TABLE Lod scores for linkage between DNA loci and Von Recklinghausen neurofibromatosis.

DNA marker	Chromosomal localisation	θ (recombination fraction)					
		0.00	0.05	0.10	0.20	0.30	0.40
NGFB	1p22.1	—	-2.28	-1.18	-0.35	-0.16	-0.03
RAF1	3p25→p24	—	-5.29	-3.18	-1.39	-0.58	-0.25
RAF2	4pter→p15	—	-3.97	-2.49	-1.08	-0.55	-0.18
ALB	4q11→q13	-2.50	-1.21	-0.72	-0.34	-0.19	-0.09
D4S1	4p15.1→q11	-3.70	-1.19	-0.27	+0.32	+0.38	+0.23
D4S35	4p15.1→q12	-15.35	-10.21	-7.27	-3.69	-1.73	-0.60
ABL	9p34	—	-0.90	-0.59	-0.28	-0.12	-0.03
HRAS1	11p15	—	-6.86	-4.02	-1.59	-0.54	-0.25
KRAS2	12p12	—	-5.40	-3.18	-1.44	-1.17	-0.23
IGF1	12q22→q24.1	—	-2.73	-1.47	-0.47	-0.04	+0.03
D22S9	22q11	—	-5.87	-3.59	-1.52	-0.67	-0.19
D22S1	22q11.2→q13	—	-2.29	-1.59	-0.86	-0.42	-0.15
SIS	22q12.3→q13.1	—	-5.49	-3.29	-1.33	-0.51	-0.07

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References

- Mulvihill J. Tentative definitions of neurofibromatosis. *Neurofibromatosis Research Newsletter*, 1986;2:1.

- Spence MA, Bader J, Parry DM, et al. Linkage analysis of neurofibromatosis (Von Recklinghausen disease). *J Med Genet* 1983;20:334-7.
- Gusella JF, Wexler NS, Conneally PM, et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983;306:234-8.
- Seizinger BR, Martuza RL, Gusella JF. Loss of genes on chromosome 22 in human acoustic neuroma. *Nature* 1986;321:644-7.
- Seizinger BR, Rouleau G, Ozelius LJ, et al. Common pathogenic mechanism for three different tumor types in bilateral acoustic neurofibromatosis. *Science* 1987;236:317-9.
- Krone W, Hogemann I. Cell culture studies on neurofibromatosis (Von Recklinghausen). V. Monosomy 22 and other chromosomal anomalies in cultures from peripheral neurofibromas. *Hum Genet* 1986;74:453-5.

Correspondence and requests for reprints to Dr B R Seizinger, Neurogenetics Laboratory, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.

(See Note added in proof on p 538.)

Linkage studies in peripheral neurofibromatosis

MARGARET A PERICAK-VANCE*, LARRY H YAMAOKA*, JEFFERY M VANCE*, ARTHUR S AYLSWORTH†, GEORGE O D ROSSENWASSER‡, P CRAIG GASKELL JR*, MARK J ALBERTS*, WU-YEN HUNG*, CAROL HAYNES*, AND ALLEN D ROSES*

From *the Division of Neurology, Department of Medicine, and ‡the Department of Ophthalmology, Duke University Medical Center, Durham, North Carolina 27710; and †the Department of Pediatrics, University of North Carolina, Chapel Hill, North Carolina, USA.

SUMMARY Peripheral neurofibromatosis (NF) is one of the most common major genetic disorders in man. Its chromosomal location is unknown and questions regarding such factors

as genetic heterogeneity remain unanswered. We have ascertained and sampled several large multi-generation families for linkage studies including one family of 66 subjects, 28 of whom were affected with NF. Recombinant DNA studies of several restriction fragment length

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polymorphisms (RFLPs) including C3, ApoC2, pBam34 (D19S6), HAUP[APRT], pE40-1[D11S21], Hp[Hp2 α], LDR92, and LDR111 failed to show a significant linkage ($\hat{Z}$ [lod score] ≥ 3.00) in this family. In addition, the results excluded areas of the genome around the marker loci ($\hat{Z}\leq -2.00$) as potential sites for linkage. The maximum $\hat{Z}$ obtained with the markers was for Hp at $\hat{\theta}$ (maximum recombination fraction)=0.20 and $\hat{Z}=0.399$. We are now in the process of screening additional RFLPs and families for linkage to NF.

Neurofibromatosis (NF) is a neurogenetic disorder with autosomal dominant inheritance characterised by variable expressivity and incomplete penetrance.¹⁻³ It is a relatively common disorder with a frequency of approximately 1 in 3000. NF is believed to have a high mutation rate and genetic heterogeneity has been postulated.

Clinically, NF is most frequently characterised by café au lait spots and tumours palpable below the skin. Other features may include pendulous tumours, scoliosis, pseudoarthrosis of the tibia, phaeochromocytoma, meningioma, glioma, acoustic neuroma, optic neuroma, mental retardation, hypertension, and hypoglycaemia.² Localised pigmentary changes may be present at birth with skin tumours arising at or around puberty. With careful examination most subjects can be diagnosed with certainty. However, documented cases of incompletely penetrant persons do exist.²

Recently, efforts have been made to localise the NF gene using linkage analysis. These attempts, while excluding substantial regions of the genome, have been unsuccessful to date. Chromosomal localisation of the NF gene would be the first step in delineation of problems such as genetic heterogeneity and in identifying the basic defect in NF.

Materials and methods

The families available were ascertained through the Department of Pediatrics at the University of North Carolina at Chapel Hill Medical School and through the Division of Neurology at the Duke University Medical Center. All family members have been examined by a neurologist or clinical geneticist or both and an ophthalmologist. Blood samples were obtained for paternity testing and quick DNA and lymphoblast cell transformation. We currently have samples on over 250 family members from seven large multi-generation NF families.

In order to circumvent the problem of genetic heterogeneity we limited our initial studies to one large pedigree. This family is sufficiently large to allow for the independent evaluation of individual markers for linkage. Once a successful linkage is identified, we will begin testing in the other families. In this family, 66 subjects were sampled (including spouses) and 28 of these were definitely affected with NF. There are approximately 50 potentially informative meioses for analysis. Standard diagnostic criteria were used to determine whether a subject was affected. These included at least two of the following findings: (1) positive family history of NF, (2) Lisch nodules, (3) five or more café au lait spots, and (4) one or more neurofibroma. Subjects, particularly prepubescent younger family members, whose clinical status could not be determined with certainty, were coded as unknown for the linkage analysis.

Linkage analysis was performed using the computer programme LIPED.⁴ The NF gene was assumed to be autosomal dominant with a penetrance of 0.95.² However, varying the estimates for the penetrance did not substantially change the results. In addition, equal recombination for males and females was assumed between NF and the marker loci. The markers used in the analyses and their chromosomal locations⁵ are C3 (19pter $\rightarrow$ q13.2), ApoC2 (19cen $\rightarrow$ q13), pBam 34 [D19S6] (19q13.3), HUAP [APRT] (16q22), pE40-1 [D11S21] (11p13 $\rightarrow$ 15), and Hp [Hp2 α and the haploglobin serum marker] (16q22.1). Hp was run as a haplotype combining the probe and serum marker data. In addition, we ran the LDR92(17) and the LDR111(17) probes. LDR111 recognises a *HindIII* polymorphism and LDR92 a *PstI* polymorphism. Both are random genomic fragments obtained from libraries constructed in our laboratory. DNA analysis followed standard laboratory protocols for restriction fragment length polymorphism (RFLP) analyses.⁶

The gene frequencies used for the marker loci were based on reported frequencies⁵ and our own typed sample of random unrelated persons. The NF gene frequency used was 0.001.

Results

The results of the lod scores are given in the table. Tight linkage ($\hat{\theta}<0.001$) could be excluded for C3, ApoC2, and Hp. LDR9 and pE40-1 could be excluded at a distance of $\hat{\theta}<0.05$ and pBam34, LDR111, and HUAP could be further excluded at $\hat{\theta}<0.10$. The highest lod score obtained was for Hp at $\hat{\theta}=0.20$ and $\hat{Z}=0.399$.

TABLE Lod scores (Z) for neurofibromatosis and the marker loci.

Marker/chromosomal location	Recombination fraction ($\hat{\theta}$) - ($\hat{\theta} M = \hat{\theta}_f$)					
	0.001	0.05	0.10	0.20	0.30	0.40
C3/19pter→q13.2	-3.740	-1.441	-0.892	-0.400	-0.166	-0.046
ApoC2/19cen→q13	-2.129	-0.430	-0.153	0.041	0.061	0.023
pBAM34(D19S6)/19q13.3	-7.300	-2.770	-1.753	-0.777	-0.318	-0.102
LDR111/17	-10.158	-4.671	-3.013	-1.376	-0.602	-2.028
LDR92/17	-7.591	-1.817	-0.713	0.107	0.273	0.167
HUAP(APRT)/16q22	-8.964	-3.581	-2.450	-1.346	-0.698	-0.258
pE40-1(D11S21)/11p13→15	-8.582	-2.094	-1.068	-0.243	0.040	0.094
Hp(Hp2α and Hp serum)/16q22-1	-2.424	-0.417	0.054	0.399	0.370	0.195

Discussion

These results represent our initial attempt to localise the gene for NF. At present, the data are inconclusive. We will continue to screen this pedigree and our other large multi-generation NF families for linkage until a successful linkage is found.

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References

- Riccardi VM. Von Recklinghausen neurofibromatosis. *N Engl J Med* 1981;**305**:1617-26.
- Carey JC, Laub JM, Hall BD. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. *Birth Defects* 1979;**XV**(5B):271-81.
- McKusick VA. *Mendelian inheritance in man*. 6th ed. Baltimore: Johns Hopkins University Press, 1983.
- Ott J. Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. *Am J Hum Genet* 1974;**26**:588-97.
- Willard HF, Skolnick MH, Pearson PL, Mandel JL. Report of the committee on human gene mapping by recombinant DNA techniques. HGM8. *Cytogenet Cell Genet* 1985;**40**:360-490.
- Davies KE. *Human genetic diseases: a practical approach*. Oxford: IRL Press, 1986.

Correspondence and requests for reprints to Margaret A Pericak-Vance, Division of Neurology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, USA.

Linkage analysis of peripheral neurofibromatosis to DNA markers on chromosome 8

S R DIEHL*, M BOEHNKE†, F S COLLINS*, R P PERICKSON*, I J KAROLYI*, L M PLOUGHMAN†, M A PERICAK-VANCE‡, A S AYLSWORTH§, AND A D ROSES‡

From *the Departments of Human Genetics and †Biostatistics, University of Michigan, Ann Arbor, Michigan 48109-0618; ‡the Division of Neurology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, and §the Department of Pediatrics and the Biological Sciences Research Center, University of North Carolina, Chapel Hill, North Carolina 27514, USA.

SUMMARY Linkage relationships of the gene for peripheral neurofibromatosis (NF) were assessed in a large American Caucasian pedigree using two DNA markers located on

chromosome 8. Linkage to the thyroglobulin locus, located at 8q24, was excluded (lod < -2.0) to 21 cM. Data obtained for the tissue plasminogen activator locus, located at 8p12, excluded linkage to 4 cM. These results exclude between 20 to 30% of chromosome 8 as a possible map location for the NF gene in this