Genetics of vascular malformation and therapeutic implications

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Purpose of review
Vascular malformations (VaMs) are a consequence of disrupted morphogenesis that may involve arterial, capillary, venous, or lymphatic endothelium alone or in a combination. VaMs can have serious health impacts, leading to life-threatening conditions sometimes. Genetic mutations affecting proliferation, migration, adhesion, differentiation, and survival of endothelial cells, as well as integrity of extracellular matrix are believed to be the pathogenesis of these disorders. Here, we present an updated review of genetic mutations and potential therapeutic targets for VaMs.

Recent findings
Increased number of genetic mutations have been discovered in vascular anomalies via targeted deep sequencing. When a genetic defect is identified, it often presents in only a small percentage of cells within the malformation. In addition, mutations within the same gene may result in different clinical phenotypes. Management of VaMs can be challenging depending on the severity and functional impairment associated. There are no standard treatment algorithms available to date for VaMs, therefore the disorder has significant unmet clinical needs. Currently, the focus of therapeutic development is to target constitutively activated intracellular signaling pathways resulted from genetic mutations.

Summary
Knowledge about the genetic mutations and altered signaling pathways related to VaMs have improved our understanding about the pathogenesis of vascular anomalies and provided insights to the development of new targeted therapies.

Keywords
genetic mutations, signaling pathways, targeted therapy, vascular malformations

INTRODUCTION

Vascular malformations (VaMs) are a consequence of defective morphogenesis involving arterial, capillary, venous, or lymphatic endothelium alone or in combination [1]. These are lifelong conditions affecting approximately 1.5% of the general population [2]. VaMs often result in disfigurement, functional impairment, and recurrent infections. They can have a profound impact on quality of life and may increase lifetime risk of morbidity and mortality. VaMs can also arise with extracutaneous abnormalities as a part of genetic syndromes [1].

Genetic mutations affecting proliferation, migration, adhesion, differentiation, and survival of endothelial cells, as well as the integrity of extracellular matrix are believed to contribute to the pathogenesis of VaMs. These mutations are often identified via targeted deep sequencing in a percentage of cells within the malformation, and majority of VaMs are caused by monogenetic mutations. It is not uncommon that VaMs with different clinical phenotypes result from mutations within the same gene [3,4▪▪]. As a mosaic disorder, VaMs can be attributed to somatic mutation alone or loss of second allele in the presence of a preexisting germline mutation [4▪▪,5,6]. This explains why such patients can present with multifocal lesions evolving over time [7].

Here, we present an updated comprehensive review about the genetic pathogenesis (Table 1), current managements and potential targeted

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KEY POINTS

- VaMs are a consequence of defective morphogenesis involving arterial, capillary, venous, or lymphatic endothelium alone or in a combination during embryonic development. Pathogenic mutations of VaM result in altered proliferation, migration, adhesion, differentiation of endothelial cells, as well as integrity of extracellular matrix.
- VaMs can be attributed to either somatic mutation alone or somatic mutation of the second allele in the presence of an inherited germline mutation of the other allele.
- Majority of VaMs are monogenic disorders, although VaMs with mutations in the same gene may have different clinical phenotypes.
- Recent discovery of specific pathogenic mutations associated with VaMs has revealed possible targets for therapeutic interventions. These therapies are designed against constitutively activated proangiogenic pathways in VaMs.

therapies for VaMs (Table 2). We have only included VaMs based on 2018 classification by the International Society for the Study of Vascular Anomalies (https://www.issva.org/classification), excluding the entities provisionally unclassified. Mutations with unknown functional effect or unknown clinical significance are included but not discussed.

GENETICS OF VASCULAR MALFORMATION

Mutations associated with VaMs often occur in genes encoding proteins that are part of the RAS/MEK/ERK (also known as MAPK/ERK) and/or PI3 kinase/mTOR signaling pathways (Fig. 1). Such genetic changes lead directly to altered endothelial cell proliferation, differentiation, and survival [3]. Un-controlled angiogenesis because of increased endothelial cell proliferation results in dysmorphogenesis of vascular network and vascular malformation. Even though many VaMs share commonly signaling pathways, their clinical phenotypes clearly vary. Although genotype–phenotype correlation remains an area of active investigation currently, studies have suggested that the origins of cells affected during embryogenesis may play a role in determining the final clinical phenotype of VaMs [3]. Recent discovery of many inhibitors targeting key players of these signaling pathways have offered unprecedented therapeutic opportunities for vascular anomalies.

G-protein subunit α Q, G-protein subunit α 11 in capillary malformation

Mutation of G-protein subunit α Q (*GNAQ*) presents in endothelial cells of nonsyndromic and syndromic capillary malformations. For example both non-complicated cutaneous capillary malformation and capillary malformation associated with Sturge-Weber syndrome were found to have same GNAQ mutations [4▪▪,8,9–13]. The direct downstream effect of the *GNAQ* mutation is to induce activation of the MEK/ERK pathway, therefore promoting cell proliferation, migration, angiogenesis and malformation [9,11,13]. In addition, mutations within G-protein subunit α 11 have also been discovered among individuals with diffuse capillary malformations. These mutations are likely to activate MEK/ERK pathway as well, but more frequently associated with overgrowth syndrome [3,7,14].

PIK3CA, AKT1/2/3, PTEN in lymphatic malformation and Proteus syndrome

Recent studies have demonstrated that more than 70% of lymphatic malformation are associated with *PIK3CA* mutations. Gain of function mutations in *PIK3CA* lead to increased phosphorylation of AKT (AKT1, AKT2, or AKT3), therefore subsequent activation of the AKT/mTOR pathway (Fig. 1) as well as formation of disorganized vascular network because of uncontrolled cell proliferation [4▪▪,7,15]. Overactivation of *PIK3CA* has also been shown to cause loss of fibronectin in extracellular matrix, which promote increased cell migration [7]. Aside from localized lymphatic malformation, *PIK3CA* mutation was also discovered in patients with CLOVES syndrome, CLAPO syndrome, capillary malformation with megalencephaly, Klippel-Trenaunay syndrome, or mucocutaneous venous malformations [3,7,16,17]. Similarly, mutations in *AKT1, AKT2, and AKT3* have also been discovered in Proteus syndrome, venous malformations, and capillary malformation with megalencephaly patients, respectively [17–19]. PTEN exerts an inhibitory effect on AKT via dephosphorylation (Fig. 1). Mutation of *PTEN* therefore results in loss of AKT inhibition and increased activation of AKT/mTOR pathway. Bannayan-Riley-Ruvalcaba syndrome, a genetic condition with macrocephaly and multiple hamartomas, is known to associate with *PTEN* mutations [15,18,20].

FLT-4 (VEGFR3), VEGFC, CCBE1, ADAMTS3 in lymphatic anomalies

VEGFR3, encoded by *FLT4,* and its ligand VEGFC play a major role in lymphangiogenesis during the embryonic development. Loss of function (LoF)

Table 1. Vascular malformations and associated genetic mutations

Vascular malformation	Gene	Mutation type	Inheritance	References
CM				
Nevus simplex/salmon patch	Unknown	Unknown	Unknown	
Port-wine stain				
Nonsyndromic CM	*GNAQ*	GoF	Somatic	[3,4▪▪,7]
Sturge-Weber syndrome	*GNAQ*	GoF	Somatic	[3,4▪▪,7,10]
CM with overgrowth	*GNA11*	GoF	Somatic	[3,7,14,66]
Reticulate CM				
CM of MIC	*STAMBP*	LoF	Germline. Unknown Mendelian pattern of inheritance	[67,68]
CM of MAC-polymicrogyria	*1)PIK3CA* *2)AKT3* *3)PIK3R2*	GoF	1)Somatic and somatic mosaicism 2)Germline and mosaic mutations. Unknown Mendelian pattern of inheritance 3)Germline. Unknown Mendelian pattern of inheritance	[3,19,55,59]
CMTC	*ARL6IP6*. Novel candidate gene	LoF	AD or mosaic[a]	[69–71]
Lymphatic Anomaly				
Micro or Macrocystic LM	*PIK3CA*	GoF	Somatic	[3,7,62]
Generalized lymphatic anomaly	NRAS	GoF	Somatic	[72]
Kaposiform lymphangiomatosis	1)*NRAS* 2)*BAD* 3)*TSC1*	1)GoF 2–3)LoF[a]	1)Somatic 2–3)Somatic	[73,74]
LM in Gorham-Stout disease	1)*TNFRSF11A* 2)*TREM2* 3)*PTEN*	Unknown	1–2)Somatic[a] 3)Germline. Unknown Mendelian pattern of inheritance	[75]
Channel type LM	*EPHB4*	LoF	AD	[76]
Acquired progressive lymphatic anomaly	Unknown	Unknown	Unknown	————
Primary lymphedema				
1)Nonne-Milroy syndrome or Milroy's disease and 2)Milroy –like lymphoedema	1)*FLT4*(VEGFR3) 2)*VEGFC*	LoF	1)AD, AR or *de novo* 2)AD	[22,77,78]
Primary hereditary lymphedema or Meige disease	1)*GJC2* 2)*MET* 3)*HGF*	1)GoF[a] 2–3)LoF	1)AD[a] 2–3)Unknown	[77,79]
Lymphedema-distichiasis	*FOXC2*	LoF	AD	[77,80]
Hypotrichosis-lymphedema-telangiectasia	*SOX18*	LoF	AD or AR or *de novo*	[24,81,82]
Primary lymphedema with myelodysplasia or Emberger syndrome	*GATA2*	LoF	AD	[77,79]
Primary generalized lymphatic anomaly or dysplasia (Hennekam lymphangiectasia-lymphedema syndrome)	1)*CCBE1* 2)*ADAMTS3* 3)*FAT4* 4)*PIEZO1* 5)*EPHB4*	1–2)LoF 3)LoF[a] 4–5)Unknown	1–4)AR 5)AD	[21,22,77,83]

Table 1 (*Continued*)

Vascular malformation	Gene	Mutation type	Inheritance	References
Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome	*KIF11*	LoF[a]	AD	[77,84]
Lymphedema-choanal atresia	*PTPN14*	LoF	AR	[81,85]
VM				
- *Common VM*	1)*TEK (TIE2)* 2)*PIK3CA* 3)*GANQ* 4)*IRS2* 5)*MAP2K1/MEK1* 6)*AKT2* 7)*AKT3* 8)*NF1*	1–7) GoF 8)LoF	Somatic	[4▪▪,12]
- *Familial VMCM*	*TEK (TIE2)*	GoF	AD	[3,4▪▪]
- *BRBNS*	*TEK (TIE2)*	GoF	Somatic	[4▪▪]
- *GVM*	*Glomulin*	LoF	AD and somatic (second hit)	[3,4▪▪]
- *CCM*	1)*CCM1/KRIT1* 2)*CCM2/malcavernin* 3)*CCM3/PDCD10*	LoF	AD or sporadic	[3,4▪▪]
- *FIVM*	*ELMO2*	LoF	AR	[86]
- *VVM*	*MAP3K3/MEKK3*	GoF	Somatic	[3,7]
-*MVM*	*TEK (TIE2)*	GoF	Mosaic and sporadic somatic	[7]
AVM or AVF				
- *Sporadic and non syndromic cerebrospinal AVM*	1)*MAP2K1/MEK1* 2)*SMAD9* 3)*KRAS* 4)*BRAF* 5)*NARFL* 6)*PITPNM3* 7)*SARS* 8)*LEMD3* 9)*ALK1/ACVRL1* 10)*CLDN14*	1)GoF 2)LoF 6–9)LoF 10)GoF[a]	1)Somatic 2) Germline mutationUnknown Mendelian pattern of inheritance 6–9)De novo 10)Germline. Unknown Mendelian pattern of inheritance	[3,43,48,87–89]
- *HHT*	1)*ENG* 2)*ALK1/ACVRL1* 3)*BMP9/GDF2* 4)*SMAD4/MADH4* 5)*BMPR2*	1–5) LoF	1–2)AD and mosaicism 3–4)AD 5–6)Germline mutation. Unknown Mendelian pattern of inheritance	[3–7,88]
- *CM of CM-AVM 1 and 2*	1)*RASA1* 2)*EPHB4*	LoF	AD or somatic	[3,4▪▪,7]
- *Extracranial AVM*	*MAP2K1/MEK1*	GoF	Somatic	[3,43,44]
- *pulmonary AVM*	*NARFL*	LoF	Germline. Unknown Mendelian pattern of inheritance	[46]
Other vascular malformation associated with other anomalies				
Klippel-Trenaunay syndrome	*PIK3CA*	GoF	Somatic and somatic mosaicism	[4▪▪,62]
Parkes Weber syndrome	*RASA1*	LoF	AD and somatic	[4▪▪,7]
Limb CM + congenital nonprogressive limb overgrowth	*GNA11*	GoF	Somatic	[14,66]
Maffucci syndrome	*Isocitrate dehydrogenase 1 and/or 2*	Unknown	Somatic and mosaic	[3,20]

Table 1 (*Continued*)

Vascular malformation	Gene	Mutation type	Inheritance	References
CLOVES syndrome	*PIK3CA*	GoF	Somatic and somatic mosaicism	[3,17,62]
Proteus syndrome	*AKT1*	GoF	Somatic and somatic mosaicism	[3,15,17,55]
Bannayan-Riley-Ruvalcaba syndrome	*PTEN*	LoF	AD and mosaic	[15,81,90]
CLAPO syndrome	*PIK3CA*	GoF	Somatic	[17]
MOVLDS	*DDX24*	Unknown	Germline. Unknown Mendelian pattern of inheritance	[2]

AD, autosomal dominant; AR, autosomal recessive; AVF, arteriovenous fistula; AVM, arteriovenous malformation; BRBNS, Blue rubber bleb nevus syndrome; CCM, cerebral cavernous malformation; CM, capillary malformation; CMTC, cutis marmorata telangiectatica congenita; FIVM, familial intraosseous vascular malformation; GoF, gain of function; GVM, glomuvenous malformation; HHT, hereditary hemorrhagic telangiectasia; LM, lymphatic malformation; LoF, loss of function; MAC, macrocephaly; MIC, microcephaly; MOVLDS, multiorgan venous and lymphatic defect syndrome; MVM, multifocal venous malformation; VM, venous malformation; VMCM, venous malformation cutaneo-mucosal; VVM, verrucous venous malformation.
[a]it needs more studies to be conducted in this field.

mutations of VEGFR3 and VEGFC have been reported in Milroy's disease and Milroy-like lymphedema, respectively. CCBE1 and ADAMTS3 are also known to be required for the activation of VEGFC, as autosomal recessive LoF mutations in *CCBE1 and ADAMTS3* have been identified in Hennekam lymphangiectasia–lymphedema syndrome [21,22]. Mutations within any of the four genes *(FLT-4 (VEGFR3), VEGFC, CCBE1, ADAMTS3)* may impair signaling via the MAPK/ERK and AKT/PI3K/mTOR pathways, which present potential targets for therapeutic interventions [23,24].

TEK (TIE2) in Venous Malformation

Blue rubber bleb nevus syndrome (BRBNS), as well as familial mucocutaneous venous malformation and more than 50% sporadic venous malformations can be attributed to activating mutations in *TEK*, which encodes the TIE2 receptor expressed primarily on venous endothelial cells [4▪▪,16,25]. Individuals with venous malformation and BRBNS may carry activating mutation (s) in the same allele, though double mutations are more common in BRBNS [26]. Multifocal venous malformation and familial venous malformation cutaneomucosal are because of a somatic second-hit mutation among individuals who already carry a germline *TEK* mutation [7,16]. Mutation of *TEK* results in ligand-independent phosphorylation of TIE2 receptor and constitutive activation of TIE2-mediated signaling via pathways including AKT/PI3K/mTOR and MAPK/ERK [7,27]. Pathogenic *TEK* mutations also give rise to activities of c-ABL tyrosine kinase that results in subsequent activation of PI3K/AKT/mTOR and PLCγ/ERK1/2 pathways. The activation of these pathways directly enhance angiogenesis and reduce endothelial cell apoptosis, therefore leads to abnormal vascular morphogenesis [25].

Glomulin and glumovenous malformation

Recent study has shown that germline glomulin *(GLMN)* LoF mutation and somatic mutation of second allele are often associated with glumuvenous malformation [3,7]. Germline mutations affecting both alleles are most likely incompatible with life. *GLMN* are expressed in endothelial and perivascular smooth muscle cells. Mutation in *GLMN* blocks TGF-ß signaling, but enhance signaling via the PI3K/mTOR pathway that results in abnormal differentiation of the vascular smooth muscle cells and defective formation of vascular bed because of inappropriate remodeling [4▪▪,7].

DDX24 in extacutaneous veno-lymphatic malformation

A missense mutation of *DDX24* was recently described in a large family with multiorgan venous and lymphatic defect syndrome. Veno-lymphatic malformation lesions were found in multiples organs of the affected individuals, in addition to portal and hepatic vein stenosis. The same mutation was reported in another group of patients with sporadic portal and hepatic vein stenosis and increased risk of inferior vena cava occlusion. DDX24 regulates endothelial cell migration and tube formation, thus affecting generalized venous and lymphatic development especially in liver and heart [2].

Table 2. Targeted therapies for vascular anomalies

Signaling pathway	Vascular anomalies	Targeted therapy[a]	References
PI3K-AKT-mTOR	HHT, GVM, Proteuss, CMMAC, BRRS, CLM, LM in Gorham-Stout disease CLOVESS, CLAPOS, and KTS.	*PI3K inhibitors:* Alpelisib, Dactolisib, Idelasib, copanlisib/ BAY 80–6946, and taselisib/ GDC-0032 *PI3K p110α Inhibitor:* Pictilisib/ GDC-0941 *PIK3CA Inhibitors*: Buparlisib/ BKM-120, Wortmannin, Ly294002, and Alpelisib *AKT inhibitor:* MK2206 *Pan-AKT inhibitor:* ARQ092 *mTOR inhibitors:* sirolimus, everolimus and temsirolimus *mTORC1/C2-PI3K inhibitor:* BEZ235	[7,16,54–56,59,62,63,65]
RAS-BRAF-MEK-ERK	NSCM, CM with OG, CM with bone and/or OG, DCM with or without OG, LCMCNPLOG, SWS, PWwM, CCM, Kaposiform lymphangiomatosis, VVM, EAVM, and SAVM	BRAF inhibitor: Vemurafenib *MEK inhibitors:* Trametinib, Cobimetinib, PD98059, U0126 MEK5 inhibitor: BIXO2189 *ERK5 inhibitors:* XMD8–92, XMD17–105 *Multiple kinase inhibitors* (VEGFR1, RAF-ERK, others): Sorafenib *VEGF inhibitor:* Bevacizumab *ALK5 inhibitor:* SB-431542	[7,16,31,44,47,60,61]
PI3K-AKT-mTOR and RAS-BRAF-MEK-ERK	PWS, CM-AVM 1 and 2, Channel type LM, MD, HLLS, MLL, BAVM, CVM, MVM, FVMCM, and BRBNS.	*TIE2 inhibitor*: CAS 948557–43–5 *ABL inhibitor:* Ponatinib	[16,25]
Smad1/5/8	HHT	*Calcineurin inhibitor and activator of Smad1/5/8:* Tacrolimus	[54]

BAVM, brain arteriovenous malformation; BRBNS, blue rubber bleb nevus syndrome; BRRS, Bannayan-Riley-Ruvalcaba syndrome; CCM, cerebral cavernous malformation; CLAPOS, CLAPO syndrome; CLM, cystic lymphatic malformation; CLOVESS, CLOVES syndrome; CM, capillary malformation; CM-AVM, capillary malformation – arteriovenous malformation; CMMAC, capillary malformation of macrocephaly; CVM, common venous malformation; DCM, diffuse capillary malformation; EAVM, extracranial arteriovenous malformation; FVMCM, familial venous malformation cutaneous-mucosal; GVM, glomuvenous malformation; HHT, hereditary hemorrhagic telangiectasia; HLLS, Hennekam lymphangiectasia-lymphedema syndrome; KTS, Klippel-Trenaunay syndrome; LCMCNPLOG, limb capillary malformation + congenital nonprogressive limb overgrowth; LM, lymphatic malformation; MD, Milroy's disease; MLL, Milroy-like lymphedema; MVM, multifocal venous malformation; NSCM, non syndromic capillary malformation; OG, overgrowth; Proteuss, Proteus syndrome; PWS, Parkes Weber syndrome; PWwM, port-wine stain with macrocheilia; SAVM, spinal arteriovenous malformation; SWS, Sturge-Weber syndrome; VVM, verrucous venous malformation.
[a]Therapies that have shown some efficacy in human studies or current in clinical trials are highlighted in bold.

CCM1/KRIT1, CCM2/malcavernin, CCM3/ PDCD10 in cerebral cavernous malformation

Most of the patients with cerebral cavernous malformation (CCM) have sporadic mutations in the *CCM* genes [3,28–30]. LoF mutations in any of the *CCM* genes result in similar clinical phenotypes. However, the most severe diseases are often because of mutations of *CCM3* gene [31]. *CCM* genes encode cytosolic proteins that form a complex downregulating MAP3K3 functions, therefore, inhibits the activity of MEK/ERK pathway. The inhibitory effect maintains quiescent state of endothelial cells and mitigates VEGF proangiogenic signaling [4▪▪,7,32]. Mutations of *CCM* gene result in loss of regulation on MEK/ERK pathway that leads to proangiogenic endothelial cells migration, impaired endothelium integrity and disrupted vascular morphogenesis [7,32]. The intracranial vasculature is therefore fragile and prone to hemorrhage [4▪▪,32,33].

RASA1, EPHB4 and capillary malformation-arteriovenous malformation

Germline autosomal dominant RASA1 mutations have been identified in 50% of capillary malformation-arteriovenous malformation (CM-AVM1) patients including those with Parkes-Weber syndrome [3,7,34]. Germline *RASA1* mutations as 'first hit' produce haploinsufficiency, and a somatic mutation of the second allele as ''second hit' results

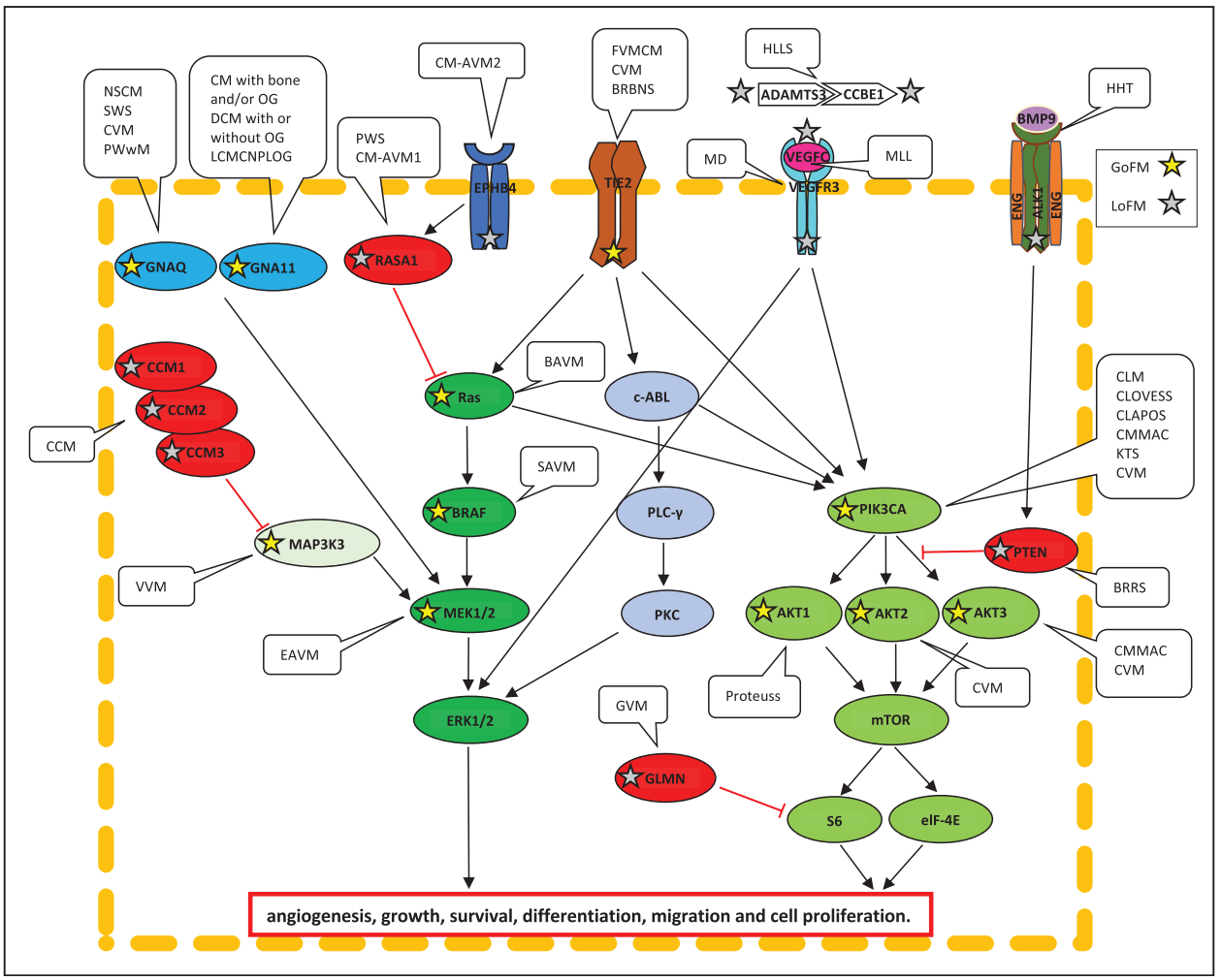

FIGURE 1. Schematic diagram of a signal transduction in endothelial cells and the main genetic mutations associated to vascular malformation. Commonly activated signaling pathways associated with vascular anomalies are color coded in green and blue. Transmembrane receptors and intracellular proteins play a role in angiogenesis are shown in color. Proteins mutated in different vascular disorders are shown. Arrows indicate direct or indirect interactions and red lines indicate inhibition. PIK3CA is part of PI3K; RAS includes HRAS and KRAS; RASA1 is one of the RASGAPs. BAVM, brain arteriovenous malformation; BRBNS, blue rubber bleb nevus syndrome; BRRS, Bannayan-Riley-Ruvalcaba syndrome; CCM, cerebral cavernous malformation; CLAPOS, CLAPO syndrome; CLM, cystic lymphatic malformation; CLOVESS, CLOVES syndrome; CM-AVM, capillary malformation-arteriovenous malformation; CMMAC, capillary malformation of Macrocephaly; CVM, common venous malformation; DCM, diffuse capillary malformation; EAVM, extracranial arteriovenous malformation; FVMCM, familial venous malformation cutaneous-mucosal; GoFM, gain of function mutation; GVM, glomuvenous malformation; HHT, hereditary hemorrhagic telangiectasia; HLLS, Hennekam lymphangiectasia-lymphedema syndrome; KTS, Klippel-Trenaunay syndrome; LCMCNPLOG, limb capillary malformation + congenital nonprogressive limb overgrowth; LoFM, loss of function mutation; MD, Milroy's disease; MLL, Milroy-like lymphedema; NSCM, non syndromic capillary malformation; OG, overgrowth; ProteusS, Proteus syndrome; PWS, Parkes Weber syndrome; PWwM, port-wine stain with macrocheilia; SAVM, spinal arteriovenous malformation; SWS, Sturge-Weber syndrome; VVM, verrucous venous malformation.

in VaMs in this disorder [4▪▪,35,36,37]. *RASA1* encodes p120-RasGAP protein that inhibits activity of RAS protein, an upstream regulator of the MEK/ERK1/2 and AKT/PI3K/mTOR pathways [7,38–40]. LoF mutations of *RASA1* therefore may lead to activation of RAS and increased downstream signaling via MEK/ERK1/2 and AKT/mTOR pathways [4▪▪,7,41] that can be targeted potentially.

Additional studies demonstrated recently that autosomal dominant *EPHB4* mutations were found in CM-AVM2 [42]. This gene encodes a transmembrane receptor expressed primarily in venous endothelial cells during vascular development interacting with its ligand, EphrinB2, on arterial endothelial cells. The interaction between EPHB4 and EphrinB2 is essential in venous and arterial

differentiation. EPHB4 also activates p120RasGAP, therefore exerts similar downstream effects to MEK/ERK pathway as RASA1. The phenotypic similarity between CM-AVM1 and CM-AVM2 suggests *RASA1 and EPHB4* play an overlapping role in vascular development during embryogenesis [7,38].

MAP2K1 (MEK1), nuclear prelamin A recognition factor-like and extracranial arteriovenous malformation

MAP2K1 or MEK1 is a well-known, downstream effector of BRAF. Mutation of *MAP2K1* has been identified in more than 50% cases of extracranial AVMs, though it has also been described in some cases of intracranial AVM [3,43,44]. The discovery has presented the possibility of using targeted MEK inhibitors that are currently available to manage complex extracranial AVMs. This has been confirmed by a recent proof-of-concept case report by Lekwuttikarn *et al.* [45].

Nuclear prelamin A recognition factor-like protein is encoded by *CIAO3* gene. LoF mutation of *CIAO3* has been described in two siblings with diffuse pulmonary AVMs from a Chinese consanguineous family. In animal model, nuclear prelamin A recognition factor-like knockdown presents with upregulation of transferrin receptor 1 expression and intracellular iron storage, which leads to increased angiogenesis via activated VEGF signaling [46].

KRAS, BRAF and SMAD9 in AVM of central nervous system

Somatic mutations of KRAS and BRAF were found in 80–100% of patients with brain and spinal AVMs [44]. These mutations activate signaling of the MAPK/ERK pathway, which leads to unregulated endothelial cells proliferation, growth, migration, and differentiation [7,44,47].

SMAD9 mutation was recently described in a 14-year-old girl with recurrent brain AVM. Phosphorylated wild type SMAD9 interacts with SMAD4, before being translocated into the nucleus to exert its proangiogenic function [48].

ENG, ALK1/ACVRL1, BMP9/GDF2, and SMAD4/MADH4 in hereditary hemorrhagic telangiectasia

Autosomal dominant mutations of *ENG and ALK1* affected approximately 96% of hereditary hemorrhagic telangiectasia (HHT) patients. The remaining populations affected were attributed to mutations of *SMAD4* and *BMP9* [3,5].

Genotype-phenotype correlation is better defined in HHT patients. Individuals with ENG mutations have a higher incidence of pulmonary AVMs, therefore increased risk of complications. *ALK1* mutations, on the other hand, are associated more frequently with hepatic AVMs and gastrointestinal bleeds [49]. Patients with aortic dilation often carry *SMAD4* mutations [50].

ENG is an endothelial transmembrane protein that can bind different types of TGF-ß and BMP, and interact with ALK1 upon activation [7,51,52]. The ENG-ALK1 complex phosphorylates SMADs, before they translocate into the nucleus to promote the transcription of proangiogenic genes [51,53]. Tacrolimus was specifically proposed as a therapy for HHT1, as it helps to mitigate aberrant AKT activation [54].

THERAPEUTIC IMPLICATIONS

Vascular anomalies with mutations affecting the AKT/PI3K/mTOR pathway (e.g. venous malformation, venous malformation cutaneo-mucosal, multifocal venous malformation, BRBNS, and lymphatic malformation) are known to respond to mTOR inhibitors (Fig. 1, Table 2) (sirolimus, everolimus, and temsirolimus) [55,56]. Recent phase II study has clearly demonstrated the efficacy of sirolimus for a variety of vascular anomalies in majority of participants [57,58]. Theoretically, PI3K inhibitors (alpelisib, dactolisib, copanlisib, idelasib, copanlisib/BAY 80-6946, and taselisib/GDC-0032) as well as AKT inhibitor (MK2206) [7,16,59] can block upstream signaling of mTOR pathway and exert better therapeutic effects. However, well-controlled perspective studies are needed to evaluate the safety as well as efficacy.

Malformations because of mutations affecting RAS/BRAF/MEK/ERK pathway (e.g. capillary malformation, CM-AVM, cerebrospinal AVM) could perhaps be targeted by BRAF inhibitor (e.g. vemurafenib) and/or MEK inhibitors (trametinib, cobimetinib, U0126 or PD98059) that are available [7,16,44,47]. The MEK inhibitor U0126 restores cadherin localization in the junction of endothelial cells in human umbilical vein endothelial cells; therefore has been proposed as a potential therapy for central nervous system AVMs [60,61]. Additional therapeutics, including sorafenib, multiple kinase inhibitors (VEGFR1, RAF-ERK), ALK5 inhibitor (SB-431542), ERK5 inhibitors (XMD8–92, XMD17–105), and MEK5 inhibitor (BIXO2189) are currently investigated for treatment of CCMs [31].

In addition, other targeted treatments for VaMs using different mechanism of actions are being studied. For example, sorafenib, PIK3CA inhibitors

(buparlisib/BKM-120, wortmannin, and Ly294002), selective PI3K p110α inhibitor (pictilisib/GDC-0941) and MK2206 have been evaluated *in vitro* for the treatment of lymphatic malformation [62,63]. In-vitro and in-vivo studies with everolimus, MK2206 and mTOR-PI3K duo inhibitor (BEZ235) are being evaluated for the treatment of venous malformation because of PIK3CA mutations [56,63]. Alpelisib (BYL719), a highly specific PIK3CA inhibitor is found to be superior than sirolimus in-vitro as well as in-vivo studies. The drug was also shown to be effective in treating cutaneous venous malformation in a mouse allotransplantation model when delivered as a topical cream. Case report of two patients with CLOVES appear to have benefited from treatment with alpelisib [16,64]. A subsequent large case series published recently [64] demonstrated marked improvement among patients with PIK3-related overgrowth syndrome. Phase 1 clinical trial is currently underway in the United States to further investigate its safety and benefit. The efficacy of new pan-AKT inhibitor, ARQ092, for the treatment of Proteus, CLOVES, and capillary malformation of macrocephaly is also being examined [59,65]. Ponatinib, a potent ABL inhibitor, was shown to be efficacious inhibiting angiogenesis in comparison to PI3K or MEK inhibitors *in vitro*. Combined therapy with ponatinib and sirolimus induces regression of the venous malformation synergistically [25]. It has been hypothesized that ponatinib blocks signaling via both TIE2 and c-ABL, therefore downregulates multiple pathways including PI3K/AKT/mTOR and PLCγ/ERK1/2. Furthermore, a new TIE2 kinase inhibitor is currently in development (CAS 948557-43-5) [16].

CONCLUSION

Knowledge about the genetics of VaMs has improved our understanding about the pathogenesis of these disorders, and provided a foundation for the development of new targeted therapy. As many of these malformations activate similar downstream angiogenic pathways, targeted therapies developed may be used for treatment of several vascular anomalies. Proof-of-concept studies are underway to investigate the safety and efficacy of these treatments. Furthermore combination therapies may be considered in the future to improve the management of complex vascular disorders.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
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