Progress toward understanding vascular malformations

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A locus for hereditary capillary malformations mapped on chromosome 5q

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Chapter 3

Abstract

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% of live births. Most capillary malformations occur sporadically and present as a solitary lesion. Capillary malformations can also occur as a component of well described syndromes. Familial occurrence of multiple capillary malformations has been described in the literature, suggesting autosomal dominant inheritance with variable expression in this subgroup. A hereditary basis underlying the development of solitary capillary malformations has not been found but may well be possible. We have mapped a locus for an autosomal dominant disorder in a three-generation family that manifested itself with multiple cutaneous capillary malformations to chromosome 5q13-22. This locus spans 48 cM between the markers D5S647 and D5S659 and harbours several candidate genes. By defining the gene(s) responsible for capillary malformations, we will gain more insight in the pathogenesis of this disorder. It is likely that genes implicated in these familial cases may be involved in the more sporadic cases.
Introduction

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% of live births (OMIM 163000)(18). The majority of capillary malformations (CMs) are located at the head and neck, with 85% occurring in a unilateral distribution that follows a dermatome (34). Most CMs occur sporadically and present as a solitary lesion (28). CMs can also occur as a component of well described syndromes, such as the Klippel-Trenaunay syndrome (OMIM14900) and Sturge-Weber syndrome (OMIM 185300) (15). With advancing age capillary malformations undergo progressive ectasia resulting in typical red-to-purple lesions (3,29). The psychological burden associated with CM is high and often warrants intervention, such as pulsed dye laser (PDL) (21). The efficacy of PDL in treating patients with CMs is very variable, in part due to the depth and the diameter of the ectatic blood vessel (20). For accurate diagnosis and subsequent optimal treatment, knowledge of the pathogenesis is of paramount importance.

Familial occurrence of multiple CM was first described in 1949 by Shelley and Livingood (32). Since then a limited number of similar families were published, suggesting autosomal dominant inheritance with variable expression (reviewed in ref. 7). A hereditary basis underlying the development of solitary CM has not been suggested. Recent studies have provided first clues for the molecular basis of vascular malformations. Mutations in the TEK gene, located at chromosome 9p21, can be the cause of familial mucocutaneous venous malformations composed of thin-walled vascular channels surrounded by deficient smooth muscle cells (OMIM 600195) (5,35). Autosomal dominant venous malformations with accompanying glomus cells (OMIM 138000) were linked to chromosome 1p21-p22 (4,17). Genetic linkage of inherited central nervous system vascular malformations has been established to three chromosomal loci; 3q25-27, 7p13-15, and 7q21-22 (CCM1 gene) (OMIM 6032851, 603284, 604214). (8,15). Hereditary hemorrhagic telangiectasia (HHT, OMIM 187300) has also been assigned to at least two genes. ENG (endoglin), a TGF-beta binding protein of endothelial cells, at chromosome 9q34.1 and ACVRL1, an activin receptor-like kinase, at chromosome 12q (25,36). Four kindreds with autosomal dominant hemangiomas (OMIM 602089) associated with vascular malformations were used to show linkage to 5q31-q33 (6,37). The localizations of genes involved in lymph vessels may also be important, as the lymphatic system develops in part from the venous system (7). Linkage studies have mapped the lymphedema-distichiasis syndrome (OMIM 153400) at 16q24.3 (24). Later on it was proven that mutations in the FOXC2 gene cause not only this entity, but may also cause several other lymphedema syndromes (9,11). Congenital hereditary lymphedema Milroy type (OMIM 153100) was localized at chromosome 5q35.3, and proven to be caused by mutations in
the *FLT4* gene (10,19). Here we report on the results of a whole genome linkage screen of a large family in which capillary malformations occurred in an autosomal mode of inheritance, and discuss some of the candidate genes within the linked region.

### Methods

After oral consent, DNA was isolated from blood samples. Whole genome linkage analysis was performed using the Linkage Mapping set MD10 (PE-biosystems). The 17 members used for genome screening are indicated with * in figure 1. This set contains 400 markers spread over the genome with an average distance of 10cM. Markers were amplified by multiplexed PCRs and fragments were analysed on an ABI310 (PE-biosystems), using genescan and genotyper software. Additional markers in the 5q14-22 (D5S2495, D5S2499, D5S2055 and D5S659) region were Cy5 labeled by PCR and analysed on an ALF sequencer (Pharmacia). 2-Point LOD scores were calculated using the MLINK program of the LINKAGE package, assuming autosomal dominant inheritance with complete penetrance.

### Results

**Clinical findings**

The pedigree is shown in Figure 1; all family members were examined by the same person (CCB). The proband (Fig 1: III 3) applied for pulsed dye laser treatment at our hospital. She was a 6-year-old girl with a CM on the upper part of her left leg (figure 2). The diameter of this lesion was 31 x 27 cm. She had several smaller CMs on both her feet and left arm ranging in size from 1 x 1 cm to 2 x 3 cm. The mother of the proband (II-7) had several CMs on her right arm, inside her left lower arm, and on her right lower leg. Dimensions varied between 1 x 1 cm and 3.2 x 4 cm. The proband had one affected sister (III 4), who had large CM (± 30 x 30 cm) on her lateral right upper leg. Several smaller CM were seen all over her body ranging in size from 1 x 1 cm to 7.5 x 4 cm. The grandmother of the proband (I 2) has deceased but was reported to have had multiple small CMs on her thorax. Three aunts and two uncles of the proband had multiple CMs. One uncle (II-3) had a large CMs on the antero-lateral side of his left upper leg (30 x 30 cm) and a smaller CM on the anterior side of his left lower leg (10 x 5 cm). When he was nine years old he had an overgrowth of this leg (9 cm leg length difference) which was subsequently treated with an epiphysodesis. This has resulted in a small leg length difference (0.5 cm) as an adult, but
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Figure 1:
Pedigree of present family with multiple cutaneous capillary malformations. The family members included in the original whole genome screen are marked by an asterisk. The segregating haplotype is indicated by the boxed regions. A recombination between D5S647 and D5S424 in III-3 determines the centromeric border and recombinations between D5S2027 and D5S659 in II-6 and II-12 determine the telomeric border. The affection status of individual III-2 is uncertain. When she is considered affected, the linked region will be reduced to the region between D5S647 and D5S428. On the other hand, when she is not affected, the linking region is between D5S424 and D5S659 (see discussion).

Figure 2:
A photograph of the CM located on upper left leg of the proband.
the difference in leg circumference remained unchanged. He had no other symptoms indicative of Klippel-Trenaunay syndrome. His daughter (III-1) had multiple CMs located all over her body. She also had a hemangioma on the back of her head. An aunt of the proband (II-6) had one daughter (III-2) that had a hemangioma on her forehead, but no signs of capillary malformations. The two boys (III-6, III-7) both had multiple CMs, III-6 also having a salmon patch ("angel’s kiss") on his forehead as an infant.

**Molecular findings**

Seventeen family members (all persons excluding 3 spouses (II-4, II-8 and II-13)) were included in a whole genome screen. Highly significant linkage was found only for D5S644 (LOD score 4.2) and D5S433 (LOD score 3.9), D5S618 (LOD score 3.9) and D5S424 (LOD score 4.2). Additional markers in this region (D5S2495, D5S2499 and D5S2027) were analysed and haplotypes were constructed (LOD scores are given in Table 1). Cosegregation with CMs is seen between markers D5S647 and D5S659. The borders of this haplotype are determined by a crossover between D4S647 and D5S424 in III-3 (proximal border) and crossovers between D5S2027 and D5S659 in II-6 and II-12 (distal border) (Fig. 1). The linking region on 5q13-22 spans 48 cM according to the

<table>
<thead>
<tr>
<th>Locus</th>
<th>cM</th>
<th>Z at q=0</th>
<th>Zmax</th>
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Table 1: Two-point LOD scores (Z) for the 5q14-22 markers at recombination fraction (q) 0 and the maximum LOD scores (Zmax) and the recombination fraction (qmax) at which this is found.
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![Schematic presentation of chromosome 5 showing the position of the linking region and the location of candidate genes.](image)

**Figure 3:**
Schematic presentation of chromosome 5 showing the position of the linking region and the location of candidate genes.


**Discussion**

We present a family in which several members had capillary malformations of variable severity. The inheritance mode fits best an autosomal dominant pattern of inheritance. We localize the gene causative for the vascular malformations in a candidate interval of 48cM at chromosome 5q13-22. This localization has not been described in other families with similar vascular malformations. This locus is further distinct from previously described loci for other vascular anomalies, like venous malformations, ataxia-telangiectasia (OMIM 208900), Fabry disease (OMIM 301500) and Rendu-Osler-Weber syndrome (OMIM 187300) (4,5,7,25,36).

Most sporadically occurring CMs are solitary lesions, but the affected family members in our study all had multiple CMs. The affected family described by Boon and co-workers to have autosomal dominantly inherited venous malformations also had multiple lesions, while most venous malformations are sporadic and isolated (5). The exact significance of this finding in
hereditary cases is still unclear but the Knudson two-hit hypothesis has been mentioned as a possible explanation (5,22).

In the present study two family members also had a hemangioma. Blei et al identified six kindreds in which hemangiomas were segregated in an autosomal dominant trait (6). In these families several members had associated vascular malformations. Walter et al showed linkage to 5q31-q33 using four of these families for a genome wide linkage screen (37). Despite hemangiomas and vascular malformations having distinct clinical characteristics, it is possible that the development of both may involve the deregulation of a common regulatory pathway (6). On the other hand, the co-occurrence can be explained as sheer coincidence. Therefore, the affection status of individual II-2 is considered unknown in this study, as she presented only with a hemangioma and did not have any CMs. The locus decreases to the region between D5S647 and D5S428 if hemangiomas and capillary malformations would arise from the same gene defect and, thus, II-2 would be affected, or decreases to the region between D5S424 and D5S659 if they are two independent trait and II-2 would be unaffected. At present vascular tumors (hemangiomas) and vascular malformations are still classified as two separate categories and maybe after molecular investigation of more families with hemangiomas and vascular malformations we will see that this distinction is not as black and white as be always assumed (28).

CMs are congenital lesions located in the upper reticular and papillary dermis (3). Several studies have failed to demonstrate a difference between endothelial cells of capillary malformations and normal vessels (12,20,23,29). Pericytes were found to be predominantly located in the inner part of the vessel wall (31). In general the cell metabolism of these pericytes did not look more active than normal. Two studies have postulated that a decreased innervation may be responsible for the progressive ectasia of the capillary malformation (30,33). In one study the capillary malformations lacked not only sympathetic innervation, but also sensory innervation (30). In combination with the absence of anomalies in endothelial cells and pericytes it is likely that a defective genesis of vascular innervation is a major cause of capillary malformations. The 5q linkage area is at present still quite large. Several candidate genes implicated in neurogenesis are located in this area. These include the FER and THBS4 genes shown to be involved with neurite outgrowth and the EFNA5 protein involved in axon guidance (1,2,38). The MEF2C gene may be involved in myogenesis and neurogenesis (26,27). In addition, EDIL3 and F2R are involved in vasculogenesis (14,16).

Although a hereditary basis for CMs is suggested, they clearly represent a minority of the total cases of CMs. In analogy to other genes it is possible that genes implicated in familial cases will also be involved through somatic mutations in the more common sporadic cases. It has been
proven that the vascular endothelium and the surrounding support cells reciprocally influence each other, and it is likely that any disruption in the cellular physiology of either cell type can result in dysfunction. Thus, multiple genes might be implicated in CMs. Further refinement of the present linkage region and subsequent mutation analysis should allow detection of a causative gene.

Acknowledgments

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References