

Linkage analysis of peripheral neurofibromatosis (Von Recklinghausen disease) and chromosome 19 markers linked to myotonic dystrophy

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SUMMARY Three chromosome 19 markers known to be linked to myotonic dystrophy have been studied in nine families with peripheral neurofibromatosis (Von Recklinghausen's disease). Clear evidence against linkage has been found for all three markers, excluding the peripheral neurofibromatosis gene from the myotonic dystrophy region of chromosome 19. Previous reports of co-inheritance of the two disorders in families cannot therefore be explained on the basis of close genetic linkage between the loci.

Peripheral neurofibromatosis (NF), first described by Von Recklinghausen in 1882,¹ is an autosomal dominant disorder with a prevalence of around 30 per 100 000.² The major defining features of the disease³ are multiple café-au-lait spots, which develop in infancy and early childhood, and multiple cutaneous neurofibromas, usually appearing around the onset of puberty and growing in number and size throughout life. There are a number of well recognised complications of the disease which include skeletal abnormalities (pseudoarthroses and scoliosis), tumours of the central nervous system, and malignant change in the neurofibromas.

The severity of NF is very variable and to many patients it merely presents a cosmetic problem. About 25% of patients,⁴ however, develop one or more of the severe complications of the disease and their occurrence can not be predicted, even within families.

There is no laboratory test diagnostic for NF and the pathogenesis of the disease is unknown. The identification of a linked genetic marker would thus be a major advance both for prediction and subsequent isolation of the gene itself.

There have been two studies^{5,6} of classical genetic markers in families with NF, neither of which identified linkage. In addition, Derby *et al*⁷ studied the segregation of polymorphisms identified by the cDNA probe for nerve growth factor (BNGF) and

found significant evidence against linkage at this locus. There have, however, been two three generation families reported in which myotonic dystrophy (DM) and NF appear to segregate together^{8,9} suggesting that the two diseases may be closely linked.

Linkage analysis using protein and DNA polymorphisms has established that the DM locus lies in the linkage group on chromosome 19 containing the loci for the Lutheran blood group,¹⁰ the ABH secretor system,¹¹ the C3 complement component,¹² the enzyme peptidase D,¹³ and the apolipoproteins E¹⁴ and CII.¹⁵

Because of the suggested linkage between DM and NF we have studied the segregation of those chromosome 19 markers closely linked to DM in NF families.

Material and methods

The families were ascertained through two sources, the records of the Department of Medical Genetics, University Hospital of Wales and the British Neurofibromatosis Patients Association, LINK.

The criteria used for the diagnosis of peripheral neurofibromatosis were as follows.

(1) In adults, six or more café-au-lait spots > 1.5 cm diameter and multiple peripheral neurofibromas.

(2) In children, an affected parent and six or more café-au-lait spots > 1.5 cm diameter.

All the family members were examined by one of

TABLE Lod scores, assuming $\theta M = \theta F$, between NF and the APOC2 and C3 polymorphisms and Secretor.

	No of families	Lod scores at various values of θ					
		0.01	0.05	0.10	0.20	0.30	0.40
NF-APOC2	6	-22.63	-11.85	-7.42	-3.48	-1.65	-0.70
NF-C3	3	-3.79	-1.82	-1.08	-0.50	-0.26	-0.11
NF-Se	4	-4.12	-2.08	-1.25	-0.52	-0.19	-0.05

us (SMH) and specimens of blood and saliva taken. A total of 57 affected subjects and 41 normal relatives was studied from six three generation and three two generation families. Three children were excluded from the study because of equivocal clinical findings. No evidence of non-penetrance of the NF gene was found in the families studied.

Family members were typed for the following markers: the ABH Secretor system, the Lutheran blood group, and the enzyme peptidase D, and the restriction fragment length polymorphisms identified by the gene probes for the third component of complement (C3) and the apolipoprotein CII (APOC2) when total human DNA is digested using the restriction enzymes *Sst*I and *Taq*I respectively. Both these polymorphisms have previously been reported in detail.^{12 15}

Linkage analysis was performed using the programme LIPED 3.¹⁶ In the case of unaffected relatives, only those over the age of 5 years were included because of the uncertainty about the age by which affected subjects will have manifested café-au-lait spots.

Results

The lod scores for various values of recombination fraction from 0.01 to 0.40 for Secretor, the C3, and APOC2 polymorphisms are shown in the table. Results are given only for equal male and female recombination frequencies. None of the families was informative for the Lutheran blood group or the enzyme peptidase D.

The three informative markers all show significant evidence against linkage (lod ≤ -2.0) for at least some values of θ . There was also no evidence of linkage when male and female recombinations were analysed separately. There was no evidence of genetic heterogeneity in the families studied.

Discussion

Genetic linkage analysis of the markers used in this study in families with DM has shown that peptidase

D and APOC2 are the closest markers for the DM locus available at present. Peptidase D gives a maximum lod score of 3.51 at $\theta=0$ ¹³ and APOC2 a maximum lod score of 7.87 at $\theta=0.04$.¹⁷ C3 and the secretor locus probably flank the DM locus at distances of approximately 5 to 10 cM¹⁸ and 10 cM,¹¹ respectively.

The present study gives clear evidence against linkage between NF and APOC2 for values of θ up to 20 cM and therefore excludes NF from a considerable region of chromosome 19 around the DM locus.

This study was prompted by previous suggestions of linkage between DM and NF from two families in which the two diseases appeared to segregate together. In the family reported by Ichikawa *et al*⁸ the origin of DM in generation I is not clear and the evidence that I.1, who had NF, also suffered from DM is anecdotal. If I.2 in fact had DM then there is no evidence of linkage between the two diseases in this family. We consider that this is the more likely situation rather than the possibility that there is more than one genetic locus for NF.

From a clinical viewpoint our results are important but disappointing. Not only would a closely linked marker for NF be useful clinically to identify the status of persons at risk, but the mapping of the gene would be a major step towards our understanding of the disease and its relationship to other forms of neurofibromatosis.¹⁹

A more general analysis of DNA and other genetic markers will now be required to map the NF gene. Samples from the families involved in this study will make an important contribution to this.

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