

## Chromosome 1 Linkage Studies in Charcot-Marie-Tooth Neuropathy Type I

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### Summary

Charcot-Marie-Tooth neuropathy type 1 (CMT1) is an autosomal dominant disorder of peripheral nerve. The gene for CMT1 was originally localized to chromosome 1 by linkage to the Duffy blood group, but it has since been shown that not all CMT1 pedigrees show this linkage. We report here the results of linkage studies using five chromosome 1 markers—Duffy (Fy), antithrombin III (AT3), renin (REN),  $\beta$ -nerve growth factor (NGFB), and salivary amylase (AMY1)—in 16 CMT1 pedigrees. The total lod scores exclude close linkage of CMT1 to any of these markers. However, individual families show probable linkage of CMT1 to Duffy, AT3, and/or AMY1. No linkage was indicated with REN or NGFB. These results indicate the possible location of a CMT1 gene between the AMY1 and AT3 loci at p21 and q23, respectively, on chromosome 1 and support the theory that there is at least one other CMT1 gene.

### Introduction

Charcot-Marie-Tooth neuropathy type 1 (CMT1), also known as hereditary motor and sensory neuropathy I (HMSN1), is an autosomal dominant disorder characterized by progressive limb weakness and muscle atrophy. Those affected display a marked slowing of nerve conduction velocities as well as hypertrophic changes in nerve biopsies (Dyck 1975). The biochemical basis of the disease is unknown, but the gene for CMT1 has been localized to chromosome 1 by linkage to the Duffy blood group (Bird et al. 1982; Guilloff et al. 1982; Stebbins and Conneally 1982), with a combined maximum lod score of 7.08 at a recombination fraction ( $\theta$ ) of .1 (Chance et al. 1987). However, it has since become apparent that not all CMT1 families show this linkage to Duffy (Bird et al. 1983; Dyck et al. 1983). Bird et al. (1983) have suggested that CMT1 is a heterogeneous disorder and categorized those families showing Duffy linkage as HMSN1b and those not showing linkage as HMSN1a. The exact locations of both the Duffy

locus and that of HMSN1b on chromosome 1 are unknown. To more precisely define the location of the chromosome 1 CMT1 gene defect and to investigate the possible heterogeneity of CMT1, we have undertaken an extensive linkage study using 16 CMT1 pedigrees, composed of 260 individuals of whom 125 are affected. These studies were performed using five chromosome 1 markers; the Duffy blood group (Fy); and gene probes for antithrombin III (AT3), renin (REN),  $\beta$ -nerve growth factor (NGFB), and salivary amylase (AMY1). Each of the DNA probes detects an RFLP that can be used for linkage studies. Also, each of the markers has been localized to chromosome 1 (fig. 1). Duffy shows linkage to the uncoiler locus (1qH) on the long arm of chromosome 1 (Donahue et al. 1968); and AT3 has been assigned to 1q23 (Bock et al. 1984, 1985), renin to 1q12→qter (Griffiths et al. 1987),  $\beta$ -nerve growth factor to 1p22.1 (Darby et al. 1985a; Münke et al. 1985), and amylase to 1p21 (Tricoli and Shows 1984; Zabel et al. 1984).

### Methods

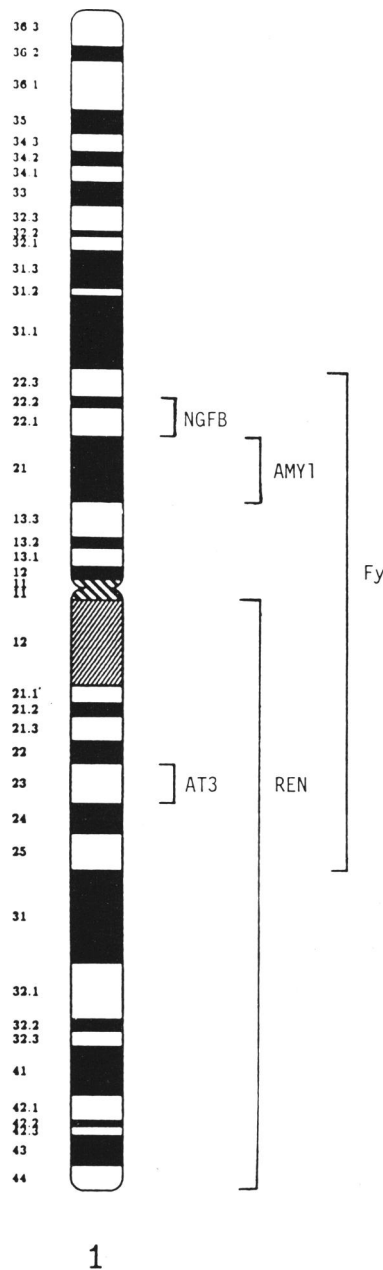
#### Families

Sixteen CMT1 families were collected for these studies (Appendix, figs. A1–A16). In total, 260

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**Figure 1** Localization of probes used in CMT1 linkage study.

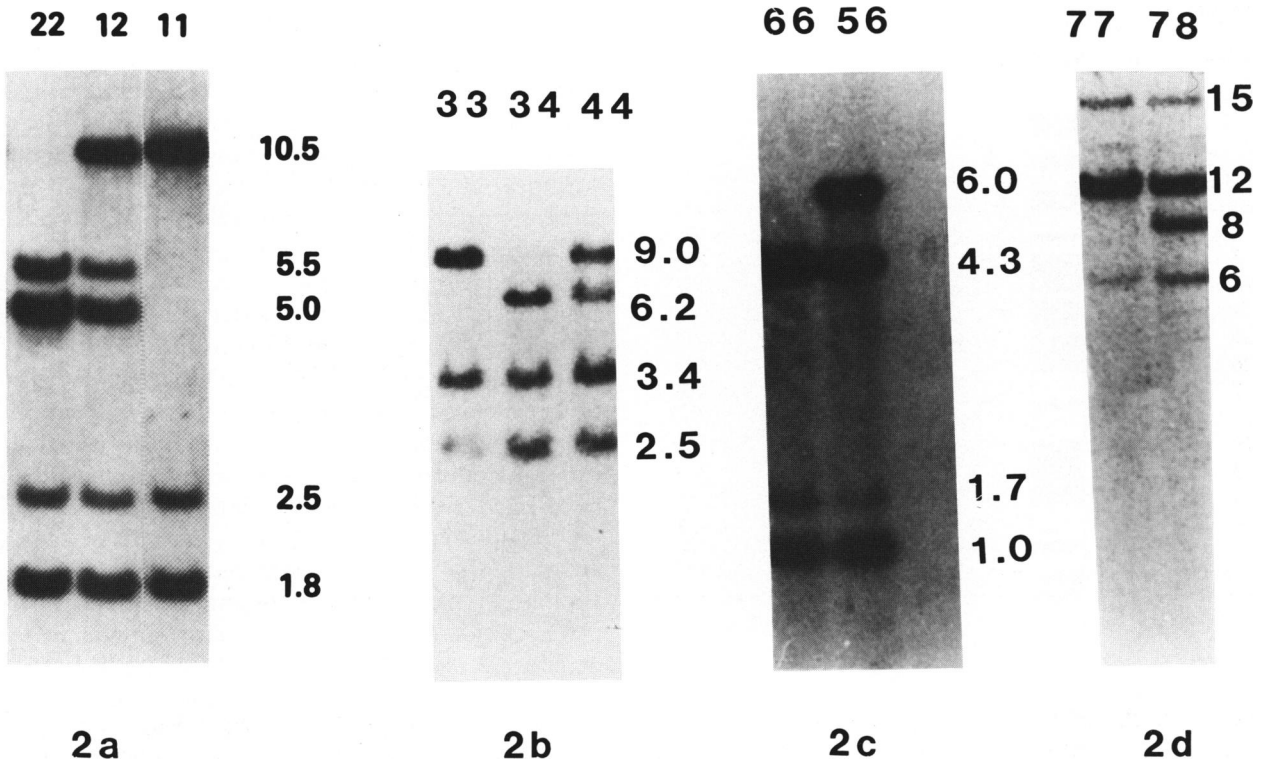
blood samples were collected, 125 from affected individuals. Both affected and unaffected family members were typed for Duffy, ABO, Rh, MNS, P, Kell, Lu, Le, and Jk to confirm parentage. Blood typing was performed by the Australian Red Cross Blood Bank and the Haematology Department, Concord Hospital. Affected family members were diagnosed

by neurologic examination and motor-nerve conduction-velocity measurements according to a method described by Bird and Kraft (1978), with all at-risk individuals examined carefully to determine disease status. Affected members showed distal muscle weakness and atrophy, loss of tendon reflexes, and slow motor-nerve conduction velocities (<30 m/s). At least one sural nerve biopsy on an affected member of each pedigree was performed. Sural nerve biopsies and motor-nerve conduction-velocity measurements were consistent with the diagnosis of CMT type 1. The possibility of an X-linked dominant form of transmission cannot be ruled out in all pedigrees. X-linked dominance will not show male-to-male transmission, and all daughters of affected males will be affected. Fischbeck et al. (1986) have also noticed that many heterozygous females in a large North Carolina CMTX family are mildly symptomatic. Families 10, 9, 34, 23, and 24 and the smaller intermarriage side of family 1 (1b) do not show male-to-male transmission. However, there are very few chances of male-to-male transmission being shown in these pedigrees (none in families 10, 9, and 34; one in families 23 and 1b; and two in family 24). In pedigrees 23 and 24 there are unaffected daughters, >35 years, of affected males; and in all six of these pedigrees affected females show severe clinical symptoms, making the possibility of X-linked dominance unlikely.

#### DNA Extractions, Southern Blotting, and Hybridizations

DNA was isolated from the peripheral blood leukocytes of 260 individuals in the 16 CMT1 pedigrees by the method of Blin and Stafford (1976). Pedigree 6 was not used for the DNA linkage studies. Fifteen micrograms of DNA from each individual was digested with the appropriate restriction enzyme (Boehringer Mannheim), electrophoresed in 1% agarose gels, and transferred to Gene Screen Plus® membranes (New England Nuclear) according to the directions, supplied by the manufacturer, for Southern blot analysis (Southern 1975).

The AT3, REN, NGFB, and AMY1 probes were radioactively labeled by nick-translation (Rigby et al. 1977) using [<sup>32</sup>P]-dCTP (New England Nuclear) and were then hybridized to the membrane filters at 42 C in 50% formamide, 0.9 M NaCl, 50 mM sodium phosphate buffer, pH 6.8, 200 μg salmon-sperm DNA/ml, 10% dextran sulfate, 3.5% SDS. All filters were washed twice in 2 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) at 22 C, once



**Figure 2** RFLPs used in CMT1 linkage studies. *2a*, AT3 *PstI* RFLP, homozygotes have either 10.5- or 5.5/5.0-kb bands. *2b*, Renin *HindIII* RFLP, homozygotes 9.0- or 6.2-kb bands. *2c*, NGFB *TaqI* RFLP, homozygotes 6- or 4.3/1.7-kb bands. *2d*, Amylase *PstI* RFLP, homozygotes 12- or 8-kb bands.

in  $2 \times$  SSC, 1% SDS at 65 C, and twice in  $0.1 \times$  SSC at 65 C. After drying in air, the filters were exposed to Kodak XAR-5 film, with a Lightning Plus intensifying screen (DuPont) for 2–7 days at  $-80$  C. Linkage results were analyzed using the LIPED computer program of Ott (1974), with lod scores presented as  $\theta_m = \theta_f$ , owing to the large amount of data.

#### Probes

The phATIII 113 probe was obtained from S. Woo (Chandra et al. 1983). This clone contains 1,479-bp of DNA encoding the entire mature antithrombin III protein and detects a *PstI* sequence polymorphism (Prochownik et al. 1983). Hybridization of the nick-translated probe to human DNA cut with *PstI* reveals the presence of the RFLP. Homozygotes have either 10.5- or 5.5/5.0-kb bands with invariant 2.5- and 1.8-kb bands, and heterozygotes show all five bands (fig. 2*a*). The 10.5- and 5.5/5.0-kb allele frequencies are .5 each.

The  $\lambda$ HRV REN probe was obtained from B. Morris (Hardman et al. 1984). This clone, whose 14-kb insert encodes exons 2–9 of human REN, detects a

*HindIII* RFLP (Frossard et al. 1986). Homozygotes have either a 9.0- or 6.2-kb band with invariant 3.4- and 2.5-kb bands (fig. 2*b*). Frequencies for the 9.0- and 6.2-kb alleles are .66 and .34, respectively.

The NGFB probe was supplied by J. Darby. This probe contains a 6.6-kb insert in the *EcoRI* site of pBR322 (Darby et al. 1985*a*). The probe detects a *TaqI* polymorphism with homozygotes having either 6- or 4.3/1.7-kb bands plus an invariant 1-kb band (fig. 2*c*). Frequencies for the RFLP are .17 (6 kb) and .83 (4.3/1.7 kb).

The pEB-8 AMY1 probe was obtained from M. Meisler (Meisler and Gumucio 1986). This probe contains a 2.1-kb insert in pUC9 and detects a *PstI* RFLP. Homozygotes have either a 12- or 8-kb band with invariant 15- and 6-kb bands (fig. 2*d*). Allele frequencies for the 12- and 8-kb bands are .85 and .15, respectively.

#### Results

The Duffy blood group marker has two common alleles  $Fy^a$  and  $Fy^b$ , (designated as a and b, respec-

Table 1

## CMT1 and Duffy Lod Scores

PEDIGREE DESIGNATION	$\theta^a$							
	0	.05	.1	.15	.2	.25	.3	.4
10 .....	0.667	0.533	0.393	0.249	0.104	-0.028	-0.128	-0.154
8 .....	$-\infty$	-5.754	-3.285	-1.980	-1.171	-0.647	-0.316	-0.033
1 .....	$-\infty$	-4.179	-2.688	-1.834	-1.260	-0.853	-0.557	-0.187
23 .....	-0.079	-0.063	-0.048	-0.035	-0.024	-0.016	-0.009	-0.002
24 .....	$-\infty$	-0.186	0.022	0.100	0.124	0.118	0.095	0.031
39 .....	0.480	0.407	0.333	0.262	0.195	0.134	0.083	0.018
26 .....	$-\infty$	-0.896	-0.609	-0.446	-0.333	-0.248	-0.180	-0.077
7 .....	$-\infty$	-0.721	-0.444	-0.292	-0.194	-0.125	-0.076	-0.018
13 .....	$-\infty$	-0.469	-0.248	-0.148	-0.093	-0.061	-0.039	-0.011
19 .....	$-\infty$	-1.288	-0.755	-0.475	-0.299	-0.183	-0.104	-0.021
3 .....	$-\infty$	-2.624	-1.763	-1.277	-0.944	-0.695	-0.498	-0.206
34 .....	$-\infty$	-0.612	-0.355	-0.223	-0.142	-0.088	-0.052	-0.012
6 .....	0.581	0.517	0.453	0.389	0.325	0.263	0.202	0.092
Total .....	$-\infty$	-15.333	-8.993	-5.709	-3.713	-2.429	-1.580	-0.579

<sup>a</sup>  $\theta_m = \theta_f$ .

tively, in the Appendix pedigree figures). Thirteen families were informative for Duffy (table 1), with the total lod scores excluding linkage of CMT1 to Duffy at  $\leq 25$  cM (lod score = -2.429 at  $\theta = .25$ ). However, families 10, 39, and 6 show probable linkage of CMT1 to Duffy, giving a combined maximum lod score of 1.728 at 0 cM. Ten other families show probable nonlinkage with families 8 and 1, giving lod scores  $< -2$  at 10 cM. When Morton's  $\chi^2$  heterogeneity testing (Morton 1956) was used, no significant level of heterogeneity in these families could be found ( $\chi^2_{12} = 9.9$ ;  $P > .1$ ), but combining these results with previously published data suggests that heterogeneity in CMT1 exists (paper in preparation). As an example of this point, a lod score of 3.03 between CMT1 and Duffy at 10 cM was obtained by Stebbins and Conneally (1982) in one family, whereas scores  $< -2$  at the same distance have been obtained in individual families in the present study.

Linkage results with both AT3 (table 2) and AMY1 (table 3) were similar to those with Duffy. Thirteen families were informative for AT3, with the total lod scores excluding linkage at  $\leq 15$  cM (lod score = -2.006 at  $\theta = .15$ ). Families 10, 1, 19, and 7, however, show probable linkage of CMT1 to AT3, with a maximum combined lod score of 2.240 at 0 cM. The nine other families give negative lod scores, with family 8 showing a score of  $< -2$  at 10 cM. Eleven families were informative for AMY1,

with the total lod scores excluding linkage at  $\leq 10$  cM (lod score = -3.062 at  $\theta = .1$ ). Families 9, 19, 3, and 34 show probable linkage of CMT1 to AMY1, with a combined maximum lod score of 1.489 at 5 cM; but the other seven families show nonlinkage, with family 8 giving a lod score of  $< -2$  at  $\theta = .15$ .

It should be noted that (1) no family gives an individual significant lod score of linkage to Fy, AT3, or AMY1 and (2) those that show probable linkage to one of these markers do not necessarily show it to all three. As an example, family 1 shows significant nonlinkage to Fy with a lod score of -2.688 at  $\theta = .1$  but probable linkage to AT3 with a lod score of 0.559 at 0 cM. Also, family 3 shows nonlinkage to Fy and AT3 but probable linkage to AMY1. The only individual significant scores are those for nonlinkage, with family 8 in particular giving lod scores of  $< -2$  at  $\theta = .1$  for all three markers.

Linkage results when the REN (table 4) and NGFB (table 5) probes are used do not indicate close linkage between CMT1 and these markers. Twelve families were informative for REN, with the combined lod score excluding linkage at  $\leq 30$  cM (lod score = -2.457 at  $\theta = .3$ ). The only family (family 24) showing positive linkage gave a small positive lod score (maximum of 0.089 at  $\theta = 0$ ). Similarly, of the 10 families informative for NGFB, two (families 1 and 24) show low positive linkage, both maximizing at  $\theta = 0$  with a lod score of  $\sim 0.1$  each. Hence no

**Table 2**  
**CMT1 and AT3 Lod Scores**

PEDIGREE DESIGNATION	$\theta^a$							
	0	.05	.1	.15	.2	.25	.3	.4
10 .....	0.602	0.535	0.465	0.393	0.318	0.243	0.170	0.049
8 .....	−∞	−4.640	−2.960	−2.036	−1.426	−0.991	−0.669	−0.241
1 .....	0.559	0.467	0.378	0.297	0.225	0.164	0.115	0.048
23 .....	−∞	−1.846	−1.063	−0.659	−0.411	−0.250	−0.143	−0.031
21 .....	−∞	−0.885	−0.377	−0.124	0.021	0.102	0.141	0.123
24 .....	−0.138	−0.116	−0.094	−0.074	−0.055	−0.039	−0.025	−0.006
39 .....	−3.398	−0.612	−0.351	−0.216	−0.133	−0.079	−0.044	−0.008
26 .....	−0.194	−0.148	−0.111	−0.081	−0.057	−0.038	−0.024	−0.006
7 .....	0.176	0.148	0.121	0.095	0.072	0.051	0.033	0.009
13 .....	−∞	−0.575	−0.308	−0.173	−0.095	−0.049	−0.022	−0.002
19 .....	0.903	0.836	0.766	0.691	0.612	0.528	0.438	0.238
3 .....	−∞	−0.380	−0.145	−0.041	0.009	0.029	0.031	0.012
34 .....	−0.176	−0.137	−0.104	−0.077	−0.056	−0.038	−0.024	−0.006
Total .....	−∞	−7.352	−3.784	−2.006	−0.977	−0.367	−0.022	0.179

<sup>a</sup>  $\theta_m = \theta_f$ .

family gave a lod score indicating probable linkage between CMT1 and REN or NGFB. The combined total lod scores for NGFB exclude linkage with CMT1 at  $\leq 20$  cM (lod score =  $-2.497$  at  $\theta = .2$ ).

Linkage results between markers are presented in table 6. These represent a sum of lod scores for all informative families for each of the marker relationships. Linkage was excluded at  $\leq 5$  cM for Fy-AT3,

Fy-REN, Fy-NGFB, REN-NGFB, and REN-AMY1. Maximum lod scores were obtained at  $\sim \theta = .2-.3$  for most marker relationships, but it is clear that more information is needed to accurately determine the distances between these loci. It is possible that multipoint linkage analysis of this data could clarify marker linkage relationships—and could more concisely map CMT1 on the Duffy-AT3-AMY1 gene map. This analysis is currently being performed.

**Table 3**  
**CMT1 and AMY1 Lod Scores**

PEDIGREE DESIGNATION	$\theta^a$							
	0	.05	.1	.15	.2	.25	.3	.4
8 .....	−∞	−4.305	−2.853	−2.023	−1.452	−1.029	−0.704	−0.255
1 .....	−∞	−1.068	−0.535	−0.272	−0.126	−0.046	−0.006	0.013
23 .....	−0.014	−0.010	−0.008	−0.005	−0.004	−0.002	−0.001	0.000
24 .....	−0.041	−0.031	−0.023	−0.017	−0.012	−0.008	−0.005	−0.001
39 .....	−∞	−0.697	−0.440	−0.302	−0.210	−0.142	−0.091	−0.023
26 .....	−∞	−0.642	−0.388	−0.248	−0.163	−0.104	−0.063	−0.015
9 .....	0.230	0.212	0.193	0.173	0.152	0.130	0.107	0.057
13 .....	−∞	−0.599	−0.344	−0.214	−0.135	−0.083	−0.048	−0.011
19 .....	−∞	0.373	0.537	0.572	0.552	0.500	0.427	0.237
3 .....	0.848	0.767	0.681	0.589	0.492	0.390	0.286	0.092
34 .....	0.164	0.137	0.112	0.088	0.066	0.047	0.031	0.008
Total .....	−∞	−5.863	−3.062	−1.658	−0.838	−0.347	−0.068	0.102

<sup>a</sup>  $\theta_m = \theta_f$ .

**Table 4**  
**CMT1 and REN Lod Scores**

PEDIGREE DESIGNATION	$\theta^a$							
	0	.05	.1	.15	.2	.25	.3	.4
10 .....	$-\infty$	-3.164	-2.030	-1.400	-0.979	-0.676	-0.449	-0.150
8 .....	$-\infty$	-6.117	-3.795	-2.540	-1.731	-1.169	-0.764	-0.253
1 .....	$-\infty$	-0.636	-0.291	-0.123	-0.034	0.008	0.023	0.012
23 .....	$-\infty$	-0.890	-0.571	-0.387	-0.261	-0.170	-0.104	-0.025
21 .....	$-\infty$	-0.721	-0.444	-0.292	-0.194	-0.125	-0.076	-0.018
24 .....	0.089	0.076	0.063	0.051	0.040	0.029	0.020	0.005
39 .....	$-\infty$	-0.515	-0.278	-0.165	-0.100	-0.061	-0.035	-0.008
26 .....	$-\infty$	-0.775	-0.468	-0.298	-0.188	-0.115	-0.065	-0.013
13 .....	$-\infty$	-0.543	-0.267	-0.122	-0.035	0.016	0.042	0.043
27 .....	$-\infty$	-4.000	-2.796	-2.092	-1.592	-1.204	-0.887	-0.388
3 .....	$-\infty$	-0.679	-0.457	-0.344	-0.265	-0.202	-0.148	-0.059
34 .....	-0.100	-0.079	-0.061	-0.046	-0.033	-0.023	-0.014	-0.004
Total .....	$-\infty$	-18.043	-11.395	-7.756	-5.373	-3.690	-2.457	-0.857

<sup>a</sup>  $\theta_m = \theta_f$ .

### Discussion

The use of closely linked RFLP markers for chromosomal localization of genetic disorders and their subsequent use in prenatal and postnatal diagnosis is well documented (Gusella et al. 1983; Bakker et al. 1985). The precise localization of the CMT1 gene and development of linked markers for diagnostic uses have been made more difficult by the intervention of heterogeneity. Heterogeneity in genetic dis-

eases is not uncommon. Charcot-Marie-Tooth neuropathy itself is a heterogeneous collection of genetic disorders, with differentiation of types based on mode of inheritance, nerve conduction-velocity measurements, and nerve biopsy morphology (Harding and Thomas 1980). The heterogeneity of elliptocytosis was first described by Morton in 1956, and Conneally et al. (1978) have described another disorder, congenital cataracts, first localized to chromosome 1 by linkage to Duffy but later shown to be

**Table 5**  
**CMT1 and NGFB Lod Scores**

PEDIGREE DESIGNATION	$\theta^a$							
	0	.05	.1	.15	.2	.25	.3	.4
10 .....	$-\infty$	-3.721	-2.541	-1.861	-1.388	-1.028	-0.741	-0.308
8 .....	$-\infty$	-2.801	-1.152	-0.391	-0.002	0.181	0.236	0.154
1 .....	0.118	0.093	0.072	0.054	0.039	0.027	0.017	0.004
23 .....	$-\infty$	-1.490	-0.921	-0.609	-0.405	-0.264	-0.163	-0.043
24 .....	0.123	0.102	0.083	0.066	0.050	0.036	0.024	0.006
39 .....	-0.242	-0.184	-0.139	-0.102	-0.072	-0.049	-0.031	-0.007
26 .....	-0.047	-0.037	-0.029	-0.021	-0.015	-0.010	-0.006	-0.002
13 .....	$-\infty$	-2.007	-1.402	-1.048	-0.797	-0.603	-0.444	-0.194
3 .....	$-\infty$	-0.230	-0.016	0.072	0.108	0.117	0.108	0.064
34 .....	-0.042	-0.034	-0.027	-0.020	-0.015	-0.010	-0.006	-0.002
Total .....	$-\infty$	-10.310	-6.070	-3.860	-2.497	-1.604	-1.006	-0.327

<sup>a</sup>  $\theta_m = \theta_f$ .

**Table 6**  
**Marker Linkage Relationships: Lod Scores**

LINKAGE RELATIONSHIP	$\theta^a$					
	0	.05	.1	.2	.3	.4
Fy-AT3 .....	$-\infty$	-3.040	-1.365	-0.090	0.225	0.166
Fy-REN .....	$-\infty$	-2.643	-1.008	0.100	0.249	0.099
Fy-NGFB .....	$-\infty$	-2.020	-1.094	-0.345	-0.089	-0.015
Fy-AMY1 .....	$-\infty$	-0.804	0.074	0.620	0.579	0.297
AT3-REN .....	$-\infty$	-1.865	-0.618	0.166	0.213	0.060
AT3-NGFB .....	$-\infty$	-0.564	0.445	0.888	0.672	0.292
AT3-AMY1 .....	$-\infty$	-0.534	-0.015	0.318	0.325	0.199
REN-NGFB .....	$-\infty$	-4.000	-1.972	-0.460	-0.022	0.044
REN-AMY1 .....	$-\infty$	-2.142	-0.796	0.054	0.177	0.076
NGFB-AMY1 .....	-0.092	0.276	0.438	0.499	0.385	0.197

<sup>a</sup>  $\theta_m = \theta_r$ .

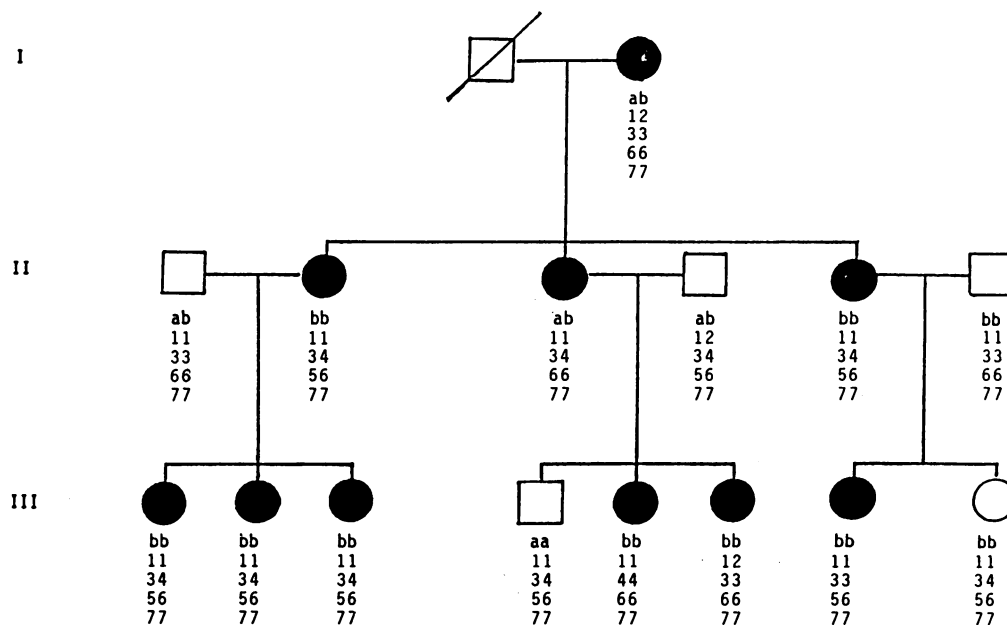
heterogeneous. Heterogeneity in bipolar affective disorders has also recently been described (Hodgkinson et al. 1987). The results presented in the present paper indicate that CMT1 is probably heterogeneous; however, other factors may be involved in producing increased recombination frequencies, e.g., the presence of hot spots, the effect of linkage across the centromere, the presence of the uncoiler locus within this region, and the large distances on chromosome 1. Regardless of the heterogeneity question, our results indicate that some families show linkage not only to Duffy but also to AT3 and AMY1. The precise localization of Duffy—and hence of CMT1—is unknown. Linkage studies with the 1qH locus (Rivas et al. 1975; Cook et al. 1978) and AT3 (Bishop et al. 1982; Winter et al. 1982) suggest that Fy is on the long arm of chromosome 1; but linkage to amylase (Hill et al. 1972) and NGFB has also been shown (Darby et al. 1985*b*), suggesting that Duffy is on the short arm. Hence, Duffy has been assigned imprecisely to 1p2→1q2. Our results with CMT1 indicate some linkage to amylase and AT3; and therefore the original localization of CMT1 to the long arm of

chromosome 1 may be questionable. The data presented here indicate the presence of a CMT1 gene defect between p21 and q23 on chromosome 1. More probes within this region will need to be tested to more precisely define this localization. Also, our data support the theory that at least one other CMT1 gene exists outside this region of chromosome 1. It is clear that a number of probes will need to be tested to find closely linked RFLPs useful for CMT1 diagnostic purposes.

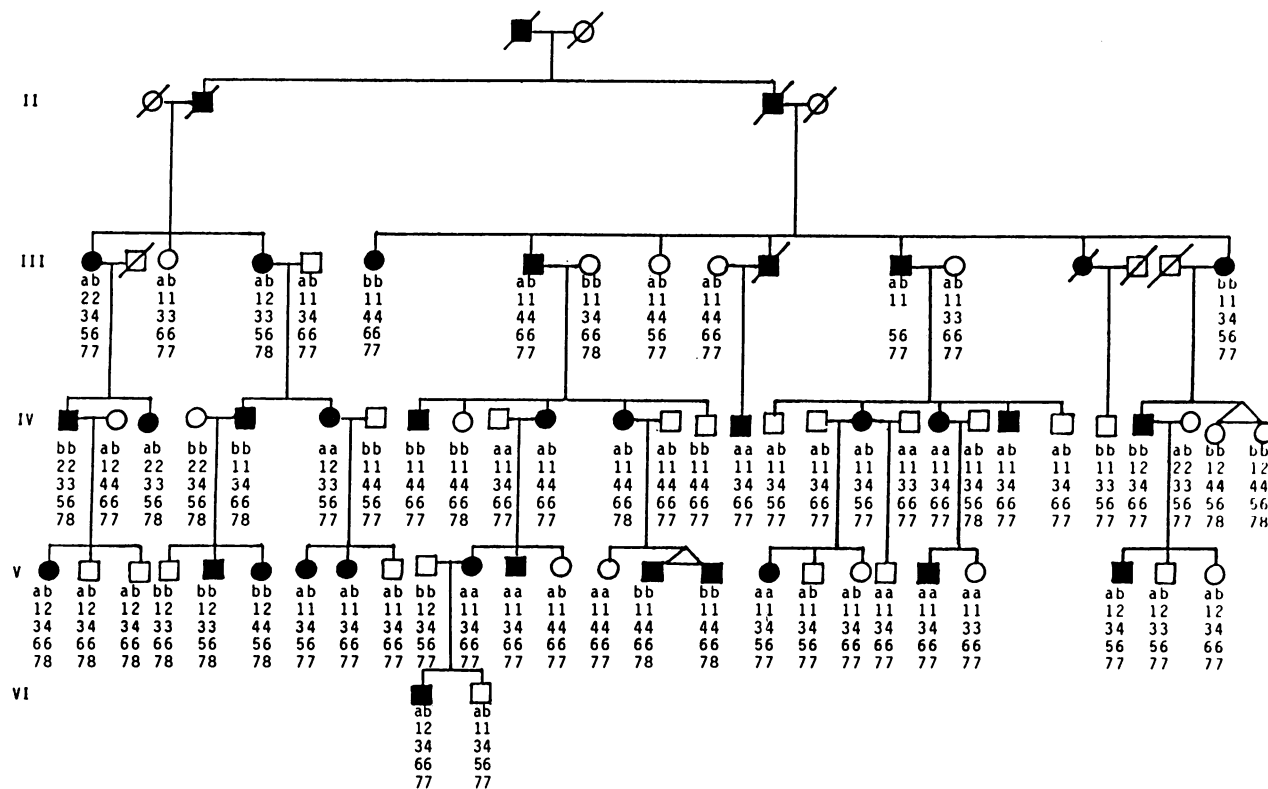
### Acknowledgments

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## Appendix

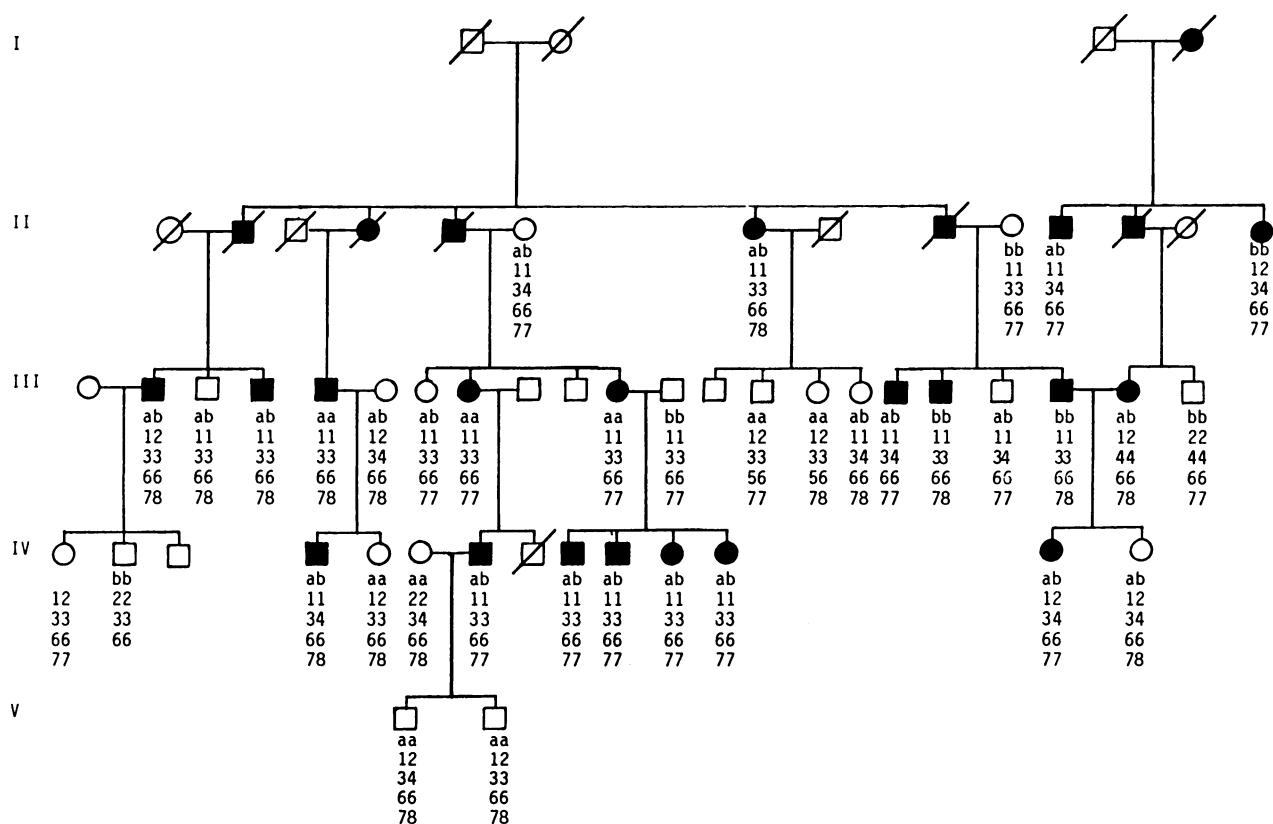


**Figure A1** Pedigree of family 10 with CMT1 showing Duffy blood typing, AT3, REN, NGFB, and AMY1 genotypes. In figs. A1–A16, Duffy genotypes are indicated by aa, bb homozygotes and ab heterozygotes; AT3 genotypes by 11, 22 homozygotes and 12 heterozygotes; REN genotypes by 33, 44 homozygotes and 34 heterozygotes; NGFB genotypes by 55, 66 homozygotes and 56 heterozygotes; and AMY1 genotypes by 77, 88 homozygotes and 78 heterozygotes.

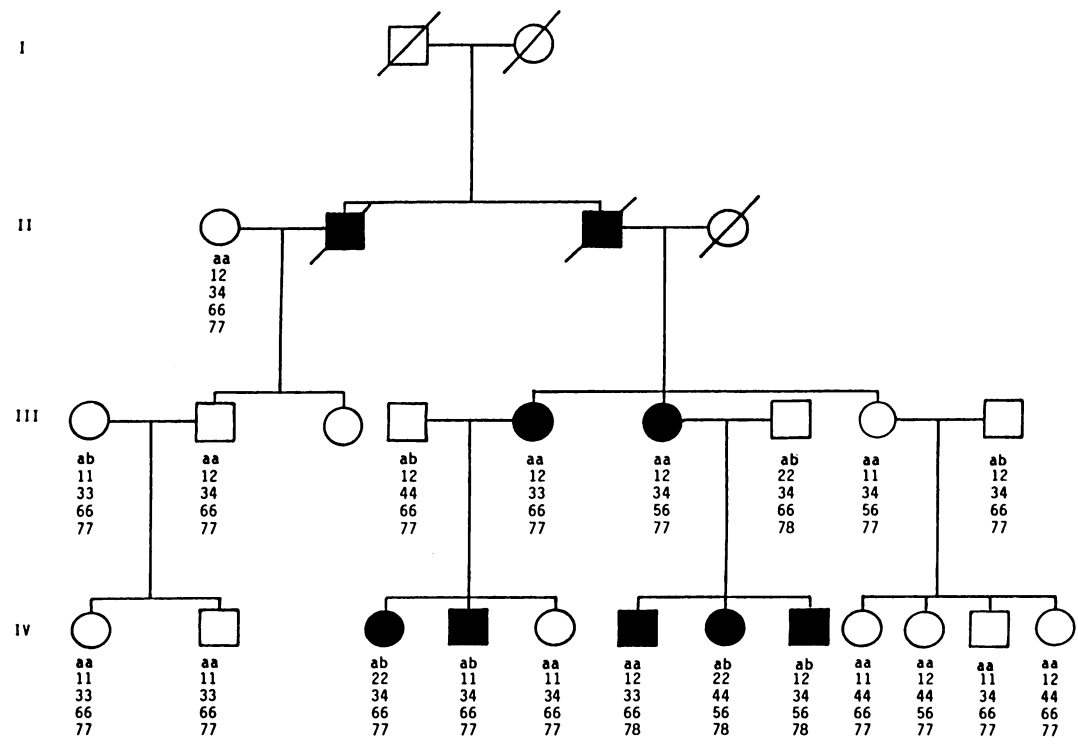


**Figure A2** Pedigree of family 8 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes

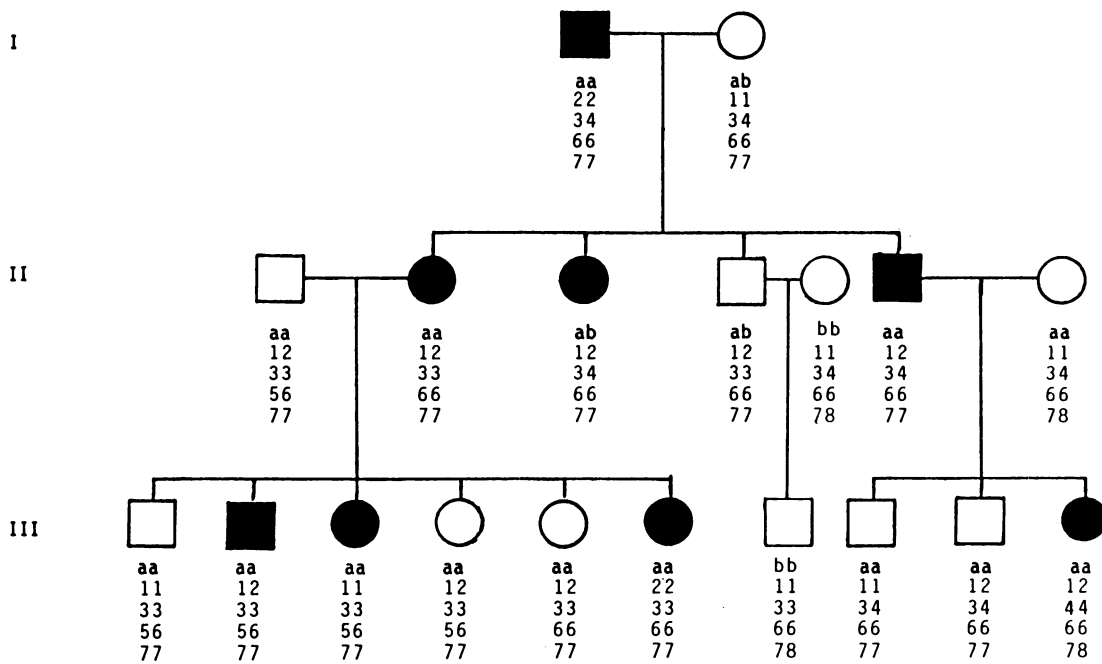




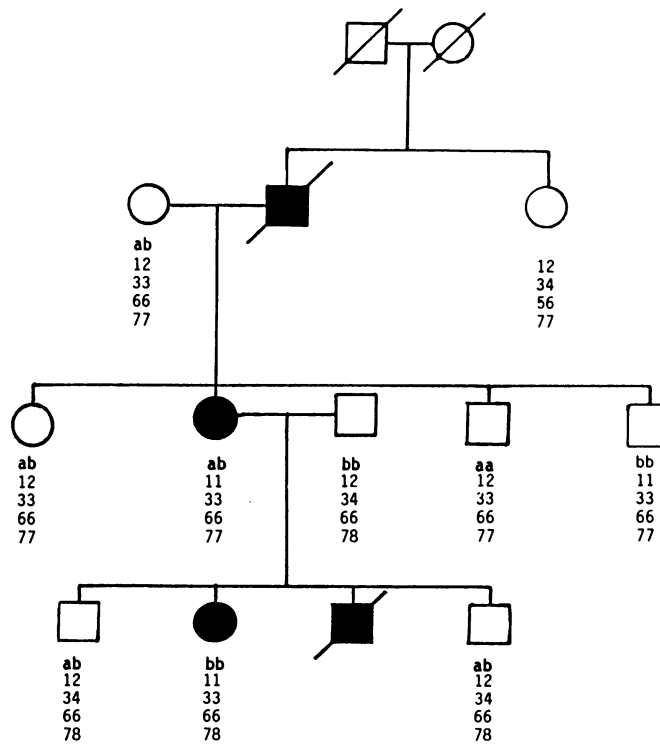
**Figure A3** Pedigree of family 1 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



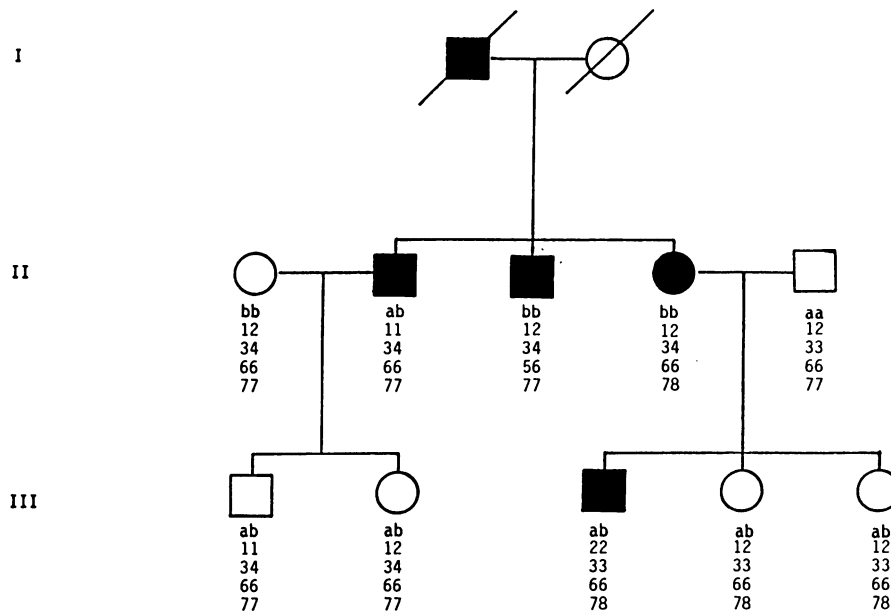
**Figure A4** Pedigree of family 23 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



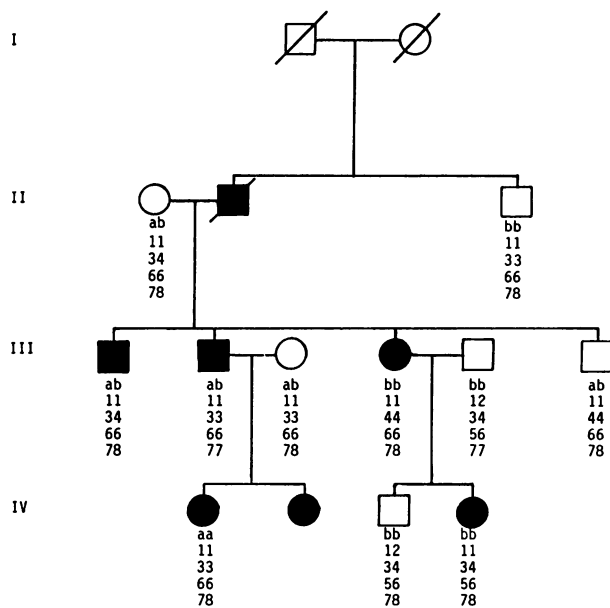
**Figure A5** Pedigree of family 21 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



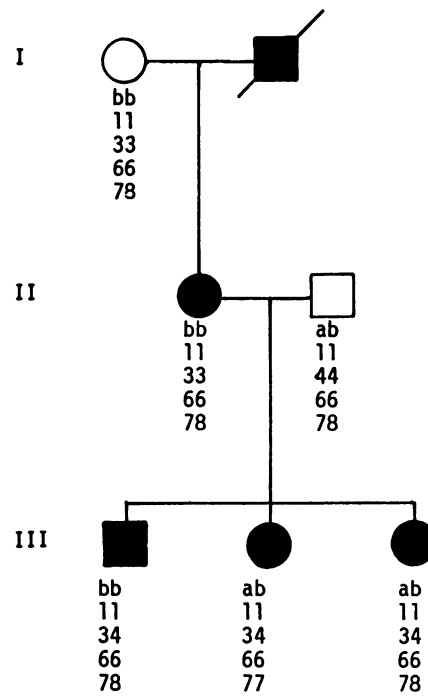
**Figure A6** Pedigree of family 24 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



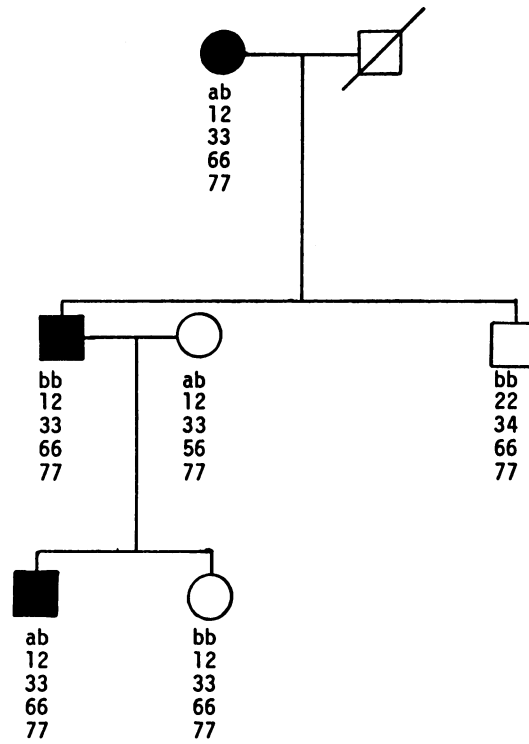
**Figure A7** Pedigree of family 39 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



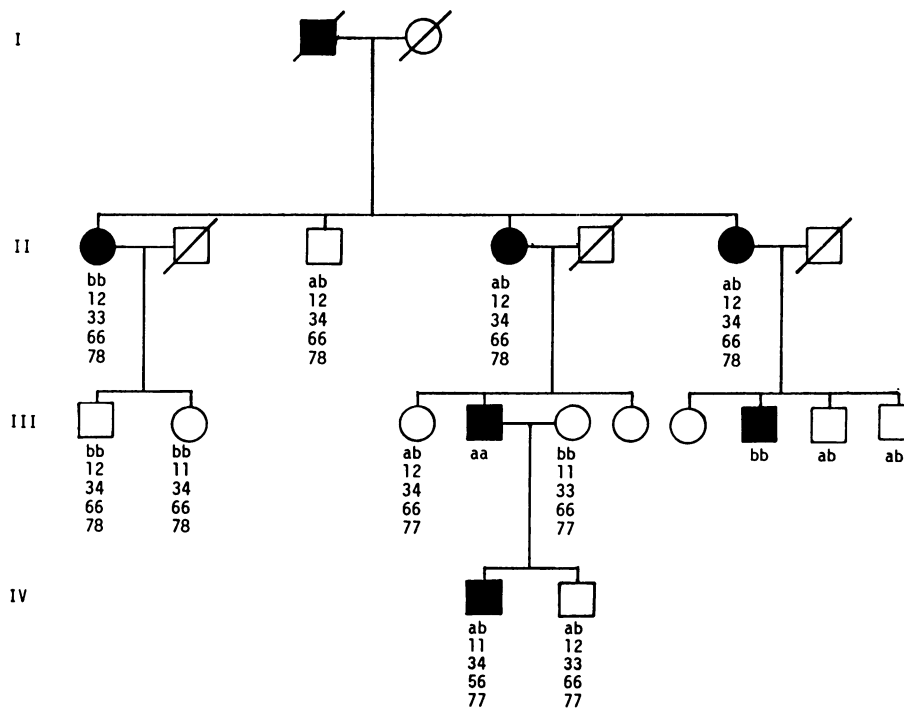
**Figure A8** Pedigree of family 26 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



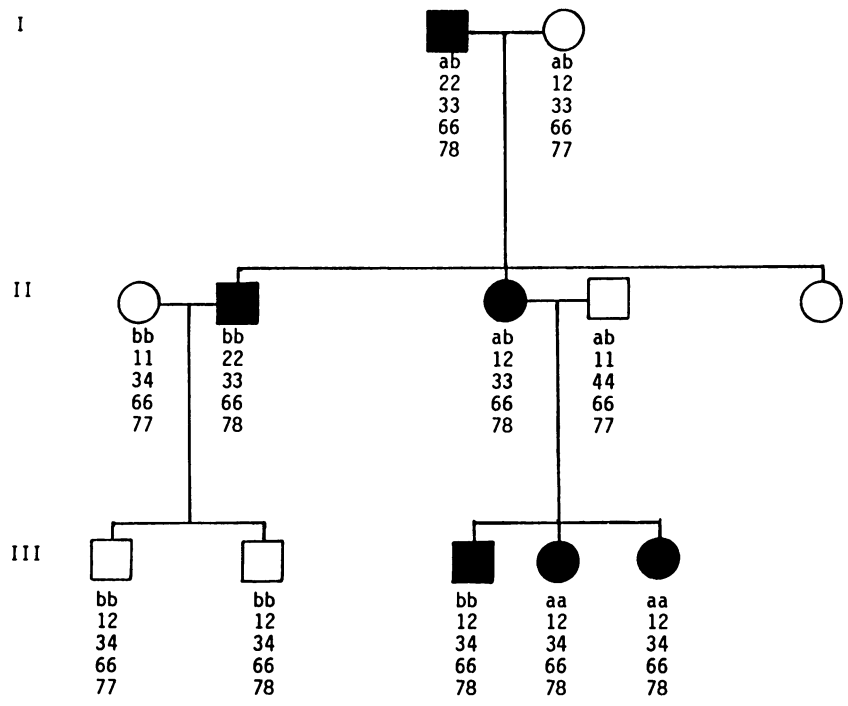
**Figure A9** Pedigree of family 9 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



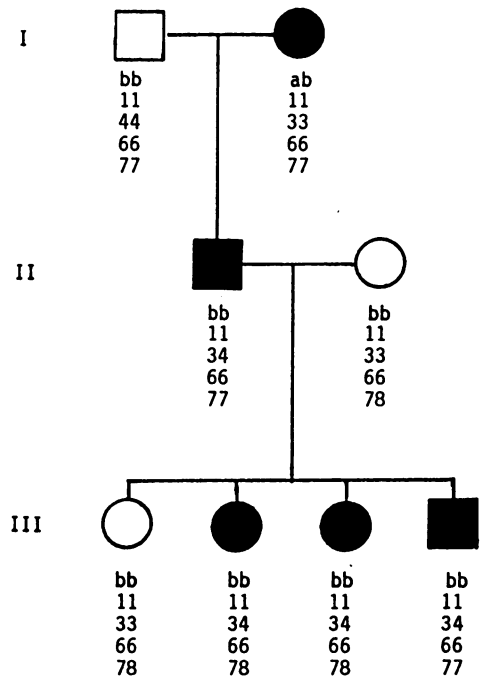
**Figure A10** Pedigree of family 7 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



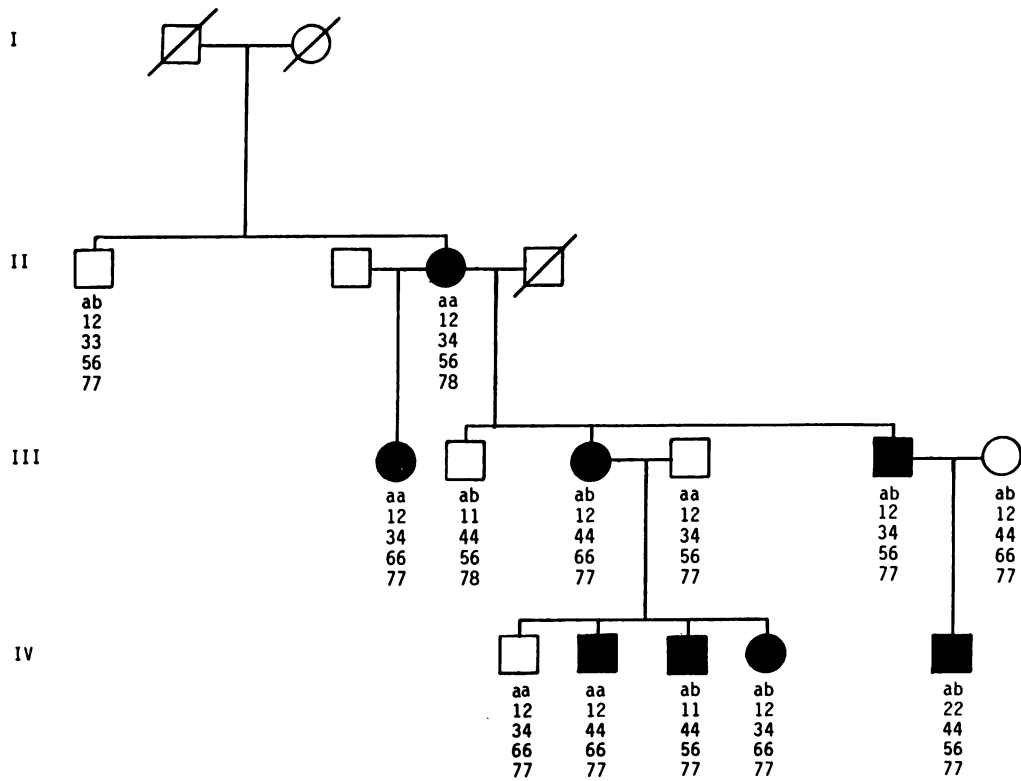
**Figure A11** Pedigree of family 13 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



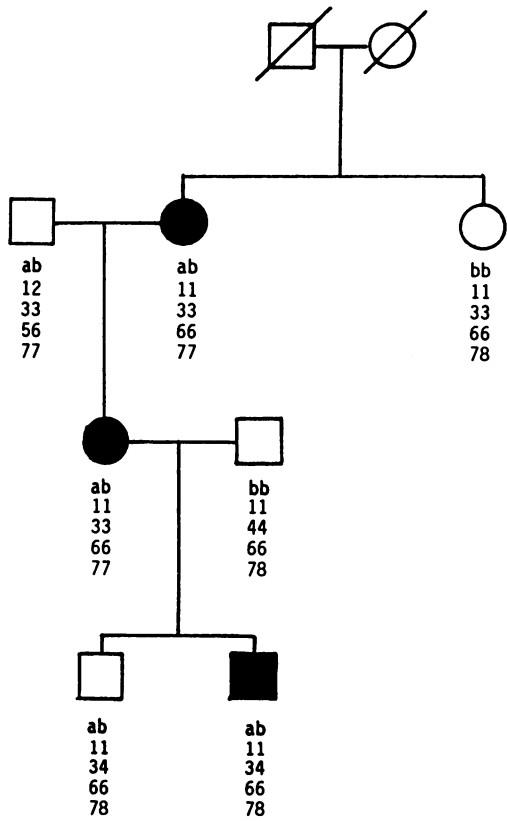
**Figure A12** Pedigree of family 19 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



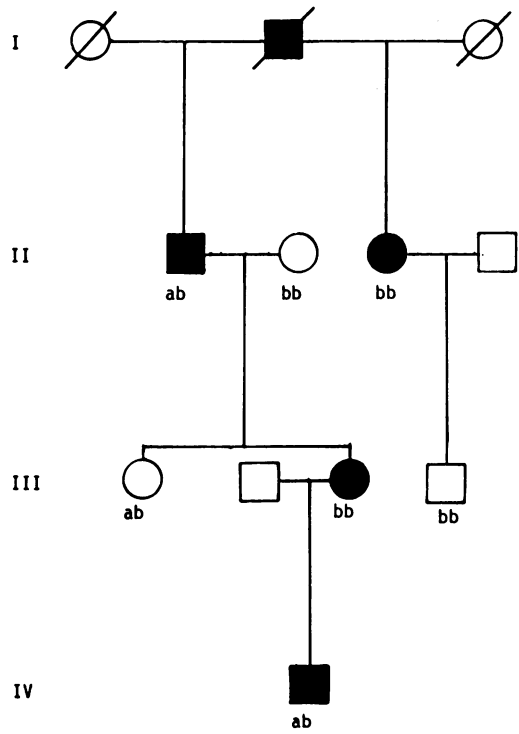
**Figure A13** Pedigree of family 27 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



**Figure A14** Pedigree of family 3 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes



**Figure A15** Pedigree of family 34 with CMT1 showing Duffy, AT3, REN, NGFB, and AMY1 genotypes.



**Figure A16** Pedigree of family 6 with CMT1 showing Duffy blood group typing.

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