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Localization of the Mutation in an Extended Family with Charcot-Marie-Tooth Neuropathy (HMSN I)

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Summary

Hereditary motor and sensory neuropathy type I (HMSN I) or Charcot-Marie-Tooth (CMT) disease is an autosomal dominant peripheral neuropathy. In some CMT families linkage has been reported with either the Duffy blood group or the APOA2 gene, both located on chromosome 1q. More recently, linkage has been found in six CMT families with two chromosome 17p markers. We extensively analyzed a multigeneration Charcot-Marie-Tooth family by using molecular genetic techniques in order to localize the CMT gene defect. First, we constructed a continuous linkage group of 11 chromosome 1 markers and definitely excluded chromosome 1 as the site of mutation. Second, we analyzed the family for linkage with chromosome 17. The two-point lod scores obtained with D17S58 and D17S71 proved that this Charcot-Marie-Tooth family is linked to chromosome 17. Moreover, multipoint linkage results indicated that the mutation is most likely located on the chromosome 17p arm, distal of D17S71.

Introduction

Hereditary motor and sensory neuropathy (HMSN) is a heterogeneous group of peripheral neuropathies comprising seven different clinical subtypes. In general, the disease is characterized by atrophy and weakness of distal muscles and nerves (Dyck 1984). The most prominent clinical form, HMSN type I or Charcot-Marie-Tooth (CMT) disease, is inherited as an autosomal dominant trait. CMT disease is associated with reduced nerve conduction velocities, pes cavus, depressed tendon reflexes, and segmental de- and remyelination of peripheral nerve trunks followed by "onion bulb" formation. The prevalence of CMT disease has been estimated as 5–40/100,000 (Skre 1974; Kurtzke 1984).

A first indication for the existence of a CMT locus on chromosome 1 came from positive linkage results obtained with the Duffy blood group (FY) in two CMT families (Bird et al. 1982). Linkage with FY was later confirmed by other investigators (Guiloff et al. 1982; Stebbins and Conneally 1982; Dyck et al. 1983). The FY blood group has been assigned to the centromeric part of the long arm of chromosome 1 by genetic linkage to the α-spectrin gene (Raeymaekers et al. 1988*b*, 1989*b*). Furthermore, physical and linkage analyses with polymorphic chromosome 1 loci indicated that the Duffy-linked CMT locus is on the proximal long arm of chromosome 1 (Lebo et al., in press).

However, in several other CMT families negative linkage relationships were observed between CMT and FY, suggesting genetic heterogeneity (Bird et al. 1983; Dyck et al. 1983; Ionasescu et al. 1987; Griffiths et al. 1988; Middleton-Price et al. 1989). Families in which the disease could not be linked to the FY locus were designated HMSN Ia; those in which the disease was linked to the FY locus were designated HMSN Ib. Nonallelic heterogeneity implies the existence of another locus on chromosome 1 or on any other autosome. The involvement of other chromosome 1 loci in the genetics of CMT has been suggested. Ionasescu et al. (1988) found close

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linkage to the APOA2 gene in four CMT families, formerly excluded for linkage with the FY locus. However, the presence of an APOA2-linked CMT locus on proximal 1q has not yet been confirmed. We reported suggestive linkage with the chromosome 1p loci D1S2 and D1S22. Linkage to this region of chromosome 1 was subsequently disproved by multipoint linkage mapping (Raeymaekers et al., in press-*a*).

More recently linkage of six CMT families with chromosome 17 has been proved (Vance et al. 1989). Although conclusive linkage results were obtained with the marker loci D17S71 and D17S58, which are 3 cM apart, the position of the CMT gene relative to the marker loci could not be determined. Since both markers are located in the pericentromeric region of chromosome 17p, the disease gene could still be located on either the short or long arm of chromosome 17.

We described a 5-generation CMT family comprising 105 family members, including 42 sampled patients with a diagnosis consistent with the HMSN I phenotype, e.g., slow motor-nerve conductions and large peripheral nerves. Linkage of the disease to FY and to the α-spectrin gene was rejected, indicating that the neuropathy in this family belongs to the HMSN Ia subtype (Raeymaekers et al. 1988*a*). In addition, linkage to six other chromosome 1 genes was found to be unlikely (Raeymaekers et al. 1989*a*).

In the present study, we constructed a continuous linkage group of 11 marker loci, encompassing 310 cM of the sex-average chromosome 1 map. Multipoint linkage analyses completely excluded chromosome 1 as the site of mutation. Furthermore, we examined linkage with chromosome 17. Two-point and multipoint linkage results indicated that the CMT gene in this family is most likely located on chromosome 17p.

Material and Methods

DNA Analysis

Blood samples were obtained from 105 family members, including 42 patients. DNA was extracted from white blood cells, digested with various restriction enzymes, transferred by Southern blotting on Hybond™ N membranes (Amersham) and hybridized according to a method described elsewhere (Raeymaekers et al. 1988*a*).

Plasmid DNA was prepared using a modified alkaline extraction procedure (Birnboim and Doly 1979). Recombinant phage DNA was isolated according to the plate lysate method of Maniatis et al. (1982). Hybridization probes were obtained by radioactive labeling of total plasmid or phage DNA by nick-translation with α-[^{32}P]dCTP. In the case of the FUCA1 and the pA10–41 probes, insert DNA was recovered by low-melting-point agarose-gel electrophoresis and labeled by oligo-priming.

FY Analysis

The FY phenotypes were determined by an indirect anti-globulin test described elsewhere (Raeymaekers et al. 1988*a*). Allele frequencies are from Race and Sanger (1975).

Linkage Analysis

Two-point and multipoint analyses were performed using the programs MLINK and LINKMAP of the computer package LINKAGE (Lathrop et al. 1984, 1985). We assumed both a CMT gene frequency of 1/10,000 and equal male and female recombination rates. Haldane's (1919) mapping functions were applied to convert recombination fractions into genetic distances.

Results

Exclusion of Chromosome 1

From the genetic maps of Donis-Keller et al. (1987) and Dracopoli et al. (1988) we selected 10 chromosome 1 markers; 9 mutually linked markers, viz. D1S47 through D1S48; and D1S68. In order to obtain a continuous chromosome 1 linkage group we analyzed the human minisatellite probe pMS32 (D1S8), physically localized to the distal 1q42-q43 chromosomal region (Royle et al. 1988). Multipoint linkage analysis indicated that the most probable location of D1S8 is between D1S48 and D1S68, bridging the gap between both linkage groups (Raeymaekers et al., in press-*b*). The 11 selected chromosome 1 markers are forming together a single linkage group of 310 cM on the sex-average map, spanning virtually the whole chromosome. The relative order of the 11 marker loci and the intermarker-distances are indicated on the X-axis of figure 1.

The two-point lod scores between the CMT locus and most of the 11 selected markers have previously been published (Raeymaekers et al. 1988*a*, 1989*a*, and in press-*a*). Six multipoint linkage analyses were carried out in order to exclude the CMT gene from chromosome 1. The marker sets used in the different analyses are given in table 1. In the consecutive sets of calculations one marker locus was held in common. In each marker interval the maximal lod score remains

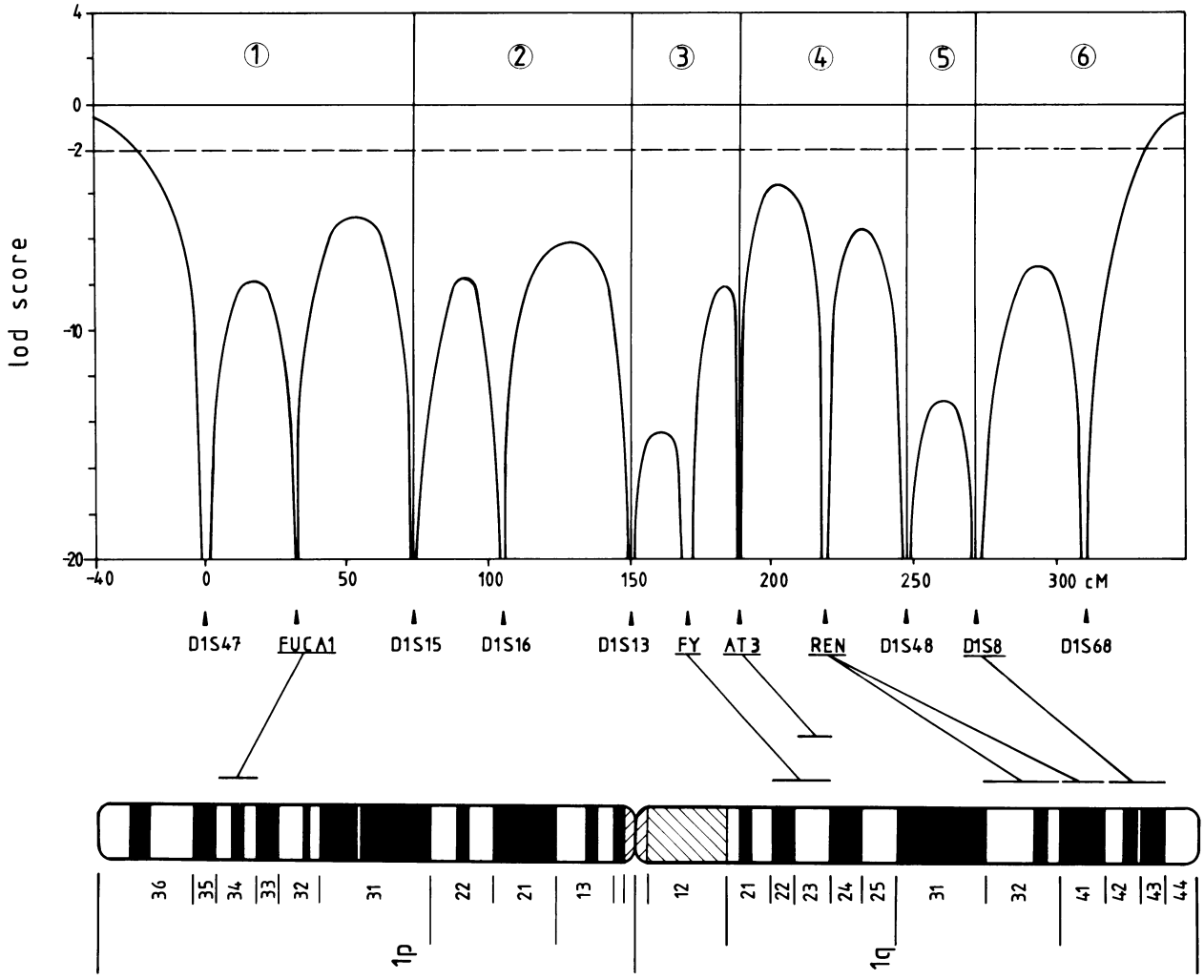

Figure 1 Exclusion of chromosome 1 as the site of mutation in the CMT family analyzed. Numbers 1–6 refer to the different LINKMAP analyses described in table 1. Chromosomal localizations indicated are from Sherman and Bruns (1988) and Royle et al. (1988).

Table 1

Multipoint Linkage Analyses in the Exclusion of Chromosome 1

LINKMAP Number	Marker Locus	Genetic Distance (cM)	Marker Locus	Genetic Distance (cM)	Marker Locus
1	D1S47	33.5	FUCA1	43.1	D1S15
2	D1S15	33.7	D1S16	42.9	D1S13
3	D1S13	19.0	FY	17.0	AT3
4	AT3	27.2	REN	27.9	D1S48
5	D1S48	22.0	D1S8		
6	D1S8	43.8	D1S68		

Table 2

Two-Point Lod Scores for CMT and Chromosome 17

Marker Locus	θ								Peak Lod Score Z at θ	
	.0	.001	.01	.05	.1	.2	.3	.4		
D1S71.....	5.26	5.26	5.20	4.78	4.27	3.18	2.02	.87	5.26	.0
D1S58.....	$-\infty$	3.40	8.11	10.50	10.58	9.02	6.49	3.32	10.67	.08

Note.—Probe pA10-41 (D17S71) detects polymorphisms with the restriction enzymes *Msp*I and *Pvu*II, and probe pEW301 (D17S58) does so with *Taq*I and *Bgl*II. Haplotype frequencies were calculated from those observed in 19 married-in individuals in the CMT family.

beneath the exclusion limit −2 (Morton 1955), excluding the presence of the CMT gene over a genetic distance of 365 cM of chromosome 1 (fig. 1).

Linkage with Chromosome 17

Two-point lod scores between the CMT locus and the marker loci D17S71 (pA10–41) and D17S58 (pEW301) were calculated at different recombination fractions (θ's) and are listed in table 2. Conclusive linkage results were obtained for both marker loci with peak lod scores of 10.67 (θ = .08) for D17S58 and 5.26 (θ = .0) for D17S71. No recombinations were observed for D17S71, a result suggesting that the CMT gene is most likely located closer to this marker.

Both D17S71 and D17S58 were localized in the pericentromeric region of chromosome 17p, separated by a θ of .03. Genetical and physical map information demonstrated that D17S58 is closer to the centromere than is D17S71 (Fain et al. 1987; Goldgar et al. 1989). We performed a multipoint linkage analysis in order to determine the position of the CMT gene relative to both marker loci (fig. 2). Peak lod scores of 12.61 and 11.36, respectively, were obtained distal and proximal of D17S58. The peak-lod-score difference of 1.25 indicates that the CMT gene is most likely located distal of D17S71 on chromosome 17p.

Discussion

The localization of genes by exclusion mapping was first applied by Cook et al. (1980) in an attempt to locate the MNSs blood group. Since then, exclusion maps have been published for cystic fibrosis (Wainwright et al. 1986), Friedreich ataxia (Chamberlain et al. 1987), and von Recklinghausen neurofibromatosis (Sarfarazi et al. 1987).

We attempted to localize the gene for CMT disease in an extended, multigeneration pedigree by exclusion mapping. Since linkage of CMT was first reported with chromosome 1, we constructed a continuous linkage group of 11 loci and definitely excluded chromosome 1 as the site of the mutation. Moreover, the exclusion map demonstrated that this one family had enough genetic potential to finally prove linkage for CMT. Regions of chromosomes 3, 5, 10, 19, and 21 were subsequently excluded (data not shown).

In the course of the present study, evidence for the existence of a CMT locus on chromosome 17 was reported (Vance et al. 1989). Therefore, we examined

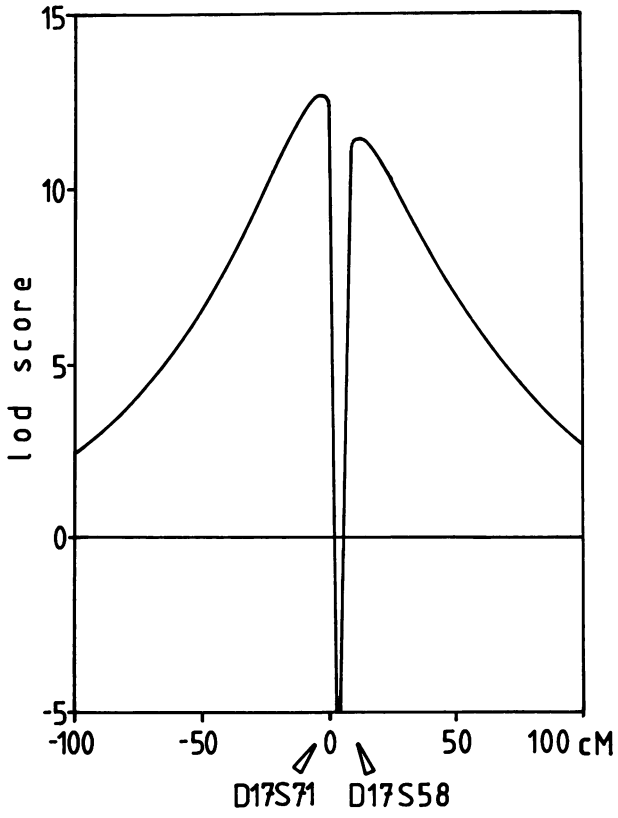

Figure 2 Multipoint localization of the CMT gene. The marker loci D17S71 and D17S58 were fixed at θ = .03.

linkage of the CMT family with the marker loci D17S71 and D17S58 and obtained conclusive lod scores, confirming the linkage results of Vance et al. (1989). Next, we determined the relative position of the CMT gene by multipoint linkage analysis. The mapping data suggested that the CMT gene is most likely located distal of D17S71 on chromosome 17p. Although our results are significant at the 95% confidence level (Conneally et al. 1986), this preliminary CMT gene localization needs to be confirmed. Information supporting the 17p localization can be gained from the genetic analysis of the CMT family by additional chromosome 17p markers.

The knowledge of the exact localization of the CMT gene will be important not only for the isolation and identification of the CMT gene but also for prenatal and presymptomatic diagnosis of affected individuals.

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