Genetic causes of vascular malformations

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Vascular malformations are localized defects of vascular development. They usually affect a limited number of vessels in a restricted area of the body. Although most malformations are sporadic, inheritance is observed, enabling genetic analysis. Usually, sporadic forms present with a single lesion whereas multiple lesions are observed in familial cases. The last decade has seen unraveling of several causative genes and beginning of elucidation of the pathophysiological pathways involved in the inherited forms. In parallel, definition of the clinical phenotypes has improved and disorders such as Parkes-Weber syndrome (PKWS), first thought to be sporadic, is now known to be part of a more common inheritable phenotype. In addition, the concept of double-hit mechanism that we proposed earlier to explain the incomplete penetrance, variable expressivity and multifocality of lesions in inherited venous anomalies is now becoming confirmed, as some somatic mutations have been identified in venous, glomuvenous and cerebral cavernous malformations. It is thus tempting to suggest that familial forms of vascular malformations follow paradoxic inheritance and that sporadic forms, the etiopathogenic causes of which are still unelucidated, are caused by somatic mutations in the same genes.

INTRODUCTION

The blood and lymphatic vessels are made of a single layer of endothelial cells (ECs) surrounded by variable number of layers of vascular smooth muscle cells (vSMCs) and/or pericytes. These mural cells are sparse in capillaries and peripheral lymphatics. The main processes through which this complex network is developed are called vasculogenesis, angiogenesis and lymphangiogenesis. Vascular anomalies, subdivided into vascular tumors (mainly the hemangiomas, of unknown etiology) and vascular malformations (named according to the type of vessel affected) are thought to be due to defects in these pathways (1). Most malformations are present at birth and grow proportionately with the child. In inherited forms, new lesions can appear, but they stay small. The etiopathological genetic defects have been elucidated for some of these, and they are discussed here with relevant functional data and development of small animal models.

Venous malformations

Venous anomalies have an incidence estimated around 1/10 000 (2). These slow-flow lesions are subdivided into venous malformations (VM) (95%, including sporadic VM and cutaneomucosal venous malformation (VMCM), i.e. mucocutaneous VM), and glomuvenous malformations (GVM, 5%). Following identification of the causative genes for VMCM and GVM, criteria for differential diagnosis were established (3). This has allowed better management. The etiopathogenesis of sporadic VM and syndromes, which associate venous anomalies, including blue rubber bleb nevus syndrome (BRBN) (MIM 112200), characterized by cutaneous and gastrointestinal VM, Maffucci syndrome (MAF) (MIM 166000), and Klippel-Trenaunay syndrome (KTS) (MIM149000) are unknown. The latter was suggested to be due to mutations in VG5Q (4), but the reported nucleotide change was later shown to be a common polymorphism (5,6).

Cutaneomucosal venous malformation and sporadic venous malformation. VM (MIM 600195) presents as a bluish-hue lesion, mainly on skin and mucosa, commonly infiltrating the underlying muscle and joints (Fig. 1A). It can be emptied by compression, it can be painful, but not on palpation, and sometimes it develops calcifications. Large size, involvement of underlying tissues and presence of calcifications

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is linked to localized intravascular coagulopathy (LIC) (A. Dompmartin et al., submitted for publication). Although mostly sporadic (~98%), VM follows autosomal dominant inheritance in VMCM (3). On histology, enlarged vein-like channels, lined by a single layer of ECs, present a patchy relative lack of surrounding vSMCs (7). The current treatments include elastic stockings, sclerotherapy and surgery (8).

The inherited VMCM is caused by mutations in the EC-specific receptor tyrosine kinase TIE2, also known as TEK, located in the VMCM1 locus on 9p21 (7). Only two mutations have been reported: R849W in four families and Y897S in one (7,9,10). We have identified six additional families with the R849W change and six with a novel substitution, all in the kinase domains (V. Wouters et al., submitted for publication). All R849W changes are not due to a single founder allele, suggesting this change to be one of the rare changes able to cause VM while remaining compatible with germline transmission (V. Wouters et al., submitted for publication). R849W and Y897S increase ligand-independent autophosphorylation of the receptor, without causing EC proliferation (7,9). Interestingly, we observed a somatic second-hit in TIE2 in a VM of a patient with inherited R849W mutation (V. Wouters et al., submitted for publication). This, like the one reported in a GVM (11), supports the idea that the inherited forms need a somatic alteration of the second allele for development of lesions.

Three TIE2 ligands are known: angiopoietins -1, -2 and -4, the latter corresponding to Angpt3 in mouse (12–14). ANGPT1 activates tyrosine phosphorylation while ANGPT2 has a weaker effect and is considered as a competitive inhibitor of ANGPT1. Upon binding of the multimeric ligand, receptors dimerize and cross-phosphorylate, triggering mainly the PI3-kinase pathway, which activates AKT and inhibits apoptosis, and the MAP-kinase pathway (Fig. 2) (15). Tie2-deficient mice die at mid-gestation with insufficient remodeling of the primary capillary plexus (12,16), and mice deficient in the catalytic subunit of the PI3K result in diminished Tie2 expression, with a strikingly similar phenotype (17). As survival, mediated by ShcA, is increased by mutant TIE2 (18), it may explain the relative excess of ECs in VM. ANGPT1, via TIE2, triggers vSMC recruitment by upregulation of hepatocyte growth factor secretion (19). HGF is also a survival factor for ECs (20) but its role in VM is not known (Fig. 2).

Glomuvenous malformation. GVMs (MIM 138000) are pink-to-purple-bluish, usually raised and nodular lesions, located on the extremities (Fig. 1B). They involve skin and subcutis, rarely the mucosa. They are commonly multifocal, often hyperkeratotic and painful on palpation. They cannot be completely emptied by compression (3). The treatment of choice is surgical resection, which sometimes can be associated with sclerotherapy. Histologically, GVM is characterized...
Figure 2. Pathways involved in vascular anomalies. Schemes on four cell types: lymphatic endothelial cell (LEC) with genes involved in lymphedema; vSMC for which the only primary defect is in glomulin; blood endothelial cell (BEC) regrouping alterations leading to arterial, capillary and VMs; and a cell which is either of endothelial or neuronal origin, affected by CCMs. The mutated genes are marked in red (refer text for details).
by abnormally differentiated vSMCs, ‘glomus cells’ in the walls of distended venous channels (21,22).

Frequently, if not always, inherited, GVM segregates as an autosomal dominant disorder due to loss-of-function mutations in glomulin, on chromosome lp21–22 (11). Of the 30 mutations discovered in 86 families (11,22–24), eight account for 70% of families, with a strong founder effect (23). There is no phenotype–genotype correlation, but undetectable glomulin expression by in situ hybridization and the identification of a double-hit mutation in a lesion, suggest predominant inheritance (11, B.A. McIntyre et al., submitted for publication).

Glomulin expression is restricted to vSMCs (25) and is involved in their differentiation (B.A. McIntyre et al., submitted for publication). When lacking, the precursors cells seem to be deviated towards the ‘glomus cell’ phenotype. As transforming growth factor beta (TGFβ) signaling is crucial for vSMC differentiation, the alteration may be due to lack of glomulin to compete with the FKBP12 binding to TGFβ type I receptor (TβRI), which is inhibiting TGFβ signaling (26,27). Glomulin also interacts with HGF receptor c-Met (Fig. 2). Upon HGF binding, glomulin is tyrosine-phosphorylated, released, and induces phosphorylation of p70S6-kinase, thereby influencing protein synthesis (27). By interaction with Cul7, glomulin may also control protein degradation via ubiquitination (22,28).

Both in VMCM and in GVM, the concerted cross-talk between ECs and vSMCs is likely altered (Fig. 2). TIE2-induced HGF triggers vSMC migration (19), and liberation of glomulin from cMET enables TGFβ signaling. Upon EC/SMC contact, latent TGFβ is activated (29), leading to vSMC differentiation and vessel maturation. Why the hereditary glomulin and TIE2 mutations cause VMs mostly in the skin is not understood.

Capillary malformation

Capillary malformations (CM) (MIM 163000) or ‘port-wine stains’, are flat, red-purple, cutaneous lesions most frequently located in head and neck (Fig. 1C). They affect ~0.3% of newborns (30). Salmon patch, Angel’s kiss or Nevus flammeus neonatorum are similar birthmarks that fade progressively, seen in up to 40% of newborns. On histology, CMs are characterized by dilated and/or increased number of capillary-like vessels (31), in which ECs seem normal, but neuronal marking is decreased (32).

Autosomal dominant inheritance of CM allowed mapping of CM1 locus on 5q13–22 (33,34). Discovery of the causative gene unraveled an unrecognized clinical entity, that we named CM-AVM for capillary malformation-arteriovenous malformation (35). Families not linked to CM1 suggest locus heterogeneity.

Capillary malformation-arteriovenous malformation

Mutations in RASA1 were identified in six families with inherited atypical cutaneous CMs (35). Some individuals with a mutation had an additional fast-flow lesion, such as an arteriovenous fistula (AVF), i.e. direct connections between arteries and veins without intervening capillaries, an AVM with an intermediary nidus, or a Parkes-Weber syndrome (PKWS) (MIM 608355). This delineated the newly recognized disorder: CM-AVM (MIM 608354) (35). A more extensive study, which identified 41 additional truncating mutations, revealed that the CMs are small, multifocal and randomly distributed, pink-to-red or brown (Fig. 1C), often with a pale halo, and associated in 30% of the cases with a fast-flow lesion (N. Revencu et al., submitted for publication). Two-thirds are AVM or AVF; the last third PKWS. In PKWS patients, large cutaneous capillary stains on an extremity are associated with multiple micro-AVFs and overgrowth of the affected limb. PKWS worsens with age and can result in congestive heart failure (35, N. Revencu et al., submitted for publication). PKWS has been considered sporadic or eventually due to post-zygotic mutations, but when associated with multifocal CMs, it is due to a germline RASA1 mutation.

CMs usually require no treatment but can be lasered. However, fast-flow lesions render CM-AVM dangerous and difficult to treat, but the identification of involvement of RASA1 gives hope for development of novel therapeutic approaches. Most AVMs are sporadic, reflecting the severity of the defects that would probably result in early embryonic lethality if transmitted.

Reduced penetrance and variable expressivity suggest a double-hit mechanism to be involved. The encoded protein, p120RasGAP, negatively regulates the Ras/MapKine pathway (Fig. 2). Upon receptor tyrosine kinase activation, it is recruited to the plasma membrane, alone or by Annexin A6, to inactivate Ras (36). It also interacts with p190RhoGAP to control cell motility (37), and binds to AKT to protect cells from apoptosis (38). It is not known which one(s) of the pathways is/are altered in CM-AVM (39). Rasa1+/− mice are normal, while knockouts die at E10.5 due to defective vascular development and increased apoptosis (40).

Hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) (MIM 187300 and 600376) also known as Rendu-Osler-Weber syndrome, is an autosomal dominant disorder with an incidence around 1/10 000 (41). It is characterized by epistaxis and cutaneous-cossal telangiectasias (Fig. 1D), often associated with AVF in the lung (PAVM, 50% of patients), the liver (40%), the brain (CAVM) and sometimes in the gastrointestinal tract (41,42). Pulmonary and hepatic AVMs are rare in CM-AVM (N. Revencu et al., submitted for publication). The other inherited AVMs that are seen in PTEN hamartoma tumor syndrome (PHTS) (MIM 153480) also differ in that they are often intramuscular, multifocal, associated with ectopic fat and cause severe destruction of tissue architecture (N. Revencu et al., submitted for publication, 43,44).

Telangiectasias are focal dilatations of post-capillary venules with excessive layers of vSMCs, likely due to progressive disappearance of the capillary bed. With AVM, they might represent a spectrum of the same defect (45). Telangiectasias are also seen in Ataxia-telangiectasia (Louis-Bar syndrome; MIM 208900), an autosomal recessive disease caused by mutations in the ATM gene, on 11q23 (46), and also in Cutis Marmorata Telangiectatica Congenita (CMTC).
(MIM 219250) and Macrocephaly Cutis Marmorata (M-CM) (MIM 602501), two sporadic disorders of unknown etiology. In Progressive Patchy Capillary Malformation (Angioma serpiginosum, MIM 106050), linked to Xp11.23-q12 (47), the cutaneous vascular lesions are more similar to capillary malformations (48).

At least four loci have been associated with HHT: HHT1 on 9q33–34, with mutations in endoglin (ENG) (49), HHT2 on 12q11–14, with mutations in the activin receptor-like kinase 1 (ALK1) (50), HHT3 on 5q (51) and HHT4 on 7p14 (52) (Table 1). Moreover, Juvenile polyposis/HHT (JPPH) (MIM 175050) is caused by mutations in JPH1 (MIM 175050) (53). The function of the CCM proteins is starting to be unraveled. CCM1 RNA has been detected in astrocytes, neurons and various epithelial cells (79,80) and the protein was detected in ECs of capillaries and arterioles in adult (81). KRIT1 interacts with the α isoform of the β1-integrin cytoplasmic domain-associated protein 1, ICAP-1α (82,83), which participates in regulation of cell adhesion and migration (Fig. 2) (84,85). By competing with this interaction, KRIT1 may control EC behavior (85). Conversely, ICAP-1α is able to sequester KRIT1 to the nucleus (82). KRIT1 also associates with microtubules (86). Interestingly, KRIT1−/− embryos die at mid-gestation due to defective vascular development associated with downregulation of arterial markers (87). The basic defect in CCM might thus be linked to arterial-venous specification.

Expression profiles of CCM2 and PDCD10 are similar to KRIT1, and CCM2 is also transiently expressed in mesenchymal and parenchymal vessels (81,88,89). The CCM2 protein contains a phosphotyrosine-binding domain similar to that of ICAP-1α and it is able to sequester KRIT1 in the cytoplasm (90), suggesting ICAP-1α, KRIT1 and CCM2 to function in the same signaling pathway (Fig. 2). Direct interaction between KRIT1 and CCM2 has also been demonstrated. The murine orthologue of CCM2 suggests Mekk3-induced p38MAPK activation to be part of it, triggered by hyperosmotic choc (91). The CCM3 protein, PDCD10, mostly contains helical structures on the basis of its amino acid sequence. Due to the similarity in phenotype, it is likely involved in the same pathway(s).

### Lymphatic malformation and lymphedemas

Lymphatic malformations (LMs) are localized lesions composed of dilated lymphatic channels or vesicles that are not connected to the lymphatic vessels and are filled with clear fluid (92). LMs are usually congenital and often enlarge when infected. No evidence for inheritance exists, suggesting that the possible genetic causes are compatible with life only as somatic mutations in a restricted area of the lymphatic network. Another lymphatic dysfunction is lymphedema, characterized by swelling, usually of the lower extremities (Fig. 1F), due to non-functional lymphatic vessels (93). Lymphedema can be primary or secondary, for example due to surgery or infection.

**Primary congenital lymphedema** (Milroy disease or type I lymphedema; MIM 153100) is usually present at birth, bilateral, and affects most commonly the feet up to the knees. Sometimes, prenatal pleural effusion or hydrops-fetalis is seen (94,95). This autosomal dominant disorder, linked to 5q35.3, is caused by missense mutations in the tyrosine-kinase domain of the vascular endothelial growth factor receptor-3, VEGFR3, also known as FLT-4 (96,97). Although familial history was considered as a requisite for this disease, de novo mutations have been reported (95,98). The mutations inhibit phosphorylation of the receptor and prevent downstream signaling (Fig. 2). Similar phenotype is seen in the Chv mouse, due to a mutation in vegfr3 (99), and in vegfr3-deficient mice, which die around E9.5 due to irregular vessels with defective lumens (100).

**Late onset lymphedema** (type II lymphedema, Meige disease or lymphedema praecox; MIM 153200) develops around puberty. Truncating and some missense mutations in the transcription factor FOXC2, on 16q24.3, were found in families with lymphedema distichiasis (LD) (MIM 153400), lymphedema and ptosis (MIM 153000) and yellow nail syndrome (MIM 153300) (101–103). As distichiasis has a high penetrance, but is not always looked for, it has been proposed...
that all families with a FOXC2 mutation may have LD (104). Foxc2−/− mice have increased recruitment of pericytes in collecting lymphatics due to lack of inhibition of PDGF expression, a potent chemoattractant for vSMCs associated with lymphatic valve dysfunction (Fig. 2) (105).


**CONCLUDING REMARKS**

The identification of several genes, mutations in which cause vascular malformations, has helped to better delineate the spectrum of signs and symptoms of each subtype and to newly recognize clinical entities. This is paving the way to understand their molecular etiopathogenesis, a fundamental step towards precise diagnosis and management.

Most of the defects disturb the function of vascular ECs. Only in GVM the primary defect is in mural smooth muscle cells, and in CCM, it is not clear which cell types are affected. The identification of several genes, mutations in which cause vascular malformations, has helped to better delineate the spectrum of signs and symptoms of each subtype and to newly recognize clinical entities. This is paving the way to understand their molecular etiopathogenesis, a fundamental step towards precise diagnosis and management.

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An interesting question is the vessel-type specificity of the localized lesions. Only peripheral small vessels are affected, and for example, the distribution of AVMs is different in CM-AVM, HHT1, HHT2 and PHTS. Thus, the mutated molecules must have vessel-type specific functions and/or interactions. The challenge is to define these and to identify the cells that express the proteins. For most of these genes, the homozygous murine knockout embryos are lethal, and the heterozygous animals are phenotypically normal. Yet, the patients with familial vascular anomalies mostly carry a germ-line heterozygous mutation. Therefore, obtention of good animals models to understand the pathophysiological processes and to develop novel therapies, will probably require inducible conditional targeting, underscoring the likelihood that the double-hit mechanism could explain the localized nature, multifocality, varied expressivity, and penetrance that reaches its maximum towards puberty, of these lesions.

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