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Flanking Markers for the Gene Causing von Recklinghausen Neurofibromatosis (NF1)

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Summary

The defective gene causing von Recklinghausen neurofibromatosis (NF1), one of the most common inherited disorders affecting the human nervous system, was recently mapped to chromosome 17. We have used additional DNA markers to further narrow and bracket the NF1 defect. A multipoint linkage analysis suggests that the NF1 gene is flanked by D17Z1 on the centromeric side and by EW 207 on the telomeric side of the long arm of chromosome 17. The identification of closely linked flanking markers should allow us to develop a reliable prenatal and presymptomatic diagnostic test for this serious neurological disorder and provides the basis for applying chromosome-specific cloning techniques for the isolation and characterization of the mutant gene.

The defective gene causing von Recklinghausen neurofibromatosis (NF1) was recently mapped to chromosome 17 by genetic linkage to the nerve growth factor receptor (NGFR) on chromosome 17q12-17q22 and to two anonymous markers, D17Z1 (p3-6) and D17S71 (pA10-41), in the pericentromeric region of chromosome 17 (Barker et al. 1987; Seizinger et al. 1987*b*). This represented the first crucial step in applying the so-called reverse genetics approach to the isolation and characterization of the defect.

To further narrow and bracket the NF1 defect, we have tested additional DNA markers, including EW 203 (D17S54), EW 206 (D17S57), EW 207 (D17S73) (Fain et al. 1987), and pHHH202 (D17S33) (White et al. 1987). These markers were used for linkage studies in the NF1 pedigrees BOS 1, NCI 1-7, and DUK 115. Table 1 shows the lod scores for linkage of NF1 to these chromosome 17 markers, on the basis of the computer program LIPED (Ott 1974, 1976), as described elsewhere (Seizinger et al. 1987*a*, 1987*b*). Obligate crossovers were observed for the marker EW 203 (two recombinants in pedigree DUK 115) and for EW 207 (one recombinant in pedigree DUK 115). pHHH202 did not show crossovers in any of the tested NF1 families.

We have recently described an obligate recombination between the NF1 gene and the centromeric marker D17Z1 in pedigree DUK 115 (Pericak-Vance et al. 1987; Seizinger et al. 1987*a*). This particular nuclear family within the DUK 115 pedigree was also informative for the marker EW 207, which did not show a crossover with the NF1 gene. This suggests that either D17Z1 and EW 207 are both located on the centromeric side of the NF1 defect, with EW 207 being closer to the disease gene, or that EW 207 is flanking the NF1 gene on its telomeric side (the markers EW 203, EW 206, and pHHH202 were not informative in this nuclear family).

To maximize the linkage information in pedigree DUK 115, we used LINKMAP from the LINKAGE package (Ott 1974, 1976; Lathrop et al. 1984) to perform a three-point linkage analysis varying the potential position of

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Table 1

Two-Point Linkage Analysis between the NF1 Gene and Markers for Chromosome 17

Marker	Recombination Fraction ($\hat{\theta}$)								
	.0	.05	.1	.15	.20	.30	.40	$\hat{z}$	$\hat{\theta}$
EW203	$-\infty$	4.71	4.67	4.35	3.89	2.70	1.27	4.75	.07
EW207	$-\infty$	2.56	2.71	2.59	2.34	1.63	.77	2.71	.09
EW206	$-\infty$	-.59	.01	.21	.28	.27	.16	.28	.22
HHH202	3.83	3.53	3.20	2.83	2.42	1.51	.65	3.83	.00
D17Z1	$-\infty$	10.61	9.62	8.43	7.11	4.29	1.61	10.86	.01

NOTE.—For two-point linkage analysis, the mutation rate for NF1 was set at .00015, the frequency of the defective allele at .0003, and penetrance at 95%. Lod scores for all markers are based on NF1 pedigrees BOS 1, NCI 1-7, and DUK 115.

the NF1 gene relative to fixed positions of D17Z1 and EW 207. D17Z1, which was arbitrarily placed at .0, was found to be genetically linked to EW 207 with a maximum lod score of $\hat{z} = 12.5$ at $\hat{\theta} = .02$ on the basis of a preliminary linkage map for chromosome 17 in our laboratory (J. L. Haines, L. J. Ozelius, B. R. Seizinger, and J. F. Gusella, unpublished data). Our primary resource for the construction of a linkage map has been the "Venezuela reference pedigree," which contains many large interrelated sibships from the Huntington disease pedigree from Venezuela. A permanent set of lymphoblastoid cell lines has been established from this pedigree to determine the linkage relationship of DNA markers (Gilliam et al. 1987). As shown in figure 1, this multipoint linkage analysis suggests (although does not formally prove) that the NF1 gene is flanked by D17Z1 (centromeric side) and EW 207 (telomeric side). The odds are approximately 18:1 in favor of the NF1 defect being located between D17Z1 and EW 207— and against a location on the short-arm side of the centromeric marker D17Z1. Similarly, the odds are 24:1 in favor of the NF1 gene being located between D17Z1 and EW 207—and against a location on the telomeric side of the chromosome 17q marker EW 207. This analysis further supports our previous suggestion that the NF1 gene resides on the long arm rather than on the short arm of chromosome 17 (Seizinger et al. 1987*a*, *1987b*). Since EW 207 was found to be very tightly linked to another chromosome 17 q marker, EW 206 ($\hat{z} = 40.2$ at $\hat{\theta} = .005$), EW 206 might be used, together with EW 207, as a flanking marker on the telomeric side of the disease locus.

Thus, the identification of closely linked flanking markers should allow us to develop a reliable prenatal and presymptomatic diagnostic test for this serious neurological disorder and provides the basis for applying chromosome-specific cloning techniques for the isolation and characterization of the defective gene.

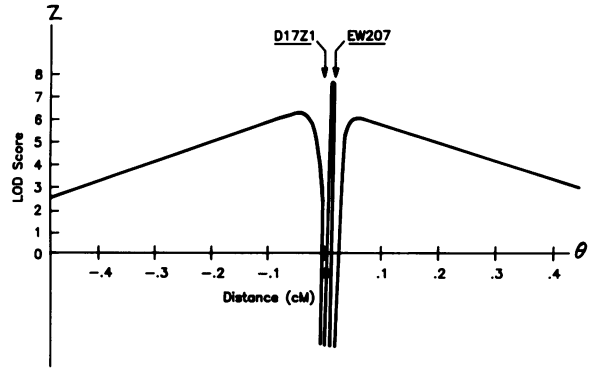

Figure 1 Multipoint linkage analysis of the NF1 gene, with the chromosome 17 markers D17Z1 (p3-6) and EW 207 (D17S73). The program LINKMAP from the LINKAGE package was used to calculate lod scores (z) for various locations of the NF1 gene relative to fixed positions for the DNA markers. θ = recombination fraction.

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