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Genetic heterogeneity in hereditary haemorrhagic telangiectasia

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Abstract

A locus causing hereditary haemorrhagic telangiectasia (HHT) has recently been mapped to 9q34 in four families and designated HHT1. In this paper, the results of a linkage study showing genetic heterogeneity in four families in whom HHT is segregating are reported. All the previously reported 9q34 linked families contain at least one affected member with a symptomatic pulmonary arteriovenous malformation. We postulate that clinical heterogeneity may also be a feature of HHT with a significantly higher predisposition to symptomatic PAVMs associated with the HHT1 linked families.

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Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterised by recurrent epistaxes and mucocutaneous telangiectases. The prevalence in the British population has been estimated as approximately 1 in 40 000¹ and is significantly higher in parts of France.² Penetrance is age dependent, being almost complete by the age of 40.¹ Mucocutaneous telangiectases in the bowel lead to significant gastrointestinal bleeding in about 20% of patients. Pulmonary arteriovenous malformations (PAVM) are a common finding occurring in about 20% of patients. They are characteristically multiple and give rise to most of the neurological complications of HHT.

Recently HHT has been mapped to 9q34 in four families.^{3,4} Genetic heterogeneity was suggested by Shovlin *et al*⁴ who found one family not to be linked, and the 9q34 locus was therefore designated HHT1.

Materials and methods

As part of a continuing collaborative study, five families with HHT and a structure suitable for linkage analysis were ascertained. Of these, one showed linkage to markers close to the HHT1 locus at 9q34 with a lod score of 2.41 (data not shown).

Fig 1 shows the pedigrees of the other four families. All family members involved were examined clinically by MP and a full medical history taken. Two of three features, recurrent epistaxes, mucocutaneous telangiectases, and a family history, were required for a positive diagnosis. Other complications are detailed in the key. Significantly, no-one in the four families had a symptomatic PAVM and there was no increased level of neurological morbidity.

Genomic DNA was extracted from peripheral white blood cells using standard techniques. Previously published PCR primers flanking short tandem repeat polymorphisms at D9S103, GSN, D9S65, and ASS were used in "hot" PCR reactions with end labelling of the forward primer.³

Unaffected persons under 30 were not included in the analysis and a penetrance of 90% was assumed in persons over 30. Two point analysis between the disease and the marker was carried out using the MLINK subprogramme of the LINKAGE package.⁵ Published allele frequencies were used. The hypothesis of genetic heterogeneity was tested using the HOMOG version 3.00 program. Multipoint analysis was performed using LINKMAP⁵ and assuming a locus order cen-D9S103-GSN-D9S65-ASS with respective

Figure 1 Pedigrees and clinical information on the four British HHT families. Note that only III-4 from family 4 has a PAVM.

Lod scores for linkage of HHT to 9q3 markers calculated assuming 9% penetrance and an HHT gene frequency of 0.0004

Marker	Recombination fraction θ						
	0	0.01	0.05	0.1	0.2	0.3	0.4
D9S103	$-\infty$	-9.53	-4.49	-2.53	-0.96	-0.35	-0.13
GSN	$-\infty$	-6.83	-3.12	-1.58	-0.27	0.19	0.23
D9S65	$-\infty$	-13.38	-7.39	-4.93	-2.48	-1.3	-0.56
ASS	$-\infty$	-9.08	-4.88	-3.00	-1.26	-0.43	-0.08

Figure 2 Multipoint analysis of linkage of HHT to GSN and D9S65 in the four families. Map distances were calculated using the Haldane function and a distance of 15 cM was assumed between the two markers.

distances between the loci of 0.1, 15, and 1 cM. Previously published map distances were assumed with D9S103 1 cM centromeric to GSN and ASS 2 cM telomeric to D9S65.³

Results

The combined two point lod scores for the four families against probes D9S103, GSN, D9S65, and ASS are shown in the table. The putative location for HHT1 is within the interval bounded by GSN and D9S65^{3,4} and the three point linkage map of HHT with regard to these markers shows significant exclusion over the interval (fig 2). HOMOG analysis was performed on the data obtained from the two point analysis of HHT against D9S65 and HHT against ASS. As a comparison, data from the two 9q34 linked American families were included (D9S65 lod 4.37 and 6.84 at $\theta=0$; ASS lod 4.02 and 6.02 at $\theta=0$).³ If homogeneity is assumed, the p value for the D9S65 results is 0.0000 and for the ASS is 0.0014 strongly favouring heterogeneity. Given that HOMOG has shown linkage with heterogeneity, the probability (p) of families 1 and 3 showing linkage to D9S65

is 0.0000 (0.0000–0.0076) and 0.0000 (0.0000–0.0004). Families 2 and 4 were only semi-informative with D9S65 but the probability of linkage to ASS is 0.0000 (0.0000–0.0004) and 0.0001 (0.0000–0.2584).

Discussion

One major locus for HHT, HHT1, has been identified at 9q34, but the data presented in this paper show that at least one more is still to be found. Clinical heterogeneity has not been formally shown in HHT although there is variation between families. In particular, certain families seem to be less prone to symptomatic PAVMs and their neurological sequelae. Two large families with a total of 261 affected subjects have been reported with no mention of PAVMs, although formal oximetry was not carried out.^{6,7} Three of the four families reported here (1, 2, and 3) were assessed for PAVMs using the transcutaneous oximetry method reported by Hughes and Allison⁸ and no evidence of desaturation was found. The oximeter was not available during the assessment of family 4 in which one person (III.4) had radiological evidence of a PAVM. Detailed family history failed to indicate any evidence of increased neurological morbidity in any of the families. Genetic counselling in HHT is based at present on all families being at risk of PAVMs and their neurological sequelae. Identification of families at either high or low risk of developing symptomatic PAVMs will enable more appropriate screening protocols to be formulated. The genes underlying HHT are worthy of continued attention in view of the importance of new vessel formation in a variety of disease processes and treatments and the future potential for gene therapy in this late onset and accessible disease.

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1 Porteous MEM, Proctor SJ, Burn J. Hereditary haemorrhagic telangiectasia: a clinical analysis. *J Med Genet* 1992;29: 527–30.
2 Plauchu H, de Chadarevian JP, Bideau A, Robert JM. Age-related clinical profile of hereditary haemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet* 1989;32:291–7.
3 McDonald MT, Papenberg KA, Ghosh S, et al. A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33–34. *Nature Genet* 1994;6:197–204.
4 Shovlin CL, Hughes JMB, Tuddenham EGD, et al. A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nature Genet* 1994;6:205–9.
5 Lathrop M, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985;37:482–98.
6 Bird RM, Hammarsten JF, Marshall RA, Robinson RR. A family reunion: a study of hereditary haemorrhagic telangiectasia. *N Engl J Med* 1957;257:105–9.
7 Davidson CH. Hereditary haemorrhagic telangiectasia: a report on a family of seven generations. *Scott Med J* 1959;4: 260–2.
8 Hughes JMB, Allison DJ. Pulmonary arteriovenous malformations: the radiologist replaces the surgeon. *Clin Radiol* 1990;41:297–8.