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Genetic Mapping of Autosomal Dominant Charcot-Marie-Tooth Disease in a Large French-Acadian Kindred: Identification of New Linked Markers on Chromosome 17

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Summary

We have performed linkage analysis in a large French-Acadian kindred segregating one form of autosomal dominant Charcot-Marie-Tooth disease (CMTD) (type IA) using 17 polymorphic DNA markers spanning human chromosome 17 and demonstrate linkage to several markers in the pericentromeric region, including DNA probes pA10-41, EW301, S12-30, pTH17.19, c11-2B, and p11-2c11.5. Linkage of markers pA10-41 and EW301 to CMTD type IA has been reported elsewhere. Four new markers, 1516, 1517, 1541, and LL101, which map to chromosome 17 have been identified. The marker 1516 appears to be closely linked to the CMTD locus on chromosome 17 as demonstrated by a maximum lod score of 3.42 at $\hat{\theta}$ (recombination fraction) = 0. This marker has been mapped to 17p11.2 using a somatic cell hybrid constructed from a patient with Smith-Magenis syndrome [46,XY, del(17)(p11.2p11.2)]. A lod score of 6.16 has been obtained by multipoint linkage analysis with 1516 and two markers from 17q11.2, pTH17.19, and c11–2B. The markers 1517 and 1541 have been mapped to 17p12-17q11.2 and demonstrate maximum lod scores of 2.35 and 0.63 at recombination values of .1 and .2, respectively. The marker LL101 has been mapped to 17p13.105-17p13.100 and demonstrates a maximum lod score of 1.56 at a recombination value of .1. Our study confirms the localization of CMTD type IA to the pericentromeric region of chromosome 17.

Introduction

The hereditary motor and sensory neuropathies (HMSN) are a clinically heterogeneous group of genetic disorders of the peripheral nerves with both motor- and sensory-nerve involvement. Differences in the patterns of inheritance, natural history, and clinical, neurophysiological, and pathological features have been demonstrated among kinships with HMSN. On the basis of these differences, Dyck has recognized at least seven types of HMSN (Dyck and Lambert 1968*a*, 1968*b*). Charcot-Marie-Tooth disease (CMTD), which is the most common inherited peripheral neuropathy, with a prevalence rate of 40/100,000, is one type of HMSN (Skre 1974; Charcot and Marie 1886). This disease is characterized by a progressive muscular atrophy that primarily affects the distal muscles, with muscles innervated by the peroneal nerve usually being the most severely affected. Autosomal dominant, recessive, and X-linked forms of CMTD have been reported, with the autosomal dominant form being the most prevalent (McKusick 1988). On the basis of electrophysiological and anatomical findings, two clinical types of autosomal dominantly inherited CMTD are distinguishable: type I, the hypertrophic form, has uniformly slow nerve-conduction velocity (NCV) with pronounced hypertrophy of the peripheral nerve, whereas type II, the neuronal form, has normal or near normal NCV with less hypertrophic change, less severe weakness, and less frequent loss of sensory function.

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The molecular basis of CMTD is currently unknown. Genetic mapping and linkage analysis can provide an objective basis for clarifying the various subtypes of CMTD, allow for a better assessment of nonallelic and allelic heterogeneity, and permit accurate diagnosis of the disease. An X-linked form of CMTD has been localized to chromosome Xq13 by using a number of polymorphic DNA markers (Beckett et al. 1986). Linkage studies have led to a subclassification of clinically defined type I CMTD. One form of autosomal dominant CMTD has been linked to the Duffy locus on chromosome 1q and has been termed type IB. A cumulative lod score (Z) of 6.06 at a recombination fraction (θ) of .1, from three separate studies (Bird et al. 1982; Guiloff et al. 1982; Stebbins and Conneally 1982) was considered significant evidence for this linkage. Subsequent studies (Bird et al. 1983; Dyck et al. 1983; Griffiths et al. 1988; Raeymaekers et al. 1988; Middleton-Price et al. 1989) have demonstrated the existence of pedigrees where there is no demonstrable linkage of autosomal dominant CMTD to the Duffy locus; this type has been termed type IA and appears to be the most prevalent form of the disease. Recently, Vance et al. (1989) have reported linkage of six CMTD type IA families to the markers EW301 (D17S58) and A10-41 (D17S71) located on the proximal short arm of chromosome 17. Thus, there appear to be at least two independent genetic loci responsible for autosomal dominant CMTD.

In order to localize the autosomal dominant CMTD type I gene, our efforts have focused on the analysis of a single, large French-Acadian pedigree. This strategy avoids the problems posed by interfamilial genetic heterogeneity, since it is likely that a single mutation is responsible for the phenotype in a given family. We demonstrate in the present report that CMTD in this pedigree is linked to several previously reported pericentromeric chromosome 17 markers, including pA10-41 (D17S71), EW301 (D17S58), and to four new genetic markers, 1516, 1517, 1541, and LL101. The marker 1516 is tightly linked to CMTD and is shown to map to 17p11.2 by using a somatic cell hybrid panel.

Material and Methods

Subjects

Fifty-three members of a 299-person French-Acadian pedigree exhibiting autosomal dominant CMTD were studied (fig. 1). The affection status of each individual was determined by demonstration of difficulty walking on their heels, leg or hand muscle atrophy, absent stretch reflexes, and, in some patients, presence of pes cavus and enlarged auricular, ulnar, or peroneal nerves. NCVs in most of the patients were <30 m/s. Sural nerve biopsy done in selected patients showed hypertrophic neuropathy. With informed consent, blood was collected from each participating family member and was used to establish Epstein-Barr virus–transformed lymphoblasts (Anderson and Gusella 1984) and for isolation of high-molecular-weight DNA (Miller et al. 1988).

DNA Probes

The probes used in the present study, their chromosomal location, the restriction enzyme displaying polymorphism, and their sources are listed in table 1. Cosmids 1516, 1517, and 1541 were isolated from a library constructed from MH22–6, a hybrid cell line retaining chromosome 17 as the only human chromosome (vanTuinen et al. 1987; Y. Nakamura, unpublished data); the phage, LL101, was isolated from a flow-sorted chromosome 17 *Not*I linking library (Wallace et al. 1989).

Somatic Cell Hybrids and Cell Lines

The rodent parent of each hybrid cell is clone 1D, a thymidine kinase–deficient mouse fibroblast cell line. The hybrid cell lines are depicted in figure 2. All hybrid cell lines except DH110-D1 have been described elsewhere (vanTuinen et al. 1987, 1988). The hybrid cell line, DH110-D1, retaining a del(17)(p11.2p11.2) chromosome was constructed by fusion of clone 1D cells to lymphoblasts from a patient with Smith-Magenis syndrome—46,XY, del (17) (p11.2p11.2) (Smith et al. 1986)—by the method of Su et al. (1984).

Southern Analysis

Thirty-two members of pedigree 1 (see fig. 1) were selected for linkage analysis. These included 22 affected individuals and their first-degree relatives, who were selected to provide the maximum linkage information. For Southern analysis, 5 μg of DNA from each of these 32 members was digested for ≥4 h with 3–4 units of restriction enzyme/μg. Samples were electrophoresed in Tris-acetate agarose gels with buffer recirculation. Gels were denatured in two changes of 0.4 N NaOH and transferred to Genescreen Plus (NEN) membrane in 10 × SSC. Entire cosmids and inserts from plasmids separated in low-melting-temperature agarose were labeled by the method of Feinberg and Vogelstein (1983). Free nucleotides were separated by precipitation of nucleic acid with 10 mM spermine (Wallace 1987). Blots were prehybridized and hybridized in 1 M NaCl, 1% SDS,

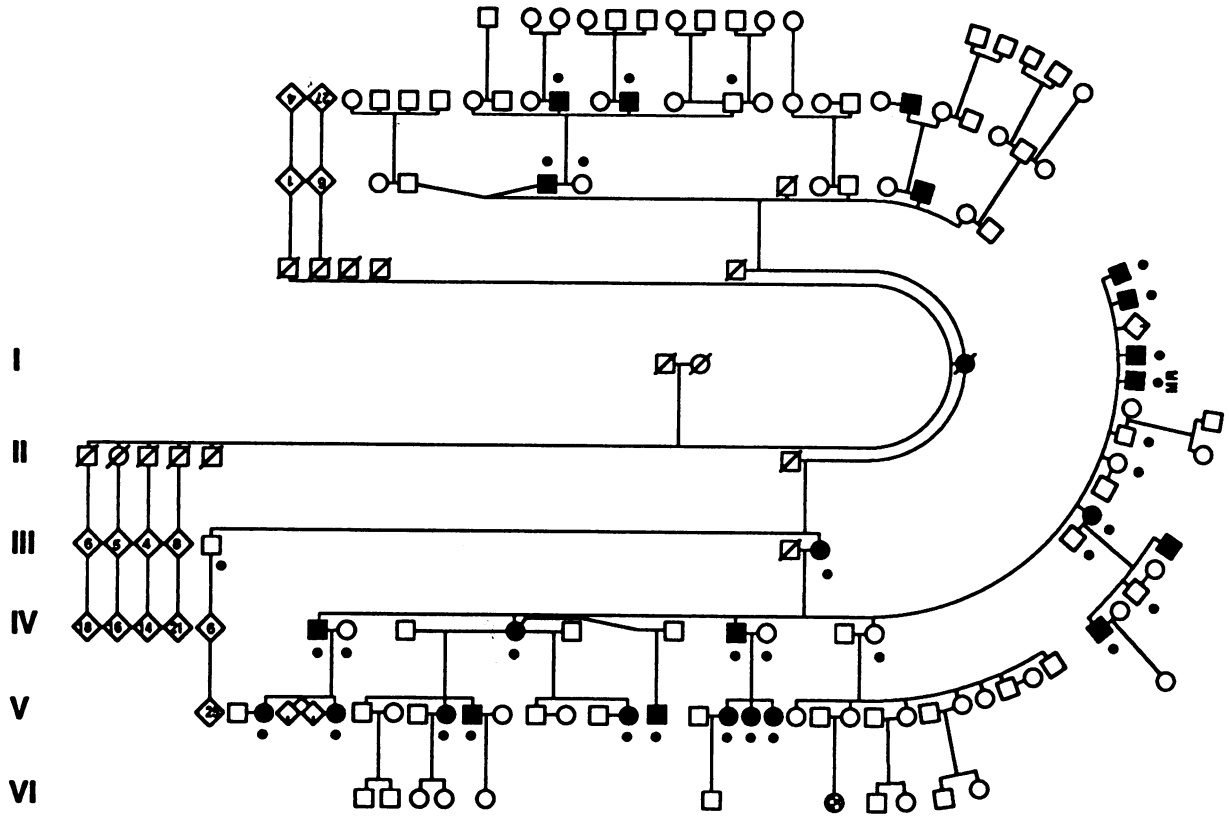

Figure 1 A French-Acadian pedigree segregating CMTD in an autosomal dominant fashion is shown. □ and ○ = Unaffected male and unaffected female, respectively; ■ and ● = affected male and affected female, respectively; ⊛ = congenital adrenal hypoplasia; MR = CMT plus mental retardation (this individual had a normal 46XY karyotype); ◇ = number of unaffected individuals; ⊘ and ∅ = deceased male and deceased female, respectively; ◇ = stillborn or miscarriage. Individuals selected for this study are indicated by a closed circle beneath the pedigree symbol. Note that neither any of the siblings of the affected female in generation II nor their descendants are affected, suggesting that CMTD in this pedigree is due to a new mutation.

10% dextran sulfate, 0.1 mg herring-sperm DNA/ml or 0.25 mg human placental DNA/ml at 65°C. Some probes required preassociation with 0.25 mg/ml human placental DNA in hybridization solution for 1–2 h at 65°C to quench repeat sequences. Blots were washed for 40 min with one change in 2 × SSC, 0.1% SDS at 65°C, followed by a stringent wash in 0.1 × SSC, 0.1% SDS at 65°C for 10–20 min. Finally, blots were exposed to Kodak XAR5 film with either one or two intensifying screens (DuPont) at −70°C.

Linkage Analysis

We performed two-point linkage analysis between CMTD and 17 DNA markers on human chromosome 17. The probe and locus names of each marker analyzed, its chromosomal location, and the restriction enzyme displaying the polymorphism are shown in table 1. We assumed an autosomal dominant mode of inheritance for CMTD, with the frequency of the mutant allele being .0001. The disease was assumed to be fully penetrant but with an age-dependent risk of affection as given by Bird and Kraft (1978). Specifically, the liability to affection was .15, .45, .75, .85, .99, and 1.00 in the age ranges 0–10, 11–20, 21–30, 31–35, 36–50, and 51+ years, respectively. Linkage analysis was performed using Z values and the maximum-likelihood method (Morton 1955) and used the computer program package LINKAGE (Lathrop et al. 1985). The θ value between disease and marker was assumed to be equal in both sexes. The maximum-likelihood estimate of the θ value ($\hat{\theta}$) and the maximum Z ($\hat{Z}$) were obtained from the standard lod table by interpolation using the methods of Rao et al. (1978). Multipoint linkage analysis was performed using the methods of Lathrop et al. (1985) and the computer program LINKAGE.

Table 1

Informative DNA Markers Used in Linkage Analysis

Probe	Locus	Location	Enzyme	References
YNH37.3	D17S28	17p13	*Taq*I	Nakamura et al. 1988
MCT35.1	D17S31	17p13	*Msp*I	Nakamura et al. 1988
LL101[a,b]	D17S251	17p13.105-17q13.100	*Pvu*II	Present report
1541b[b,c]	D17S260	17p12-17q12.3	*Bam*HI *Hin*dIII	Present report
1517[b,d]	D17S259	17p12-17q12.3	*Msp*I	Present report
pA10-41	D17S71	17p11.2	*Msp*I *Pvu*II	Barker et al. 1987
EW301	D17S58	cen-17p11.2	*Taq*I *Bgl*II	Fain et al. 1987
1516[b,e]	D17S258	17p11.2	*Hin*dIII	Present report
S12-30	D17Z1	17 cen	*Eco*RI *Pvu*II	vanTuinen et al. 1987
c11-2B		17q11.2	*Msp*I	O'Connell et al. 1989
pTH17.19		17q11.2	*Bgl*II	O'Connell et al. 1989
p11-2c11.5		17q11.2	*Taq*I *Rsa*I	O'Connell et al. 1989
CRI-L581	D17S37	cen→17q12	*Taq*I	Stephens et al. 1987
CRI-L946	D17S36	cen→17q12	*Msp*I *Bgl*II	Stephens et al. 1987
YNZ94		17q	*Pst*I	
THH59	D17S54	17q23-17q25	*Taq*I	Nakamura et al. 1988
KKA35	D17S35	17q23-17q25	*Msp*I	Nakamura et al. 1988

[a] The *Not*I linking phage clone, LL101, detects *Pvu*II alleles of 7.5 kb/1.5 kb.

[b] Requires prereassociation with placental DNA, as described in Material and Methods.

[c] The cosmid 1541 detects *Bam*HI alleles of 11 kb/10 kb and *Hin*dIII allleles of 10 kb/7 + 3 kb.

[d] The largest *Msp*I fragment (2.4 kb) from cosmid 1517 detects polymorphic alleles of 4.0 kb/2.4 kb and a constant fragment of 2.3 kb with *Msp*I.

[e] The cosmid 1516 detects polymorphic alleles of 22 kb/12 + 10 kb and a constant fragment of 18 kb with *Hin*dIII.

Results

CMTD in the French-Acadian pedigree shown in figure 1 appears to be due to a new mutation in the female in the second generation, since neither her nine siblings nor the >100 descendants of her siblings show visible signs of the disease. Nineteen of 26 patients who appeared affected on clinical examination were evaluated for NCV. Normal values for NCV were taken from Kimura (1989), with the mean ± 2 SD for the median nerve equal to 57.7 ± 4.9 and with the ulnar motor nerve equal to 58.7 ± 5.1. The mean NCV of these 19 affected individuals was 19 m/s. Seven clinically normal at-risk individuals were tested for NCV and were found to have median motor elbow-to-wrist NCV >50 m/s and ulnar-wrist-to-elbow NCV >50 m/s. Three other at-risk individuals, of ages 27, 26, and 19 years and who appeared clinically normal when subjected to a physical examination, were examined for NCV, since their ages were within the range of age at onset of CMTD. Each of these individuals was found to have symmetrically delayed NCV, consistent with the diagnosis of CMTD. This implies, from our limited study, that as many as 30% of clinically normal at-risk individuals could be affected, demonstrating a need for electrophysiological examination of all at-risk individuals to ensure accuracy in linkage analysis for CMTD. The clinical and neurophysiological details of this pedigree will be reported elsewhere.

Examination of pedigree 1 for linkage of CMTD to the Duffy locus and to other neighboring loci on chromosome 1 revealed no evidence for linkage, thereby indicating that this pedigree is type IA (data not shown). Examination of CMTD versus Duffy antigen yielded a Z of -2.0 at $\theta = .15$. Consequently, we initiated a total genome search with highly polymorphic DNA markers to identify a marker linked to CMTD in this pedigree. This process enabled us to exclude ≈ 10% of the autosomal genome as a candidate region for the disease gene. While this work was in progress, Vance et al. (1989) reported linkage of CMTD type IA to two

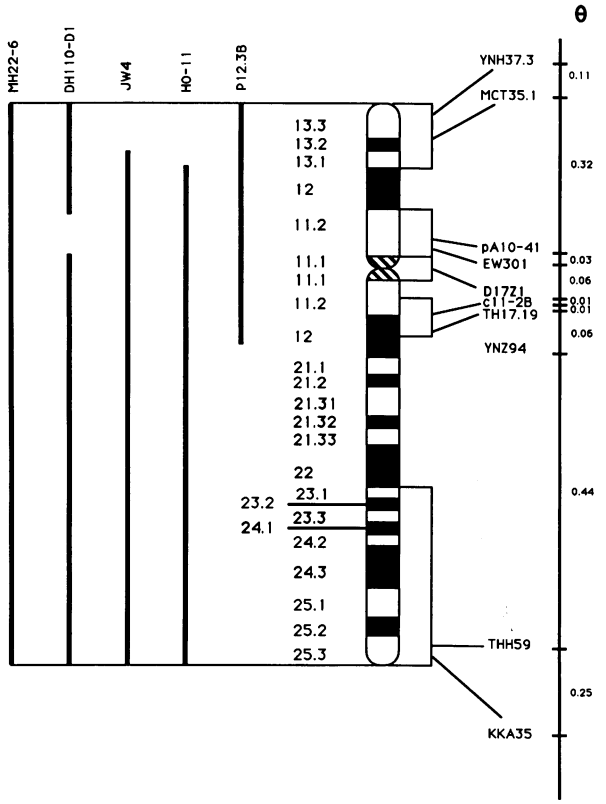

Figure 2 Idiogram of chromosome 17, showing to the left the extents of chromosome 17 retained in hybrids MH22-6, DH110-D1, JW4, HO-11, and P12.3B. Shown on the right is a sex-averaged genetic map of chromosome 17, with physical localization of some of the markers used in this study (Nakamura et al. 1988; Y. Nakamura, unpublished data). The distance between genetic markers is presented as θ.

markers, EW301 (D17S58) and pA10-41 (D17S71), in the proximal 17p region. This led to further analysis of chromosome 17–specific markers in pedigree 1.

We initially typed members of our CMTD pedigree 1 for the markers pA10-41 and EW301 in order to determine whether the CMTD gene in this pedigree was indeed linked to these markers. The results of our linkage analysis presented as Z values at various θ values are shown in table 2. The Z values obtained for pA10-41 and EW301 were 0.89 ($\hat{\theta}$ = 0) and 2.37 ($\hat{\theta}$ = .07), respectively, suggesting linkage to chromosome 17. Consequently, we examined several additional previously reported markers from the pericentromeric region of chromosome 17 for linkage to the CMTD locus. These included S12-30, the α-satellite sequence from the centromere of chromosome 17, and probes pTH17.19, c11-2B, pHHH202, p11-2c11.5, CRI-L581, and CRI-L946, which are all from the proximal 17q region near the neurofibromatosis (NF) locus. The locations and RFLPs associated with these markers are shown in table 1, and the Z values are shown in table 2. Linkage analysis with other chromosome 17 markers which are not located in the pericentromeric region (YNH37.3, MCT35.1, YNZ94, THH59, and KKA35) was performed prior to our knowledge of the report by Vance et al. (1989). Table 2 demonstrates that markers from the pericentromeric region of chromosome 17 are indeed linked to CMTD in pedigree 1.

To identify a more tightly linked marker(s), we examined several cosmids isolated from a library constructed from MH22-6, a chromosome 17–containing hybrid cell line, and a *Not*I linking clone isolated from a flow-sorted chromosome 17 library which appeared to map to the pericentromeric region. One of these markers, 1516, appeared to be tightly linked to the CMTD gene and, as shown in table 2, showed no recombination in 13 informative meiosis, providing a Z of 3.42 at $\hat{\theta}$ = 0. The *Hin*dIII RFLP observed with the cosmid 1516 is shown in figure 3.

In order to determine the map position of the marker 1516, it was hybridized to DNA from a panel of somatic cell hybrids which allow subdivision of the pericentromeric region of chromosome 17. The breakpoints within chromosome 17 in these hybrids are shown in figure 2. The results of the Southern analysis with the cosmid 1516 are shown in figure 4. The cosmid 1516 was positive in all the hybrids except DH110-D1, which is derived from a patient with Smith-Magenis syndrome who has an interstitial deletion of 17p11.2 (Smith et al. 1986). The absence of marker 1516 in this hybrid localizes it to band 17p11.2. The marker pA10-41 is also absent in this hybrid (data not shown). The Southern blot shown in figure 4 was hybridized to pTH17.19, which is mapped to 17q11.2. All hybrids were positive for this marker, as predicted from the breakpoints shown in figure 2 (data not shown).

Once 1516 was physically mapped, we utilized this information to map CMTD by multipoint methods. The markers A10-41 and D17Z1 were not sufficiently informative, and hence we used the informative genetic markers pTH17.19 and c11-2B, which map to 17q11.2 and are 0.6 cM apart (O'Connell et al. 1989). In this French-Acadian kindred, the markers pTH17.19 and 1516 demonstrate linkage with a Z value of 3.46 at $\hat{\theta}$ = .05. Thus, the order of these three genetic markers is pTH17.19–c11-2B–1516, with adjacent θ values of .006 and .044, respectively. CMTD was placed on this map by multipoint mapping, as shown in table 3. This anal-

Table 2

Lod Scores between CMTD and Chromosome 17 Markers

Probe	Location	θ .00	.05	.10	.20	.30	.40	$\hat{\theta}$	$\hat{z}$
YNH37.3	p13	−∞	−2.36	−1.54	−.78	−.35	−.12	.50	.00
MCT35.1	p13	−∞	−3.09	−1.78	−.60	−.10	−.07	.40	.07
LL101	p13.105-p13.100	−2.96	1.51	1.56	1.29	.84	.33	.095	1.56
1541	p12-q12.3	−∞	−.05	.39	.63	.58	.36	.23	.65
1517	p12-q12.3	2.04	2.33	2.39	2.08	1.44	.61	.10	2.39
pA10-41	p11.2	.89	.79	.67	.43	.20	.05	.00	.89
EW301	cen-p11.2	1.89	2.34	2.25	1.78	1.14	.45	.07	2.37
1516	p11.2	3.42	3.12	2.77	2.03	1.23	.43	.00	3.42
S12-30	cen	−1.71	.57	.91	1.01	.92	.47	.18	1.01
c11-2B	q11.2	2.31	2.15	1.98	1.60	1.15	.63	.00	2.31
pTH17.19	q11.2	−∞	2.22	2.12	1.56	.85	.20	.05	2.22
p11-2c11.5	q11.2	.72	.68	.62	.50	.35	.18	.00	.72
CRI-L581	q12	−∞	−.89	−.23	.18	.19	.08	.26	.20
CRI-L946	q12	−∞	−2.15	−1.21	−.45	−.13	−.13	.50	.00
YNZ94	q	−11.66	−2.70	−1.74	−.86	−.35	−.08	.50	.00
THH59	q23-q25	−∞	−3.18	−1.72	−.57	−.17	−.04	.50	.00
KKA35	q23-q25	−∞	−1.49	−.76	−.17	.03	.07	.40	.07

ysis provides the best location for CMTD to be completely linked to 1516, with a multipoint Z of 6.16. The likely locations of CMTD as judged by the one-lod-difference span an interval that is 0.2 cM distal to c11-2B on 17q and 10 cM distal to 1516 on 17p.

Data from three other new markers—1517, 1541, and LL101—are also shown in table 2. The markers 1517 and 1541 were present in all the hybrids shown in figure 2 (data not shown), thus confirming their location to the region between 17p12 and 17q11.2. Since they are present in DH110-D1, they appear not to be located within 17p11.2. The markers 1517 and 1541 yielded a $\hat{Z}$ value of 2.25 and 0.63 at $\hat{\theta}$ = .1 and .2, respectively. The marker LL101 was present in hybrid JW-4 but not in HO-11, thus localizing it to the interval 17p13.105-17p13.100 (data not shown). A $\hat{Z}$ of 1.56 at $\hat{\theta}$ = .1 was obtained with the marker LL101. The examination of each of these markers in other CMTD type IA pedigrees and in reference pedigrees will determine both their genetic distance with respect to the CMTD locus and their respective order on chromosome 17.

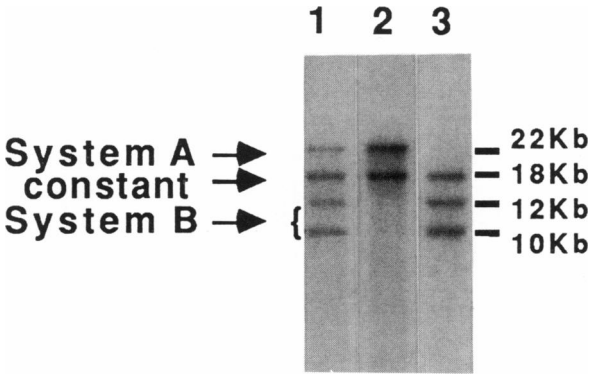

Figure 3 RFLP observed in *Hin*dIII-digested DNA with cosmid 1516. Lane 1, DNA from a heterozygous (AB) individual; lane 2, DNA from an individual homozygous for allele A; lane 3, DNA from an individual homozygous for allele B. Allele sizes are shown to the right. A constant band of 18 kb is seen in all individuals.

Discussion

The initial classification of CMTD was based on NCV measurement, pathology of peripheral nerves, and mode of inheritance and will now need to be reexamined in light of the genetic heterogeneity. It is possible that different mutations at the same locus give rise to different clinical phenotypes, as has been noted at the dystrophin locus (Kunkel et al. 1986). Different inheritance patterns may also arise by such a mechanism. These possibilities may now be tested for CMTD by examining all CMTD pedigrees with the chromosome 17– and chromosome 1–linked markers for positive or negative evidence for linkage. The likelihood of additional genetic loci responsible for the autosomal domi-

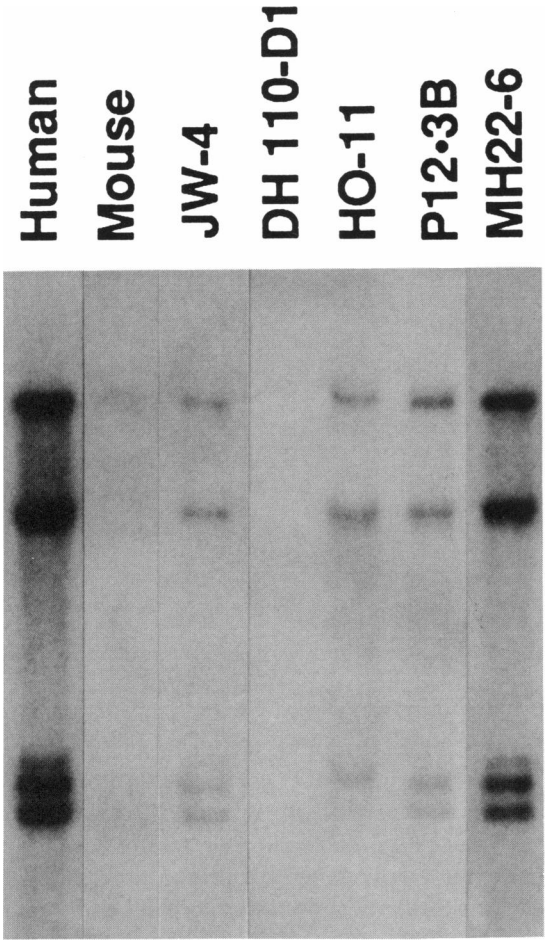

Figure 4 Southern analysis of 2.5 μg of human, 5 μg of mouse (clone 1D), and 10 μg of the indicated somatic cell hybrid DNA digested with *Eco*RI and hybridized with cosmid 1516 as described in Material and Methods. The hybrids are described in fig. 2.

nant form of the disease may also be determined by testing all pedigrees for heterogeneity on the basis of the reported linkages.

Vance et al. (1989) examined six CMTD type IA pedigrees with the markers EW301 and A10-41 and reported cumulative $\hat{Z}$ values of 10.49 at $\hat{\theta} = .05$ and 7.36 at $\theta = .06$, respectively. We report here a new marker, 1516, which could be more tightly linked to the CMTD gene than are these previously reported markers. Examination of other CMTD type IA pedigrees with 1516 will clarify the genetic distance between this marker and the disease locus. The marker 1516 is deleted in the hybrid constructed from a Smith-Magenis patient (Smith et al. 1986). It will be interesting to determine whether patients with this interstitial deletion syndrome involving band 17p11.2 are also affected with CMTD and if CMTD is therefore part of either a microdeletion or a contiguous-gene syndrome. Most currently reported patients are <10 years of age, and, since CMTD commonly presents after the first or second decade of life, this may require identification of patients that fall within this age range. Physical localization of 1516 to 17p11.2 enabled multipoint analysis with pTH17.19 and c11-2B, whose location on 17q is known (O'Connell et al. 1989). A multipoint of 6.16 confirms the location of CMTD to the pericentromeric region of chromosome 17. Further analysis will require the relative ordering of 1516 with respect to other 17p markers, such as pA10-41 and EW301.

The markers pTH17.19 and c11-2B, which are linked to the NF locus, yielded positive Z values when examined for linkage to CMTD. It is important to note that initial mapping data with the marker EW301 (D17S58) had indicated a pericentromeric and possibly a prox-

Table 3

Multipoint Mapping of CMTD within the Linkage Group pTH17.19, c11-2B, and 1516

Order	Adjacent θ Value			Multipoint Z
	θ_1	θ_2	θ_3	
CMTD–pTH17.19–c11-2B–1516	.330	.006	.044	2.12
	.167	.006	.044	3.99
pTH17.19–CMTD–c11-2B–1516	.002	.004	.044	4.86
	.004	.002	.044	5.16
pTH17.19–c11-2B–CMTD–1516	.006	.000	.044	5.33
	.006	.015	.029	5.83
	.006	.029	.015	6.04
	.006	.044	.000	6.16
pTH17.19–c11-2B–1516–CMTD	.006	.044	.167	4.38
	.006	.044	.330	2.26

imal 17p location for the NF locus (Barker et al. 1987). The identification of two NF patients with translocation breakpoints in 17q11.2 unequivocally established the location of the NF gene on the long arm of chromosome 17 (Schmidt et al. 1987). We have recently identified two patients with both CMTD and NF and are examining them for alterations or deletion of sequences which may suggest that these genes are located within close proximity of each other. Other distinct possibilities include pericentric inversion or chance alone, since NF is one of the most frequently occurring autosomal dominant human diseases with a high frequency of new mutation. However, until a larger repertoire of markers are examined in a number of CMTD pedigrees, the proximal 17p location is necessarily tentative.

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References

Anderson MA, Gusella JF (1984) The use of cyclosporin A in establishing EBV-transformed human lymphoblastoid cell lines. In Vitro 20:856–858

Barker D, Wright E, Nguyen K, Cannon L, Fain P, Goldgar D, Bishop DT, et al. (1987) Gene for von Recklinghausen neurofibromatosis is in the pericentromeric region of chromosome 17. Science 236:1100–1102

Beckett J, Holen JJA, Simpson NE, White BN, MacLeod PM (1986) Localization of X-linked dominant Charcot-Marie-Tooth disease (CMTD) to Xq13. J Neurogenet 3:225–231

Bird TD, Kraft GH (1978) Charcot-Marie-Tooth disease: data for genetic counseling relating age to risk. Clin Genet 14:43–49

Bird TD, Ott J, Giblett ER (1982) Evidence of linkage of Charcot-Marie-Tooth disease neuropathy to the Duffy locus on chromosome number 1. Am J Hum Genet 34: 388–394

Bird TD, Ott J, Giblett ER, Chance PF, Sumi SM, Kraft GH (1983) Genetic linkage evidence for heterogeneity in Charcot-Marie-Tooth neuropathy (HMSN type I). Ann Neurol 14:679–684

Charcot JM, Marie P (1886) Sur une forme particulière d'atrophie musculaire progressive souvent familiale débutant par les pied et les jauntes et atteignant plus tord les mains. Rev Med 6:97–138

Dyck PJ, Lambert EH (1968*a*) Lower motor and primary sensory neuron disease with peroneal muscular atrophy. I. Neurologic, genetic and electrophysiological findings in hereditary polyneuropathies. Arch Neurol 18:603–618

——— (1968*b*) Lower motor and primary sensory neuron disease with peroneal muscular atrophy. II. Neurologic, genetic and electrophysiological findings in various neuronal degenerations. Arch Neurol 18:619–625

Dyck PJ, Ott J, Moore SB, Swanson CJ, Lambert EH (1983) Linkage evidence for genetic heterogeneity among kinships with hereditary motor and sensory neuropathy, type I. Mayo Clin Proc 58:430–435

Fain PR, Barker DF, Goldgar DE, Wright E, Nguyen K, Carey J, Johnson J, et al (1987) Genetic analysis of NF1: identification of close flanking markers on chromosome 17. Genomics 1:340–345

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling fragments to high specific activity. Anal Biochem 132: 6–13

Griffiths LR, Zwi MB, McLeod JG, Nicholson GA (1988) Chromosome 1 linkage studies in Charcot-Marie-Tooth neuropathy type 1. Am J Hum Genet 42:756–771

Guiloff RJ, Thomas PK, Contreras M, Armitage S, Schwartz G, Sedgwick EM (1982) Linkage of autosomal type I hereditary motor and sensory neuropathy to the Duffy locus on chromosome 1. J Neuro Neurosurg Psychiatry 45:669–674

Kimura J (1989) Electrodiagnosis in diseases of nerve and muscle: principles and practice, 2d ed. FA Davis, Philadelphia

Kunkel LM, Hejtmancik JF, Caskey CT, Speer A, Monaco AP, Middlesworth W, Colletti CA, et al (1986) Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy. Nature 322:73–77

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

McKusick VA (1988) Mendelian inheritance in man, 8th ed. Johns Hopkins University Press, Baltimore and London

Middleton-Price HR, Harding AE, Berciano J, Pastor JM, Huson SM, Malcolm S (1989) Absence of linkage of hereditary motor and sensory neuropathy type I to chromosome 1 markers. Genomics 4:192–197

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Morton NE (1955) Sequential tests for the detection of linkage. Am J Hum Genet 7:277–318

Nakamura Y, Lathrop MM, O'Connell P, Leppert M, Barker D, Wright E, Skolnick M, et al (1988) A mapped set of DNA markers for human chromosome 17. Genomics 2:302–309

O'Connell P, Leach RJ, Ledbetter DH, Cawthon RM, Culver M, Eldridge JR, Frej A-K, et al (1989) Fine structure DNA mapping studies of the chromosomal region harboring the genetic defect in neurofibromatosis type I. Am J Hum Genet 44:51–57

Raeymaekers P, De Jonghe P, Swerts L, Muylle L, Gheuens J, Martin JJ, vanBroeckhoven C, et al (1988) Absence of linkage with the Duffy blood group in a family with Charcot-Marie-Tooth neuropathy. J Neurol Sci 88:145–150

Rao DC, Keats BJB, Morton NE, Yee S, Lew R (1978) Variability of human linkage data. Am J Hum Genet 30: 516–529

Schmidt MA, Michels VV, Dewald GW (1987) Cases of neurofibromatosis with rearrangements of chromosome 17 involving band 17q11.2. Am J Med Genet 28:771–777

Skre H (1974) Genetic and clinical aspects of Charcot-Marie-Tooth disease. Clin Genet 6:98–118

Smith ACM, McGavran L, Robinson J, Waldstein G, Macfarlane J, Zonona J, Reiss M, et al (1986) Interstitial deletion of (17)(p11.2p11.2) in nine patients. Am J Med Genet 24:393–414

Stebbins NB, Conneally PM (1982) Linkage of dominantly inherited Charcot-Marie-Tooth neuropathy to the Duffy locus in an Indiana family. Am J Hum Genet 34:195A

Stephens K, Riccardi VM, Rising M, Ng S, Green P, Collins FS, Rediker KS, et al (1987) Linkage studies with chromosome 17 DNA markers in 45 neurofibromatosis 1 families. Genomics 1:353–357

Su T-S, Nussbaum RL, Airhart S, Ledbetter DH, Mohandas T, O'Brien WE, Beaudet AL (1984) Human chromosomal assignments for 14 argininosuccinate synthetase pseudogenes: cloned DNAs as reagents for cytogenetic analysis. Am J Hum Genet 36:954–964

Vance JM, Nicholson GA, Yamaoka LH, Stajich J, Stewart CS, Speer MC, Hung W-Y, et al (1989) Linkage of Charcot-Marie-Tooth neuropathy type 1a to chromosome 17. Exp Neurol 104:186–189

vanTuinen P, Dobyns WB, Rich DC, Summers KM, Robinson TJ, Nakamura Y, Ledbetter DH (1988) Molecular detection of microscopic and submicroscopic deletions associated with Miller-Dieker syndrome. Am J Hum Genet 43: 587–596

vanTuinen P, Rich DC, Summers KM, Ledbetter DH (1987) Regional mapping panel for human chromosome 17: application to neurofibromatosis type 1. Genomics 1:374–381

Wallace DM (1987) Precipitation of nucleic acids. Methods Enzymol 152:41–48

Wallace MR, Fountain JW, Brereton AM, Collins FS (1989) Direct construction of a chromosome-specific *Not*I linking library from flow-sorted chromosomes. Nucleic Acids Res 17:1665–1677