We wish to thank Drs R White, A Ullrich, R Williamson, C Gilliam, T Bonner, G Bell, and D Kurmit for providing DNA probes, and K Griffin for typing the manuscript. BRS is supported by a fellowship and grant from the National Neurofibromatosis Foundation (USA) and GR by the Fonds de Recherche en Santé du Quebec and the Medical Research Council of Canada. JFG is a Searle Scholar of the Chicago Community Trust. Funds for this research were provided by NINCDS grants NS22224 and NS20012, the National Neurofibromatosis Foundation, the McKnight Foundation, the Dystonia Medical Research Foundation, and the Julieanne Dorn Fund for Neurological Research.

References


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 lengths...
polymorphisms \textit{(RFLPs)} including C3, ApoC2, pBam34 (D19S6), HAUP[APRT], pE40-[D11521], Hp[Hp2α], LDR92, and LDR111 failed to show a significant linkage (Z [lod score]\geq 3.00) in this family. In addition, the results excluded areas of the genome around the marker loci (Z \leq -2.00) as potential sites for linkage. The maximum Z obtained with the markers was for Hp at \( \theta \) (maximum recombination fraction)=0.20 and 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{4} The NF gene was assumed to be autosomal dominant with a penetrance of 0.95.\textsuperscript{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 locations\textsuperscript{5} are C3 (19pter→q13-2), ApoC2 (19cen→q13), pBam34 [D19S6] (19q13-3), HAUP [APRT] (16q22), pE40-1 [D11S21] (11p13→15), and Hp [Hp2α 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.\textsuperscript{6}

The gene frequencies used for the marker loci were based on reported frequencies\textsuperscript{5} 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 (\( \theta \leq 0.001 \)) could be excluded for C3, ApoC2, and Hp. LDR9 and pE40-1 could be excluded at a distance of \( \theta \leq 0.05 \) and pBam34, LDR111, and HAUP could be further excluded at \( \theta \leq 0.10 \). The highest lod score obtained was for Hp at \( \theta = 0.20 \) and Z=0.399.
Table 1. Lod scores (Z) for neurofibromatosis and the marker loci.

<table>
<thead>
<tr>
<th>Marker/chromosomal location</th>
<th>Recombination fraction (θ) – (0 M=μh)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-001 0-05 0-10 0-20 0-30 0-40 0-50 0-60 0-70 0-80 0-90</td>
</tr>
<tr>
<td>C3/19pter–q13-2</td>
<td>-3-74 -1-44  -0-89  -0-40  -0-16  -0-06  -0-02  -0-00  -0-00  -0-00  -0-00</td>
</tr>
<tr>
<td>pBAm34(D19S6)/19q13-3</td>
<td>-7-30  -2-77  -1-75  -0-77  -0-31  -0-10  -0-02  -0-04  -0-06  -0-08  -0-10</td>
</tr>
<tr>
<td>LDR11/17</td>
<td>-10-15 -4-67  -3-03  -1-37  -0-60  -0-23  -0-08  -0-04  -0-02  -0-00  -0-00</td>
</tr>
<tr>
<td>LDR92/17</td>
<td>-7-59  -1-81  -0-71  -0-10  -0-23  -0-17  -0-10  -0-06  -0-02  -0-00  -0-00</td>
</tr>
<tr>
<td>HUAP(APRT)/16p22</td>
<td>-8-96  -3-58  -2-45  -1-34  -0-69  -0-25  -0-12  -0-03  -0-01  -0-00  -0-00</td>
</tr>
<tr>
<td>pE40-1(D11S21)/11p13–15</td>
<td>-8-58  -2-09  -1-06  -0-24  -0-04  -0-09  -0-04  -0-03  -0-01  -0-00  -0-00</td>
</tr>
</tbody>
</table>

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.

Funding for this project was provided by NIH Research Grant No 1 R01 NS23008-01. The authors would like to thank the following for generously supplying us with DNA probes for analysis: Drs Stephen Humphries (ApoC2), George Fey (C3), Peter Stambrook (HUAP), Nubuyo Maeda (Hp2α), and Thomas Glaser (pE40-1). The authors would also like to thank Laurita Melton, Patricia Watson, and Peggy Pate for their technical assistance in the preparation of this manuscript and the pedigree data. Finally, we would like to acknowledge the many NF family members without whose participation these studies would not have been possible.

References

Correspondence and requests for reprints to Drs D. 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 ERICKSON*, 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 score < −2.0) to 21 cM. Data obtained for the tissue plasminogen activator locus, located at 8p12, also excluded linkage to 4 cM. These results exclude between 20 to 30% of chromosome 8 as possible map location for the NF gene in this family.