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Disorders of the Venous System

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9.1 INTRODUCTION

The vasculature is the first organ system to develop during embryogenesis, delivering nutrients, growth factors, and oxygen to tissues and removing wastes. It is made up of four major types of vessels: arteries, capillaries, veins, and lymphatic vessels, all of which have a single layer of endothelial cells (ECs) forming the innermost layer. In blood vessels, the endothelial tubes are supported by a layer of vascular smooth muscle cells (vSMCs) and/or pericytes (together called mural cells) of variable thickness. A basement membrane separates the endothelial and vSMC layers, with an extracellular matrix (ECM) of fibrous and elastic proteins and carbohydrate polymers forming the outermost sheath. Lymphatic vessels lack the outer ECM sheath, and continuous vSMC and basement membrane layers are only present in certain large vessels.

The mechanisms that give rise to the mature vascular network are complex. These developmental processes, termed vasculogenesis, angiogenesis, and lymphangiogenesis, involve a plethora of signaling molecules and their receptors, acting at different time points: the angiopoietins (ANGPTs), ephrins, fibroblast growth factors, platelet-derived growth factors (PDGFs), transforming growth factors (TGFs), bone morphogenetic proteins (BMPs), vascular endothelial growth factors (VEGFs), and many others.

Vascular anomalies are localized defects that occur during vascular development [1–5]. They are subdivided into proliferative vascular tumors (mainly infantile hemangioma) and more slow-growing vascular malformations. The latter are subcategorized according to the type(s) of vessel(s) altered [5–7] into capillary, venous, arteriovenous, lymphatic, and combined malformations.

9.2 THE VENOUS SYSTEM

Veins collect CO_2-rich blood from the capillary network and contain about 75%–80% of the total volume of blood in the body. They have larger lumens than arteries, with thinner, less muscular walls. Venous flow is passive, essentially mediated by physical movements of the body and the aspirating effect exerted by the heart. The presence of valves ensures correct orientation of blood flow. Some veins, especially in the legs, are exposed to high pressure due to gravitational forces and subject to frequent distortions (i.e., varicose veins).

During vascular development, a primary capillary plexus is formed early on by in situ differentiation of hemangioblasts, originating from the mesoderm, into ECs that assemble to form endothelial tubes (vasculogenesis). In response to the hemodynamic forces of local blood flow, this plexus is progressively remodeled during angiogenesis to form the three types of blood vessels. At the cellular level, the "venous" phenotype of the ECs is determined very early, even before the vessel is committed to being a vein [8]. In the trunk of the embryo, the venous system is first present as symmetrical bilateral vessels. The veins on the left then regress

Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics. https://doi.org/10.1016/B978-0-12-812532-8.00009-4

progressively while those on the right evolve into the superior and inferior vena cava. Defective remodeling may lead to retention of embryonic veins, such as persistence of superior vena cava [9,10], the right umbilical vein [11], or saphenous vein [12].

9.3 DISORDERS OF THE VENOUS SYSTEM

Venous malformations (VMs) are the most frequent malformations seen in interdisciplinary vascular anomaly centers. Several inherited or sporadically occurring subtypes exist. They are discussed here. Varicose veins and varicosities are discussed with the disorders affecting lymphatic vessels (Chapter 8). Malformations with a capillary or arteriovenous component are described in Chapter 10. Diagnostics and management of acute and chronic venous diseases have been extensively reviewed elsewhere [13].

9.3.1 Glomuvenous Malformation

The most frequently inherited VM, glomuvenous malformation (GVM, OMIM 138000) accounts for roughly 5% of VMs [14] and is often improperly called "glomangioma" or "(multiple) glomus tumor" although it is not a tumor and never becomes malignant. GVM is characterized by the presence of a variable number of "glomus cells" around distended venous channels [15,16]. Similar rounded cells are present in paraganglioma (OMIM 115310, 168000, 601650 and 605373) and (subungual) solitary glomus tumor. All are sometimes referred to as "glomus tumor." However, paraganglioma are tumors of the parasympathetic ganglia, most commonly located in the head and neck area, caused by mutations in subunits of the succinate dehydrogenase enzyme complex [17]. Solitary glomus tumors are usually subungual painful lesions histologically characterized by the presence of glomus cells, but without important vascular component [18].

GVM segregates as an autosomal dominant disease, with incomplete penetrance and variable expressivity [19]. Frequently, a single individual in a family is more severely affected and is brought to the medical attention, whereas most of the other affected individuals have small lesions and never consider treatment. As penetrance is below 100% and many individuals have tiny asymptomatic lesions, it is likely that the inherited nature of patient's lesions is overlooked.

Clinical distinction of GVM from VMs and mucocutaneous venous malformations (VMCMs) can be difficult in patients with few small lesions and without familial history of the disease [20]. Yet, a series of clinical criteria and a few biomarkers have been defined [21]. GVM is usually raised, nodular, present at birth, and slowly expands during childhood (Fig. 9.1). It is often multifocal and hyperkeratotic. Its color varies from pink to purplish dark blue [20]. Sometimes, it is flat and purple in color, especially in the newborn. This plaque-like GVM usually darkens with time [22]. GVMs are mainly located on the extremities, involve skin and subcutis, and are often painful on palpation. It cannot be completely emptied by compression. Contrary to VM, GVM is rarely encountered in mucosae [20]. Patients with GVM have normal mental and physical development, normal blood cell counts, coagulation, liver and renal function, electrolytes, plasma proteins, and urinalysis. They do not have intestinal hemorrhage either, what distinguishes them from patients suffering from blue rubber bleb nevus (BRBN) syndrome (MIM 112200) [23]. D-dimer level is normal in GVM [24]. In two patients, thoracic plaque-type GVM was associated with pleural effusion [25].

The alterations in the mutated gene seem to cause specifically abnormal cutaneous vascular development. Currently,

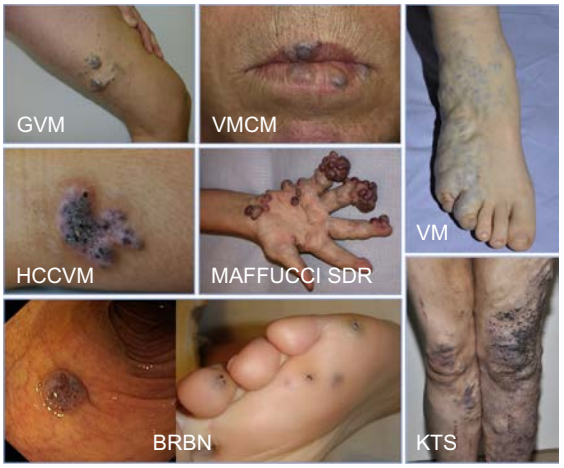

Figure 9.1 Venous anomalies: GVM on leg; cutaneous and mucosal venous malformations (VMCMs) on lips; sporadic VM on foot; HCCVM on arm; multiple spindle cell hemangioendotheliomas and enchondromas on hand of patient with Maffucci syndrome; intestinal and cutaneous VMs of patient with BRBN syndrome; CLVM on legs of patient with KTS (left side more affected). *BRBN*, blue rubber bleb nevus; *CLVM*, capillaro–lymphatico–venous malformation; *GVM*, glomuvenous malformation; *HCCVM*, hyperkeratotic cutaneous capillary–venous malformation; *KTS*, Klippel–Trenaunay syndrome; *VM*, venous malformation.

the best therapy is surgical resection of the entire GVM, which is curative, and is facilitated by the noninfiltration of GVM in underlying tissues [20]. Alternatively, sclerotherapy can be used for a GVM that is soft on palpation.

GVMs are caused by loss-of-function mutations in a gene named glomulin (*GLMN*), the exact function of which is still unknown [26]. It encodes a protein of 594 amino acids. Forty distinct mutations have been reported in 162 families [19,26,27]. Sixteen of these mutations were detected in several families, accounting for 85% of the pedigrees with a *GLMN* mutation. The most frequent mutation is *c.157*_161del (aka *157delAAGAA*), present in 72 kindreds (44.4%). Screening first for these shared mutations is therefore recommended to accelerate genetic diagnosis.

There is no correlation between the position of a mutation in the *GLMN* gene and characteristics of the disorder, such as the number of glomus cells in the lesion, the extent of the lesion, or the number of lesions. Yet, for the same mutation, the expressivity is variable from patient to patient [27]. Several observations suggest a paradominant mode of inheritance for GVM, instead of a simple autosomal dominant transmission [26]. GVM has an age-dependant variation in penetrance, which reaches its maximum (92.7%) by 20 years of age. Unaffected mutation carriers have been identified in GVM families and affected individuals develop new lesions by time, albeit they stay small [19,20]. Variable penetrance and multifocality can be explained by the occurrence of double-hit mutations in *GLMN* [26,28]. Out of 17 somatic second hits discovered, 12 were acquired uniparental isodisomies of chromosome *1p*, rendering the inherited *GLMN* mutation homozygote in the cells of the affected tissues [28].

In GVMs, the mural glomus cells are round or polygonal, instead of elongated like the normal vSMCs [15,29]. Glomus cells stain positively for smooth muscle α-actin and vimentin, whereas they are negative for desmin, von Willebrand factor, and S-100 neuronal marker [29]. During murine development, *GLMN* RNA was first detected at E10.5 dpc in cardiac outflow tracts and later, strong expression was seen in vSMCs [30]. In contrast, glomus cells do not express late markers of vSMC differentiation, *GLMN*, and smoothelin-b, whereas two earlier markers, smooth muscle myosin heavy chain and h-caldesmon, were detected [28,31]. Thus, it seems that glomus cells have deviated in their differentiation process due to lack of *GLMN* expression.

GLMN has no known motif or conserved domain and no paralogue exists. It may act synergistically to control both TGF beta (TGFβ) and hepatocyte growth factor (HGF) pathways (Fig. 9.2), which are crucial for vSMC differentiation [32,33]. In vitro, *GLMN* interacts with FKBP12 and might impede its binding and inhibition of TGFβ receptor signaling [34,35]. FKBP12 also inhibits downstream signaling of mTOR [36,37]. Complete loss of *GLMN* in GVMs could thus result in inhibition of TGFβ and mTOR signaling.

GLMN also interacts with the intracellular part of the HGF receptor c-Met. Upon HGF binding, *GLMN* is tyrosine phosphorylated, released, and induces phosphorylation of p70S6-kinase [35], thereby controlling protein synthesis [36,37]. As HGF triggers downstream signaling mediating vascular SMC migration [33], lack of *GLMN* in GVMs likely alters this signaling.

GLMN was also reported to interact with Cul7, with which it forms an E3-ubiquitin–ligase–SCF-like complex [38]. *GLMN*-deficient mice appeared as fleshy masses at E7.5 or showed severe developmental defects and died between E10.5 and E12.5 [39,40]. The defects included decreased growth, delayed neural tube closure, incomplete turning, pericardial effusion, hemorrhages, and defective/absent yolk sac vasculature. Lack of *GLMN* resulted in decreased levels of Fbw7 (a substrate of the E3 ubiquitin ligase complex), inducing higher levels of cyclin E and c-Myc. This increase was also seen in GVM lesions. The crystal structure of GLMN–CRL-like complex revealed that GLMN adopts an HEAT-like structure (mainly helices, resembling a slightly bent finger), binds RBX1-containing CRLs via its C-terminal tail, and blocks the access of the E2-ubiquitin ligase to the substrate [41]. GLMN might also have some protective effects against macrophage cell death [39].

9.3.2 Inherited Venous Malformation

A second, less common inherited VM is autosomal dominant VMCM (OMIM 600195), which accounts for 1%–2% of VMs [14]. It is characterized by multiple small, compressible blue lesions on the skin and mucosa, commonly located in the cervicofacial region and the limbs, and less often on the trunk (Fig. 9.1). Histologically, distended venous channels are lined by a single EC layer surrounded by sparse, irregularly distributed vSMCs [42]. VMCM is caused by mutations in the gene *TEK* [42–44], encoding TIE2, the EC tyrosine kinase receptor for the ANGPTs: ANGPT 1, 2, and 4 (the last corresponding to Angpt3 in mice) [45–47]. ANGPT–TIE2 signaling through pathways including PI3K/AKT and MAPK [48,49] is critical to EC survival and function (Fig. 9.2) [46,50,51].

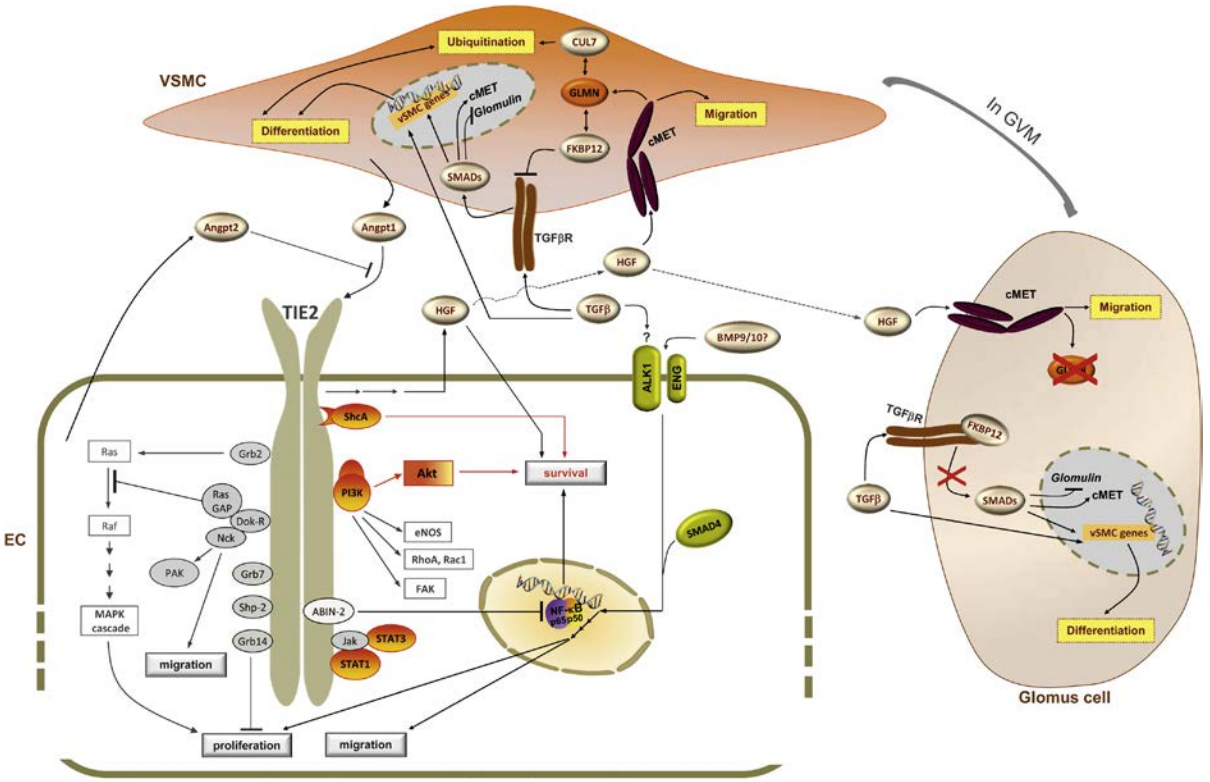

Figure 9.2 Signaling pathways involving glomulin (GLMN) and TIE-2 in normal and pathological endothelial and vSMCs. Lack of GLMN signaling results in aberrant differentiation toward glomus cells. Pathways chronically activated by TIE-2 mutations highlighted in orange; with the exception of STAT1, all are also activated by wild-type receptor, upon stimulation. *vSMCs*, vascular smooth muscle cells.

Eight missense *TEK*/TIE2 mutations have been identified in VMCM. All are located in the intracellular region of the TIE2 receptor and cause varying degrees of ligand-independent phosphorylation when overexpressed in vitro [42–44]. The most commonly identified change, R849W (10/17 families reported), has been demonstrated to be accompanied by somatic second-hit mutations in the same gene, in affected tissues from two unrelated patients [52,53]. As in the case of GVM, this likely explains why the germline mutations do not cause generalized vascular abnormalities despite being ubiquitous, instead resulting in highly focal malformations only where their effects are compounded by somatic events.

9.3.3 Sporadic Unifocal Venous Malformation

Over 95% of VMs are sporadic, that is, they occur in individuals with no family history of the disorder. Histologically similar to VMCMs, sporadic VMs are typically extensive, single lesions that affect the skin and mucosa (Fig. 9.1), but can also infiltrate underlying tissues in various organ systems [14]. They can cause significant morbidity due to their size, localization, or expansion, requiring treatment at centers specializing in vascular anomalies [14]. Localized intravascular coagulopathy, characterized by elevated levels of D-dimers, is observed in about 42% of patients and correlates with the size and depth of lesions and the presence of phleboliths [24,54]. Current therapies are compression, sclerotherapy, and surgical removal; however, large or inaccessible lesions can be problematic, with incomplete resection resulting in regrowth [21].

Somatic mutations in *TEK*/TIE2 cause >60% of sporadic unifocal VM [52,53,55,56]. As in the case of VMCM, these mutations cause ligand-independent receptor phosphorylation in vitro. The most frequent change is L914F, identified in >73% of TIE2

mutation-positive samples [52,55,56]. L914F has never been identified as a germline change in VMCM families, suggesting it is lethal when ubiquitous. The sporadic VM-causative somatic changes also include a series of double mutations that occur in *cis* (i.e., on the same allele of *TEK*) [52,56]. Most often, they consist of a substitution of Y897 (to residues including F, C, and S), accompanied by a second substitution at another residue (e.g., R915(C/L), R918(C)). They include premature truncations of the C-terminal tail (e.g., R1099*, G1115*) that cause receptor phosphorylation [56], likely by abolishing the inhibitory conformation of this domain [57,58].

About half of TIE2 mutation-negative sporadic unifocal VMs (i.e., 20% overall) are caused by somatic, activating mutations in PIK3CA, the gene encoding the Class I p110α catalytic subunit of PI3K. Three mutations together account for >92% of PIK3CA mutation-positive cases: E542K (30%), E545K (15%), and H1047R (48%). These "hotspot" mutations are also frequent in (endometrial, breast, ovarian, colorectal, and other) cancers [59–61], PIK3CA-related overgrowth syndromes ("PROS"); [62,63], several of which have vascular components (reviewed by Blei et al. [64]) and lymphatic malformation [65–67].

9.3.4 Multifocal Sporadic Forms of Venous Malformation

Multifocal forms of sporadic VM are extremely rare and fall into two categories: BRBN syndrome and multifocal sporadic VM (MSVM).

BRBN syndrome is a severe congenital disorder characterized by the presence of one extensive, "dominant" lesion (that can be cutaneous or subcutaneous) accompanied by dozens to hundreds of scattered cutaneous VMs. Cutaneous lesions tend to be small, hyperkeratotic, and often palmoplantar in localization and can increase in size and number with age. Gastrointestinal VMs, diagnosed by endoscopy, colonoscopy, or MRI, are pathognomonic and can cause chronic bleeding and anemia. Like other VM forms, BRBN is associated with high D-dimer levels [53]. MSVM is a milder phenotype, characterized by cutaneous or subcutaneous VMs, typically up to a dozen rather than hundreds. These VMs do not tend to be hyperkeratotic or enriched on the palms and soles, and gastrointestinal VMs are very rare [53].

Both BRBN and MSVM are caused by postzygotic mutations in the intracellular exons of *TEK*/TIE2 [53]. Unlike unifocal VM, mutations are almost always double (*cis*) mutations (>83%). The combination T1105N-T1106P is recurrent in BRBN (57% of mutation-positive individuals); the remainder are largely Y897-containing combinations. One combination: Y897C-R915C, seems to be specific to, and recurrent in, MSVM. Thus, the clinical distinction between these entities is supported by differences in mutation identity. In both disorders, the same mutations are observed in multiple VMs from the same individual, suggesting they originate from a common cellular progenitor [53].

9.3.5 Molecular Basis of VM Pathogenesis

Ligand/growth factor–independent activation of PI3K/AKT signaling seems to be the principal pathogenic molecular mechanism, shared by all VM-causative *TEK*/TIE2 and PIK3CA mutations. Human umbilical vein endothelial cells (HUVECs) overexpressing mutant forms of TIE2 or PIK3CA show increased phosphorylation of AKT as compared to their wild-type protein-expressing counterparts [55,68,69]; certain TIE2 mutant forms (but not all) also cause increased ERK1/2 and STAT1 phosphorylation [52,68–71]. Activated AKT phosphorylates and inhibits the transcription factor FOXO1, causing dysregulation of its targets including PDGFB, a major EC-secreted vSMC attractant [69]. This likely contributes to the sparse, misaligned, irregularly distributed vSMCs observed in VMs. AKT activation has also been linked to improved survival of mutant ECs [69,72], perhaps explaining why they persist despite the lack of appropriate support and cross talk with surrounding vSMCs.

TEK/TIE2 and PIK3CA mutations change the morphology of HUVECs from organized, cobblestone monolayers to elongated and overlapping, with long cell extensions [55,68]. Correspondingly, ultrastructural images of patient VMs show the presence of elongated ECs with a "ragged" appearance due to the presence of extensions [68]. Abnormal endothelial morphology may be caused by ECM defects: mutant ECs show a lack of ECM, but not intracellular, fibronectin in vitro [55,68]. VMs surgically excised from patients also show ECM irregularities, with sparse, disordered collagen fibrils and abnormal, multilayered basal membranes surrounding the ectatic lumens [68]. Degradation of ECM components may be accomplished by metalloproteases of the ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) family, several of which are upregulated in mutant HUVECs [69]. The proteases

PLAT (tissue plasminogen activator) and PLAU (urokinase plasminogen activator) are upregulated in mutant HUVECs, while their inhibitor PAI-1 (plasminogen activator inhibitor 1) is downregulated, suggesting activation of plasminogen-plasmin conversion [55,68,69]. The proteases of this system, as well as plasmin itself, are capable of degrading ECM components including fibronectin. Increased plasminogen-plasmin processing may also lead to increased coagulation and subsequent fibrinolysis, contributing to the high D-dimer levels observed in VM. Significantly, these pathological in vitro phenotypes are normalized by PI3K-AKT pathway inhibition, downstream of both TIE2 and PIK3CA mutations [55,69,73].

The molecular causes of VM formation seem to be intrinsic to ECs: TIE2 (L914F or Y897F-R915L)-mutant HUVECs form large, blood-filled venous channels with occasional host-derived mural cells when implanted in immunodeficient mice [68,73]. Activated (H1047R) PIK3CA causes VMs (but not LMs or other tumors, surprisingly) in genetically engineered mice, even when its expression is not restricted to the VM effectors, that is, ECs [74,75]. The mouse xenograft model of TIE2-mediated VM has been used to demonstrate the capacity of rapamycin (sirolimus; an mTOR inhibitor that acts on the PI3K/AKT pathway) to control VM growth [73]. A pilot study showed that it ameliorated symptoms such as pain, bleeding, oozing, and reduced mobility in six severely affected patients with VM or BRBN, caused by TIE2 or PIK3CA mutations [73]. It is therefore in clinical trials as the first pharmacological therapy for VM and BRBN (EudraCT Number: 2015-001703-32).

9.3.6 Hyperkeratotic Cutaneous Capillarovenous Malformation

Multiple cutaneous venous malformations and capillarovenous malformations can occur in association with cerebral cavernous malformation (see Chapter 11). Characterized by distended capillary-like channels without any intervening brain parenchyma, CCM can be asymptomatic, but often causes headaches, focal neurological defects, seizures, or hemorrhage [76]. Loss-of-function mutations in one of three genes: *CCM1*, *CCM2*, and *CCM3*, are responsible for disease [77–80]. A systematic analysis of the cutaneous lesions associated with familial forms of the anomaly in a large cohort showed that hyperkeratotic cutaneous capillary–venous malformations (HCCVMs) are exclusively associated with mutations in *CCM1* (KRIT1) [81]. Nodular VMs are associated with mutations in *CCM3* (PDCD10) and sometimes *CCM1*, albeit less frequently [81]. Mutations in *CCM2* (malcavernin/MGC4607) are rarely if ever associated with cutaneous VMs. HCCVMs are plaque-like, irregular, black or crimson lesions, with the ECs of the venous channels surrounded by two or more layers of smooth muscle cells (Fig. 9.1). Nodular VMs are more similar to MSVM [82]. Histologically, they are similar to the cerebral lesions of CCM and consist of several thin-walled channels packed together and surrounded by a fibrous layer containing vSMCs [81].

The *CCM1*, *CCM2*, and *CCM3* proteins form an adaptor complex that regulates endothelial barrier function, inhibiting RhoA-ROCK activity downstream of cell–cell adherens junctions. Increased RhoA–ROCK activity due to CCM loss causes increased stress fiber formation and disruption of adherens junctions, impairing endothelial integrity. In addition, a KLF2/4 transcription factor-dependent increase in BMP6-SMAD signaling is observed, which contributes to endothelial to mesenchymal transition [83–86]: a key pathological feature of CCM [87].

9.3.7 Verrucous Venous Malformation

Sporadic, cutaneous verrucous venous malformation (VVM, also known as "verrucous hemangioma") is very similar to HCCVM in appearance: typically raised, reddish, and hyperkeratotic. Single or multiple lesions may be observed and can extend into the subcutis. Histologically, they consist of clustered venous channels lined with faintly or in a spotty-like manner GLUT1-positive endothelium; they are not known to be associated with cerebral lesions. A total 60% of VVM is caused by a single, somatic mutation: I441M, in the *MAP3K3* gene, which encodes the MAP kinase kinase kinase (MEKK3) [88]. MAP3K3 acts upstream of MEK5-ERK5 in the MAPK cascade, and the VVM-associated mutation is hypothesized to have a gain-of-function or neomorphic effect, although this remains to be demonstrated.

MAP3K3 participates in ANGPT1–TIE2 signaling, perhaps explaining why activating mutations in either cause venous phenotypes [89]. Whereas PI3K/AKT signaling seems to predominate in VMs (with ERK1/2 activation being more mutation-specific and variable), MAP3K3 would primarily affect the MAPK signaling, which may account for phenotypic and clinical differences between the disorders. Importantly,

MAP3K3–MEK5–ERK5 activation is responsible for the pathogenic effects of CCM mutations [83,84,86,90], accounting for the similarity of the cutaneous lesions mediated by CCM1/2 loss and MAP3K3 gain. MAP3K3 therefore represents an important node between the VM-associated TIE2–PI3K–AKT–FOXO1 axis and the CCM-associated CCM1/2/3–ERK5–KLF2/4 axis.

9.3.8 Other VM-Associated Syndromes

VMs are observed, in combination with multiple other clinical features, in certain sporadically occurring syndromes. These include Proteus syndrome (described in Chapter 8), characterized by progressive segmental overgrowth, particularly skeletal and dermatologic, with a variety of vascular anomalies, and caused by a mosaic, activating E17K hotspot mutation in AKT1 [91]. Several PIK3CA-related overgrowth syndromes (PROS), caused by the somatic hotspot E542K, E545K, and H1047R PIK3CA mutations, include VMs among their clinical features: CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and skeletal anomalies), FAVA (fibro-adipose vascular anomaly), and capillaro–lymphatico–venous malformation without (CLVM) or with (Klippel–Trenaunay syndrome; KTS) hypertrophy of an extremity [66,92] (later volumes will address such and such topic more fully).

9.3.9 Conclusion

The identification of the genetic causes of the majority of VMs, and the functional dissection of their pathological molecular and cellular effects have opened the door to improved diagnostics, clinical management, and for the first time, the use of targeted pharmacological therapies. The availability of animal models of glomuvenous and VMs is in addition critical to our understanding of how these disorders develop, and for preclinical testing of potential therapies.

ACKNOWLEDGMENTS

The authors' studies are partially funded by the Belgian Science Policy Office Interuniversity Attraction Poles (BELSPO-IAP) program through the project IAP P7/43-BeMGI; Concerted Research Actions (A.R.C.)—Convention No 16/21-073 of the Belgian French Community Ministry; the F.R.S.-FNRS (Fonds de la Recherche Scientifique) grants T.0026.14 and T.0146.16; WELBIO grant number: WELBIO-CR-2015A, and la Communauté française de Wallonie-Bruxelles et de la Lotterie nationale, Belgium. NL is a "Chercheur Qualifié" of the Fonds de la Recherche Scientifique-FNRS. PB is a Genomics Platform manager of UCL.

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