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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Linkage analysis of peripheral neurofibromatosis to DNA markers on chromosome 8

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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

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family. Comparison of the two DNA markers excluded their linkage to 0.5 cM.

As documented in the accompanying papers, the exclusion map for the location of the gene responsible for peripheral neurofibromatosis is becoming substantial. We have been investigating the possibility that this gene is located on chromosome 8, a chromosome for which until recently very few markers were available. This chromosome is of interest for two reasons. (1) It is the site of two known oncogenes, the *mos* and *myc* proto-oncogenes. (2) NF has recently been observed in association with a potentially de novo complex chromosomal rearrangement (Kittur, Valle, and Kazazian, personal communication) involving chromosomes 1q, 8q, and 15q with two breakpoints on 8q. The affected subject, a 13 year old female, exhibits symptoms of NF including multiple café au lait spots, macrocephaly, and thoracolumbar scoliosis. While the presence of features of NF could represent the simultaneous but unrelated occurrence of two different genetic conditions in the child, it is also possible that one of the breakpoints involved in the rearrangement has interrupted or activated a gene which is responsible for NF. In a possibly related observation, Palmer *et al*¹ have recently reported a child with trisomy 8 mosaicism who developed features consistent with NF as well as chronic granulocytic leukaemia. Although this evidence is highly circumstantial, it can be regarded as useful in that it suggests possible chromosomes on which to seek linkage.

We are also concerned about the possibility of locus heterogeneity in NF. Therefore, we have chosen to do our linkage analyses using a very large American Caucasian pedigree segregating for NF, in order to avoid the risk of failing to detect linkage due to pooling families that have different loci responsible for the NF disease phenotype.

Methods

The pedigree segregating for NF used in this study and the criteria for diagnosis of NF are described elsewhere.²

DNA was isolated either directly from venous blood or from EBV transformed B lymphocyte cultures.² Restriction endonuclease digestions, agarose electrophoresis, and Southern blotting were carried out using standard methods.³ Linkage to NF was tested for two DNA markers located on chromosome 8 that exhibit restriction fragment length polymorphisms. We used a genomic DNA clone containing part of the thyroglobulin (TG)

locus (pCHT16/8-0) that has been mapped to 8q24⁴ to detect a two allele *TaqI* polymorphism.⁴ We also used a cDNA clone (pTPA) containing the entire coding region of the tissue plasminogen activator (PLAT) locus. This gene has been mapped to 8p12⁵ and detects a two allele *EcoRI* polymorphism.⁶

Since both DNA markers used here are located on chromosome 8, we also assessed linkage between these marker loci in order to begin to develop a linkage map for this chromosome. Adult subjects of uncertain NF diagnosis were not included in the NF linkage analysis. Prepubescent subjects were also excluded from the NF linkage analysis, because of the age dependent penetrance of this disease.⁷ Both of these groups were used in the analysis of linkage between the two DNA markers. Therefore, for evaluating linkage to NF, we used 45 subjects (25 affected) with known TG genotypes and 36 subjects (19 affected) with known PLAT genotypes. Genotypes were determined for 44 subjects for both TG and PLAT irrespective of NF status, and these were used for assessing linkage between these DNA markers.

Linkage to NF was assessed by the method of lod scores⁸ using the programme LIPED.⁹ The following allele frequencies were used in our analyses: 0.8 for the 5.2 kb TG allele⁴; 0.5 for both PLAT alleles⁶; and 0.0003 for NF.⁷ We assumed penetrance of 95% for NF for post-pubescent subjects. Equal recombination fractions were assumed for males and females. An estimate of the number of potentially informative meioses within this complex pedigree is discussed elsewhere.² Customarily, a lod score less than -2.0 is considered sufficient to exclude linkage between loci.⁸

Results

Lod scores estimating linkage between each of the DNA markers and NF and between the two DNA markers are presented in the table. Linkage to NF can be excluded up to a recombination fraction of 0.21 for the TG locus and 0.04 for the PLAT locus. Additional analyses indicated that inclusion of apparently unaffected prepubescent subjects and the assumption of 100% penetrance for NF among adults had only small effects on lod scores.

TABLE Lod scores testing linkage of chromosome 8 DNA markers to the NF locus and each other.

Loci		θ					
		0.0	0.05	0.1	0.2	0.3	0.4
NF	TG	-∞	-5.93	-4.35	-2.23	-1.01	-0.31
NF	PLAT	-10.32	-1.88	-0.88	0.07	0.16	0.13
TG	PLAT	-∞	-0.69	-0.23	0.12	0.17	0.09

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

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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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