Clin Genet 2003: 63: 340–351
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CLINICAL GENETICS
ISSN 0009-9163

Developmental Biology: Frontiers for Clinical Genetics

Section Editor:
Roderick R. McInnes, e-mail: mcinnes@sickkids.on.ca

Vascular malformations: localized defects in vascular morphogenesis

Brouillard P, Vikkula M. Vascular malformations: localized defects in vascular morphogenesis.
Clin Genet 2003: 63: 340–351. © Blackwell Munksgaard, 2003

Vascular anomalies are localized defects of the vasculature, and usually affect a limited number of vessels in a restricted area of the body. They are subdivided into vascular malformations and vascular tumours. Most are sporadic, but Mendelian inheritance is observed in some families. By genetic analysis, several causative genes have been identified during the last 10 years. This has shed light into the pathophysiological pathways involved. Interestingly, in most cases, the primary defect seems to affect the characteristics of endothelial cells. Only mutations in the *glomulin* gene, responsible for hereditary glomuvenous malformations, are thought to directly affect vascular smooth-muscle cells.

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Key words: angiogenesis – artery – capillary – gene – genetic – hemangioma – lymphatic – vasculature – vascular anomaly – vein

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Received 11 February 2003, revised and accepted for publication 20 February 2003

A common feature to all blood and lymphatic vessels is the presence of endothelial cells (ECs) as the luminal cell layer. In blood vessels, these endothelial tubes are supported by a layer of variable thickness of vascular smooth muscle cells (VSMCs) and/or pericytes (together called mural cells), whereas only certain areas of large lymphatic vessels contain VSMCs. The main processes through which this complex network is developed are divided into vasculogenesis, angiogenesis and lymphangiogenesis.

Vasculogenesis, the formation of blood vessels in the embryo, is based on *in situ* differentiation of precursor cells (1, 2). These cells, called hemangioblasts, aggregate progressively to form blood islands (Fig. 1). The outermost cells differentiate into primordial endothelium, whereas the inner ones form blood-cell precursors (3). The newly differentiated endothelial cells assemble together to form tube-like structures, creating the primary capillary plexus (4). Many factors that play a role in this process are already known (see Fig. 1).

Angiogenesis denotes the growth and remodelling of this primary capillary plexus into a complex network of vessels composed of capillaries, arteries and veins (Fig. 1) (reviewed in ref. 5). Four distinct phenomena are observed:

1) *sprouting* of capillaries from pre-existing vessels;
2) *non-sprouting angiogenesis* resulting in enlargement, fusion or splitting of pre-existing vessels by transcapillary pillars;
3) *pruning* (the loss of certain endothelial tubes and cells); and
4) *maturation* – recruitment of pericytes and SMCs, which is tightly associated with the appearance of circulation and dependent on metabolic demands.

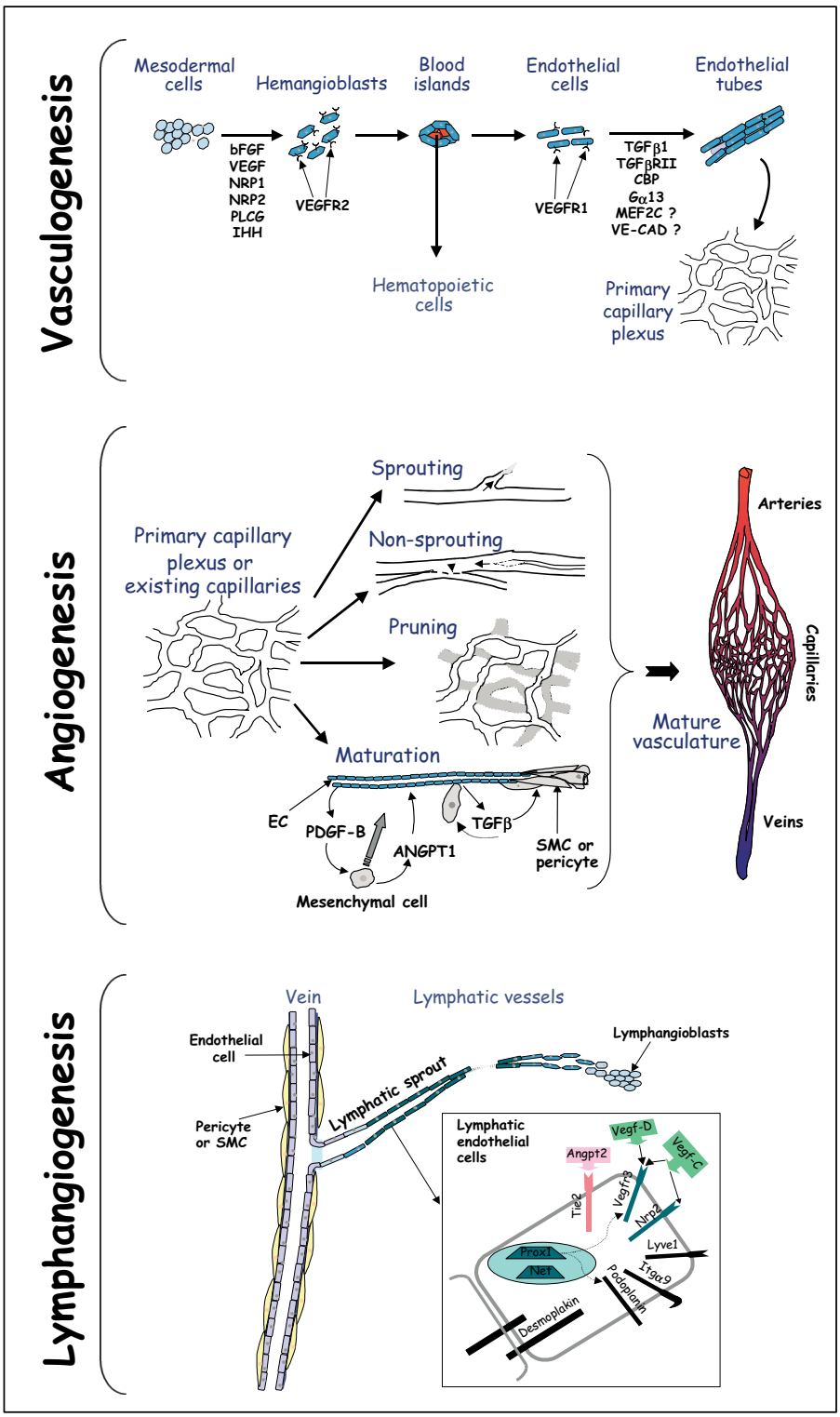

Fig. 1. Development of blood and lymphatic vessels. Only the most important factors are shown. ANGPT1, angiopoietin 1; Angpt2, angiopoietin 2; bFGF, basic fibroblast growth factor; CBP, CREB-binding protein, EC, endothelial cell; Gα13, GTP-binding protein α-13; IHH, Indian hedgehog; Itgα9, integrin α9; Lyvel, lymphatic vessel endothelial HA-receptor 1; MEF2C, mad-box elongation factor 2c; Net, new Ets transcription factor; NRP1, neuropilin 1; NRP2, neuropilin 2; PDGF-B, platelet-derived growth factor B; PLCG, phosphatidylinositol-specific phospholipase C; Prox 1, prospero-homologous homeobox gene 1; SMC, smooth muscle cell; TGFβ, transforming growth factor β; TGFβRII, transforming growth factor β receptor 2; Tie2, tyrosine kinase receptor with immunoglobulin-like loops and epidermal growth factor homology domain; Vegf, vascular endothelial growth factor (C and D); VEGFR (1, 2, 3), vascular endothelial growth factor receptor 1, 2 and 3.

Non-perfused blood vessels regress, whereas circulating factors and shear-stress induce modifications in cell–cell and cell–matrix interactions. Once the mature vasculature has been formed, it seems relatively stable in time and endothelial cells present an especially low turnover (6). However, in response to physical damage (in wound repair) or to pathologies (e.g. atherosclerosis, retinopathy, psoriasis and cancer), neovascularization can be induced.

Lymphatic vessels develop very soon after angiogenesis and are essential for the recovery of fluid leaking from the vasculature (7). The first theory on lymphangiogenesis proposed that primitive lymph sacs would arise from endothelial cells, which are derived from embryonic veins, and assemble to form lymphatic capillaries (Fig. 1) (8). The alternative theory suggests that lymph sacs are derived from lymphangioblasts, i.e. mesenchymal precursor cells independent of veins, in a process similar to vasculogenesis (Fig. 1) (9). Currently, it seems that lymphangiogenesis is a combination of both a venous-derived process and *in situ* differentiation of lymphatic endothelial cells (Fig. 1) (7).

As these morphogenic processes are controlled by the interactions and ordered effects of numerous angiogenic and antiangiogenic factors, it is not surprising that developmental defects can occur. These defects, called *vascular malformations*, are usually localized and generally divided according to the type of vessel affected (Fig. 2). They include capillary, venous, arteriovenous, lymphatic and combined malformations (10, 11). These vascular malformations are usually present at birth and grow proportionately with the patient. Another group of vascular anomalies is formed by *vascular tumours*. The most common is hemangioma, which is a benign tumour that rapidly grows during the first year of life followed by spontaneous regression within 5–10 years (Fig. 2) (10). Hemangiomas occur in 10–12% of 1-year-old children. Although no clear evidence exists regarding the causes of their appearance, some recent studies have characterized abnormalities in hemangioma-derived endothelial cells, suggesting the presence of an intrinsic cellular defect (12; see ref. 13 for review). This is supported by the identification of a possible locus on chromosome *5q31-33* (associated with hemangiomas) that seemed to be inherited as an autosomal-dominant trait (14), and the loss of heterozygosity observed on *5q* in some microdissected sporadic hemangiomas (15).

Whereas hemangioma research has only started to focus on the identification of molecular causes of these vascular tumours, several causative factors have already been identified for vascular malformations. In the following text, we discuss these discoveries regarding the malformations of capillaries of the skin and the nervous system, followed by those affecting arteries, and those of veins. Finally, disorders of the lymphatic system are described.

Capillary malformations

Capillary malformations (CM; MIM 163000), often called 'port-wine stains', are flat, red/purple cutaneous lesions that occur in about 0.3% of newborns (Fig. 2) (16). CMs are typically located in the head and neck region. They often thicken with age and darken from pink to dark red (11). Similar stains, called 'salmon patch', 'angel's kiss' or 'nevus flammeus neonatorum', occur in up to 40% of newborns, but fade progressively during infancy (16). Histologically, CMs are composed of capillary-like vessels that are dilated and/or increased in number. They have been suggested to be remnants of unmodified primitive capillary plexus (11). The vascular walls and endothelial cells seem normal, as detected by immunohistochemistry (17–19). In contrast, neuronal marking is significantly decreased (20, 21), suggesting that the lack of innervation may be the cause of dilatation of cutaneous capillaries (20). Alternatively, the reduced density of nerves may be a consequence of abnormal circulation and progressive ischaemia (22). A concerted development of vasculature and innervation may be a common phenomenon, and vascular endothelial growth factor (VEGF), secreted by, e.g. cutaneous nerves, has been suggested to be important for this (23).

Despite the high frequency of CMs in the general population, only few families that show familial segregation of CMs as an autosomal-dominant trait have been reported (24–28). Linkage analysis recently led to the identification of a locus, *CMC1*, on *5q13-22* (24, 25). Preliminary data also indicate that locus heterogeneity exists, suggesting that a number of genes are involved in CM pathogenesis (24, and I. Eerola et al., unpublished). Whether the affected gene(s) has(have) a role only in angiogenesis, neurogenesis, or both, awaits elucidation.

Cerebral cavernous malformations

Vascular malformations can also occur in the central nervous system. Cerebral cavernous (or capillary-venous) malformations (CCM; MIM 116860) present as lace-like structures composed of dilated capillary-like vessels and/or large

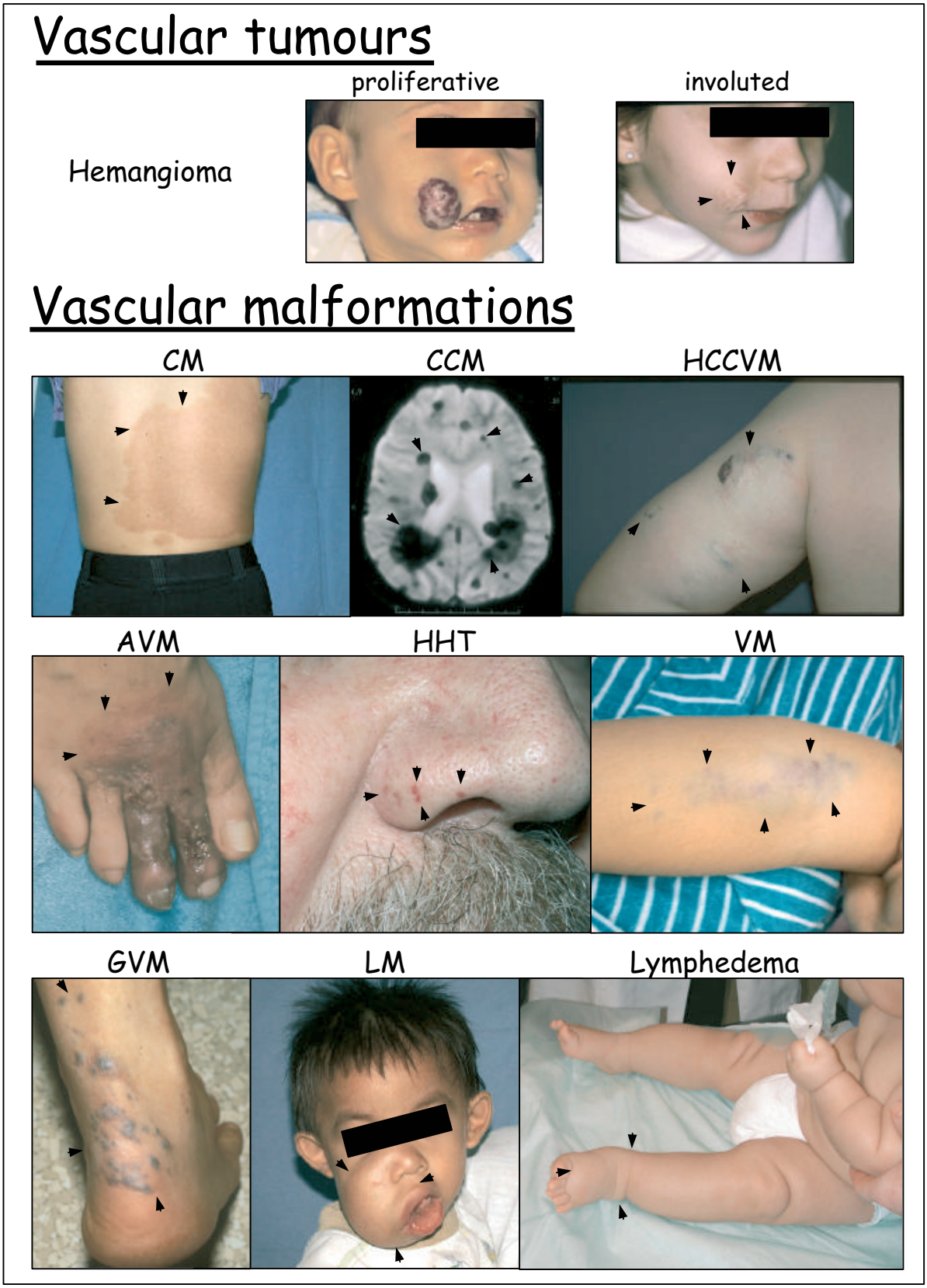

Fig. 2. Classification of vascular anomalies. AVM, arteriovenous malformation; CCM, cerebral cavernous (or capillary-venous) malformation; CM, capillary malformation; GVM, glomuvenous malformation ('glomangioma'); HCCVM, hyperkeratotic cutaneous capillary-venous malformation; HHT, hereditary hemorrhagic telangiectasia; LM, lymphatic malformation; VM, venous malformation. Arrows indicate (limits of) vascular malformations.

cavernous channels, in the brain parenchyma (Fig. 2). They can cause seizures, headaches and various neurological problems (29). CCMs occur in about 0.5% of the population and thus represent a major cause for neurological signs and symptoms (30, 31). They also show a high frequency of familial aggregation with autosomal-dominant inheritance (29). The lesions consist of endothelial-lined vascular sinusoids embedded in a collagen matrix, with the basal lamina sometimes presenting multiple layers. No tight junctions are observed at endothelial cell interfaces, whereas gaps are seen between endothelial cell processes. Heavy hemosiderin deposits within the basal lamina and the absence of astrocytic foot processes can also be noted (32). Interestingly, an increase in the number of lesions can be observed in patients (33, 34), which may be a result of the identified slight increase in the proliferative capacity of CCM-derived endothelial cells (35). This could also be the cause for the lack of pericytes that has been observed (36).

Genetic analysis enabled identification of the *CCM1* locus on *7q11-22* (37, 38) and of the mutated gene, *KREV1 interaction trapped 1* (*KRIT1*) (39, 40). All of the known mutations lead to premature truncation of KRIT1, probably resulting in loss of function (39–44). As mutated transcripts are present in Epstein–Barr virus (EBV)-transformed lymphoblasts, although at lower levels than wild-type transcripts, it cannot be excluded that truncated proteins with residual function are produced (41, 44). Interestingly, two

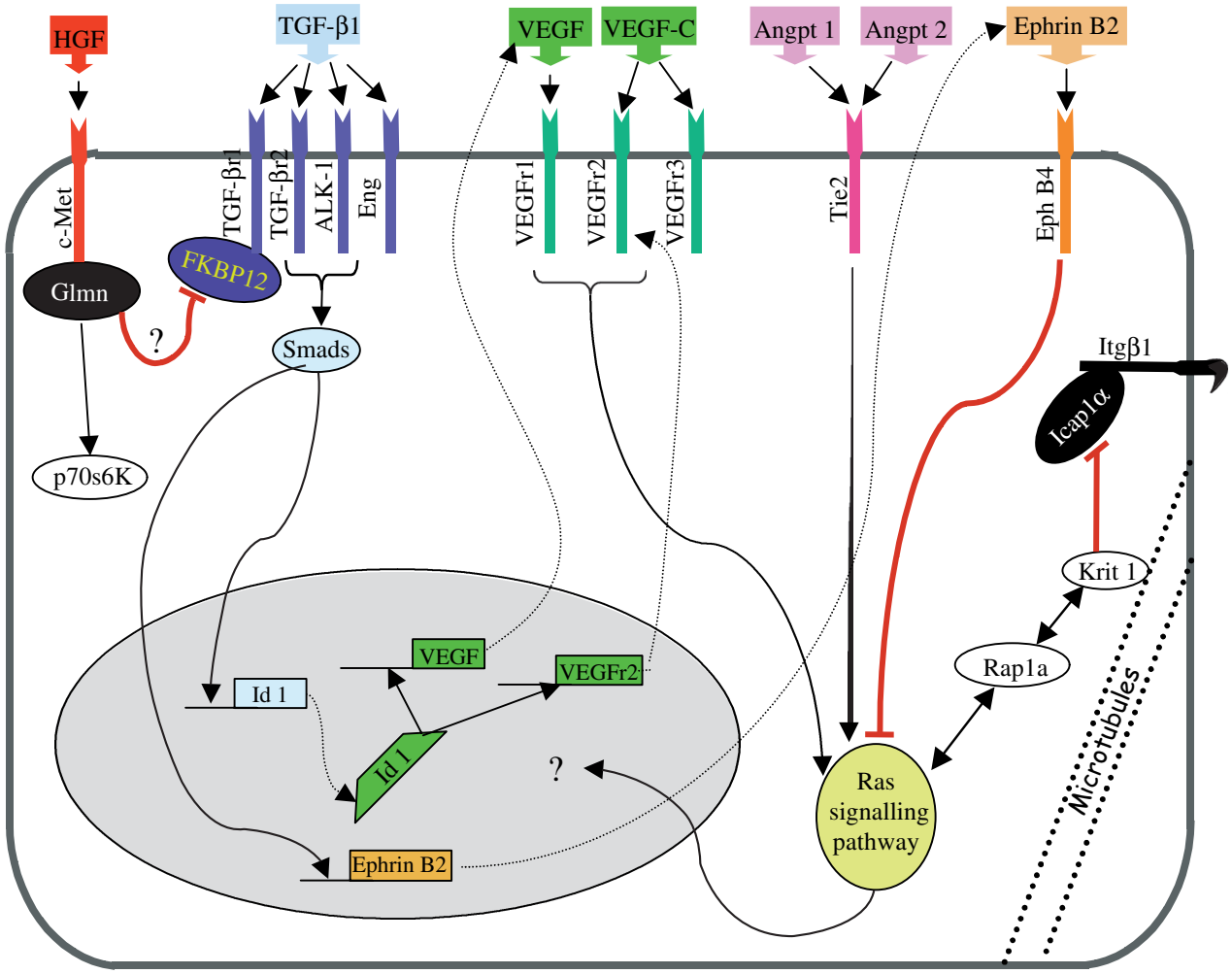

Fig. 3. Schematic presentation of molecular pathways involved in vascular malformations or with related function. Transforming growth factor-β (TGF-β) signalling via the Smads can regulate expression of Ephrin B2 and, via Id1, that of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2). See the text for other details. →, activation; ⊥, inhibition; ....., transfer of product; genes are shown in a box. ALK-1 activin receptor-like kinase 1; Eng, endoglin; FKBP12, FK506-binding protein of 12kDa; Glmn, glomulin; HGF, hepatocyte growth factor; TGF-β1, transforming growth factor-β1.

somatic mutations were identified in a CCM lesion of one sporadic patient (45). Even if it is not clear whether these mutations were on different alleles, this indicates that a double-hit (i.e. targeting of the two alleles) may be needed for CCMs to appear.

The mutated protein, KRIT1, was originally identified in a yeast two-hybrid screen (46) because of its interaction with RAP1A (KREV1), a member of the RAS-GTPase family of proteins that are involved in morphogenesis and cell differentiation. Thus, mutations on *KRIT1* may cause altered Ras signalling (Fig. 3). Other yeast two-hybrid experiments, using KRIT1 (or part of it) as bait, retrieved the α isoform of the integrin cytoplasmic domain-associated protein 1, ICAP-1α (47, 48). ICAP-1α is known to bind the intracellular part of β_1 integrin and to participate in cell adhesion and migration (49, 50). KRIT-1 binding competes with this interaction and may therefore constitute a regulatory mechanism controlling integrin-mediated endothelial cell behaviour (50). As KRIT1 was also found to associate with microtubules, it may have a role in determining endothelial cell shape and function in response to cell–cell and cell–matrix interactions (Fig. 3) (51). Studies on the recently reported *Krit-1* knockout mouse, which is homozygous lethal (around E11) but viable as a heterozygote, with scattered, abnormally dilated blood vessels (52), should contribute to the understanding of the mechanisms underlying the pathogenic processes of CCMs. Two additional possible loci have also been identified: *CCM2* on chromosome *7p13-35* and *CCM3* at *3q25.2-27* (53).

Hyperkeratotic cutaneous capillary-venous malformations

In certain CCM families, some patients (e.g. 10 individuals in four out of 57 French CCM families) also present cutaneous capillary-venous malformations with hyperkeratotic epidermis (HCCVM; MIM 116860) (Fig. 2) (41, 54, 55). These crimson-coloured and irregularly shaped lesions are distinct from CCM and venous malformations. The dilated capillaries extend into the dermis and hypodermis, and larger venous-like channels are also observed (41,54). A truncating mutation was identified in *KRIT1* in one family with CCMs and HCCVMs (41). Interestingly, this mutation is located the most 5′ in the *KRIT1* sequence as compared to all the CCM mutations. This could explain the difference in phenotype, and suggests a role for the N-terminal part of KRIT1 in cutaneous vessels (41). This possibility, as well as the role of KRIT1 in vasculogenesis, angiogenesis (and lymphangiogenesis), may be examined in the knockout mice, in which the truncation targets the first exons encoding the protein (i.e. exons 5 and 6), a modification similar to that occuring in HCCVM (52). In addition, a large series of genetically analysed CCM patients would enable better genotype–phenotype comparison.

Arteriovenous malformations

Although capillaries normally form a dense network of small vessels between arterioles and venules, sometimes arteries enter to a so-called 'nidus', which is directly emptied by a number of draining veins. Therefore, no capillary network separates arterioles from venules. These arteriovenous malformations (AVM) appear as pink-to-red, warm and pulsatile lesions in the skin (Fig. 2) (11). AVMs are the most dangerous and difficult to treat vascular anomalies. They may worsen at any time of life, especially after trauma or surgical treatment, and can cause congestive heart failure (56).

Biological data from AVM-derived endothelial cells show a high proliferation rate and absence of sensitivity to inhibitory cytokines, such as interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β) and interferon-γ (IFN-γ) (57). Therefore, genes that regulate angiogenic processes, and more specifically proliferation and/or apoptosis, may be involved in the etiopathogenesis of AVMs. Other probable candidates are genes encoding proteins that are essential for vessel identity, such as ephrin B2, mostly expressed in arteries, and its specific receptor, Eph-B4, present in veins (Fig. 3) (58).

As most AVMs are sporadic, genetic predisposition seems unlikely. However, AVMs have been observed in families with inherited CMs (I. Eerola et al., unpublished). This suggests a genetic predisposition for AVMs in families with inherited capillary malformations and indicates that the same gene could be involved in the pathogenesis of CMs and AVMs. Such a gene probably resides in the recently reported *CMC1* locus on *5q* (24). Could it be that on the basis of the location or identity of the altered capillaries, either CMs or AVMs are formed?

Hereditary hemorrhagic telangiectasia

Arteriovenous defects are also present in hereditary hemorrhagic telangiectasia (HHT; MIM 187300 and 600376), an autosomal-dominant

vascular disorder otherwise known as Rendu–Osler–Weber syndrome. It is characterized by telangiectasias in skin and mucosa (Fig. 2), and often associated with arteriovenous fistulas (i.e. direct connections between arteries and veins), especially affecting lung, brain and sometimes the gastrointestinal tract (59). Telangiectasias are focal dilatations of post-capillary venules, with excessive layers of SMCs, owing to frequent direct connections to dilated arterioles resulting from progressive disappearance of the capillary bed (59, 60).

By linkage analysis, two different loci were identified: *HHT1* on *9q33-34* (61, 62) and *HHT2* on *12q11-14* (63, 64). Several premature stop codons were found in the genes encoding endoglin (*ENG*) and the activin receptor-like kinase 1 (*ALK-1*), respectively (65, 66). Both endoglin and ALK1 take part in the TGF-β receptor complex in vascular endothelial cells. Endoglin is a TGF-β-binding glycoprotein and ALK1 is a TGF-β receptor-associated serine–threonine kinase (Fig. 3) (66, 67). The loss of function resulting from the mutations in these receptors implies that TGF-β signalling has an important role in the stability of the capillary bed between arteries and veins. This suggests that TGF-β signalling may also be altered in inherited CMs and the associated AVMs.

Some of the heterozygous mice deficient for endoglin or Alk-1 have telangiectasias and nosebleeds similar to the heterozygous HHT patients (68–73). Thus, they at least partially recapitulate the human disease. As they also show differences in phenotypic severity, depending on the genetic background, these mice could help unravel modifier genes that are important for TGF-β function in the vascular system.

Venous malformations

Venous malformations (VMs; MIM 600195) present as blueish-purple lesions, mainly localized on skin and mucosae (Fig. 2). Their spectrum varies from cutaneous varicosities, ectasias or localized spongy masses, to complex lesions that infiltrate soft tissues or bones (11). VMs represent more than 50% of patients referred to centres for vascular anomalies (74) and their incidence is estimated to be around 1 in 10,000 (75). Most appear to be sporadic (76) but they can also be inherited, in which case multiple lesions are frequent (74, 77). Histologically, VMs are characterized by enlarged vein-like channels of variable size (10). Immunohistochemistry revealed a relative lack of SMCs around flat endothelial cells (78), probably resulting from defective recruitment of SMCs, as ECs were not proliferative (79, 80).

A locus for autosomal-dominant multiple cutaneous and mucosal venous malformations, *VMCM1*, was identified on chromosome *9p21* (74,77). A causative mutation (R849W) was found in the endothelial cell-specific receptor tyrosine kinase TIE-2 (TEK). At the protein level, this results in increased ligand-independent autophosphorylation of the receptor, but does not induce proliferation of endothelial cells *in vitro* (78). The same R849W mutation has been found in altogether three families, and a second mutation (Y897S) leading to a similar activating effect, has been discovered in a fourth kindred (78, 81). This limited number of mutations and families is probably a result of the small number of amino-acid changes that can lead to specific activation and alterations of the function of the angiopoietin receptor, TIE-2.

Endothelial cells that express mutant TIE-2 probably exhibit multiple downstream alterations, as mutant TIE-2 activates more strongly than the wild-type receptor signal transducers and activators of transcription STAT-1, STAT-3 and STAT-5 (82), and additional alterations are not excluded. The relative lack of VSMCs could thus be linked to alterations in EC adhesion, SMC recruitment by chemoattractants, a combination of both, or even other events. A paradigm is the fact that VMs are restricted to cutaneous and mucosal vessels. This could be explained by the protective effect of vascular endothelial-protein-tyrosine phosphatase (VE-PTP), which acts specifically on TIE-2 and is expressed at lower levels in small capillaries and veins than in SMC-invested large vessels (83). Alternatively, environmental factors or additional genetic alterations, e.g. in the Eph/ephrin pathway (Fig. 3), may also play a role.

Mice deficient in Tie-2 or its activating ligand, Angpt-1, die embryonically as a result of generalized angiogenic defects (84–86). Similar disruption of blood vessels is observed when the inhibitory ligand, Angpt-2, is overexpressed in skin (87). Therefore, it seems that any alteration of Tie-2 signalling perturbs vascular development (Fig. 3), and that mutations in TIE-2 or its ligands are likely to occur in vascular malformations, unless loss of their activity is lethal.

Glomuvenous malformations

Glomuvenous malformations (GVM; MIM 138000) represent a subtype of VMs, also known as ‘glomangioma’ or ‘multiple glomus

tumours'. GVM can be distinguished clinically from other venous malformations by their raised, blueish-purple and cobblestone appearance, and by their painfulness on palpation (Fig. 2) (76). Moreover, they are rarely encountered in mucosae and are frequently inherited. The lesions are histologically characterized by the presence of 'glomus cells' around distended venous channels (88, 89). Electron microscopic and immunohistochemical studies revealed resemblance between glomus cells and smooth-muscle cells, as well as a lack of desmin, a late marker of SMC differentiation, in these abnormal mural cells (89, 90). Therefore, glomus cells in GVM can be considered as incompletely differentiated or maldifferentiated VSMCs. Similar cells are present in paragangliomas and solitary glomus tumours, but the pathogenic molecular pathways are distinct (91–96).

GVM segregate as an autosomal-dominant disease, with incomplete penetrance and variable expressivity (76, 88, 97–99). We identified a linked locus, *VMGLOM*, on *1p21–22* (100, 101) and identified, by positional cloning, 16 different mutations in a novel gene that we named *glomulin* (102). Most of the mutations are truncating. A somatic 'second hit' mutation was identified in one lesion of a patient, suggesting that the lesions may be caused by a complete localized loss-of-function of glomulin (102). As we found a *glomulin* mutation in all GVM families tested, locus heterogeneity is unlikely.

To date, little is known about glomulin. It has no homologies with any known protein or any conserved domain. In contrast, glomulin RNA was found in many cells and tissues, which is in contrast with the phenotype restricted to cutaneous vessels (102). Based on the interaction of FAP48 (a truncated form of glomulin) with the immunophilins, such as FKBP12 (103), we suggested involvement of glomulin in the TGF-β pathway. Glomulin could act as a competitor for FKBP12, an inhibitor of TGF-β signalling (102, 104). Therefore, glomulin could regulate signalling through TGF-β receptors. A second hypothesis involves the hepatocyte growth factor (HGF) pathway. FAP68 – a protein identical to glomulin – was shown to interact specifically with the inactive form of the HGF receptor c-Met, and to be phosphorylated and released upon activation of the receptor (105). This phosphorylated glomulin/FAP68 stimulated phosphorylation of the p70S6 kinase, a downstream target of phosphoinositide 3-kinase (PI3 kinase) (105). We are currently generating *glomulin* knockout mice in order to study the role of this gene during development.

Lymphedemas

Besides defects in the vascular system, alterations of lymphatics are also encountered. The most frequent are lymphedemas (Fig. 2), which can be primary or secondary (e.g. as a result of surgery or infection). Primary lymphedema is characterized by subcutaneous swelling, usually of the lower extremities, with aplasia, hypoplasia or hyperplasia of lymphatic vessels in lymphangiography (106). They often segregate as an autosomal-dominant trait, and are subdivided into early-onset and late-onset forms. They can also be associated with other clinical features or be part of a syndrome.

Table 1. Loci and genes involved in hereditary vascular malformations

Malformation	Locus	Locus name	Mutated gene	Type of mutation	References
Hereditary capillary malformation (CM)	5q13-15	CMC1	?	?	24,25
Cerebral cavernous (or capillary)	7q11-22	CCM1	*KRIT1*	Inactivating?	37–40
malformation (CCM)	7p13-35	CCM2	?	?	53
	3q25.2-27	CCM3	?	?	53
Hyperkeratotic cutaneous capillary-venous malformation (HCCVM)	7q11-22	CCM1	*KRIT1*	Inactivating?	41
Arteriovenous malformation (AVM)	5q13-15?	CMC1?	?	?	24, unpublished
Hereditary hemorrhagic telangiectasia (HHT)	9q33-34	HHT1	*ENG*	Inactivating	61,62,65
	12q11-14	HHT2	*ALK1*	Inactivating?	63,64,66
Venous malformation (VM)	9p21	VMCM1	*TIE2 (TEK)*	Activating	74,77,78
Glomuvenous malformation (GVM)	1p21-22	VMGLOM	*GLOMULIN*	Inactivating?	100–102
Primary congenital lymphoedema	5q34-35	?	*FLT4(VEGFR3)*	Inactivating	107,108, 115,116
Milroy's disease					
Lymphoedema-distichiasis (LD)	16q24.3	?	*FOXC2*	Inactivating?	109–111, 117
Meige lymphoedema					
Lymphoedema and ptosis					
Yellow nail syndrome					
Hypotrichosis-lymphedema-telangiectasia	20q	?	*SOX18*	Inactivating	112

Primary congenital lymphedema, the early-onset form, is also known as Milroy's disease or type I lymphedema (MIM 153100). Several missense mutations were found in the *FLT-4* (*VEGFR3*) gene (107, 108). These mutations affect the catalytic domain of the receptor and inhibit autophosphorylation, therefore preventing downstream signalling (107, 108). Late-onset lymphedema, also called type II lymphedema, Meige lymphedema or lymphedema praecox, develops around puberty (MIM 153200). It is caused by inactivating mutations in the forkhead transcription factor *FOXC2* (109, 110). Mutations in the same gene were also found in families with lymphedema distichiasis (MIM 153400), lymphedema and ptosis (MIM 153000) and yellow nail syndrome (MIM 153300) (111). Recently, we discovered the involvement of another transcription factor, SOX18, in the pathogenesis of lymphedema, by identification of mutations in dominant and recessive forms of hypotrichosis-lymphedema-telangiectasia (112).

Lymphatic malformations

In contrast to lymphedemas, lymphatic malformations (LMs) are composed of dilated lymphatic channels or vesicles, which are filled with clear fluid and not connected to the lymphatic vessels (113). LMs are usually present at birth, and typically swell and enlarge when there is an infection. No evidence for inheritance of LMs exists, indicating that if genetic alterations play a role in their pathogenesis, germline mutations are lethal and sporadic lesions may occur as a result of 'localized' somatic mutations.

Concluding remarks

The identification of several chromosomal loci linked to inherited forms of vascular malformations has shed light into their pathogenesis. This has also helped to recognize distinct molecular forms of the disorders (Table 1). In future, we may need to name each vascular malformation on the basis of its molecular background. Already, the current knowledge permits genetic diagnosis in a selected number of patients, enabling a more precise evaluation of clinical prognosis.

In most cases, the mutations are truncating or missense, presumably resulting in loss of function. The only exception is *TIE2*, in which only activating mutations have been found. In either case, unaffected mutation carriers exist and the lesions are usually well localized. Therefore, the 'double-hit' mechanism seems to be an intriguing explanation. To date, this has only been shown for one GVM lesion of one patient (102), and might also be the case for one CCM lesion (45). It could also be that the secondary event affects another gene which interacts with the disease-causing one, rendering the identification difficult.

The variety of genes that are implicated in vascular malformations can be grouped into a limited number of functional pathways (Fig. 3). These include the major kinase receptor signalling pathways, i.e. TGF-β, VEGF, ANGPT and Ephrins, and their intracellular effectors that commonly relay to RAS signalling (Fig. 3). They mostly seem to disturb vascular endothelial cells, which is not surprising, as ECs are considered to be the basis of angiogenic vessel formation (114). Glomulin, which is expected to have its primary role in VSMCs, is an exception. Overall, it seems probable that other mutations, which cause vascular anomalies but remain to be discovered, relate to these genes and pathways that are the major controllers of vascular development.

The next step towards understanding the etiopathogenic mechanisms of vascular malformations will largely depend on *in vivo* analysis of murine models. In such mice, the partial, complete and induced loss of the targeted gene can be evaluated with a control of the genetic background. Some will hopefully sufficiently mimic the human disorders, enabling experimental testing of novel therapeutic approaches. As the genes that are mutated in vascular malformations are often prime targets or candidates for (anti)angiogenic therapy for common disorders, the human (and murine) phenotypes that are caused by mutations in these genes, also pinpoint alterations that may be induced by their use as therapy or therapeutic target.

Acknowledgements

We are grateful to all family members who participated in the studies. These studies were supported, in part, by the Fonds Spéciaux de Recherche-Université Catholique de Louvain, the Belgian Federal Service for Scientific, Technical and Cultural Affairs, the Fonds National de la Recherche Scientifique (F.N.R.S) and the Actions de Recherche Concertées-Communauté Française de Belgique. P.B. was supported by a fellowship from F.R.I.A. (Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture) and M.V. is a 'chercheur qualifié du F.N.R.S.'. We thank Dr Laurence M. Boon for the clinical pictures and Ms Liliana Niculescu for secretarial help.

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