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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 paradominant 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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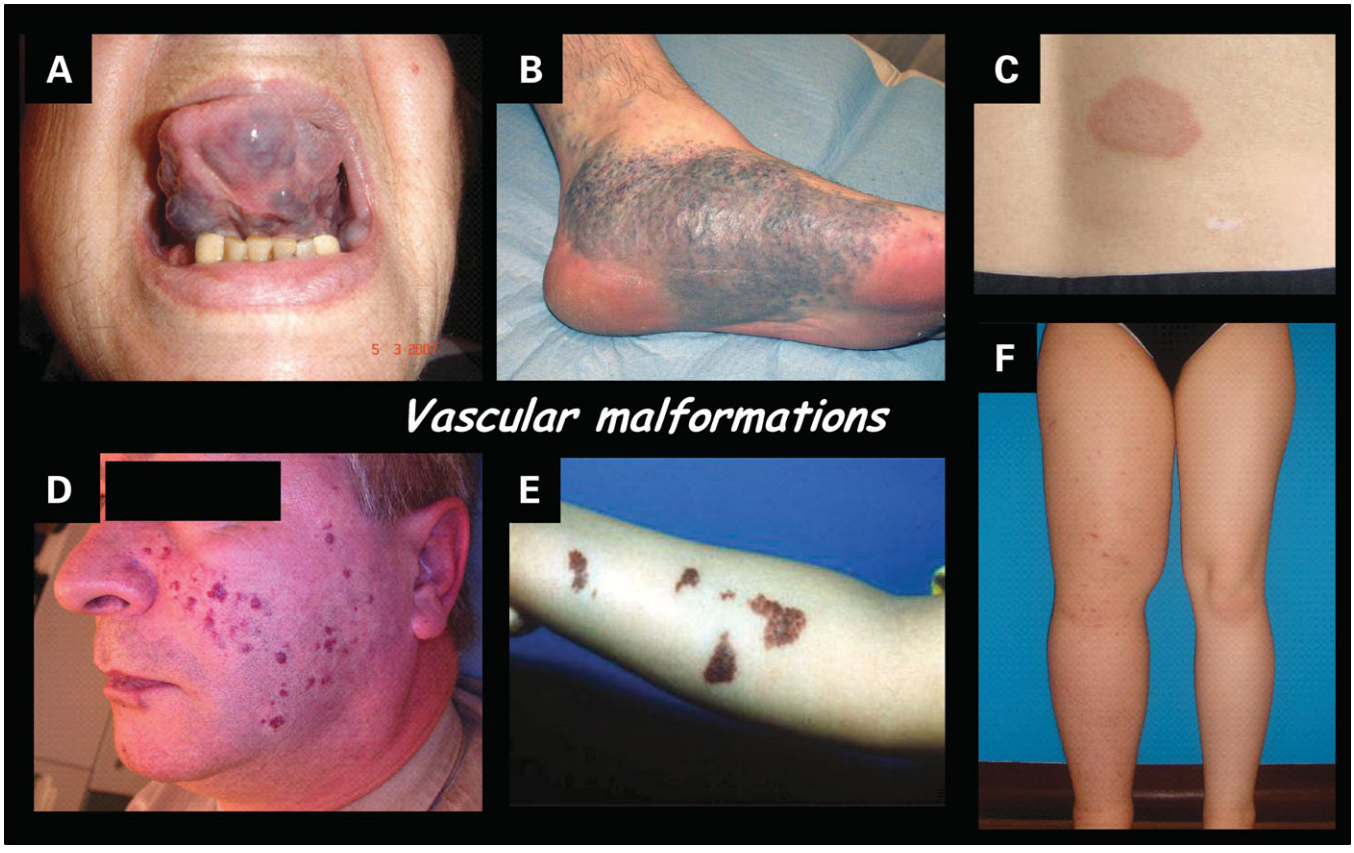

Figure 1. Selected vascular malformations: (**A**) VM on tongue; (**B**) GVM on foot; (**C**) capillary malformation of CM-AVM on back; (**D**) HHT on the cheek; (**E**) hyperkeratotic cutaneous capillaro-venous malformation on arm of a patient with CCM; (**F**) lymphedema on right leg.

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

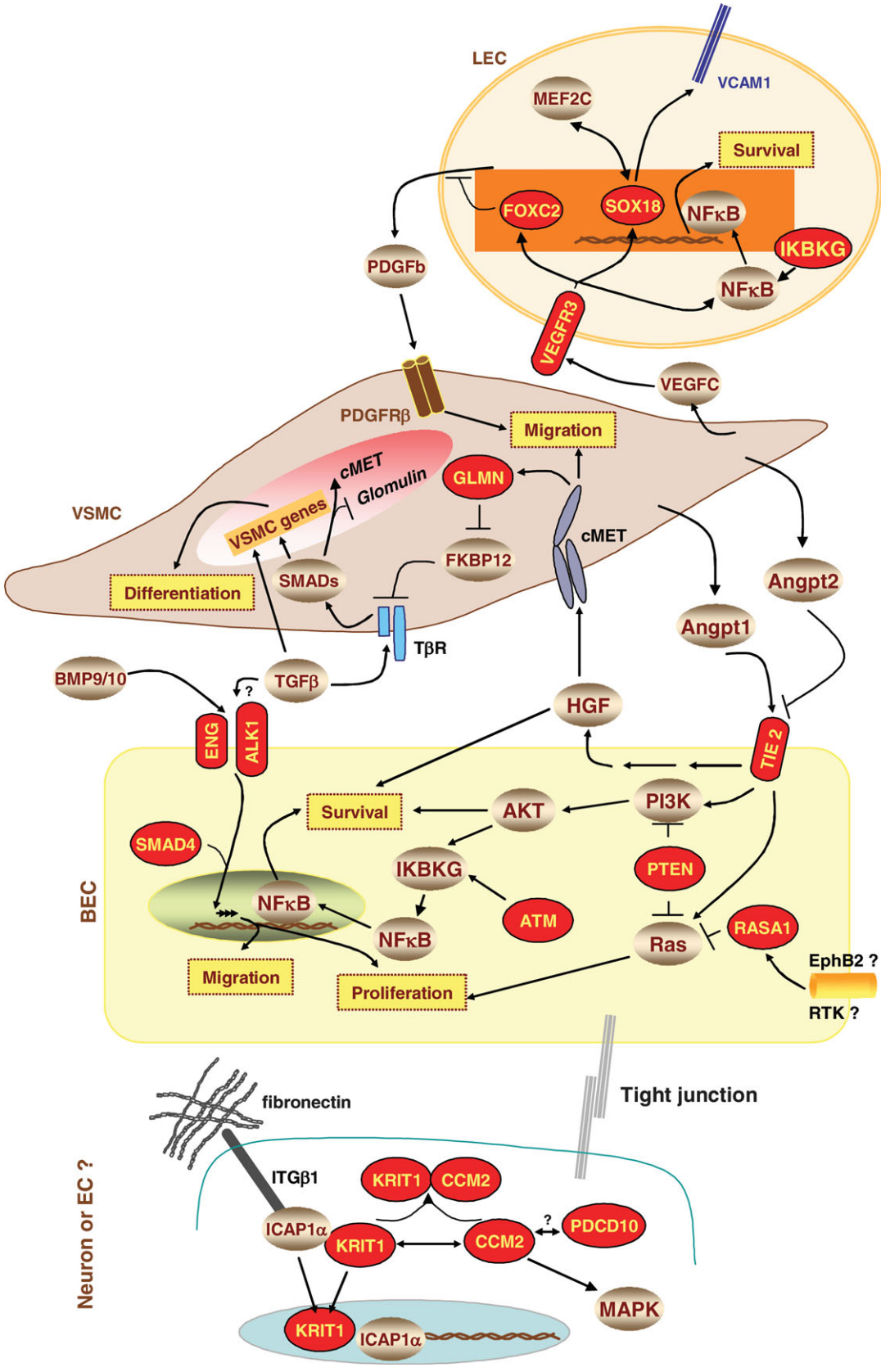

Figure 2. Pathways involved in vascular anomalies. Schemes on four cell types: lymphatic endothelial cell (LEC) with genes involved in lymphedema; vSMC for which the only primary defect is in glomulin; blood endothelial cell (BEC) regrouping alterations leading to arterial, capillary and VMs; and a cell 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 *1p21–22* (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 paradominant 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 CMC1 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 *CMC1* 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/MAPkinase 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 cutaneomucosal 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 *Xp11.3-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* syndrome (JPHT) (MIM 175050) is caused by mutations in *MADH4*, which encodes SMAD4 (53). Pulmonary AVMs are more common in HHT1, whereas hepatic AVMs are characteristic of HHT2. HHT2 also has a later onset and lower penetrance. More than 150 *ENG* mutations and 120 *ALK1* mutations have been reported (41). Mutations in both genes, expressed in ECs, likely result in haploinsufficiency. TGFβ signaling via ALK1 induces migration and proliferation (54) and ENG modulates this response (Fig. 2) (55). Although *Alk1* or *Eng*-deficient mice are lethal (56–60), heterozygotes are viable, and some develop HHT-like lesions (61,62). ALK1 ligands involved in HHT seem to be BMP9 and BMP10 rather than TGFβ (Fig. 2) (63,64). They inhibit EC proliferation and migration (64). The ubiquitously expressed SMAD4 is an intracellular TGFβ receptor signal transducer, but its knockout causes early lethality due to failure in gastrulation (65). The *HHT3* and *4* genes are like other players in the same signaling pathway.

Cerebral cavernous malformation

Cerebral cavernous (or capillary-venous) malformation (CCM) (MIM 116860) has a prevalence of about 0.5% (66). Seizures, headaches and neurological problems are the common symptoms, although many can be asymptomatic (67). Histologically, CCM consists of dilated capillary-like vessels mixed with large saccular vessels with thickened walls in the brain parenchyme. ECs lack tight junctions, resulting in gaps between them (68). CCM follows autosomal dominant inheritance, and four loci have been reported: *CCM1* on *7q11–22* with mutations in *KRIT-1* (KREV1 interaction trapped 1) (69,70); *CCM2* on *7p13*, with *MGC4607* or *malcavernin* mutations (71,72); *CCM3* on *3q26.1* with mutations in *PDCD10* (73); and *CCM4* in *3q26.3–27.2* (74).

Close to hundred mutations have been identified in *CCM1*, representing about 40% of the CCM families (75). Most result in loss-of-function, and double-hits have been discovered in two samples (76,77). In three families with KRIT1 mutations, the patients presented hyperkeratotic cutaneous capillary-venous malformations (HCCVM) (MIM 116860) (Fig. 1E) in addition to CCMs (78, N. Limaye *et al*., submitted for publication).

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 β_1-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 *Chy* mouse, due to a mutation in v*egfr3* (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

Table 1. Loci and genes involved in vascular malformations

Malformation	Acronym	Locus	Locus name	Mutated gene
Cutaneomucosal venous malformation	VMCM	*9p21*	*VMCM1*	*TIE2 (TEK)*
Glomuvenous malformation	GVM	*1p21–22*	*VMGLOM*	*GLOMULIN*
Blue rubber bleb nevus syndrome	BRBN	?	?	?
Maffucci syndrome	MAF	?	?	?
Klippel-Trenaunay syndrome	KTS	?	?	?
Capillary malformation-arteriovenous malformation	CM-AVM	*5q13–22*	*CMC1*	*RASA1*
Hereditary capillary malformation	CM	?	?	?
Arteriovenous malformation	AVM	?	?	?
PTEN hamartoma tumor syndrome	PHTS	*10q23*	*PHTS*	*PTEN*
Hereditary hemorrhagic telangiectasia	HHT	*9q33–34*	*HHT1*	*ENG*
	HHT	*12q11–14*	*HHT2*	*ALK1*
	HHT	*5q*	*HHT3*	?
	HHT	*7p14*	*HHT4*	?
Juvenile polyposis/HHT syndrome	JPHT	*18q21.1*	*JPHT*	*SMAD4*
Progressive patchy capillary malformation (Angioma serpiginosum)	PPCM	*Xp11.3–q12*	?	?
Ataxia-telangiectasia	AT	*11q23*	*AT1*	*ATM*
Cutis Marmorata Telangiectatica Congenita	CMTC	?	?	?
Macrocephaly Cutis Marmorata	M-CM	?	?	?
Cerebral cavernous (or capillary) malformation	CCM	*7q11–22*	*CCM1*	*CCM1 (KR1T1)*
	CCM	*7p13*	*CCM2*	*CCM2 (Malcavernin)*
	CCM	*3q26.1*	*CCM3*	*CCM3 (PDCD10)*
	CCM	*3q26.3–27.2*	*CCM4*	?
Hyperkeratotic cutaneous capillary-venous malformation	HCCVM	*7q11–22*	*CCM1*	*KR1T1*
Primary congenital lymphedema/Milroy disease	PCL	*5q35.3*	*PCLI*	*FLT4*(*VEGFR3*)
Lymphedema-distichiasis/lymphedema-ptosis/yellow nail	LD	*16q24.3*	*LD*	*FOXC2*
Hypotrichosis-lymphedema-telangiectasia syndrome	HLTS	*20q13.33*	*HLT*	*SOX18*
Lymphedema-cholestasis/Aagenaes syndrome	LCS	*15q*	*LCSI*	?
Osteoporosis Lymphedema Anhydrotic Ectodermal Dysplasia Immunodeficiency	OLEDAID	*Xq28*	*IP2*	*IKBKG*(*NEMO*)

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).

Hypotrichosis lymphedema telangiectasia syndrome (HLTS) (MIM 607823), is characterized by lymphedema, which is associated with sparse hair and cutaneous telangiectasias. Both autosomal dominant and recessive inheritance have been observed (106,107). By phenotypic homology to the *ragged* mice, caused by four different premature truncations in the transcription factor *Sox18* (108), a dominant nonsense mutation in the transactivation domain and homozygous recessive substitutions in the DNA-binding domain of *SOX18* (*20q13.33*) were discovered in three families (106). *Sox18* is expressed in ECs, hair and feather follicles and the heart (109). It has two close homologues, SOX7 and SOX 17. It is regulated by VEGFR3 and it is an early marker of lymphatic differentiation. SOX18 interacts with transcription factor MEF2C, and directly regulates expression of VCAM1, an EC adhesion molecule (Fig. 2) (110). Yet, its function awaits unraveling.

Lymphedema is also observed in *Osteoporosis Lymphedema Anhydrotic Ectodermal Dysplasia with Immunodeficiency* syndrome, abbreviated OLEDAID, a rare syndrome associated with Incontinentia Pigmenti (MIM 308300). Replacement of the termination codon of the NFκB essential modulator *IKBKG* (*NEMO*, *Xq28*) by a tryptophane, was identified in two independent patients. The mutation leads to an enlarged protein with reduced NFκB activation (111,112). *Ikbkg*$^{-/-}$ mice die from severe apoptosis due to defective NFκB activity (113). As VEGFR3 has been shown to activate NFκB it may be the pathway involved in Milroy disease. *Lymphedema-cholestasis* syndrome, also known as Aagenaes syndrome (MIM 214900), is an autosomal recessive disorder (114), although *de novo* autosomal dominant mutation was also suggested (115). An haplotype-shared region has been identified in *15q* (116), and the search for the defective gene is ongoing.

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 by the primary defect. In addition, the pathogenic mechanisms that lead from the mutations to development of lesions are still far from being understood. Figure 2 schematizes the factors identified to be involved in vascular and lymphatic anomalies. All except the TIE2 mutations presumably result in nonfunctional alleles, which may cause either haploinsufficiency and/or dominant-negative effects.

An interesting question is the vessel-type specificity of the localized lesions. Only peripheral small vessels are affected, and for exemple, 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 germline heterozygous mutation. Therefore, obtention of good animal 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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Conflict of Interest statement. None declared.

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