

Linkage of hereditary haemorrhagic telangiectasia to chromosome 9q34 and evidence for locus heterogeneity

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Abstract
Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant disorder with unknown pathophysiology that is characterised by arteriovenous lesions and recurrent haemorrhage in virtually every organ. Linkage of HHT to markers on chromosome 9q has recently been reported. In this study we report confirmation of this localisation in three unrelated families of Dutch origin. A fourth unrelated HHT family, in which considerably fewer pulmonary arteriovenous malformations (PAVM) were present, yielded evidence for non-linkage to this region. We conclude that HHT is a genetically heterogeneous disorder and our results indicate that the presence of PAVM may be more common in patients with a chromosome 9 linked form of HHT than in patients with the non-linked form.

mostly appear as easily bleeding telangiectases in the skin and mucosa, but may also give rise to larger arteriovenous malformations, for example, in the lung or in the brain. Clinical manifestations depend on the location and size of the vascular anomaly: epistaxis is frequently present, intestinal bleeding can occur, dyspnoea and cyanosis can arise from arteriovenous malformations in the pulmonary circulation, and bleeding of cerebral lesions may cause serious complications.^{1,2} Since therapy can prevent a number of complications, case finding is useful.

The first patient with HHT was described by Babington³ in 1865; Rendu⁴ suggested a hereditary trait as the cause for familial epistaxis and telangiectasia, which was confirmed by Osler⁵ and Weber.⁶ The prevalence of HHT ranges between 1 to 2 per 100 000 and 1 per 10 000 with almost complete penetrance by the age of 40 years.⁷⁻⁹ The mode of inheritance is autosomal dominant. The disease occurs in all races, and is equally frequent in both sexes.^{7,10} One homozygous child has been described who died of bleeding and hypoxaemia soon after birth.¹¹ Several earlier linkage studies led to inconclusive results owing to the low informativeness of the markers used.¹¹⁻¹⁵

In order to perform a systematic genome

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Hereditary haemorrhagic telangiectasia (HHT), also known as Osler-Rendu-Weber disease, is a rare disorder characterised by arteriovenous communications which may develop in virtually every organ.¹ These lesions

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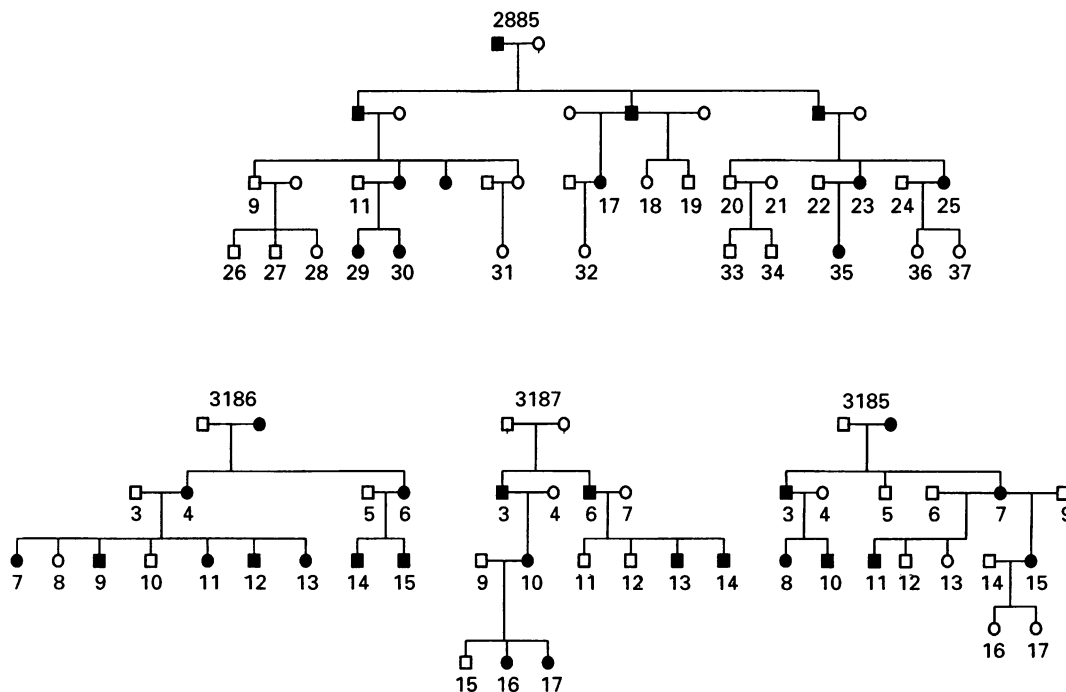


Figure 1 Pedigrees of four HHT families. Blood samples were obtained from numbered persons.

search, as a first step in the "positional cloning" of the HHT gene, we collected blood samples from four extended families (fig 1). Power calculations using SLINK¹⁶ confirmed that the family material should provide sufficient information to detect linkage.

The genome search was undertaken with a selection of 350 highly polymorphic microsatellite markers evenly distributed over the human autosomes. During the course of our genome search, two reports of linkage of HHT to markers on chromosome 9q were published.^{17,18} This prompted us to test chromosome 9q markers in our families. We report here linkage of HHT in three Dutch families to markers on chromosome 9q and recombination events that allow us to define boundaries for the candidate region between two flanking markers on 9q34. The fourth family was not linked to the same chromosomal region. Heterogeneity analysis clearly indicated that HHT is a genetically heterogeneous disorder.

Subjects and methods

FAMILY ASCERTAINMENT

The pedigrees of all four families are shown in fig 1. Blood was obtained from HHT patients

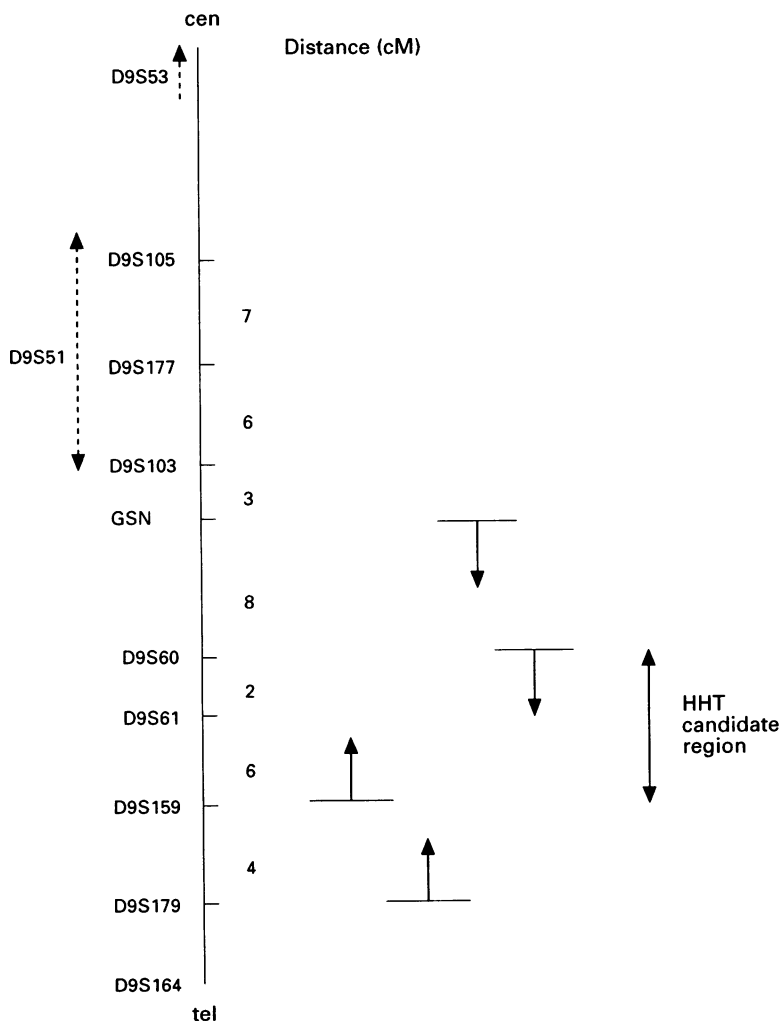


Figure 2 Schematic map of the HHT linked region on chromosome 9q34 with sex average genetic distances based on previously published linkage maps.²⁰⁻²³ Each arrow indicates one meiotic recombination event in an affected person in one of the linked families. Dotted arrows indicate the possible location of a marker. The double sided arrow indicates the minimum candidate region for HHT.

and family members participating in a family screening study (manuscript in preparation). All were investigated for the presence of mucocutaneous telangiectasia, including inspection of the nasal mucosa. All affected persons were screened for pulmonary arteriovenous malformations (PAVM) by chest radiography and measurement of arterial oxygenation. Intravenous digital subtraction angiography (iv-DSA) of the pulmonary circulation was performed in patients with abnormalities on either chest radiography or in arterial oxygenation. Also, iv-DSA of the cerebral circulation was done in all affected persons to screen for cerebral arteriovenous malformations (CAVM).

DNA STUDIES

Genomic DNA was isolated from peripheral blood as described by Miller *et al.*¹⁹ Chromosome 9q microsatellites were selected based on available linkage maps.²⁰⁻²³ Microsatellite markers were amplified and analysed essentially as described by Weber and May.²⁴ Briefly, markers were amplified in multiplex reactions in a total volume of 11 μ l using 96 well plates (Costar Thermowell 6509). Oligonucleotides for radioactive amplification were obtained with a grant from the Netherlands Organization for Scientific Research (NWO). Additional oligonucleotide primers were labelled during synthesis with Fluorescein Amidite (FluorePrime, Pharmacia, Sweden). Reactions were performed with 50 ng of genomic DNA, 40 ng fluorescein primer, 40 ng non-fluorescein primer, 10 mmol/l Tris pH 8.3, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 0.01% gelatin, 200 μ mol/l each dATP, dCTP, dGTP, dTTP, and 0.4 units *Taq* DNA Polymerase (Life Technologies).

PCR products were resolved according to size by denaturing gel electrophoresis (5.5% Hydrolink, 40 W) using an ALF automated sequencer (Pharmacia LKB Biotechnology AB). Data were analysed with the Fragment Manager software package version 1.00 (Pharmacia LKB Biotechnology AB).

LINKAGE ANALYSIS

Pairwise lod scores were calculated for each family using the MLINK program of the LINKAGE package, version 5.1²⁵ assuming HHT to be an autosomal dominant disease with a gene frequency of 0.0001. At risk subjects were grouped into three liability classes (30 years of age or younger, 31 to 45 years, and older than 45 years) with penetrances of 0.5, 0.7, and 0.9 respectively. No allowance was made for phenocopies.

Mutation rate was set at zero and recombination rates in males and females were assumed to be equal. Marker allele frequencies were set equal. Calculation of pairwise lod scores with allele frequencies calculated from spouses did not substantially alter the results (<10%).

In order to make multipoint analysis manageable, the five most relevant markers were recoded to no more than five alleles each and

Table 1 Two point lod scores between chromosome 9q markers and HHT families

Marker	Family	Recombination fraction (θ)					
		0.00	0.01	0.05	0.10	0.20	0.30
D9S53	3185	-4.113	-0.675	-0.395	-0.143	-0.037	-0.003
	3186	1.958	1.792	1.613	1.226	0.826	0.415
	3187	0.612	0.559	0.504	0.388	0.266	0.138
	2885	-4.576	-0.822	-0.544	-0.289	-0.158	-0.071
	Totals	-6.119	0.854	1.178	1.183	0.891	0.478
D9S105	3181	0.139	0.196	0.215	0.179	0.095	0.023
	3186	1.971	1.819	1.654	1.288	0.872	0.406
	3187	NI	NI	NI	NI	NI	NI
	2885	2.632	2.377	2.111	1.545	0.946	0.360
	Totals	4.742	4.392	3.979	3.012	1.913	0.798
D9S177	3185	-3.756	-1.721	-0.119	0.083	0.178	0.138
	3186	2.107	2.104	1.930	1.746	1.358	0.941
	3187	0.670	0.668	0.590	0.513	0.370	0.246
	2885	-7.265	-3.641	-0.473	-0.058	0.157	0.125
	Totals	-8.244	-2.590	1.928	2.284	2.063	1.450
D9S51	3185	-0.065	-0.050	-0.038	-0.020	-0.009	-0.002
	3186	2.782	2.552	2.313	1.806	1.257	0.660
	3187	1.082	0.953	0.822	0.556	0.294	0.076
	2885	-7.872	1.063	1.125	0.925	0.575	0.194
	Totals	-4.073	4.518	4.223	3.267	2.117	0.928
D9S103	3185	1.112	0.981	0.845	0.564	0.298	0.099
	3186	0.602	0.558	0.511	0.408	0.292	0.158
	3187	1.520	1.339	1.152	0.768	0.392	0.101
	2885	0.077	0.063	0.050	0.030	0.015	0.005
	Totals	3.310	2.939	2.558	1.770	0.996	0.363
GSN	3185	0.862	0.860	0.753	0.640	0.406	0.189
	3186	2.782	2.778	2.552	2.313	1.806	1.257
	3187	1.346	1.342	1.187	1.022	0.680	0.346
	2885	0.415	0.414	0.368	0.324	0.239	0.156
	Totals	5.405	5.394	4.860	4.298	3.130	1.948
D9S60	3185	-3.657	-1.586	-0.018	0.152	0.192	0.129
	3186	1.436	1.435	1.376	1.297	1.090	0.812
	3187	1.612	1.609	1.457	1.295	0.951	0.587
	2885	-2.621	-1.717	0.175	0.455	0.501	0.324
	Totals	-3.230	-0.260	2.989	3.198	2.733	1.852
D9S61	3185	-3.840	-1.324	-0.716	-0.473	-0.209	-0.050
	3186	0.961	0.935	0.835	0.718	0.518	0.354
	3187	1.140	1.115	1.015	0.887	0.622	0.351
	2885	2.342	2.299	2.122	1.888	1.382	0.840
	Totals	0.603	3.026	3.262	3.020	2.313	1.496
D9S159	3185	-3.657	-1.415	-0.776	-0.226	-0.018	0.033
	3186	0.670	0.612	0.552	0.430	0.300	0.159
	3187	-3.138	0.439	0.569	0.527	0.371	0.190
	2885	2.314	2.065	1.807	1.266	0.719	0.252
	Totals	-3.812	1.700	2.152	1.997	1.372	0.634
D9S179	3185	-3.657	-0.363	-0.084	0.053	0.069	0.046
	3186	2.782	2.552	2.313	1.806	1.257	0.660
	3187	-3.519	0.104	0.284	0.346	0.286	0.170
	2885	-∞	-1.117	-0.774	-0.392	-0.169	-0.044
	Totals	-∞	1.236	1.739	1.812	1.443	0.832
D9S164	3185	-4.143	-0.826	-0.509	-0.200	-0.060	-0.008
	3186	NI	NI	NI	NI	NI	NI
	3187	-3.102	0.472	0.600	0.549	0.382	0.193
	2885	-∞	0.841	0.945	0.813	0.519	0.188
	Totals	-∞	0.488	1.035	1.162	0.842	0.374

run in subsequent four point analyses against the disease using the LINKMAP program. The HOMOG programs were used in the analysis of heterogeneity.²⁶ Distances between markers were based on previously published linkage maps (fig 2).²⁰⁻²³

Results and discussion

Recently, linkage of HHT to markers on chromosome 9q was reported.^{17,18} In the study presented here, positive lod scores on chromosome 9q were obtained with three families (table 1). None of the families reached significance individually, but combined lod scores reached significance for markers D9S105, D9S51, D9S103, and D9S61. Our results therefore confirm linkage of HHT to this region of chromosome 9.

Multipoint analysis was performed to narrow down the minimal genetic region for HHT. Overlapping four point analyses were combined into a single curve (fig 3A). Multipoint analysis per family suggested that the HHT gene is located on chromosome 9q in families 2885,

3186, and 3187 but not in family 3185. Assuming genetic homogeneity we obtained a maximum cumulative lod score (Zmax) of 3.95 between GSN and D9S61. Analysis of genetic heterogeneity showed odds of 33:1 in favour of locus heterogeneity, with a peak lod score of 5.47 between D9S61 and D9S159 in support of both heterogeneity and linkage. The results of the HOMOG2 analysis showed that family 3185 is not linked to the chromosome 9q locus ($p < 0.0010$).

We performed recombination analysis in the linked families by the construction of haplotypes for all markers along the chromosome, because in the linkage analysis not all meiotic events were informative. The haplotype analysis in the linked families showed recombination events between markers D9S60 and D9S61 on the proximal side and between D9S159 and D9S61 on the distal side of the HHT locus (fig 2). This places the HHT locus distal to D9S60 and proximal to D9S159 defining the candidate region to an 8 cM interval on the sex average linkage map. The localisations for HHT reported by Shovlin *et al*¹⁸ and McDonald *et al*¹⁷ both fall into the critical region that is reported here. However, the localisations reported in these studies are in disagreement with each other. Unless there are two tightly linked loci for HHT on chromosome 9q34, one of the reported recombinants must be the result of a mistyping of marker D9S61 or a clinical misdiagnosis of one of the patients.

The results of the clinical investigations of the four families described in this report are summarised in table 2. Considerably fewer PAVM were found in the unlinked family 3185 compared to the other families. Statistical significance for this observation was not reached but this might be because of the small number of persons available from this family. Shovlin *et al*¹⁸ reported one family with HHT that was not linked to chromosome 9 but found no systematic differences in clinical phenotype between patients of the unlinked family and patients of the two linked families; there is, however, a difference in clinical methodology between that study and the study reported here. We have rigorously investigated all patients in our four families by chest radiography, measurement of arterial oxygenation, iv-DSA of the pulmonary circulation, and iv-DSA of the cerebral circulation. Our results suggest that the PAVM may be more common in patients with a chromosome 9 linked form of HHT. The clinical methodology used in this study will probably detect PAVM at an earlier stage than the methodology used by Shovlin *et al*.¹⁸ This could be the reason that they did not detect clinical differences between the linked

Table 2 Clinical features of affected HHT family members

Family No	No of affected persons	PAVM*	CAVM†
3185	5	1	0
3186	11	6	2
3187	9	8	2
2885	9	4	0

* Number of persons with at least one PAVM.
† Number of persons with at least one CAVM.

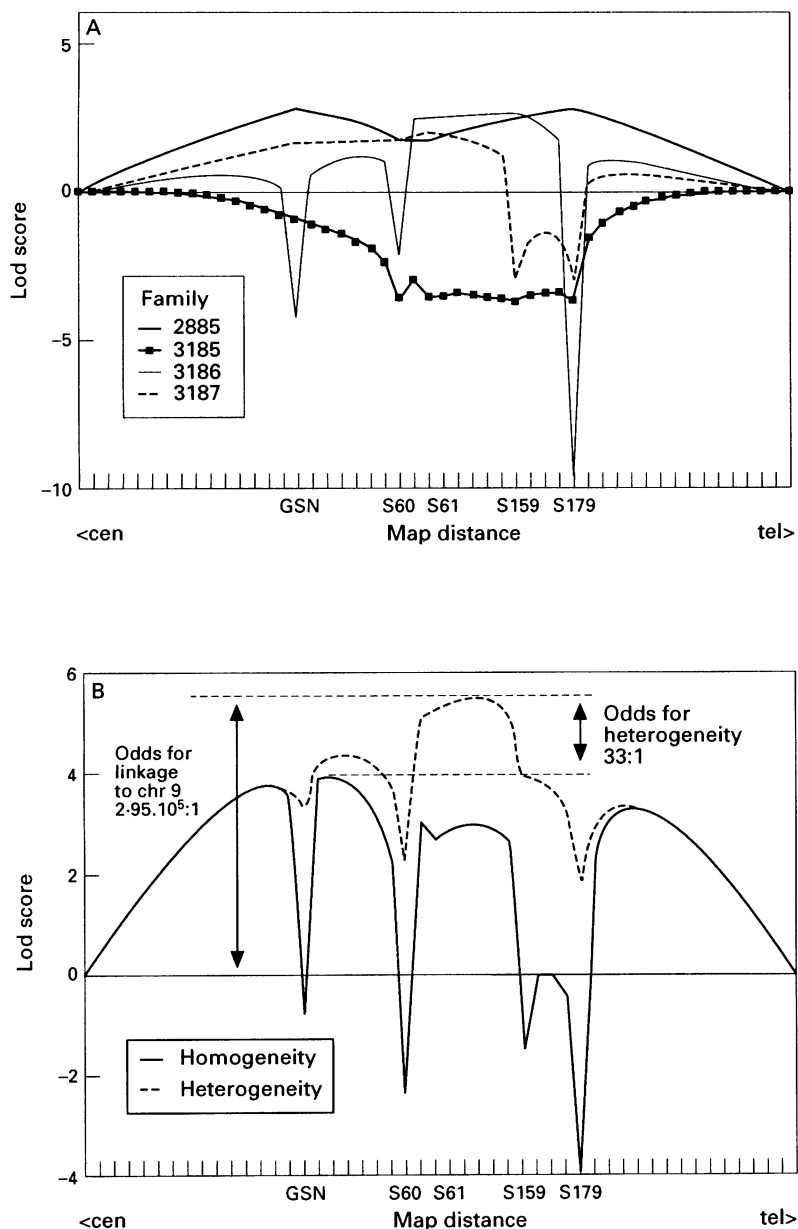


Figure 3 (A) Multipoint linkage analysis per family for five markers on chromosome 9q34. (B) Combined multipoint linkage analysis for all families for five markers on chromosome 9q34 under genetic homogeneity and under genetic heterogeneity.

and unlinked families. Analysis of additional HHT families with the methodology reported here will be necessary to explore this intriguing finding further.

The clinical features of HHT suggest that the gene product is a structural component of blood vessels or that it plays a role in blood vessel development. Several candidate genes can be suggested on chromosome 9q34. The α -1 polypeptide of human collagen type V (COL5A1) has been mapped to chromosome 9q34 and is a structural component that is expressed in blood vessels.^{27,28} ZNF79, a zinc finger motif containing gene, is a candidate gene because of its predicted regulatory function of transcription.²⁹

Linkage of HHT with a locus on chromosome 9q34 has now been reported in three independent studies. However, two studies reported genetic heterogeneity. This will limit the applicability of linked DNA markers in small

families for presymptomatic testing. Only extended pedigrees will be informative enough to determine whether or not the chromosome 9 locus is responsible for disease onset in the patient.

The eventual isolation of the gene responsible for HHT on chromosome 9 will help to give insight into the processes that take place in the development and remodelling of the vasculature.

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