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Close Flanking Markers for Neurofibromatosis Type I (NF1)

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Summary

A genetic linkage study with 16 polymorphic DNA markers spanning the region 17p11–17q24 in 22 NF1 families is presented. Close linkage between NF1 and eight pericentromeric markers (HHH202, EW206, CRI-L946, EW203, EW301, FG2, p17H8, and CRI-L581) has been found, probe HHH202 being the closest marker to NF1. Genetic heterogeneity has been excluded. The study of multiply informative meioses suggests that the probes HHH202 and RW206 are flanking markers for NF1. The most likely order on the basis of multiply informative meioses and multipoint mapping is pter-pA10.41-EW301-cen-HHH202-NF1-EW206-EW207-qter.

Introduction

Von Recklinghausen neurofibromatosis (NF1) is one of the most frequent autosomal dominant disorders, with an incidence of around 1/4,000 individuals. The gene for NF1 was mapped to chromosome 17 by two independent groups (Barker et al. 1987; Seizinger et al. 1987). Subsequently, DNA markers have been studied in a large number of NF1 families, and it has been possible to narrow the localization of the NF1 gene to the pericentromeric region (Skolnick et al. 1987). Tight flanking markers for the centromere have been reported, but the localization of the NF1 gene to the short or long arm has not until now been resolved by genetic linkage studies (Skolnick et al. 1987). Finer localization of the gene and the availability of flanking markers will allow presymptomatic carrier detection and prenatal diagnosis.

In the present paper, we report a further linkage study with 16 polymorphic DNA markers in 22 families, which maps NF1 to the proximal long arm of chromosome 17 and defines flanking markers.

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Material and Methods

The family panel has been reported elsewhere (Upadhyaya et al. 1987). It has now been extended to 22 families, of which 14 are 3 generations and eight are 2 generations. The panel comprises 148 potentially informative meioses, 64 of which are phase known. All the family members were examined using the diagnostic criteria agreed on at the 1987 NIH consensus conference on neurofibromatosis (Neurofibromatosis 1988). In the case of unaffected relatives, only those under the age of 5 years were excluded, since the NF1 locus has a complete penetrance by this age.

In the analysis we used 16 polymorphyic DNA markers spanning region 17p11-17q24. The physical localization and allele frequencies for each marker are summarized in table 1. We have used the LINKAGE (MLINK, LODSCORE, ILINK, and LINKMAP) package (Lathrop 1984), LIPED (Ott 1974), and HOMOG (Ott 1983) in the analysis. Two-point linkage analysis was done with the computer program MLINK. Differences in recombination between males and females were analyzed using LIPED, and a test of heterogeneity was done with the program HOMOG. The program LODSCORE was used for two-point linkage analysis between the marker loci. ILINK and LINKMAP were used for multipoint linkage analysis.

Approximately 20 ml peripheral blood were obtained from each family member. DNA was extracted from

Table 1

DNA Markers Used for Linkage Analysis

Probe	Locus	Physical Localization	Enzyme	Allele Frequencies
pA10–41	D17S71	17p11	*Msp*I	.42, .58
			*Pvu*II	.28, .72
EW301	D17S58	17p	*Taq*I	.36, .64
			*Bgl*II	.14, .86
p17H8	D17Z1	17 cen	*Eco*RI	A: .40, .60
				B: .11, .89
			*Pvu*II	.26 (2.2 present)
pHHH202	D17S33	17q	*Rsa*I	.36, .64
EW207	D17S73	17q	*Bgl*II	.11, .89
			*Hin*dIII	.20, .80
EW206	D17S57	17q	*Msp*I	.14, .86
EW203	D17S54	17q	*Bgl*II	.42, .58
EW204	D17S55	17q	*Msp*I	.15, .85
EW205	D17S56	17q	*Rsa*I	.19, .81
CRI-L581	D17S37	17	*Taq*I	.10, .38, .52
CTI-L946	D17S36	17	*Msp*I	.12, .16, .72
FG2	COL1A1	17q21.3-q22	*Msp*I	.46, .54
heA1	ERBA1	17q11-q21	*Pvu*II	.04, .96
BS3	HOX2	17q21-22	*Sac*I	.18, .82
pE51	NGFR	17q22	*Xmn*I	A: .06, .04
				B: .10, .90
			*Hinc*II	.20, .80
hGH	GH	17q22-24	*Bgl*II	.23, .77

Table 2

Two-Point Linkage Analysis between NF1 and 16 Markers

	R/NR				
PROBES	Phase Known	Phase Unknown	$\hat{Z}$	$\hat{\theta}$	CI
HHH202	1/18	0/31	9.68	.02	0–.10
EW206	0/1	1/19	4.16	.04	0–.18
CRI-L946	0/16	2/32	9.46	.06	.02–.15
CRI-L581	1/7	4/35	6.06	.06	.02–.17
EW203	0/8	2/21	2.56	.06	0–.28
EW301	1/19	2/37	11.79	.08	.03–.16
p17H8	0/6	2/35	4.53	.08	0–.22
FG2	2/10	2/25	3.38	.10	.03–.26
EW207	2/12	2/29	6.39	.13	.06–6.24
pA10-41	2/8	6/44	3.58	.16	.07–.29
pE51	8/33	0/2	2.21	.24	.14–.39
EW205	. . .	1/0	.27	0	0–.11
heA1	0/9	2/9	2.16	.12	0–.34
EW204	. . .	1/5	.16	.17	.08–.35
BS3	. . .	4/14	.19	.33	. . .
hGH	No linkage	. . .	.39	.31	. . .

NOTE.—R = Number of recombinants; NR = number of nonrecombinants, CI = confidence interval; $\hat{Z}$ = maximum lod score at $\hat{\theta}$; $\hat{\theta}$ = estimated recombination fraction.

lymphocyte nuclei (Kunkel et al. 1977). Five micrograms of DNA were digested with the appropriate restriction endonucleases and transferred by Southern blotting to Hybond-N (Amersham). DNA probes were labeled with P^{32} by nick-translation and primer extension (Feinberg and Vogelstein 1984) and hybridized to the Hybond-N filters at 65 C. The repetitive sequences in DNA probes HHH202, EW203, EW204, EW205, FG2, heA1, and pe51 were competed out with total genomic DNA (0.5 μg/μl).

Results

Two-point linkage analysis between NF1 and each marker (table 2) showed that eight pericentromeric markers (HHH202, EW206, CRI-L946, CRI-L581, EW203, EW301, p17H8, and FG2) were closely linked ($\theta \leq .10$) to NF1. Loose linkage ($\theta > .10$) with a further three markers (EW207, pA10.41, and pe51) was found, and four markers (EW205, heA1, EW204, and BS3) were not sufficiently informative within our families. No significant linkage was observed with hGH.

The data on the analysis of recombination frequencies in males and females are summarized in table 3. The apparent difference in the estimation of male and female recombination fractions for closely linked markers is mainly due to the limited number of recombinations in each sex.

Heterogeneity in our family panel has been analyzed by the program HOMOG, which assumes that there are two family types, one linked and the other unlinked (table 4). Among the various markers analyzed, the only slight indication for heterogeneity was noted with probe CRI-L581 at a 4% level of significance. On further analysis using program HOMOG2, which assumes that there are two family types—both with linkage, and one with θ_1, and the other with θ_2, where $\theta_1 < \theta_2 < .5$—heterogeneity was no longer observed.

Two-point linkage analysis between the marker loci (table 5) reveals that HHH202 does not cross over with p17H8, EW204, and EW205. No recombination is observed between EW206 and marker loci EW203, EW204, EW205, and CRI-L946. A look at the short arm markers shows that pA10–41 and EW301 had not recombined.

Pedigree CAR17 (fig. 1) provides information on the order of the NF1 gene in relation to markers EW301, p17H8, HHH202, and EW206. Probes EW207 and EW204 were not informative in this family. Our typing of individual 3, who is dead, was inferred on the basis of data from her children and husband. Individual 8

Table 3

Observed Recombination Fractions in Males and Females for Chromosome 17 Markers

Probe	θ_m	Z_m	θ_f	Z_f
HHH202	.00	3.6	.05	5.14
EW206	.20	.38	.00	4.21
CRI-L946	.00	5.85	.10	4.37
EW203	.00	1.45	.10	1.34
EW301:				
*Bgl*II	.00	2.82	.10	4.32
*Taq*I	.00	1.87	.10	3.88
FG2	.00	4.36	.30	.36
p17H8	.10	.79	.10	3.03
CRI-L581	.00	2.96	.20	1.91
EW207:				
*Bgl*II	.20	.56	.20	1.85
*Hin*dIII	.10	2.12	.20	1.53
pA10–41:				
*Msp*I	.20	.52	.20	1.90
*pvu*II	.00	.66	.10	.40
pe51:				
*Xmn*I-A	.10	.78	.40	.19
*Xmn*I-B	.30	.05	.40	.11
*Hin*cII	.10	1.34	.40	.11
heAl	.00	1.20	.20	1.16
EW204	.00	.58	.50	.0
EW205	.00	1.48	.00	.11

Table 4

Analysis of Heterogeneity, Using Programs HOMOG and HOMOG2

	HOMOG[a] ($\theta = \theta_1$ and $\theta = ½$)		HOMOG2[b] ($\theta_1 < \theta_2 < .5$)	
PROBE NAME	X_1^2	*P* Value	X_2^2	*P* Value
pA10-41	2.72	.05	2.72	.12
EW301	.02	.44	.15	.46
p17H8	.00	.50	.00	.50
HHH202	.00	.50	.00	.50
EW207	.32	.28	.31	.42
EW206	.00	.50	.15	.46
EW203	.00	.50	.00	.49
EW204	.008	.46	.00	.49
EW205	.00	.50	.00	.50
CRI-L581	3.02	.04	3.02	.11
CRI-L946	.028	.43	.06	.48
FG2	1.19	.13	1.19	.27
heA1	.00	.50	.00	.50
BS3	1.84	.08	1.86	.19
pe51	.282	.29	.33	.42
hGH	.00	.50	.00	.50

[a] HOMOG assumes that there are two family types, one linked and the other unlinked.

[b] HOMOG2 assumes that there are two family types, both with linkage, one with θ_1 and the other with θ_2, where $\theta_1 < \theta_2 < .5$.

Table 5

Two-Point Linkage Analysis Between Marker Loci: Observed Recombination (Lod Score)

	pA10.41	EW301	p17H8	HHH202	EW207	EW206	EW203	EW204	CRI-L581	CRI-L946	heA1	FG2
EW301	.001											
	(5.12)											
p17H8.....	.12	.14										
	(1.42)	(3.60)										
HHH202.....	.15	.11	.001									
	(1.30)	(5.15)	(4.14)									
EW207	.14	.13	.15	.16								
	(3.87)	(5.29)	(2.59)	(2.24)								
EW206	.12	.18	.17	.19	.26							
	(.62)	(1.19)	(5.74)	(8.07)	(.30)							
EW203	.16	.10	.001	.28	.10	.001						
	(.67)	(2.74)	(1.50)	(3.23)	(1.48)	(1.10)						
EW204	.00	.00	.001	.001	.001	.001	.001					
	(.04)	(.95)	(1.00)	(6.96)	(1.32)	(2.56)	(5.8)					
CRI-L581	.50	.19	.10	.21	.11	.16	.12	.001				
	(.00)	(2.33)	(1.75)	(1.32)	(4.73)	(3.53)	(2.20)	(1.15)				
CRI-L946	.29	.16	.06	.12	.06	.001	.12	.49	.09			
	(.36)	(3.34)	(2.86)	(2.34)	(6.72)	(2.72)	(1.56)	(−1.07)	(4.07)			
heA1	.44	.10	.001	.06	.00	.15	.48	.001	.50	.001		
	(.00)	(1.09)	(8.25)	(2.59)	(1.03)	(2.6)	(−5.52)	(5.97)	(1.95)	(2.11)		
FG2.....	.25	.28	.22	.12	.18	.73	.04	.15	.76	.17	.001	
	(.52)	(.56)	(5.02)	(1.12)	(1.84)	(2.97)	(1.44)	(1.87)	(2.71)	(1.1)	(3.8)	
EW205	.00	.07	.14	.001	.001	.001	.55	.001	.001	.001	.001	.49
	(1.30)	(1.09)	(2.36)	(1.36)	(1.75)	(1.23)	(.001)	(5.63)	(9.02)	(6.50)	(5.97)	(−2.55)

is a recombinant for the centromeric probe p17H8. Her grandmother is homozygous for the polymorphism identified by probe EW301. Individual 3 is heterozygous for the polymorphism, having received the disease with allele 1, but had passed allele 2 to her effected daughter (individual 8)—whereas affected individuals 11 and 12 have received the disease and allele 1 from the mother. Individual 8 is therefore also a recombinant for EW301 (17p11). She is also a recombinant for probe HHH202 (17q11) in a phase-known situation. However, she is not a recombinant for probe EW206 (17q). On the basis of this recombinant family, the likely order is pter-EW301-cen-HHH202-NF1-EW206-qter or pter-EW301-cen-HHH202-EW206-NF1-qter. NF1 is linked to HHH202 at a distance of 2 cM and to marker EW206 at 4 cM (table 2). Linkage data for the marker loci suggest that HHH202 is linked to EW206 at θ = .19, Z = 8.07 (table 5). If NF1 is assigned to a location distal to EW206, the expected genetic distance between NF1 and HHH202 will be >19 cM. This is not compatible with our data; therefore, the most likely order is EW301-cent-HHH202-NF1-EW206. The above inferred order is supported by the results of study of other multiply informative meioses (table 6).

The likely order on the basis of multipoint mapping based on three-point analysis is p17H8-HHH202-EW206. The relative likelihood of order p17H8-HHH-202-EW206 vis-à-vis order p17H8-EW206-EW202 is 300:1. The analysis based on four-point mapping is given in table 7. In constructing this table we initially assumed that the order of p17H8, HHH202, and EW206 is fixed. Subsequently we tested for the location of the NF1 gene and other marker loci in relation to the above order. Table 7 shows that when compared with the next likely order the NF1 gene is between HHH202 and EW206 with the likelihood of 83:1.

Discussion

We have found close linkage between NF1 and eight pericentromeric DNA markers on chromosome 17. Probe HHH202 (D17S33) is the marker closest to NF1 (θ = .02, Z = 9.68). Probe EW206 (D17S57) is linked

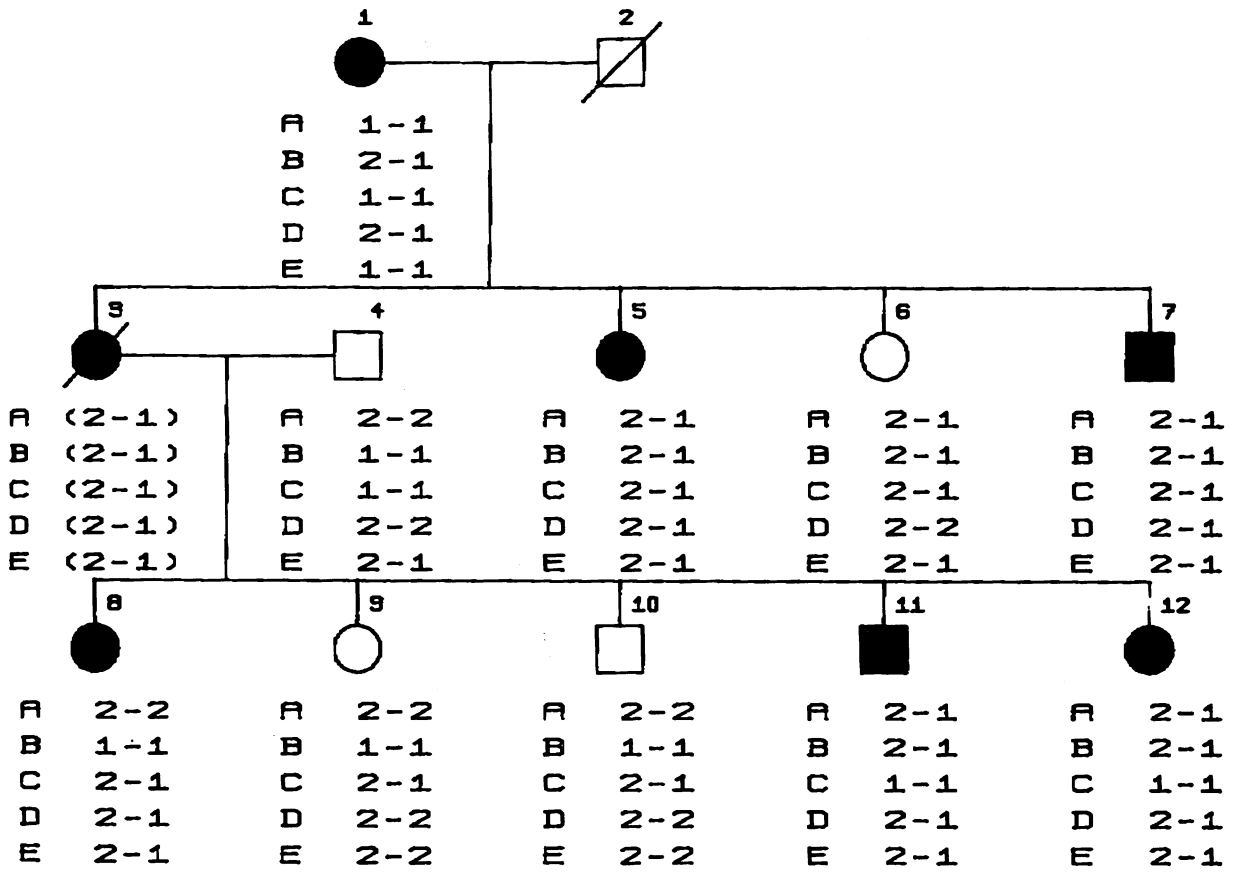

Figure 1 Pedigree typed with DNA probes EW301 (*A*), p17H8 (*B*), HHH202 (*C*), EW206 (*D*), and EW207 (*E*). Individual 8 is a recombinant for centromeric probe p17H8 (B). Her grandmother is homozygous for EW301 (*TaqI*) polymorphism. Individual 3 is dead, but her typing can be inferred from the DNA analysis of her spouse and children. She is clearly heterozygous, having received the disease with allele 1, but had passed allele 2 to her affected daughter (individual 8)—whereas affected individuals 11 and 12 have received the disease and allele 1 from the mother. Individual 8 is therefore also a recombinant for EW301 (17p11). She is also a recombinant for marker HHH202 (17q11) but not a recombinant for probe EW206 (17q). The likely order based on data for this recombinant family is pter-EW301-cen-HHH202-NF1-EW206-qter.

to NF1 at a distance of 4 cM but was less informative than HHH202 in our family panel.

Recombination is observed between all 16 markers and the NF1 locus, thus excluding any of these markers as representing the NF1 gene itself. Genetic heterogeneity has been excluded using the program HOMOG2, indicating that there is only one NF1 locus in our families.

Localization of the NF1 gene to either the proximal short or the proximal long arm of chromosome 17 was not definitely established by genetic linkage analysis at the last neurofibromatosis meeting (Skolnick et al. 1987). Our results now clearly indicate a localization of the NF1 gene to the proximal long arm. The finding is also supported by the constitutional reciprocal translocation involving chromosomes 17q11.2 and 1 in an NF1 patient (Schmidt et al. 1987).

The study of meioses multiply informative with the pericentromeric probes suggests that probes HHH202 and EW206 are flanking markers for NF1. This information will be useful for clinical applications. However, caution will still be required until the distance between HHH202 and EW206 has been well established by linkage analyses on additional pedigrees.

The most likely order based on multiply informative meioses and multipoint mapping is pter-pA10.41-EW301-cen-HHH202-NF1-EW206-EW207-qter. The biochemical and molecular defect responsible for NF1

Table 6

Inferred Order of Probes, Using Multiple Informative Meioses

Family	Sex	Status	Recombinant for	Nonrecombinant	Phase	Likely Order
CAR 12	M	AFF	EW207, FG2	...	Known	NF1-(EW207, FG2)
CAR 51	F	AFF	EW207 CRI.L581	pA10.41	Not known	pA10.41-NF1-(EW207, CRI.L581) or NF1-pA10.41-(EW207, CRI.L581)
CAR 59	M	AFF	EW207 CRI.L946 BS3, pe51	pA10.41, EW301	Not known	(pA10.41, EW301)-NF1-(EW207, CRI.L946, BS3, pe51) or NF1-(pA10.41, EW301) × (EW207, CRI.L946, BS3, pe51)
CAR 59	F	AFF	pe51	pA10.41, EW207	Known	pA10.41-EW207-NF1-pe51 or NF1-pA10.41-EW207-pe51
CAR 107	F	AFF	pe51	EW301, EW206	Known	EW301-p17H8-EW206-NF1-pe51 or NF1-EW301-p17H8-EW206-pe51
CAR 202	M	AFF	pA10.41, p17H8	EW207, CRI.L581 CRI.L946, pe51	Not known	pA10.41-p17H8-NF1-(EW207, CRI.L581, CRI.L946, pe51)
CAR 203	M	UNAFF	EW301	HHH202	Not known	EW301-NF1-HHH202 or EW301-HHH202-NF1
CAR 206	F	AFF	EW301, CRI.L946	...	Known	EW301-NF1-CRI.L946 or NF1-(EW301, CRI.L946)
CAR 207	M	UNAFF	pA10.41	EW206, EW301	Not known	pA10.41-EW301-NF1-EW206

Table 7

Multipoint Mapping Based on Four-Point Analysis

Order	−2 ln Like	Relative Likelihood
p17H8-HHH202-NF1-EW206	1266.97	781 E + 15
NF1-p17H8-HHH202-EW206	1275.81	9 E + 15
p17H8-HHH202-EW206-NF1	1281.64	509 E + 12
p17H8-NF1-HHH202-EW206	1349.37	1.00
pA10.41-p17H8-HHH202-EW206	1052.71	300 E + 41
p17H8-HHH202-pA10.41-EW206	1055.20	864 E + 40
p17H8-pA10.41-HHH202-EW206	1063.29	151 E + 39
p17H8-HHH202-EW206-pA10.41	1252.93	1.00
p17H8-HHH202-EW206-EW207	972.81	295.89
p17H8-EW207-HHH202-EW206	980.58	6.08
EW207-p17H8-HHH202-EW206	980.82	5.39
p17H8-HHH202-EW207-EW206	984.19	1.00
p17H8-heA1-HHH202-EW206	931.22	17.03
heA1-p17H8-HHH202-EW206	934.23	3.78
p17H8-HHH202-heA1-EW206	935.72	1.79
p17H8-HHH202-EW206-heA1	936.89	1.00
p17H8-HHH202-EW206-CRI.L946	960.82	3.71
CRI.L946-p17H8-HHH202-EW206	961.16	3.13
p17H8-CRI.L946-HHH202-EW206	963.40	1.02
p17H8-HHH202-CRI.L946-EW206	963.44	1.00
p17H8-HHH202-EW206-CRI-L581	983.38	5.87
p17H8-CRI.L581-HHH202-EW206	986.58	1.19
p17H8-HHH202-CRI.L581-EW206	986.66	1.14
CRI.L581-p17H8-HHH202-EW206	986.92	1.00

remains unknown, so that the "reverse genetics" approach will probably be essential for our understanding of the disorder at the molecular level. As part of a general strategy directed toward the isolation of the NF1 gene, our current work includes development of our family data base, linkage analysis with other closely linked markers, molecular analysis of new mutations by pulsed-field gel electrophoresis, and documentation of the phenotypic features for future comparison with specific mutations. Continuing collaboration between the different groups working on the genetics, biochemistry, pathology, and neurobiology of NF1 will be essential for the isolation and the characterization of the NF1 gene, for the development of accurate tests for prediction and prevention, and, ultimately, for effective therapeutic strategies.

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References

Barker, D., E. Wright, K. Nguyen, L. Cannon, P. Fain, D. Goldgar, D. T. Bishop, J. Carey, B. Baty, J. Kiuhin, H. Willard, M. Leppert, J. M. Lalouel, R. White, and M. Skolnick. 1987. Gene for von Recklinghausen neurofibromatosis in the pericentromeric region of chromosome 17. Science **236**:1100–1102.

Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabelling DNA restriction fragments to a high specific activity. Anal. Biochem. **137**:266–267.

Kunkel, L. M., K. D. Smith, S. H. Boyer, D. S. Borgoankar, S. S. Watchel, O. J. Muller, W. R. Breg, H. W. Jones, and J. M. Kay. 1977. Analysis of human Y chromosome variants. Proc. Natl. Acad. Sci. USA **74**:1245–1249.

Lathrop, G. M., J. M. Lalouel, C. Julier, and J. Ott. 1984. Strategies for multilocus linkage analysis in humans. Proc. Natl. Acad. Sci. USA **81**:3443–3446.

Neurofibromatosis 1988. Conference statement. Arch. Neurol **45**:575.

Ott, J. 1974. Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am. J. Hum. Genet. **26**:588–597.

——— 1983. Linkage analysis and family classification under heterogeneity. Ann. Hum. Genet. **47**:311–320.

Schmidt, M. A., V. V. Michels, and W. Denard. 1987. Cases of neurofibromatosis with rearrangements of chromosome 17 involved band 17q11.2. Am. J. Hum. Genet. **26**:771–777.

Seizinger, B. R., and 32 coauthors. 1987. Genetic linkage of von Recklinghausen neurofibromatosis to the nerve growth factor receptor gene. Cell **49**:589–594.

Skolnick, M., B. Ponder, and B. Seizinger. 1987. Linkage of NF1 to 12 chromosome 17 markers: a summary of eight concurrent reports. Genomics **1**:382–383.

Upadhyaya, M., M. Sarfarazi, S. M. Huson, K. Stephens, W. Broadhead, and P. S. Harper. 1987. Chromosome 17 markers and von Recklinghausen neurofibromatosis: a genetic linkage study in British population. Genomics **1**:358–360.