years/0.80, 41–50 years/0.93, and 51–80 years/0.98.) A 50% disease penetrance was reached at age 12 years in family CMT-160 and at age 24 years in family CMT-176. Autosomal dominant inheritance, equal male and female recombination rates, and a DI-CMT gene frequency of 0.0001 were assumed in the linkage calculations. For each STR marker, the number of alleles in the calculations was set at the observed allele numbers in the pedigrees (*N*), and the allele frequencies were set at 1/*N*.

Second, CMT2A is flanked by D1S244 and D1S228 in sequence contigs NT_021937 and NT_004873, and the *KIF1B* gene is contained in NT_021937, thus not overlapping with the sequence contigs in the DI-CMTC region (Ben Othmane et al. 1993; Saito et al. 1997; NCBI Nucleotides Database [build 33]). Several pedigrees have been linked to the CMT2A locus (Ben Othmane et al.

1993; Pericak-Vance et al. 1997; Saito et al. 1997; Muglia et al. 2001). It is interesting that no disease-causing mutations in *KIF1B* have been reported in these families so far. On the basis of the linkage data available in the literature, we cannot exclude the possibility that at least some families with CMT2A without *KIF1B* mutations could map to the DI-CMTC locus. Furthermore, with

regard to NCV measurements, families with CMT currently classified as having “CMT2” but with only a limited number of affected individuals could also be considered as having “intermediate CMT.”

It is interesting that several families with autosomal dominant CMT have been reported in which affected members have highly variable NCVs, ranging from normal to severely reduced. The electrophysiological phenotype thus overlaps the NCV ranges of CMT1 and CMT2. In some of these patients, specific mutations in *MPZ* (De Jonghe et al. 1999; Mastaglia et al. 1999) or *NF-L* (De Jonghe et al. 2001; Jordanova et al. 2003) were found. In addition, patients with CMTX1 with mutations in the *GJB1/Cx32* gene display variable nerve conduction. Median NCVs in affected males are in the intermediate range of 30–40 m/s, whereas median NCVs are often >40 m/s in female carriers (references in Birouk et al. [1998]). Recently, a mixed demyelinating and axonal phenotype with variable NCVs has been observed in an autosomal recessive type of CMT (CMT4A [MIM 606598]) that is due to mutations in the ganglioside-induced differentiation-associated protein 1 (*GDAP1* [MIM 214400]) (Baxter et al. 2002; Cuesta et al. 2002; Nelis et al. 2002; Boerkoel et al. 2003; Senderek et al. 2003).

In conclusion, we report a novel DI-CMT locus on 1p34-p35 in two unrelated pedigrees with patients having intermediate NCV. Mapping the DI-CMTC locus provides new evidence that intermediate CMT presents a genetically heterogeneous entity. Clinical and genetic analysis of additional families with CMT could better delineate the DI-CMT phenotype and will help to identify the disease-causing gene in DI-CMTC.

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Electronic-Database Information

URLs for data presented herein are as follows:

- Inherited Peripheral Neuropathies Mutation Database, <http://molgen-www.uia.ac.be/CMTMutations/> (for genes and mutations in CMT)
- Marshfield Center for Medical Genetics, <http://www.marshfieldclinic.org/research/genetics/> (for Marshfield Map location of markers)
- NCBI Map Viewer, <http://www.ncbi.nlm.nih.gov/mapview/> (for finding known genes, ESTs, and putative novel genes in the DI-CMTC region)
- NCBI Nucleotides Database, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide> (for NT contig numbers)
- NCBI SNP Database, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Search=snp/> (for searching for SNPs)
- Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for CMT, CMT1A, PMP22, MPZ/P0, CMT1B, LITAF/SIPMLE, CMT1C, EGR2, CMT1D, Cx32/GJB1, CMTX1, KIF1B, CMT2A, RAB7, CMT2B, GARS, CMT2D, NF-L, CMT2E, CMT2C, CMT2F, DI-CMTA, DI-CMTB, HMSN-Lom, CMT4A, and GDAP1)
- VIB Genetic Service Facility, <http://www.vibgeneticservicefacility.be/>

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