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Report

Localization of the Gene for the Intermediate Form of Charcot-Marie-Tooth to Chromosome 10q24.1-q25.1

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Intermediate Charcot-Marie-Tooth neuropathy (CMT) is an inherited sensory motor neuropathy characterized by motor median nerve conduction velocities of 25–45 m/s. We performed a genomewide search in an Italian family with autosomal dominant intermediate CMT and mapped the locus on chromosome 10q. Analysis of key recombinants maps the gene for autosomal dominant intermediate CMT to a 10.7-Mb interval on chromosome 10q24.1-q25.1, between simple tandem repeat markers D10S1709 and D10S1795.

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous disorder of the peripheral nervous system, characterized by progressive weakness and atrophy, initially of the peroneal muscles and later of the distal muscles of the arms. CMT neuropathy is subdivided into CMT1 and CMT2 on the basis of electrophysiological and neuropathological criteria (Dyck et al. 1993). CMT1 or hereditary motor and sensory neuropathy type I (HMSN I) is a demyelinating neuropathy, whereas CMT2 (or HMSN II) is an axonal neuropathy. Most patients with CMT can be classified as having either CMT1 or CMT2 by use of a cut-off value of 38 m/s for the motor median nerve-conduction velocity (NCV). However, in some families with CMT, patients have motor median NCVs ranging from 25 to 45 m/s. It has been proposed to designate this form as "intermediate CMT" (Davis et al. 1978).

Genetic-linkage studies have demonstrated that CMT1 and CMT2 are extremely genetically heterogeneous. The majority of CMT1 patients have a 1.4-Mb tandem duplication in chromosome 17p12 (CMT1A [MIM 118220]) harboring the peripheral myelin protein 22 (*PMP22* [MIM 601097]) gene (Lupski et al. 1991; Raeymaekers et al. 1991; Inoue et al. 2001). Furthermore, disease-causing mutations in at least nine genes have been identified for CMT neuropathy and related HMSN disorders: *PMP22* in CMT1A (Valentijn et al. 1992), myelin protein zero (*MPZ/P0* [MIM 159440]) in CMT1B (MIM 118200; Hayasaka et al. 1993), early growth response element 2 (*EGR2* [MIM 129010]) in CMT1 (Warner et al. 1998); connexin 32 (*Cx32/GJB1* [MIM 304040]) in CMT1X (MIM 304040; Bergoffen et al. 1993); myotubularin related protein 2 (*MTMR2* [MIM 603557]) in CMT4B (MIM 601382; Bolino et al. 2000), N-myc downstream regulated gene 1 (*NDRG1* [MIM 605262]) in HMSN-LOM (MIM 601455; Kalaydjieva et al. 2000), periaxin (*PRX* [MIM 605725]) in CMT4F (MIM 145900; Boerkoel et al. 2001), kinesin family member 1B (*KIF1B* [MIM 605995]) in CMT2A (MIM 118210; Zhao et al. 2001); and neurofilament light chain (*NEFL* [MIM 162280]) in CMT2E (MIM 162280; Mersiyanova et al. 2000). The mutations and the corresponding phenotypes are summarized in the Inherited Peripheral Neuropathy Mutation Database. Also, other loci harboring currently unknown gene defects have been identified for CMT1 and CMT2 (De Jonghe et al. 2000). So far, autosomal dominant intermediate CMT has not been mapped in the human genome. Interestingly, in some affected families with very variable NCVs ranging from normal to severely reduced, specific mutations in the *MPZ* gene have been described (De Jonghe et al. 1999). A similar phenotype is associ-

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ated with mutations in *NEFL* (De Jonghe et al. 2001). Interestingly, mutations in the *Cx32* gene causing CMT1X lead to an intermediate type of CMT in male patients, whereas female mutation carriers often have normal NCVs (Nicholson and Nash 1993; Timmerman et al. 1996; Birouk et al. 1998).

We previously studied a large Italian family diagnosed with autosomal dominant intermediate CMT. Detailed clinical and neurophysiological studies of this family have been reported (Rossi et al. 1985; Villanova et al. 1998). In brief, this type of CMT is clinically characterized by difficulties in walking on the heels from age 6 years. In the second decade of life, patients experience weakness in the lower limbs, with difficulties in running. From 25 to 30 years of age, the disease progresses slowly but steadily. From 40 to 50 years, the disease progresses very rapidly, and patients develop severe weakness and atrophy of distal leg and intrinsic hand muscles, steppage gait, and pes cavus. Later in life, the disease seems to stabilize. After age 70 years, crutches are needed to walk. However, none of our patients is wheelchair bound. Neurophysiological studies performed on 10 affected family members, with ages ranging from 11 to 72 years, show motor median NCVs between 25 and 45 m/s. NCV slowing is proportionate to the severity of the clinical deficits. A peripheral nerve biopsy shows the simultaneous presence of demyelinating features, such as onion-bulb formations and uncompacted enlarged myelin lamellae, and signs of chronic axonal degeneration with regeneration, such as large fiber loss and regeneration clusters (Malandrini et al., in press).

In this Italian family with autosomal dominant intermediate CMT, we previously excluded the CMT1A tandem duplication on chromosome 17p12, and no

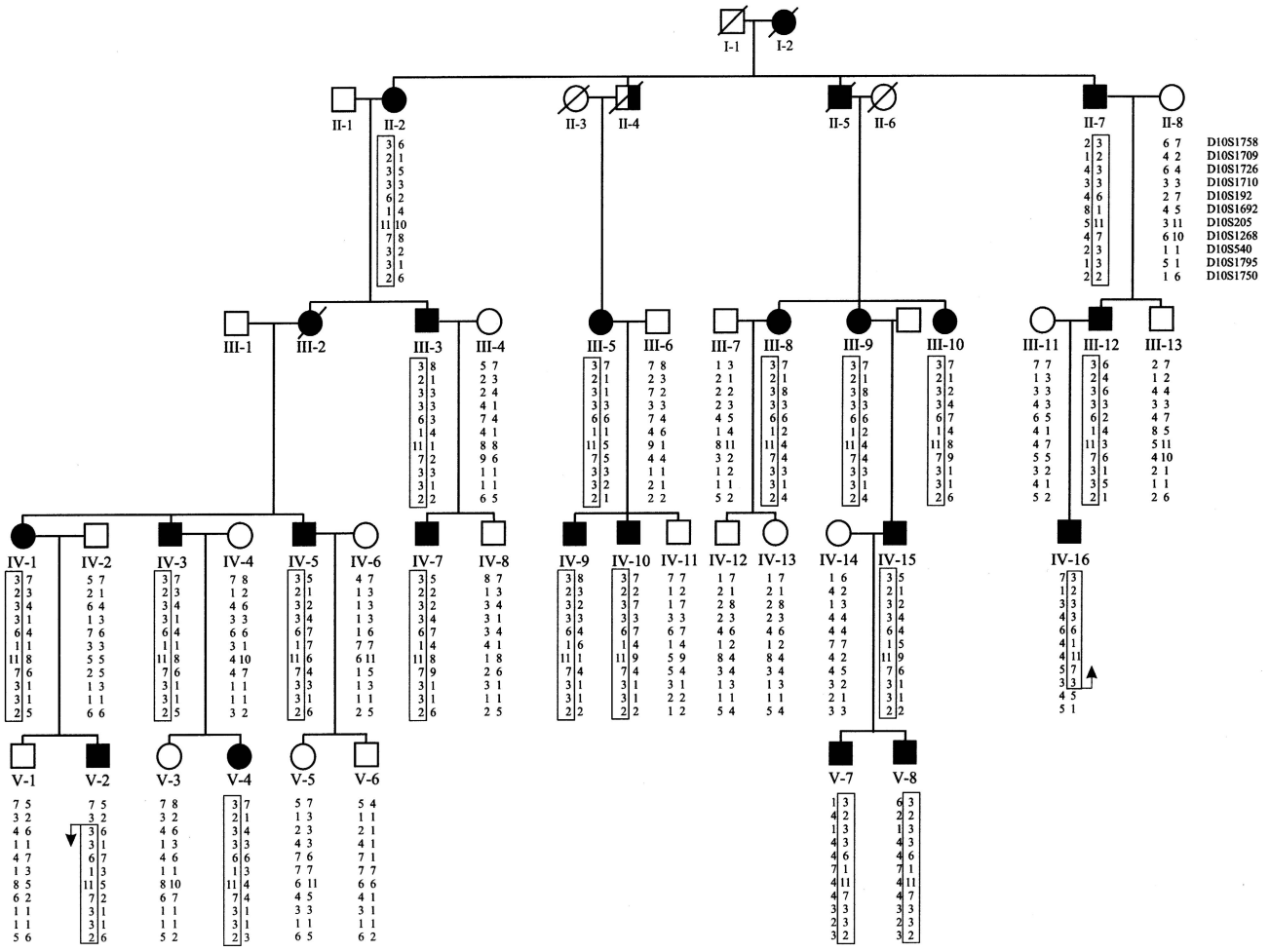

Figure 1 Linkage pedigree of the Italian family with autosomal dominant intermediate CMT (Rossi et al. 1985), showing the most likely haplotypes for the chromosome 10q markers. The haplotype segregating with the disease is boxed. Blackened symbols represent affected individuals, unblackened symbols represent unaffected individuals, and half-blackened symbols represent individuals with unknown disease status. Arrows indicate recombinants.

Table 1

Two-Point LOD Scores between the Intermediate CMT Locus and Chromosome 10q Markers

	LOD AT θ =							
MARKER	.00	.001	.01	.05	.10	.20	.30	.40
D10S1758	1.94	4.83	5.77	5.94	5.56	4.39	2.97	1.42
D10S1709	−.60	2.33	3.23	3.46	3.16	2.21	1.17	.36
D10S1726	1.05	2.83	3.76	4.13	3.97	3.21	2.17	1.01
D10S1710	−2.32	−.35	.59	1.11	1.18	1.01	.72	.37
D10S192	.28	2.21	3.15	3.57	3.46	2.80	1.88	.86
D10S1692	4.20	4.19	4.11	3.77	3.33	2.42	1.49	.62
D10S205	1.65	3.59	4.51	4.83	4.60	3.68	2.47	1.13
D10S1268	1.89	3.83	4.74	5.05	4.80	3.84	2.57	1.18
D10S540	−1.24	.72	1.65	2.08	2.03	1.60	1.03	.44
D10S1795	−4.46	−.68	1.21	2.20	2.27	1.72	.92	.27
D10S1750	−5.54	−2.22	−.27	.93	1.25	1.20	.84	.40

mutations were found in the peripheral myelin genes *PMP22* and *MPZ*. Furthermore, we performed genetic linkage studies and excluded all known CMT1 and CMT2 loci (Villanova et al. 1998). Here, we report the results of a genomewide scan.

The family contains a total of 38 individuals, 20 of whom are affected (fig. 1). Genomic DNA of all 38 family members was isolated and purified from peripheral blood samples using standard techniques. The study was approved by the institutional review boards at the Universities of Siena and Bologna (Italy) and Antwerp (Belgium), and informed consent was obtained from all family members. A genomewide scan was performed using all 400 markers of the ABI Prism Linkage Mapping Set MD-10 (PE Biosystems), which have an average intermarker distance of 10 cM. PCR products were amplified on an MJ Research PTC 200 thermocycler, were pooled using a Beckman Biomek 2000 workstation, and were loaded on an ABI 3700 automated sequencer (PE Biosystems). Data were analyzed by means of ABI Genescan 3.1 and ABI Genotyper 2.1 software. Linkage analysis was carried out using the Linkage 5.1 program package and the Fastlink computer program (Lathrop and Lalouel 1984; Cottingham et al. 1993). Mlink two-point linkage analysis was performed between the disease gene and each simple tandem repeat (STR) marker. Intermediate CMT was coded as a 90% penetrant autosomal dominant trait with a gene frequency of .0001. Equal recombination rates between males and females were assumed. For each STR marker, the number of alleles in the LOD score calculations was set at the observed number of alleles in the pedigree (*N*), and the allele frequencies were set at 1/*N*. No markers of the ABI linkage panel gave LOD scores >2.5, except for marker D10S192, localized on the long arm of chromosome 10. With this STR marker, a significant LOD score of 3.15 at $\theta = .01$ was obtained (table 1). Therefore, additional markers spanning this region were selected from the Genome Database (fig. 2). The two-point LOD scores obtained with these markers are shown in table 1. Several markers gave LOD scores >4.

The most likely disease haplotype was constructed (fig. 1). In all affected family members, a linked haplotype was found. Two key recombinants were identified. In patient IV-16, a recombination was present between markers D10S540 and D10S1795, placing the intermediate CMT gene centromeric of marker D10S1795. Another recombination event occurred in patient V-2, between markers D10S1709 and D10S1726. Combining the information of these two recombinants places the intermediate CMT gene in a 10.7-Mb region (Human Genome Browser) between markers D10S1709 and D10S1795. Finally, to find other chromosome 10q24.1-25.1 linked families, we performed a genetic linkage study in four large families compatible with intermediate CMT or CMT2 and were able to exclude the chromosome 10q locus in these families (data not shown).

The 10.7-Mb critical region has been almost completely sequenced, and >70 genes have been mapped (Human Genome Browser) between D10S1709 and D10S1795. Therefore, a refinement of the candidate region will be required before a systematic mutation screening of the genes located in the region can be undertaken. As a start, we selected the internexin neuronal intermediate filament protein alpha (*INA* [MIM 605338]) gene (GenBank accession number NM_032727) as the most obvious functional candidate gene for intermediate CMT. Alpha-internexin is a type IV intermediate filament protein that is expressed abundantly in neurons during development of the peripheral and central nervous system. It has been suggested that alpha-internexin may play a role in stabilizing small-diameter axons and may act as a scaffold on which the neurofilament-light, -medium (NEFM [MIM 162250]), and -high (NEFH [MIM 162230]) proteins coassemble during development (Ching et al. 1999; Julien 1999). Unexpectedly, overexpression of (as well as the absence of) alpha-internexin does not seem to interfere with normal axonal growth (Levavasseur et al. 1999). As the effects of missense and nonsense mutations in alpha-internexin have not been investigated, and mutations in the *NEFL* gene have been shown to cause CMT type 2E (CMT2E), *alpha-internexin* still seemed to be a good candidate gene for intermediate CMT. However, mutation analysis of the coding sequence of the *alpha-internexin* gene did not reveal any disease-causing mutations (data not shown). Since chromosome 10q has many genes associated with neuronal function, other possible candidate genes are under investigation for mutation analysis, such as *ACTR1A* (*actin-related protein 1* [MIM 605143]) and *NEURL* (*neuralized Drosophila-like* [MIM 603804]).

The autosomal dominant intermediate CMT in this Italian family is the third locus for an inherited periph-

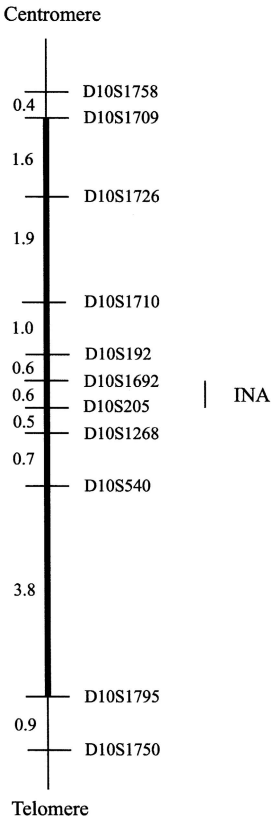

Figure 2 Genetic map of the chromosome 10q region containing the intermediate CMT locus (Human Genome Browser). Physical distances between the markers are given in megabases. The intermediate CMT region is indicated by the thick black line. The *INA* gene is located between markers D10S1692 and D10S205.

eral neuropathy that has been located on the long arm of chromosome 10. Previously, the *EGR2* gene, a transcription factor with a key role in peripheral nerve myelination, was located on 10q21.1-q22.1 (Warner et al. 1998). Mutations in the *EGR2* gene have been shown to cause congenital hypomyelinating neuropathy (CHN [MIM 605253]), CMT1, and Dejerine-Sottas Syndrome (DSS [MIM 145900]) (Warner et al. 1998; Timmerman et al. 1999). Recently, a novel locus for a recessively inherited form of CMT, named "hereditary motor and sensory neuropathy–Russe" (HMSN-R [MIM 605285]), was located on 10q23.2. It is a severe disabling form of CMT with prominent sensory loss, moderately reduced motor NCV, and a high threshold for electrical nerve stimulation (Rogers et al. 2000). However, exclusion of the intermediate-CMT locus from both regions is supported by the physical map of the region which places the HMSN-R locus ± 30 cM centromeric of the proximal boundary of the intermediate-CMT locus.

The term "intermediate CMT" was initially proposed by Davis et al. (1978) but never gained general acceptance. They distinguished three types of CMT: a hypertrophic form with motor median NCVs <25 m/s, an intermediate form with NCVs ranging from 25 to 45 m/s, and a neuronal form with NCVs >45 m/s. Intermediate NCVs are often observed in X-linked CMT1 with *Cx32* mutations (Nicholson and Nash 1993; Timmerman et al. 1996; Birouk et al. 1998). Presumably, the intermediate group of Davis et al. (1978) included mainly male patients with CMT1X, since the male:female ratio in this group was almost 2:1. The existence of an X-linked form of CMT1, however, was not generally accepted at that time (Harding and Thomas 1980). Furthermore, intermediate NCVs are also found in patients with a specific mutation in the *MPZ* gene (Thr124Met) or the *NEFL* gene (Pro8Arg) (De Jonghe et al. 1999, 2001). Our Italian family with CMT and intermediate NCVs is, however, clearly autosomal dominant, and no mutations were found in the *MPZ* and *NEFL* genes.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Human Genome Browser, http://genome.ucsc.edu/goldenPath/septTracks.html

Inherited Peripheral Neuropathy Mutation Database, http://molgen-www.uia.ac.be/CMTMutations/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CMT1A [MIM 118220], *PMP22* [MIM 601097], *MPZ/P0* [MIM 159440], CMT1B [MIM 118200], *EGR2* [MIM 129010], *Cx32/GJB1* [MIM 304040], CMT1X [MIM 304040], *MTMR2* [MIM 603557], CMT4B [MIM 601382], *NDRG1* [MIM 605262], HMSN-LOM [MIM 601455], *PRX* [MIM 605725], CMT4F [MIM 145900], *KIF1B* [MIM 605995], CMT2A [MIM 118210], *NEFL* [MIM 162280], CMT2E [MIM 162280], *INA* [MIM 605338], NEFM [MIM 162250], NEFH [MIM162230], CHN [MIM 605253], DSS [MIM 145900], HMSN-R [605285], ACTR1A [MIM 605143], and *NEURL* [MIM 603804])

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