A gene for familial venous malformations maps to chromosome 9p in a second large kindred

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Abstract

Venous malformations are a common form of vascular anomaly that cause pain and disfigurement and can be life threatening if they involve critical organs. They occur sporadically or in a familial form, where multiple lesions are usually present. We have identified a large kindred showing autosomal dominant inheritance of venous malformations. Using this family we confirm linkage of a familial form of venous malformations to chromosome 9p. We suggest that blue rubber bleb naevus syndrome can be considered a particular manifestation of this form of familial venous malformations. The candidate region for this gene encompasses the interferon gene cluster and the MTS1 (p16) tumour suppressor gene.

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Venous malformations occur as single or multiple lesions that have a nodular or tumour-like appearance and can be present anywhere on the body surface as well as in internal organs. They constitute several syndromes, one of which is blue rubber bleb naevus syndrome,[1] that can be inherited as an autosomal dominant trait or occur sporadically. The cutaneous venous malformation in this syndrome is characteristically bluish in colour, spongy, and easily compressible. They usually present at birth although in some cases they appear during childhood or later in life. In addition to cutaneous lesions, subcutaneous, intramuscular, and visceral lesions may occur. They are soft, elastic, smooth in surface without skin discolouration. Vascular lesions in the oral, nasal, and gastrointestinal tract may bleed.

In past nomenclature, venous malformations have been termed venous or cavernous haemangiomas. They are composed of vascular, large, lacunar spaces or slit-like irregular lumens with thin walls, lined by a single layered, flat, mature endothelium. The vessels lack the normal surrounding smooth muscle cells and elastic tissue.

The underlying cause of these venous malformations, in either familial or sporadic cases, has not yet been determined. The presence of a number of families with vascular lesions inherited in an autosomal dominant fashion suggests that genetic linkage analysis and positional cloning could be used to identify the responsible gene(s). Recent analysis has identified a locus on chromosome 9p responsible for venous malformations in a single large kindred.[2] In the present report, a separate, large family is described in which a predisposition to multiple lesions is inherited in an autosomal dominant fashion with high penetrance. This large kindred provides unique opportunities for studying the genetic basis of this inherited vascular defect and for further comparative studies with other families.

Methods

SUBJECTS

After obtaining informed consent, each participant (38 members of the family, fig 1) was interviewed and examined by the same physician (KAP). Physical examinations were performed and family histories were taken. Affected status was assigned on the basis of one or more venous malformations. Other vascular anomalies, such as lateral telangiectatic naevi (port wine stains), were occasionally noted but not included as part of the affected phenotype. Blood was drawn from appropriate subjects and DNA was extracted using established methods.[3]

GENOTYPES AND LINKAGE ANALYSIS

Analyses of simple repeat markers were performed as previously described.[3] The venous malformation phenotype was analysed as an autosomal dominant disorder with age dependent penetrance. For estimating the age dependent penetrance, four liability classes were created with age ranges 0–12 (penetrance 0·5), 12–21 (penetrance 0·75) 21–45 (0·90), and >45 (0·95), based on observations from more than 10 years of clinical contact with the entire extended family. Disease frequency was set at 0·0001. Two point linkage analysis was performed on a Sun Sparc station 10 using the MLINK subprogram of the LINKAGE computer package (version 4·9).[4] The analysis was repeated using the phenotypic information on only the affected subjects to ensure that information was coming only from those with a definitive disease status. Allele frequency was estimated for the markers using the pedigree maximum likelihood estimation from the family data by the method of Boehnke[5] and did not differ appreciably from the allele frequencies listed in GDB.

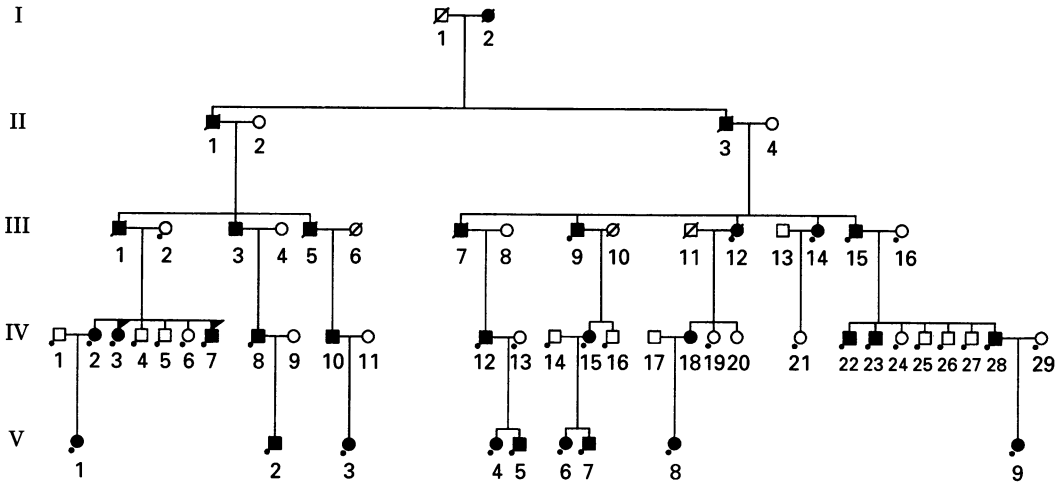

Figure 1 Pedigree of the family with multiple venous malformations. Filled symbols represent affected subjects and a dot below a symbol indicates a person who was sampled for this study.

Results

CLINICAL DESCRIPTION

This family (fig 1) with venous malformations was previously described by Pasyk *et al.*[6] Familial distribution was compatible with autosomal dominant inheritance with variable age of onset. Of 337 members of this very large, seven generation family, 56 people (29 males and 27 females) were affected. Fig 1 shows the portion of the family which participated in this study.

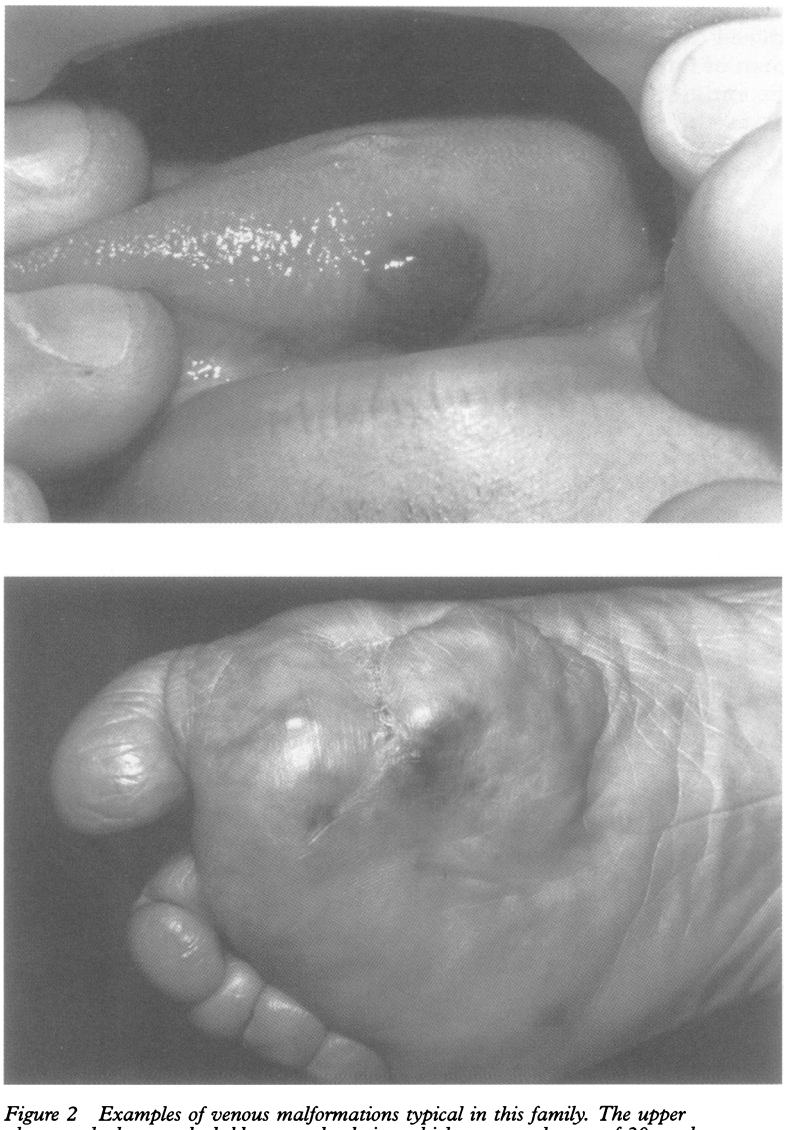

Figure 2 Examples of venous malformations typical in this family. The upper photograph shows a dark blue vascular lesion which arose at the age of 30 on the tongue of IV·8. The lower photograph shows vascular lesions on the sole of III·9's foot that have been present since birth.

Some of the members were born with lesions which never regressed, whereas others manifested new lesions later in life. Some of the affected members observed development of new vascular lesions after mechanical trauma. For example, in one subject, a large vascular mass appeared in the vagina after childbirth.

Most of the affected members had multiple venous malformations in various anatomical locations. In most cases, the lesions were present on the face and mucous membranes including the lips, the tongue, the cheeks, tonsils, and larynx (fig 2A, B). Lesions were also noted on the trunk, the abdomen, and on the extremities including the fingers and feet. Some members had venous malformations within internal organs. One developed two vascular tumours in the large intestine and required blood transfusions because of excessive bleeding. Two members of this family died from complications of these vascular abnormalities. Pathological examination of one of these people showed vascular tumours within the stomach, liver, pancreas, and spleen.

The location of these venous malformations was almost identical for several family members. In a majority of cases the lesions were small, enlarged with time, and were asymptomatic. A few females observed an increase in size of the vascular lesions approximately one week before menstruation and a subsequent decrease in size during menstruation. A few patients required surgical procedures. No-one in this family has had any symptoms suggestive of central nervous system involvement. Lymphocytic chromosome karyotypes, performed in five affected members of this family, were normal.

GENETIC LINKAGE ANALYSIS

Simple sequence repeat markers were chosen that map to a broad 60 cM interval on the short arm of chromosome 9 in approximately 10 cM intervals.[7] Preliminary results suggested linkage

Pairwise lod scores between FVM and 9p marker loci lod score (Z) at different recombination fractions (θ)

Locus	*0·00*	*0·05*	*0·10*	*0·15*	*0·20*	*0·30*	*0·40*	$\hat{Z}$	$\hat{\theta}$
D9S178	1·64	1·62	1·53	1·38	1·21	0·80	0·34	1·64	0·02
D9S168	$-\infty$	5·35	5·03	4·53	3·95	2·64	1·24	5·36	0·04
D9S285	5·41	5·02	4·57	4·07	3·55	2·39	1·12	5·41	0·00
D9S157	7·86	7·26	6·58	5·85	5·08	3·44	1·69	7·86	0·00
D9S162	5·87	5·40	4·86	4·29	3·69	2·43	1·12	5·87	0·00
D9S171	9·09	8·42	7·67	6·86	5·99	4·11	2·08	9·09	0·00
D9S169	7·90	7·29	6·62	5·90	5·14	3·52	1·76	7·90	0·00
D9S161	3·47	5·89	5·54	5·01	4·38	2·91	1·23	5·92	0·04
D9S152	$-\infty$	0·91	1·63	1·80	1·73	1·22	0·47	1·80	0·16

to some of the markers, and then additional markers in the interval were analysed (table). A maximum lod score of 9·09 was seen with marker D9S171, at a recombination fraction of 0·00, under the assumption of age dependent penetrance. With affecteds only analysis, D9S171 gives a maximum lod score of 7·40, indicating that most of the information is coming from subjects with a definitive disease status. Only one person is discordant, a 34 year old unaffected female (IV·19). Various explanations can be given for her disease haplotype which excludes the entire candidate region. The haplotype may represent double recombination between markers that flank the disease locus, a gene conversion event near the disease locus, or, most likely, non-penetrance for the disease phenotype. The maximum likelihood estimate of the recombination fraction is 0·00, despite the potential recombination event between the disease locus and D9S171 in IV·19. This results from two important factors: first, the disease is incompletely penetrant and this person still faces a 10% chance of carrying the disease gene; and, secondly, the analyses were performed assuming equal recombination between males and females. When we allowed gender specific differences in recombination, the lod score peaked at 9·10 with theta-male equal to 0·00 and theta-female equal to 0·02. Finally, the multiple additional recombination events provide strong support for the localisation of the venous malformation gene at D9S171.

Markers D9S285, D9S157, D9S162, and D9S169 also show no obligate recombinants with the disease locus in this family. The candidate region is bordered by D9S157 distally based on a recombinant in the family described by Boon *et al*[2] and D9S161 proximally based on a recombinant in this family, a distance of approximately 19 cM. Attempts are under way to contact additional members of this family in order to provide additional meioses for finer mapping of the locus.

Discussion

A previous study implicated a locus on 9p in a single family with venous malformations inherited in an autosomal dominant fashion.[2] The lesions in the family described here are similar to those in the earlier report. To determine whether the initial family was an orphan genetic disorder, or whether this locus is indeed involved in inherited venous malformations, we performed linkage analysis on another large family.

We confirm a locus on chromosome 9p responsible for an autosomal dominant form of multiple venous malformations. The relationship of this family to that described in the original report of blue rubber bleb naevus syndrome[1] is of interest. Both families present with similar vascular lesions in an autosomal dominant pattern. In addition, blue rubber bleb naevus syndrome is associated with gastrointestinal bleeding from vascular lesions. Six members of the present family have exhibited some evidence for gastrointestinal bleeding, and two had evidence of vascular lesions in the gastrointestinal tract. We suggest that the gene responsible for autosomal dominantly inherited venous malformations may be identical to that for blue rubber bleb naevus syndrome. More accurately, blue rubber bleb naevus syndrome may be a subset in the general category of familial venous malformations.[8] Formal proof of this hypothesis would require identification of the gene responsible in a family such as this and in a family with a phenotype identical to that described by Bean.[1]

The broad candidate region previously described for venous malformations coincides with that from the present report. The additional meioses provided by this family narrowed the candidate region to 19 cM. This region contains a number of potential genes including the interferon gene cluster and the MTS1 (p16) tumour supressor gene proposed to be involved in melanoma as well as other tumours.[9,10] A search of the GDB and OMIM databases showed that none of the numerous angiogenic factors that have been described maps to this region.[11] We have suggested a Knudson-like tumour suppressor hypothesis for the development of lesions seen in hereditary haemorrhagic telangiectasia.[3] We propose a similar hypothesis for the development of the venous malformations in this family, also inherited in an autosomal dominant fashion. We suggest that this locus may represent a new (or unmapped) gene involved in vasculogenesis, and that development of a venous malformation requires a second somatic mutation in a discrete progenitor cell.

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