Discussion

The possibility that NF-I is linked to markers on chromosome 4q appears to have been excluded by the data in table 2, a conclusion consistent with the findings of other investigators. In addition, the genes for TGFA, EGF, GRL, and EGFR, which may be considered 'candidate genes' for NF-I, show no evidence of close linkage. Eleven other loci selected on the basis of convenience also show no evidence of linkage.

References


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Linkage analysis of British and Indian families with Von Recklinghausen neurofibromatosis

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Summary: Linkage analysis has been undertaken in two British and three South African Indian families with Von Recklinghausen neurofibromatosis. Eleven polymorphic DNA probes were studied, including both random DNA sequences and candidate oncogenes. Although no evidence for linkage of these probes to the disease was detected, substantial exclusion regions were established on six of the chromosomes studied.
Linkage analysis of British and Indian families with Von Recklinghausen neurofibromatosis

Von Recklinghausen neurofibromatosis (VRNF) is a relatively common autosomal dominant disorder characterised by café au lait spots, cutaneous neurofibromas, and an increased risk of central nervous system tumours.1-3 The pathogenesis of the disorder is unknown and no laboratory diagnostic test is available. Several genetic linkage studies have been undertaken in order to map the locus of the VRNF gene,4-8 but none of these has detected a significant linkage between a marker and the disorder. We have therefore tested a further 11 polymorphic DNA markers as part of the international effort to locate the affected gene.

Materials and methods

Families were ascertained through the British Neurofibromatosis Patients' Association, LINK, and the records of the Department of Human Genetics, University of Cape Town Medical School. The criteria for the diagnosis of VRNF were as follows. (1) In adults, six or more café au lait spots >1.5 cm in diameter and multiple cutaneous neurofibromas. (2) In children, an affected parent and six or more café au lait spots >1.5 cm in diameter.

All subjects included in the linkage analysis were more than five years old. Blood specimens were obtained from two British families and three South African families of Indian descent. A total of 73 subjects was sampled, which included 37 affected subjects and 62 potentially informative meioses.

DNA isolation, blotting, and hybridisation were performed as described previously.9 The probes used were as follows: seven locus specific minisatellite sequences9-10 (unpublished data), the hypervariable region 3' to the α globin genes,11 the erb A212 and sis13 oncogenes, and 22c1-18,14 which is a random sequence on chromosome 22.

Likelihood computations were carried out using a modification of the LINKAGE programme.15 The VRNF gene was assumed to have a population frequency of \(2 \times 10^{-4}\) and a penetrance of 100%. Rapid computation of lod scores for the minisatellite sequences, which have multiple alleles, was facilitated by recoding the genotypes of some subjects so that at most four alleles were used.16

Results and discussion

The lod scores at recombination fractions from 0 to 0.4 are shown in the table. No evidence for linkage of any of the probes tested to the VRNF gene was obtained. However, use of the highly polymorphic minisatellite probes10-11 has established large regions of exclusion on chromosomes 1, 5, 6, 7, 11, and 12. Assuming that the exclusions do not overlap and that none of the probes is located near the end of a chromosome, a total of 236 cM (approximately 8% of the human genome) has been excluded for VRNF at a lod score of \(-2\) or less. The data also appear to exclude the sis and erb A2 oncogenes as candidate genes for VRNF. Future work will concentrate on the use of probes with high frequency restriction fragment length polymorphisms from regions of the genome where large exclusions for the VRNF gene have not been reported. The exclusion map published in this issue17 will facilitate the search.

We would like to thank Margaret Ponder for assistance in obtaining the family material, D Higgs for the hybridisations with the α globin gene HVR probe, and M Ormerod for advice on a linkage computer programme. We also thank P Middleton, A Hall, and C Buys for the erb A2, sis, and 22c1-18 probes. This work was supported by grants from the Cancer Research Campaign, the Medical Research Council, and ‘LINK’, the neurofibromatosis associa-

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**TABLE Lod scores for linkage between VRNF and 11 polymorphic DNA probes.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe</th>
<th>Chrom</th>
<th>at recombination fraction</th>
<th>0</th>
<th>0-0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Zmax</th>
<th>Exclusion at (\theta)</th>
<th>Z</th>
<th>(\theta)</th>
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<tbody>
<tr>
<td>D157</td>
<td>λM51</td>
<td>1p</td>
<td>x</td>
<td>-20.38</td>
<td>5.40</td>
<td>3.02</td>
<td>1.02</td>
<td>0.22</td>
<td>0.05</td>
<td>0.06</td>
<td>0.43</td>
<td>0.13</td>
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<tr>
<td>D158</td>
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<td>1q</td>
<td>x</td>
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<td>3.79</td>
<td>2.20</td>
<td>0.85</td>
<td>0.31</td>
<td>0.12</td>
<td>0.00</td>
<td>0.50</td>
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<td>x</td>
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<td>-0.81</td>
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<td>-5.32</td>
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<td>-0.91</td>
<td>-0.21</td>
<td>0.00</td>
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<td>7pter-q22</td>
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<td>-0.73</td>
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<td>D11</td>
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<td>11p14-pter</td>
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<td>x</td>
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<td>-0.06</td>
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<td>0.00</td>
<td>0.01</td>
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<td>V-SIS</td>
<td>22q12-13</td>
<td>x</td>
<td>-2.40</td>
<td>0.72</td>
<td>-0.44</td>
<td>-0.19</td>
<td>-0.08</td>
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<td>0.00</td>
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<td>D22S10</td>
<td>22c1-18</td>
<td>22</td>
<td>x</td>
<td>-2.10</td>
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<td>-0.06</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>0.001</td>
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*These minisatellite probes have not yet been assigned HGM symbols. The regional assignments of λM51 and λM52 are provisional.9

Chrom = chromosomal localisation.
SUMMARY Linkage analysis of neurofibromatosis was performed using genes on chromosomes 1, 8, 11, and 12. No linkage was found between NF and C-myc, AT3, IGF-1, PTH, and gamma globin genes. Evidence for linkage was found between C-ets 1, on the long arm of chromosome 11 and NF in two families with a lod score of 1.88 at θ=0. More families are being studied to confirm this linkage.

Neurofibromatosis (NF) is one of the common autosomal dominant neurological disorders with a frequency of approximately 1 in 3000. The disease is mainly characterised by café au lait spots and neurofibromas. These patients have an unusually high frequency of malignancy compared to the normal population. The intriguing question is, what gene do they carry that predisposes them to malignancy? In order to localise the gene for NF, linkage analysis using DNA markers was performed.

Three multi-generation families shown in table 1 were used.

Oncogene C-myc, insulin like growth factor-1, antithrombin-3, parathyroid hormone, and gamma globin genes were not linked to neurofibromatosis in our families. The lod scores are shown in table 2.

We found a positive lod score of 1.88 with oncogene C-ets 1 in two informative families (no recombinants observed in six informative meioses). These data provide suggestive evidence that the NF1 gene is linked to chromosome 17.