Linkage of the TG and PLAT loci to each other can be excluded up to a recombination fraction of 0.005. These relatively small recombination fractions are converted into essentially equal genetic map distances by applying any of the commonly used mapping functions that allow for interference. The smaller range of exclusion associated with both linkage tests involving the PLAT marker is attributable to the smaller sample of subjects at this locus and the occurrence of fewer informative heterozygotes among those subjects sampled.

Discussion

The results of this linkage study exclude a significant portion of chromosome 8 as the potential site of the NF locus. Since the entire human genome consists of about 3300 cM of map distance,¹⁰ and since chromosome 8 is an average size chromosome, we estimate that chromosome 8 spans about 150 cM of map distance. If the TG locus is actually located very close to the telomere in 8q24, we can only exclude linkage to NF in one direction. However, if it is located more proximally, our data would permit exclusion of 21 cM on either side of this locus. Since the PLAT marker excludes linkage in both directions, the combination of these DNA markers excludes between 29 to 50 cM (20 to 30%) of chromosome 8. The application of additional chromosome 8 markers to this NF pedigree is therefore necessary to exclude this chromosome fully as a potential location for the NF gene.

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Correspondence and requests for reprints to Dr S R Diehl, Department of Human Genetics, University of Michigan Medical School, Medical Science Institute, M4708/0618, 1500 E Medical Center Drive, Ann Arbor, Michigan 48109-0618, USA.

Further exclusion data for the Von Recklinghausen neurofibromatosis gene: a genetic linkage study of 19 polymorphic markers

MEENA UPADHYAYA*, MANSOOR SARFARAZI*, SUSAN M HUSON†, AND PETER S HARPER*

From *the Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN; and †the Division of Inherited Metabolic Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

SUMMARY Linkage analysis on a panel of 15 Von Recklinghausen neurofibromatosis (VRNF) families with 19 polymorphic markers was carried out using the computer programme M-Link. Our findings have excluded VRNF from a significant proportion of the genome.

Von Recklinghausen neurofibromatosis (VRNF) is one of the commonest autosomal dominant disorders in man with a prevalence of at least 20/100 000¹ of the population. The mapping of the VRNF gene will be an important step towards our eventual understanding of the pathogenesis of the disease and a closely linked marker would be immediately applicable in prenatal diagnosis where appropriate.

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Our initial studies² excluded the possibility of linkage between VRNF and myotonic dystrophy. The exclusion of linkage between β nerve growth factor and VRNF³ discounted the most obvious candidate gene. In continuing our linkage studies, we have chosen systematically to exclude the gene from given chromosomes using highly polymorphic DNA probes available in our laboratory and from various colleagues. We report here analysis of the first 15 DNA polymorphisms tested and four enzyme polymorphisms, the segregation of three of which had not been previously analysed in VRNF families.

Materials and methods

Our family data base has been extended¹ and now comprises 15 families with 88 affected subjects and 71 unaffected relatives. For the purpose of linkage analysis, there are 112 potentially informative meioses (50 phase known). The families have all been personally examined (by SMH) and the following criteria were used for diagnosis. (1) In children, an affected parent and six or more café au lait spots >1.5 cm in diameter (>0.5 cm in children under five years). (2) In adults, six or more café au lait spots and multiple peripheral neurofibromas. Several of the older family members had only one or two café au lait spots but the diagnosis was accepted because of their very numerous neurofibromas.

Venous blood samples were collected into EDTA for DNA extraction. Five of the largest families

have also given samples so that lymphoblastoid cell lines could be established (courtesy of Dr J Gusella).

Family members were typed by Southern blot analysis of the RFLPs identified by the DNA probes listed in the table and the enzyme polymorphisms were typed by the MRC Human Biochemical Genetics Unit in London.

Linkage analysis was performed using the computer programme M-link⁴ and penetrance assumed to be complete for this purpose. In the case of unaffected relatives, only those over the age of five years were included because of the uncertainty as to when café au lait spots are manifested.

Results

The table gives the physical localisation and the results on the various genetic markers used in the present study; the DNA probes D19S9 and C-Mos were uninformative. The remaining 17 markers all showed significant evidence against linkage. In no case was there any evidence of heterogeneity when the family results were reviewed individually.

Discussion

These results exclude VRNF from a significant proportion of the genome. Our previously published work on chromosome 19 has been confirmed and extended by Pericak-Vance *et al.*,⁵ so that VRNF can now be confidently excluded from this chromosome.

Chromosome 20 is also now excluded in view of

TABLE Linkage analysis between VRNF and marker loci.

[illegible]

its small size and the negative data for the three RFLPs reported here, two of which have been localised to 20p and one to 20q. Negative linkage between VRNF and the IGLC locus on the long arm of chromosome 22 is interesting in view of the recent work of Seizinger *et al*⁶ which suggests that the locus for bilateral acoustic neurofibromatosis (BANF) may be in this region.

Our negative results, taken with the previously published negative results of linkage analysis between VRNF and the locus SIS (22q12→13), suggest that VRNF and BANF are genetically as well as phenotypically distinct.

The negative linkage between VRNF and the chromosome 16 DNA markers, α globin and APRT, is in agreement with the previously published data⁸ on protein markers PGP (16pter→p12) and Hp (16q22).

Although the VRNF gene has not been localised, the combined data have already excluded significant areas of the genome and illustrate the value of collaborative studies to maximise information. In future, one needs to concentrate on the genomic regions which have not been studied. Pooling of data will also allow the detection of possible genetic heterogeneity in VRNF.

Localisation of the VRNF gene to a specific chromosome will be a significant advance towards isolation of the gene. Closely linked markers will not only be useful for assessing the status of the subject at risk for the disease but will also be able to provide prenatal diagnosis for at least a proportion of those families who request this.

We are grateful to the families and to the neurofibromatosis patients' association, LINK, for their help with this study; to the staff of the MRC Human

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Correspondence and requests for reprints to Dr Meena Upadhyaya, Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN.

A genomic search for linkage of neurofibromatosis to RFLPs

D BARKER*, E WRIGHT*, K NGUYEN*, L CANNON*, P FAIN*, D GOLDGAR*, D T BISHOP*, J CAREY†, J KIVLIN‡, H WILLARD§, Y NAKAMURA||, P O'CONNELL||, M LEPPERT||, R WHITE||, AND M SKOLNICK*

From *the Departments of Medical Informatics, †Pediatrics, and ‡Ophthalmology, University of Utah Medical Center, Salt Lake City; §the Department of Medical Genetics, University of Toronto, Canada; and ||the Howard Hughes Medical Institute and Department of Human Genetics, University of Utah Medical Center, Salt Lake City, Utah, USA.

SUMMARY Our initial attempt to map NF was directed towards chromosomes 4 and 19, both of which had provided positive evidence for

linkage in previous reports. This analysis showed no evidence in support of either hypothesis. Our second attempt at mapping NF was a general search of the genome analysing a set of markers selected according to