

Linkage Analysis of von Recklinghausen Neurofibromatosis to DNA Markers on Chromosome 17

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Several recent studies indicate that the von Recklinghausen neurofibromatosis (NF1) gene is located near the centromere of chromosome 17 in some families. However, variable expressivity and a very high mutation rate suggest that defects at several different loci could result in phenotypes categorized as NF1. In order to assess this possibility and to map the NF1 gene more precisely, we have used two polymorphic DNA markers from chromosome 17 to screen several pedigrees for linkage to NF1. We ascertained a large Caucasian pedigree (33 individuals sampled, 17 NF1 affected) as well as eight smaller pedigrees and nuclear families (50 individuals sampled, 30 NF1 affected). Here, we report strong evidence of linkage of NF1 to the centromeric marker D17Z1 (maximum lod = 4.42) and a weaker suggestion of linkage to the ERBA1 oncogene (maximum lod = 0.57), both at a recombination fraction of zero. Since obligate crossovers with NF1 were not observed for either marker in any of the informative families tested, the possibility of NF1 locus heterogeneity is not supported.

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INTRODUCTION

We have previously excluded linkage of NF1 to a substantial portion of chromosome 8 in a large American Caucasian pedigree (Diehl *et al.*, 1987). These data contributed to an exclusion map for NF1 (Sarfrazi *et al.*, 1987). The results of this pooled set of mapping data, especially a positive lod score of 1.17 at a recombination fraction of 0.1 (Barker *et al.*, 1987a) for linkage near the centromere of chromosome 17 (D17Z1), suggested this chromosome as one of the most likely map locations for the NF1 gene. This suggestion was confirmed by conclusive reports of linkage to markers from chromosome 17, including the

centromeric marker D17Z1 and pA10-41 (Barker *et al.*, 1987b) and the nerve growth factor receptor (Seizinger *et al.*, 1987). However, variable expressivity and a very high mutation rate of NF1 may be indicative of locus heterogeneity for this disease. Among the individual families examined in these previous studies, the largest two-point lod score was 1.46, and some families exhibited slightly negative lod scores. Therefore, we have undertaken additional linkage studies using chromosome 17 markers. Our goal is to evaluate further the possibility of locus heterogeneity and to map precisely the NF1 gene for improved diagnostic accuracy and to facilitate its cloning and characterization.

METHODS AND STUDY SUBJECT

A total of nine American Caucasian NF1 pedigrees were ascertained and diagnostic examinations were performed. Individuals were categorized as affected with NF1 if two or more of the following criteria were satisfied: (1) six café-au-lait macules greater than 1.5 cm in diameter for postpubertal individuals or five macules greater than 0.5 cm in diameter before puberty; (2) one or more neurofibromas (one, if histologically confirmed), including plexiform; (3) multiple freckles of axilla or groin; (4) two or more Lisch nodules; (5) one or more optic nerve gliomas; (6) characteristic bony changes (Holt, 1978); and (7) a first-degree relative affected with NF1. In no instance did we observe nonpenetrant individuals who passed the NF1 gene to an affected offspring. Blood samples were drawn and immortalized cell lines were established using Epstein-Barr virus transformation. DNA was extracted using a method described by Bell *et al.* (1981). Restriction endonuclease digestions, Southern blotting, and hybridizations were carried out using conventional methods (Davis *et al.*, 1986; Feinberg

TABLE 1
Lod Scores for von Recklinghausen
Neurofibromatosis Chromosome 17 Markers

Family	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
	D17Z1 (<i>EcoRI</i>)						
UMI1	2.60	2.54	2.33	2.05	1.49	0.92	0.41
UMI2	0.09	0.08	0.07	0.05	0.03	0.01	0.00
UMI3	0.84	0.82	0.73	0.63	0.41	0.20	0.05
UMI4	0.30	0.29	0.26	0.21	0.13	0.06	0.02
UMI5	0.30	0.29	0.26	0.21	0.13	0.06	0.02
UMI8	0.30	0.29	0.26	0.21	0.13	0.06	0.02
Total	4.42	4.32	3.90	3.38	2.32	1.33	0.52
	erbA1 (<i>PvuII</i> and <i>EcoRI</i>)						
UMI1	0.54	0.53	0.49	0.44	0.32	0.21	0.10
UMI2	0.03	0.03	0.02	0.02	0.01	0.00	0.00
Total	0.57	0.56	0.51	0.45	0.33	0.22	0.10

and Vogelstein, 1984). Linkage to NF1 was tested for the centromeric marker D17Z1 using the clone p17H8, which is polymorphic when tested with the restriction enzyme *EcoRI* (Willard *et al.*, 1986), and the ERBA1 oncogene, which is polymorphic for *EcoRI* when tested with the clone pE2A3D (Mathieu-Mahul *et al.*, 1985) and for *PvuII* when tested with the clone pHE-A1 (Rider *et al.*, 1987). Linkage analyses to other chromosome 17 markers using some of our NF1 families are reported by Stephens *et al.* (this volume).

A total of 77 individuals (46 NF1 affected) were codominantly genotyped for D17Z1, 80 individuals (46 NF1 affected) for the ERBA1 *EcoRI* polymorphism, and 59 individuals (37 NF1 affected) for the ERBA1 *PvuII* polymorphism. *EcoRI* and *PvuII* polymorphisms for ERBA1 were combined into haplotypes for linkage analysis. We assumed a linear increase of penetrance of NF1 from 0% at birth to 95% at age 6 years and thereafter.

RESULTS

The results of our linkage analysis, presented as lod scores (Morton, 1955) calculated using the program LIPED (Ott, 1974), are presented in Table 1. No obligate crossovers were observed between either of the markers and NF1 in any of the families tested. Maximum lod scores consequently were estimated at a recombination fraction of zero for both markers. There was no obvious difference in the number of informative male and female meioses in the data, so the observed absence of crossovers cannot be attributed to sex-specific differences in recombination rates. The centromeric marker D17Z1 was most informative and

yielded a lod score of 4.42 for all families combined. Family UMI1 alone (33 individuals scored, 17 NF1 affected) provided a lod score of 2.60 for linkage between D17Z1 and NF1, which is close to the accepted criterion of 3.0 for demonstration of linkage. The ERBA1 polymorphisms were much less informative, yielding only a combined lod score of 0.57. The degree to which these markers were informative corresponds to the observed frequencies of their alleles. Based on the counting of about 50 independent alleles, the three alleles that we observed for D17Z1 (2.7 kb only; 2.7, 2.0, and 1.5 kb; 2.7 and 1.5 kb) exhibited frequencies of 0.71, 0.27, and 0.02, respectively. In contrast, both ERBA1 polymorphisms consisted of two alleles with frequencies of about 0.96 and 0.04 (respectively, *EcoRI*: 8.5 or 20.0 kb; *Pvu II*: 1.2, 0.8, or 2.0 kb). Polymorphisms at the two enzymes utilized for the ERBA1 probe were not in complete disequilibrium.

DISCUSSION

Our results broaden the conclusion of linkage of NF1 to the pericentromeric region of chromosome 17. The complete absence of obligate crossovers in any of the informative families here analyzed reduces the likelihood that locus heterogeneity is a common source of the observed variable expressivity or high mutation rate of NF1.

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