Vascular Malformations Genetics

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98.1 Introduction

Vasculogenesis, lymphovasculogenesis, angiogenesis, and lymphangiogenesis are processes tightly regulated by several signaling pathways [1]. Disruptions in these signaling pathways can result in the development of vascular anomalies [2–4]. Although the majority of cases arise sporadically, several are caused by inherited genetic mutations that predispose patients to forming lesions. The identification of the responsible genes, as well as subsequent functional studies in vitro and in animal models, has provided insight into the underlying signaling mechanisms that govern development and maintenance of the vasculature.

Vascular anomalies are generally categorized into two main groups [5]. Vascular tumors, consisting largely of hemangiomas (infantile or congenital), are the most common benign tumors of endothelial cells (ECs). However, the genetic causes of infantile hemangiomas are not known, thus, most of our knowledge about genetics and pathophysiology of vascular anomalies refers to vascular malformations. They encompass a broad spectrum of vascular defects that affect specific blood vessel types (arteries, capillaries, veins, and lymphatics) or a combination of vessel types (complex or combined lesions). Occasionally, vascular malformations can be found associated with other non-vascular defects in various syndromes.

The majority of vascular malformations are non-inherited, appearing as an isolated vascular lesion; they are mainly caused by a somatic activating mutation with a gain-of-function (GOF) effect that results in sustained and aberrant activation of intracellular signaling involved in vasculo/angiogenesis. There is high variability in the severity of lesions among patients. This is also true in families with inherited predisposition; penetrance is not 100%. We have explained this familial variability in some vascular

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malformations by Knudson's two-hit hypothesis, which implies that a germline mutation alone is not sufficient for lesions to arise and that an additional post-zygotic change resulting in complete localized loss-of-function (LOF) is needed. The familial cases, amenable to genetic linkage studies, have led the way to unravelling the pathophysiological bases of vascular malformations. Moreover, we postulated the « somatic-basis-hypothesis » for sporadic lesions based on the second-hit studies that proved the applicability of Knudson's double-hit theory towards inherited vascular malformations. According to this hypothesis, the much more common sporadic malformations would be due to somatic mutations alone [2, 4, 6, 7].

This has led to a plethora of discoveries and allowed to obtain an overview of the different causative defects that underlie vascular malformations. It appears that the identified signaling pathway defects play variable roles in different cell and vessel types. The two most important signaling pathways involved in vascular malformations are: the Phospho-inositol-3-Kinase (PI3K)-Protein B (AKT)-mammalian Target of Rapamycin (mTOR) pathway and the Mitogen Activated Pathway Kinase (MAPK). These pathways are regulated upstream by tyrosine kinase receptors located on the surface of EC, such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), or Tyrosine kinase with immunoglobulin and EGF homology domains (TIE2), binding to circulating growth factors, including VEGF, PDGF, or angiopoietins (ANGPT-1 or 2), respectively.

The activated tyrosine kinase receptors stimulate, by phosphorylation of phosphatidy linositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphospate, the lipid kinase PI3K, which in turn recruits and phosphorylates AKT (Threonine 308). To be fully activated, AKT requires a second phosphorylation (Serine 473) by mTOR complex 2 (mTORC2). Fully activated AKT can phosphorylate and activate mTORC1. Both AKT and mTORC1 stimulate cell proliferation, survival, migration, and angiogenesis. PTEN (phosphatase and tensin homolog) negatively regulates this cascade by decreasing the PI3K-induced activation of AKT [2, 4, 6]. The receptors can also activate the MAPK pathway starting with the stimulation of the protein kinase RAS through the exchange of Guanosine Diphosphate (GDP) with Guanosine Triphosphate (GTP) by the guanine nucleotide exchange factor. Subsequently, RAS activates the signaling protein kinase RAF which, in turn, phosphorylates MAP-extracellular signal-regulated kinase 1 (MEK1), resulting *in fine* in the phosphorylation and translocation of Extracellular Regulated Kinase (ERK) 1 and 2 in the nucleus. Nuclear ERK activates multiple transcription factors (c-fos, c-jun, c-myc), which are important for cell proliferation and metabolism. Multiple isoforms exist at each step; for example, the 3 RAS isoforms (H-, N-, and KRAS) cooperatively regulate angiogenesis. RASA1 is a negative regulator of RAS, as it converts GTP to GDP, converting active RAS to its inactive form [2, 4, 6].

Uncontrolled activity of these two pathways is involved in the development of some vascular malformations. PIKopathies and RASopathies include the vascular anomalies in which the PI3K/AKT/mTOR and the MAPK signaling pathway are affected, respectively. Another pathway involves the G protein coupled receptor (GPCR), a family of cell surface receptors implicated in signal transduction. These proteins are characterized by a 7-transmembrane domain structure with an extracellular N-terminus and an intracellular C-terminus. GPCRs play a key role in important physiological processes (cardiac function, immune responses, neurotransmission, etc.). Their excessive activation also contributes to the development of some vascular anomalies [8]. Table 98.1 summarizes a list of mutations reported in vascular malformations.

Table 98.1 Vascular malformations and related genetic variants

Malformations	Mutated gene	Type of mutation	Target
VENOUS ANOMALIES (VMs)			
Sporadic VM (sporadic VM) [10, 12–14, 16]	80% *TEK* (L914F) 20% *PIK3CA (E542-E545-H1047)*	Somatic GOF mutation	Direct activation of PI3K-AKT-mTOR
Inherited cutaneo-mucosal venous malformation (VMCM) [7, 15]	*TEK* (R849W)	Germline GOF mutation (Second-Hit required)	
Multifocal venous malformation (MSVM) [14]	*TEK* (double mutation Y897C-R915C)	Somatic GOF mutation (Second-Hit required)	
Blue rubber bleb nevus syndrome (BRBN) [14]	*TEK* (double mutations T1105N-T1106P and Y897F-R915L)	Somatic GOF mutation	
Glomuvenous malformation (GVM) [17–21]	*Glomulin*	Germline LOF mutation (Second-Hit required)	Activation of PI3K downstream targets Inhibition of the TGFß signaling Increased protein ubiquitination
Verrucous venous malformation (VVM)	*MAP3K3*	Somatic GOF mutation	Increased expression of *MEK1*
Hyperkeratotic cutaneous capillary-venous malformation (HCCVM)	*KRIT1*	Germline LOF mutation	Increased expression of *MAP3K3*
CAPILLARY ANOMALIES (CMs)			
Sporadic CM [22–25] Sturge–Weber syndrome [24, 25]	*GNAQ/GNA11*	Somatic GOF mutation	Activation of PLCβ and ERK
CM-arteriovenous malformation 1 (CM-AVM1) [25, 45]	*RASA1*	Germline LOF mutation (Second-Hit required)	Increased expression of *RAS*
CM-arteriovenous malformation 2 (CM-AVM2) [46]	*EPHB4*	Germline LOF mutation	Decreased expression of *RASA1*
CEREBRAL CAVERNOUS MALFORMATIONS (CCMs)			
CCM [28–33]	*CCM1 (KRIT1), CCM2 (Malcavernin), CCM3 (PDCD10)*	Germline LOF mutation with second -hit	Activation of integrin ➔ disruption of normal tissue development. Increased expression of *MAP3K3*

(continued)

Table 98.1 (continued)

Malformations	Mutated gene	Type of mutation	Target
LYMPHATIC ANOMALIES (LMs)			
Cystic LM [47–49]	80% *PIK3CA* (hotspot *E542-E545-H1047*)	Somatic GOF mutation	Direct activation of PI3K-AKT-mTOR
Some Generalized Lymphatic Anomalies [50–52]	Around 55% *PIK3CA* (hotspot *E542-E545-H1047*)	Somatic GOF mutation	Direct activation of PI3K-AKT-mTOR
Gorham–Stout disease (GSD) [51, 57, 58]	*KRAS*	Somatic GOF mutation	Direct activation of RAS-RAF-MEK
Kaposiform lymphangiomatosis (KLA) [53, 54]	*NRAS* *CBL*	Somatic GOF mutation	Direct activation of RAS-RAF-MEK Activation of tyrosine kinase receptors
Central Conducting Lymphatic Anomaly (CCLA) [59]	*EPHB4*	Germline LOF mutation	Decreased expression of *RASA1*
Generalized Lymphatic Dysplasia Hennekam syndrome [62–65]	*CCBE1* *FAT4* *ADAMPTS3* *PIEZO1*	Germline LOF mutation Germline LOF mutation Germline LOF mutation Germline LOF mutation	Decrease activation of VEGF-C Decrease activation of VEGF-C Decrease activation of VEGF-C Decrease in the role of mechanosensitive ion channel
Nonne–Milroy Disease [47, 66, 67]	*FLT4*	Germline LOF mutation	Downregulation of VEGFR3
Hypotrichosis-Lymphedema-Telangiectasia (HLT) [68, 69]	*SOX18*	Somatic or germline LOF	
Lymphedema-distichiasis [70]	*FOXC2*	Germline LOF	
Emberger syndrome [71, 72]	*GATA2*	Germline LOF mutation	
Osteopetrosis-lymphedema-anhidrotic ectodermal dysplasia-immunodeficiency (OLEDAI)D [76, 77]	*NEMO*	X-linked/ hypomorphic	Link with VEGFR axis more tenuous
Microcephaly associated with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR) [76, 77]	*KIFF11*	Germline LOF	
ARTERIO-VENOUS ANOMALIES (AVMs)			
Sporadic extracranial AVM [37–40]	MAP2K1 *KRAS* *BRAF*	Somatic GOF mutation	Direct activation of RAS-RAF-MEK

(continued)

Table 98.1 (continued)

Malformations	Mutated gene	Type of mutation	Target
Brain AVM [38, 39]	*KRAS*	Somatic GOF mutation	Direct activation of RAS-RAF-MEK
PTEN hamartoma tumor syndrome (PHTS) [82, 83, 90–94]	*PTEN*	Germline LOF mutation	Direct activation of PI3K-AKT-mTOR
Hereditary hemorrhagic telangiectasia (HHT) [38, 41–44] Juvenile polyposis HHT	*ENG*, *ALK1*, *HHT3*, *HHT4* *SMAD4*	Germline LOF mutation	Increase activity of PI3K and decrease activity of PTEN
PIK3CA-RELATED OVERGROWTH SYNDROME (PROS)			
Congenital Lipomatous Overgrowth with Vascular Anomalies, Epidermal Nevi and Scoliosis (CLOVES) [78–80]	*PIK3CA*	Somatic GOF mutation	Direct activation of PI3K-AKT-mTOR
Fibroadipose hyperplasia (FH) [81]			
Klippel–Trenaunay syndrome [83–89]			
Megalencephaly-capillary malformation (MCAP) [82]			

ADAMTS3 ADAM metallopeptidase with thrombospondin type 1 motif 3, *AKT* protein kinase B, *ALK* activin receptor-like kinase, *CCBE1* collagen and calcium binding EGF-domain, *DLL 4-NOTCH* delta-like canonical notch ligand), *EC* endothelial cell, *EphB4* ephrin B4, *FKBP12* FK506 binding protein 12, *GOF* gain of function, *GNAQ* G protein subunit alpha Q, *FAT4* FAT atypical cadherin 4, *ICAP* integrin cytoplasmic-associated protein, *KRIT* Krev interaction trapped, *LOF* loss of function, *LM* lymphatic malformation, *MAP3K3* mitogen-activated protein kinase kinase kinase 3, *PDCD* programmed cell death 10, *PIK3CA* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, *PTEN* phosphatase and tensin homolog, *RASA* RAS p21 protein activator, *SMAD* mothers against decapentaplegic homolog, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor

98.2 Venous Malformations

Sixty percent of venous malformations (VMs) have an activating mutation of *TEK*, encoding TIE2. Approximately 20 different GOF *TEK* mutations have been described, resulting in a ligand-independent hyperphosphorylation of TIE2 and a sustained activation of downstream PI3K-AKT-mTOR signaling cascade; this leads to reduced PDGFβ secretion and a decreased smooth muscle cell (SMC) coverage [9]. Murine model with *TEK*-mutated ECs drastically improved our knowledge of VM and opened the era of precision medicine [10].

Different variants of *TEK* mutations are related to a specific condition (Fig. 98.1a–d) [11].

- The vast majority of VMs are sporadic and unifocal, characterized by the presence of a single variable-sized lesion. The most common mutation, detected in 60% of these VMs,

is a somatic leucine914-to-phenylalanine change (L914F) [12–14].

- Inherited cutaneo-mucosal venous malformation (VMCM; OMIM 600195) is characterized by the presence of multiple small lesions and is transmitted in a para-dominant manner [9]. An arginine849-to-tryptophan substitution (R849W) is found in more than half of the cases as the inherited mutation but which requires a somatic second-hit in *TEK* to trigger development of VMCM [7, 15].
- The multifocal sporadic venous malformation (MSVM) is clinically similar to VMCM but without family history; patients are often mosaic for the first mutation (commonly R915C) associated with a second-hit (typically Y897C) in affected areas [14].
- The blue rubber bleb naevus syndrome (BRBN; OMIM 112200) is characterized by a single congenital, dominant lesion along with multiple smaller ones found cutaneously. Additionally, small protruding multifocal rubbery, venous-type lesions in the gastrointestinal tract can bleed and cause anemia. BRBN is caused by a somatic *TEK* mutation, represented by a carboxy-terminal double mutation T1105N-T1106P but also sometimes with Y897F-R915L or other combinations [14].

Twenty-five percent of VMs have a somatic GOF *PIK3CA* mutation [10]. Through aberrant activation of the PI3K-AKT axis, this results in reduced PDGFβ secretion. Even if *TIE2* and *PIK3CA* mutations play a key role in the development of VMs, they do not induce similar effects in the downstream pathways, as HUVECs overexpressing mutant *PIK3CA* showed lower levels of p-STAT (phosphorylated signal transducer and activator of transcription) 1 and low phospho-ERK1 compared to *TIE2*-L914F mutation [16]. The hot-spot mutations detected in *PIK3CA* are similar to those observed in cancer and include p.Glu542Lys (E542K) and p.Glu545Lys (E545K) in the helical domain, and pHis1047Leu (H1047R) in the kinase domain [16]. These mutations are also observed in various *PIK3CA*-related overgrowth syndromes (PROS), in which vascular malformations are associated with hypertrophy of soft and sometimes bony tissues [16].

Glomuvenous malformation (GVM; OMIM 138000) is inherited in an autosomal dominant manner (Fig. 98.1e). It is caused by loss of Glomulin [17]. Glomuvenous malformation, like VMCM, follows para-dominant inheritance, with the most common second-hit being acquired uniparental isodisomy. Somatic mutation early in development would result in larger lesions, while late-onset mutation would only cause small punctate lesions [18, 19]. Not much is known about the function of Glomulin; however, the specificity of the lesions indicates that it plays an important role in SMC differentiation. Glomulin is expressed by ECs and SMCs, and is suggested to be linked to inactivated tyrosine kinase receptor c-MET. According to this hypothesis, activation of c-MET by Hepatocyte Growth Factor (HGF) leads to phosphorylation of Glomulin and its release from c-MET to trigger activation of PI3K downstream targets, such as p70S6K. Glomulin may also interact with FK506 binding protein 12 (FKBP12), which is known to, by binding to Transforming Growth factor ß (TGFß) type I receptor, inhibit the TGFß signaling and the subsequent SMC maturation [15, 16]. More generally, it was demonstrated that Glomulin plays a role in Fbw7-mediated protein ubiquitination and degradation of Cyclin E and c-Myc. LOF of Glomulin activity results thus in sustained inhibition of the TGFß signaling, and in excessive levels of cyclin E and c-Myc [19–21].

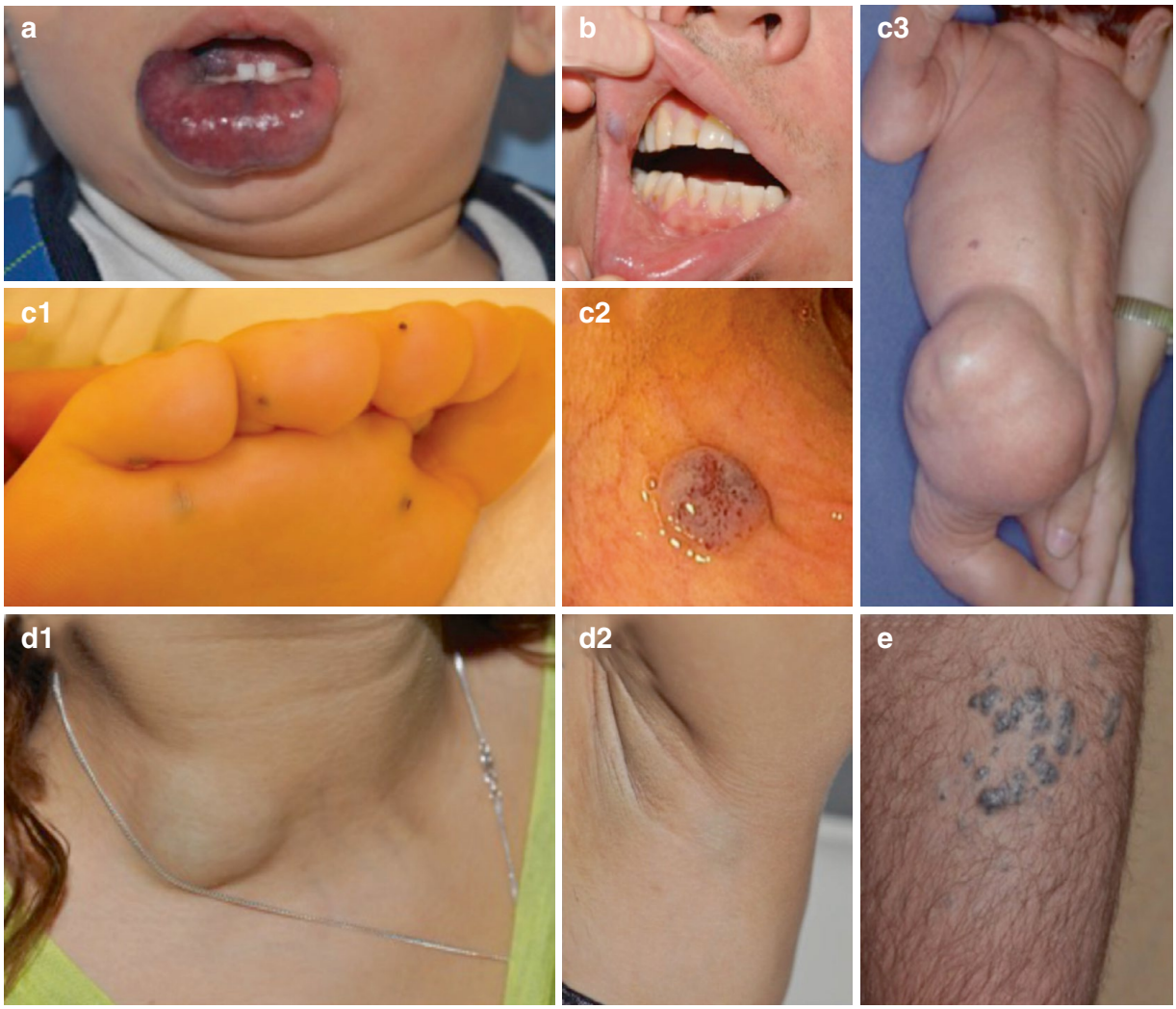

Fig. 98.1 Venous malformations. (**a**) VM. Venous malformation of lower lip; (**b**) VMCM. A small mucosal venous malformation on upper lip; (**c**) BRBN. Five pathognomonic, rubbery, hyperkeratotic, small venous malformations on right sole (**c1**), venous malformation of gastrointestinal track (**c2**), and dominant venous malformation on left buttock (**c3**) with additional small lesions on back; (**d**) MSVM. A subcutaneous venous malformation on right neck (**d1**) and left axilla (**d2**); (**e**) GVM. Glomuvenous malformation on left anterior thigh

98.3 Capillary Malformations

Capillary malformations (CM; OMIM 163000) often arise sporadically (Fig. 98.2a). Affected patients have a single small-to-large cutaneous stain that can vary from bright red to dark purple.

Guanine nucleotide-binding protein G(q) subunit alpha GNA is a family of heterotrimeric proteins that couple cell surface receptors to intracellular signaling pathways. A somatic GOF arginine183-to-glycine (R183Q) mutations in *GNAQ* has been identified in around 90% of patients with common CM [22]. Somatic pathogenic variants in *GNA11* and *GNA14* have also been identified in patients with extremity CM and overgrowth [23]. The GOF mutation of *GNAQ* results in activation of phospholipase C-β (PLCβ) and ERK [22–25]. The major issue is to recognize CM as part of a specific syndrome. CM may be observed e.g. in Sturge–Weber syndrome (SWS), in the CM-arteriovenous malformation (CM-AVM), in Diffuse capillary malformation with overgrowth (DCMO) and in other rare entities.

- Capillary malformation is a prominent feature in Sturge–Weber syndrome (SWS; OMIM 185300) (Fig. 98.2b), characterized by a

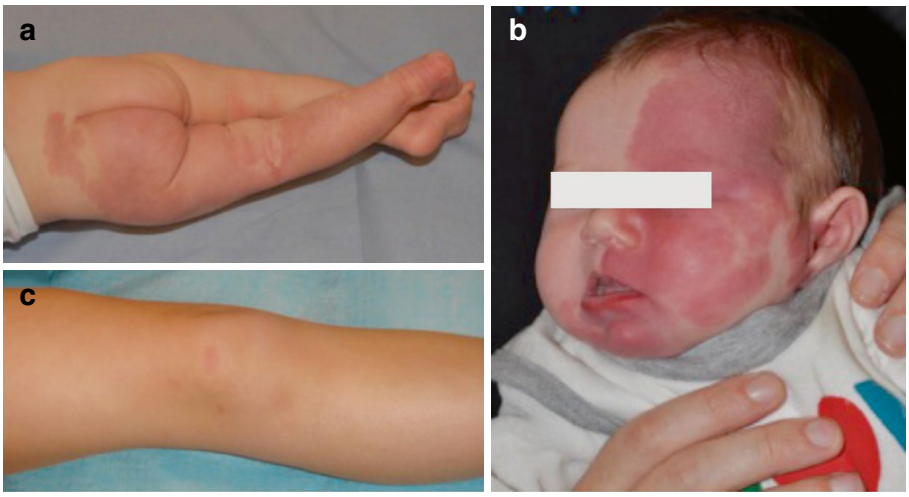

Fig. 98.2 Capillary malformations. (**a**) CM. Extensive capillary malformation of left lower extremity; (**b**) Sturge-Weber syndrome. Left hemifacial capillary malformation; (**c**) CM-AVM. Small oval capillary malformation with white halo on right knee

congenital CM involving the face (facial port-wine birthmark), as well as the ipsilateral leptomeninges and ocular choroid. GOF (R183Q) *GNAQ* mutation was found in lesion tissues (but not in normal skin) in over 90% of patients with SWS. A GOF mutation in the subunit Alpha 11 (*GNA11*) has been also reported, with distinct neurological and dermatological features; all these patients present with disseminated CM and hyper- or hypotrophy of an extremity. CM in this *GNA11*-SWS subgroup is more reticulate and darkens with time and the neurological symptoms seem less severe than in the *GNAQ* SWS [24].

- In CM-AVM, patients have multiple pink-red cutaneous capillary malformations that are lighter in color, often smaller in size compared to classic CMs. They can be surrounded by a halo, and they are even confounded with *café-au-lait* lesions or mastocytosis. Most lesions are located in the head and neck region, and many are intracerebral or within the spinal cord. These CMs occur in combination with AVMs (Fig. 98.2c). Sometimes, Vein-of-Galen aneurysmal malformation is also part of this entity, as well as Parkes–Weber Syndrome (PWS; OMIM 608355), the association of numerous arteriovenous microfistulas with a large cutaneous capillary blush and limb hypertrophy [25, 26]. Importantly, CM-AVM is not driven by a *GNAQ* mutation (see more genetic details in the next section).
- DCMO is characterized by diffuse CM of the skin accompanied by proportional, non-progressive enlargement of soft and/or bony tissue. The CM is characterized by widespread reticulated erythematous patches that may be confluent in some areas. Patients with DCMO might have prominent subcutaneous veins that are not considered true venous malformation. Syndactyly, sandal-gap or macrodactyly can be observed in 30% of DCMO patients. *GNA11* mutation has been reported in some patients [27].
- Other entities presenting CM include cutis marmorata telangiectatica congenita (CMTC), macrocephaly-CM (M-CM) syndrome.

98.4 Cerebral Cavernous Malformations

The majority of Cerebral cavernous malformations (CCM; OMIM 116860) are non-inherited, presenting sporadically at any age and consisting of an asymptomatic single cerebral lesion (sometimes in the spinal cord). However, twenty percent of CCMs are inherited, transferred in an autosomal dominant manner, and somatic second-hits have been described in lesions. Inherited forms are associated with multiple lesions in the central nervous system (supratentorial lesions more frequent than infratentorial lesions) [28, 29]. Histologically, CCMs vary

from capillary-type lesions to large venous-like cavernomas [28]. LOF of three genes have been linked to CCMs: *Krev Interaction Trapped-1* (*KRIT1*), *malcalvernin* (*CCM2*), and *Programmed cell death 10* (*PDCD10*). The three encoded proteins form a complex that interacts with Integrin Cytoplasmic-Associated Protein 1 (ICAP1), suppressing activation of Integrin [30]. Mutation in one of these genes is sufficient for causing the disease, with changes most commonly resulting in a truncated protein. CCM LOF destabilizes the function of ICAP1, leading to activation of integrin and disruption of normal tissue development. Furthermore, due to interaction between CCM complex and the delta-like canonical notch ligand (DLL) 4, LOF of CCM results in indirect activation of the MEK-ERK cascade [31–33] .

Cutaneous vascular malformations are seen in 9% of patients with familial CCM, existing as three distinct phenotypes: hyperkeratotic cutaneous capillary venous malformation (HCCVM, 39%), CM (34%), and VM (21%) CCM. Almost all these cutaneous malformations occur in association with *KRIT1*-CCM, suggesting that *KRIT1* has an important function also in cutaneous angiogenesis. No HCCVM, CM, or VM was detected in patients with *CCM2* mutation, whereas VMs were sometimes observed in *CCM3* patients [29, 30]. Somatic activating PIK3CA mutations have been identified in sporadic CCMs [34].

98.5 Arteriovenous Malformations

Most arteriovenous malformations (AVM) are sporadic (Fig. 98.3a). They are dangerous fast-flow lesions due to the absence of a capillary bed between arterioles and venules. They are warm, characterized by thrill, and can affect any tissue [35, 36].

The majority of AVMs occur sporadically and manifest as an isolated lesion. Somatic mutations in *MAP2K1*, the gene encoding MEK1, have been detected in 70% of patients in a series of extracranial AVMs [37]. These mutations consist of missense or small in-frame deletions that affect the negative regulatory domain of *MAP2K1*, resulting in increased MEK1 activity. *KRAS* and *BRAF* mutations have also been reported in AVMs, including brain AVMs, supporting the role of MAPK in this entity [38, 39]. Using mouse models, activating *KRAS* within vascular ECs allowed to generate AVM lesions in multiple soft tissues including brain, liver, heart, confirming the role of MAPK cascade in AVM development. The MEK inhibitor trametinib was able to improve survival, and normalize vessel sizes and morphology in this model [40].

There are at least two hereditary entities in which AVM can be found: hereditary hemorrhagic telangiectasia and CM-AVM.

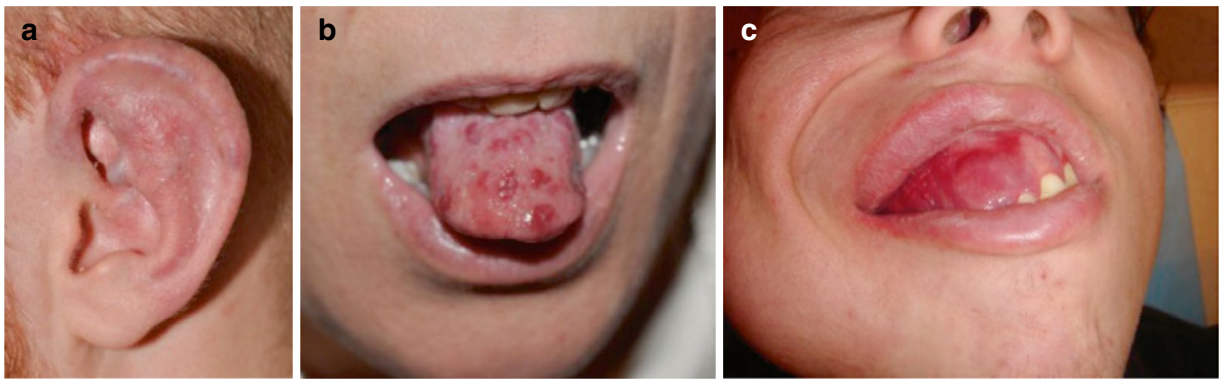

Fig. 98.3 Arteriovenous malformations. (**a**) AVM. Arteriovenous malformation of left ear; (**b**) HHT. Telangiectasias on tongue; (**c**) CM-AVM. Arteriovenous malformation of maxilla

- Hereditary hemorrhagic telangiectasia (HHT; OMIM 187300) is one of the most common inherited vascular disorders (Fig. 98.3b). It is characterized by subcutaneous telangiectasias and AVMs in various organs. Ninety-five percent of HHT cases are caused by mutations in TGF-β/bone morphogenetic protein (BMP) signaling pathway genes, including *Endoglin* (*ENG*, mutated in HHT1), *Activin receptor-Like Kinase 1* (*ALK1*, mutated in HHT2), and *MADH4 (*encoding Smad4), the latter mutated in the Juvenile Polyposis and HHT syndrome (JPHT). ENG and ALK1 are expressed predominantly at the surface of ECs, where they bind members of TGFβ/BMP family ligands, including TGF-β, BMP9 and BMP10. ALK1 is a type I serine/threonine kinase receptor and ENG acts as an auxiliary co-receptor that promotes, through recruitment of TGFβ type I and type II receptors, phosphorylation of SMADs 1,5, and 8. Phosphorylated R-Smads form complexes with Smad4, and translocate into the nucleus where they regulate the transcriptional activity of genes involved in the remodeling and angiogenesis [41]. LOF of these genes results in destabilization of the vascular structure with enhanced disorganized angiogenesis. These mutations induce also an indirect activation of the mTOR pathway by increased activity of PI3K and decreased activity of PTEN [42]. Additional loci were identified on chromosome 5q31.3–32 (HHT3) and 7p14 (HHT4) [43, 44]. There appears to be some phenotype/genotype correlation between the major types of HHT. For example, HHT1 leads to more severe symptoms, and the lungs and brain are more frequently affected. In contrast, the liver is more frequently involved in patients with HHT2 [38].
- CM-AVM (OMIM 608354) (Fig. 98.3c) is also hereditary. In CM-AVM, the CMs are associated in one-third of cases with AVMs. Two types of CM-AVMs exist:
  - CM-AVM1, caused by *RASA1* LOF mutations in 70% of cases; more than 40 truncating mutations have been described [25, 45]. In one patient, a somatic second-hit on the second *RASA1* allele was also identified in the Parkes–Weber affected arm [45].
  - CM-AVM2, caused by truncating mutations or amino acid substitutions leading to diminished or absent expression of EPHB4, so-called haploinsufficiency [46]. Telangiectasia and bier spots seem to be more characteristic to CM-AVM2 than CM-AVM1. Physiologically, the tyrosine kinase receptor EPHB4, by binding to EphrinB2, allows the subsequent recruitment of RASA1. The LOF mutations of *EPHB4* and *RASA1* lead to diminished inhibition of RAS, and thus a constitutive activation of RAS/MAPK/ERK signaling.

98.6 Lymphatic Malformations and Lymphedema

98.6.1 Lymphatic Malformations

Lymphatic malformations (LMs) are isolated micro- or macrocystic lesions, most commonly observed in the skin (Fig. 98.4a). They are usually congenital and there is no evidence that LMs are familial. Some LMs occur in syndromes such as the Turner syndrome. Thus, a cytogenetic abnormality may also be the cause of the LM in these cases [47, 48].

Similar to VMs, resected LMs have been studied for somatic genetic changes. Recurrent activating mutations were identified in the catalytic alpha-subunit of PI3K. Eighty percent of LMs have a somatic GOF hot-spot mutation in *PIK3CA*, including the three same hot-spot variants as seen in VMs and PROS (E542 and E545 in the helical domain and H1047R in the kinase domain of p110α), resulting in potent increase in PI3K activity. These hot-spot pathogenic variants cause lethality in heterozygous state, likely explaining why inherited isolated LMs have not been reported. Moreover, the timing of activation of the $PIK3CA^{H1047R}$ mutation during murine development determined the LM type; early $PIK3CA^{H1047R}$ expression was associated with large macrocystic LMs, while expression of the mutation in postnatal lymphatic vasculature caused microcystic LMs [49].

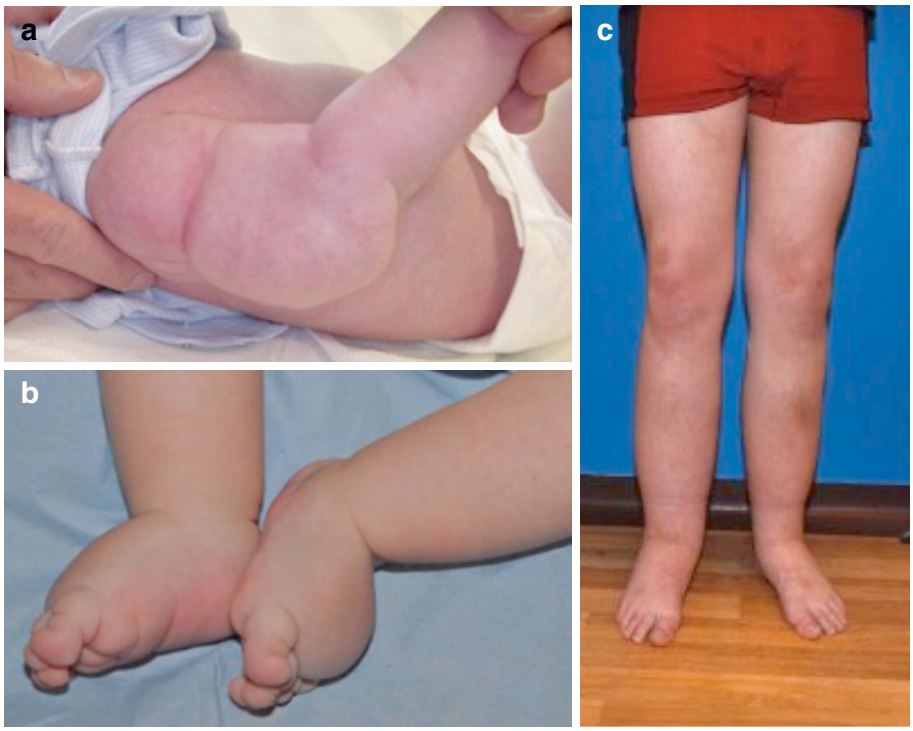

Fig. 98.4 Lymphatic malformations and lymphedema. (**a**) LM. Lymphatic malformation of right arm; (**b**) Bilateral primary lymphedema of feet (VEGFR3); (**c**) Bilateral primary lymphedema of feet and legs; more pronounced on the right (FOXC2)

98.6.2 Complex Lymphatic Anomalies

Complex Lymphatic Anomalies (CLAs) are rare, commonly involve multiple localizations and result in accumulation of fluid (chylous ascites, chylothorax), malnutrition by enteropathy, and disruption of fluid homeostasis and immune function. CLAs include Generalized Lymphatic Anomaly (GLA), Kaposiform Lymphangiomatosis (KLA), Gorham–Stout Disease (GSD), and Central Conducting Lymphatic Anomaly (CCLA); all these entities present overlapping clinical features that render the diagnosis highly challenging [50, 51].

Somatic activating *PIK3CA* mutations were detected in up to 55% of patients with GLA [52], while RASopathy has been reported in other CLAs. Somatic pathogenic mutations in *NRAS* and *KRAS* have been described in some patients with KLA and GSD, respectively [53–58]. Activating variant of *ARAF* has been reported in two patients with CCLA [59]. In a KLA patient, a somatic LOF mutation was also identified in *Casitas B lineage lymphoma* (*CBL*), a ubiquitin ligase that induces degradation of receptor tyrosine kinase [54]. Germline heterozygous kinase-dead (LOF) mutations in the gene *EPHB4*, were found in a few patients with CCLA. All these mutations lead to a sustained activation of the RAS-RAF-MEK pathway, suggesting that these lymphatic anomalies may be responsive to RAS-pathway inhibitors [40, 60].

98.6.3 Primary Lymphedema

Primary lymphedemas are often present at birth and, even if the majority of cases are sporadic, 35% of primary lymphedemas are familial [61]. Lymphedema is characterized by chronic accumulation of interstitial fluid, most commonly in the lower limbs. Around 30 genes or loci have been reported to cause post-natal primary lymphedema, with or without preceding Non-Immune Hydrops Fetalis (NIHF). These genes or loci explain about up to 30% of primary lymphedema.

- Generalized Lymphatic Dysplasia (GLD) *is* a rare form of primary lymphedema, characterized by a uniform, widespread developmental abnormality of the lymphatic system. A number of affected individuals have a positive

family history of lymphedema usually suggestive of autosomal recessive inheritance. Hennekam syndrome (HS) is an example of GLD characterized by lymphedema of all four limbs, intestinal lymphangiectasia, variable degrees of learning difficulties, and characteristic facies. Three mutated genes for GLD and HS affect *CCBE1* (collagen and calcium-binding EGF-domain 1), *FAT4* (FAT Atypical Cadherin 4), and *ADAMTS3* (ADAM metallopeptidase with thrombospondin type 1 motif 3); these mutations result in impairment in VEGF-C-induced lymphangiogenesis [62–64]. Another mutated gene is *PIEZO1*, which encodes a calcium permeable mechanically activated ion channel, and results in an autosomal recessive form of GLD with a high incidence of NHIF and childhood onset of facial and four limb lymphedema [65].

- Familial congenital lymphedema, or Nonne–Milroy disease, is linked to mutations in the VEGF receptor-3 (VEGFR3; OMIM 153100) [47, 66, 67]. VEGFR3 is known to have essential roles in angiogenesis and, later in lymphangiogenesis, during embryonic development. Mutations are found within the intracellular tyrosine kinase domain of VEGFR3 and are largely transmitted in an autosomal dominant manner [66, 67]. The mutations lead to severely reduced phosphorylation of the receptor.
- Other mutations involving transcription factors playing a role in VEGFR3-dependant lymphangiogenesis have been reported in some lymphedema syndromes. SRY-containing box (SOX18) regulates the initiator of lymphangiogenesis PROX1, which regulates VEGFR3; recessive mutations within the DNA-binding domain or dominant nonsense alterations in the transactivation domain of SOX18 is detected in Hypotrichosis–Lymphedema–Telangiectasia (HLT; OMIM 607823) [68, 69]. The forkhead box protein C2 (FOXC2) regulates many endothelial targets, such as Angiopoetin-2 and Dll4, and is a downstream target of VEGFC/VEGFR3 signaling; missense and truncating changes in FOXC2 are responsible for lymphedema–distichiasis [70]. The zinc-finger transcription factor GATA2 is highly expressed in ECs and hematopoietic stem cells, as well as the lymphatic valves; mutations in *GATA2* have been detected in the Emberger syndrome (OMIM 614038) [71, 72]. However, the role of GATA2 in lymphangiogenesis remains to be elucidated, as no vascular defect was seen in *GATA2*-null mice [73].
- Noonan and Noonan-like syndromes represent a spectrum of autosomal dominant disorders affecting 1/1000 individuals. These disorders display an overlap of clinical features including developmental delay, intellectual disability, craniofacial dysmorphism, and cardiac defects. Lymphatic disorders are well-recognized complications of these syndromes, presenting at childhood or adulthood as bilateral lower limb lymphedema, genital edema, and chylous reflux with lymphorrhea, particularly in the genital area. There is frequent systemic involvement including intestinal lymphangiectasias and/or chylothorax. Germline missense mutations have been identified in *PTPN11, KRAS, HRAS, RREB1, and MAP2K1/2*, all inducing excessive activation of the MAPK cascade [74, 75].
- Other gene mutations have been reported in some entities associated with primary lymphedema; however, their interaction with the VEGFR axis seems more tenuous. Hypomorphic changes in inhibitor of kappa light polypeptide gene enhancer in B-cells (IKBKG), or NEMO, cause the X-linked osteopetrosis–lymphedema–anhidrotic ectodermal dysplasia–immunodeficiency syndrome (OLEDAID; OMIM 308300). Microcephaly associated with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR; OMIM 152950) is due to mutations in the homotetrameric kinesin-like protein 11 (KIF11)/EG5 [76, 77].

The identification of the various genes that are mutated in inherited primary lymphedemas has given unprecedented possibilities in classifying and diagnosing patients more precisely. This is a prerequisite for detailed characterization of each

sub-phenotype. It also provides the basis for the development of future clinical trials, even if our comprehension of the pathophysiological mechanisms is not complete.

98.7 Vascular Malformations-Related Syndromes (PROS and Others)

Vascular malformations are sometimes part of a more complex phenotype. Many of these syndromes include overgrowth, wherein connective tissue and/or bone hyperplasia is present. The pathophysiological causes of these syndromes have started to be unraveled on the basis of the « somatic-basis-hypothesis » and next-generation sequencing, which allows sensitive detection of low-frequency mutations in heterogenous tissues.

Somatic/mosaic *PIK3CA* mutations have been implicated in various syndromic phenotypes that can associate vascular anomalies with hypertrophy, leading to name this spectrum "*PIK3CA*-Related Overgrowth Syndrome" or PROS. The core features are congenital or early-childhood onset of segmental/focal overgrowth with or without cellular dysplasia in the absence of a family history of similarly affected individuals (i.e., single occurrence in a family). The causative mutation leads to *PIK3CA* GOF, resulting in elevated AKT phosphorylation in the affected tissue. PROS includes CLOVES, Fibroadipose Hyperplasia (FH), Fibroadipose infiltrating lipomatosis, Hemihyperplasia multiple lipomatosis (HHML), Klippel–Trenaunay syndrome (KTS), Megalencephaly–Capillary Malformation-Polymicrogyria syndrome (MCAP), Macrodactyly, Hemimegalencephaly, and Muscle hemihyperplasia.

- Congenital lipomatous asymmetric overgrowth with vascular malformation, epidermal naevi, and skeletal anomalies (CLOVES; OMIM 612918) is due to mosaic missense mutations in *PIK3CA* (Fig. 98.5a) [78, 79]. There is great clinical heterogeneity within this disorder. Truncal lipomatous mass(es) evident at birth are a defining feature. Fast-flow lesions (AVMs) may be present within the fatty truncal masses, which contributes to the morbidity seen in this disorder. Other clinical features include characteristic triangular shape of extremities with broad-based toes, macrodactyly, and a "sandal-gap" deformity of the first and second toes. Scoliosis, spinal, and skeletal abnormalities can also be found [80].
- Fibroadipose hyperplasia (FAH) is characterized by segmental overgrowth of skeletal, visceral, fibroadipose, subcutaneous and muscular tissues with lipomatous infiltration of muscles, accompanied by adipose tissue dysregulation and regional lipohypoplasia. Additional findings may include vascular malformations, epidermal nevi, polydactyly and testicular or epididymal cysts, and hydrocele [81]. Due to overlapping features, FAH and CLOVES may be considered different phenotypes of a single overgrowth syndrome.
- The MCAP or M-CM (Macrocephaly or Megalencephaly—Capillary Malformation) is also highly variable in clinical presentation. The hallmark is an unusual growth dysregulation of the brain and multiple somatic tissues. The typical features are congenital or early postnatal progressive megalencephaly (MEG) or hemi-megalencephaly (HMEG), hypotonia, mild to severe intellectual disability and segmental to generalized somatic overgrowth with single or multifocal capillary malformations. Usually, midline facial CMs—especially persistent naevus flammeus or cutis marmorata—are present. Megalencephaly may be accompanied by secondary overgrowth of the ventricles, corpus callosum, and cerebellum followed by cerebellar tonsillar ectopia [82].
- Klippel–Trenaunay syndrome (KTS; OMIM 149000) is a sporadic capillaro-lymphatico-venous malformation associated with hypertrophy (Fig. 98.5c). *PIK3CA* mutation is detected in 90% of cases [83]. Another gene, AGGF1, has also been suggested, but it needs further validation. The *AGGF1* (angiogenic factor with G patch and FHA domains 1,

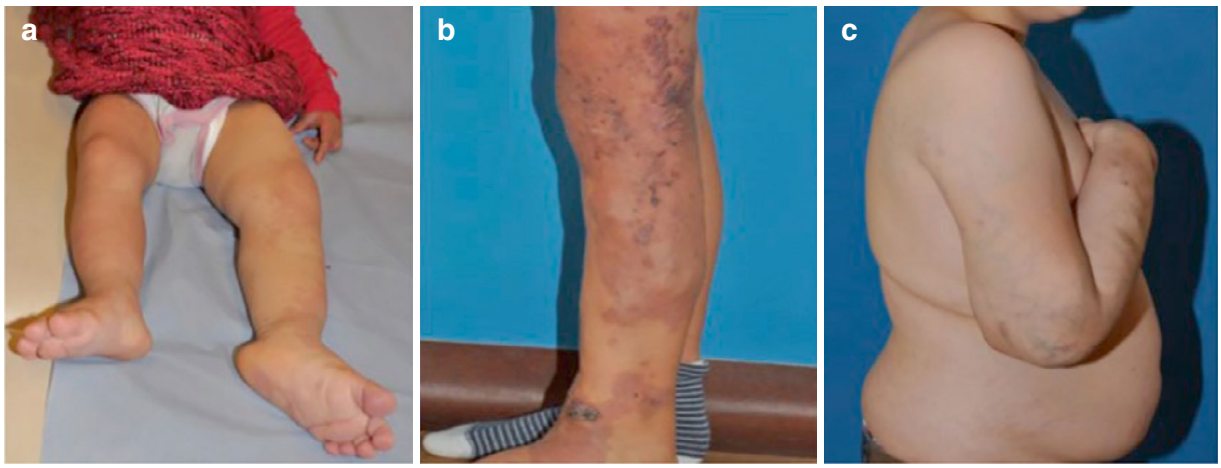

Fig. 98.5 Syndromes. (**a**) Congenital lipomatosis with overgrowth, vascular malformation, epidermal naevi, and scoliosis syndrome (CLOVES). Bilateral capillary malformation of lower extremities; overgrowth with lipomatosis of left lower extremity; (**b**) Klippel–Trenaunay syndrome (KT). Capillaro-lymphatico-venous malformation of left lower extremity; (**c**) PTEN hamartoma tumor syndrome (PHTS). Deep venous malformation with small capillary malformations and clear, visible, enlarged veins of right arm; overgrowth

OMIM 608464), also known as *VG5Q*, encodes a proangiogenic factor AGGF1, which binds to the surface of endothelial cells and promotes their proliferation by activating AKT, GSK3β, and p70 S6K [84]. In vitro, AGGF1 promotes ECs' proliferation, adhesion, migration, and capillary tube formation, and interaction with integrin α5 and β1 is required to promote its action [84–87]. The *AGGF1* variant (c.397G > A, p.Glu133Lys) can though be found in 4% of KTS patients and also in control group, suggesting that this variant in *AGGF1* is probably a nonpathogenic polymorphism [88] . The absence of RASA1 mutation suggests that *RASA1* testing could be a diagnostic method for differentiation between PWS, CM-AVM, and KTS [89].

- PTEN hamartoma tumor syndrome (PHTS) (Fig. 98.5b) is caused by loss of PTEN and combines several entities, such as the Cowden syndrome (OMIM 158350), the Banyan–Riley–Ruvalcaba syndrome (OMIM 153480), the PTEN-related Proteus syndrome, and the Proteus-like syndrome. Loss of PTEN likely leads to increased PI3K/AKT signaling, as it normally acts to counteract PI3K [82, 83]. Family history of PHTS is not always present, as de novo mutations are frequent. Only 10–50% of individuals with PHTS have an affected parent and the majority of the cases are simplex [90]. Some patients have acquired a mosaic or somatic *PTEN* pathogenic variant limited to affected organs [91]. Detecting PHTS patients is of importance as this condition increases significantly the risk of cancer and as genetic counseling is mandatory for the family. In patients without a *PTEN* mutation, a germline mutation in *KLLN* should be looked for and may be associated with a vascular anomaly such as AVM [90, 92]. This mutation leads to downregulation of KLLN that shares a bidirectional promoter with PTEN. Other mutations include germline variants in *SDHB* and *SDHD* [93], increasing the stabilization of hypoxia-inducible factor-1 (HIF1) and destabilization of p53. Germline *PIK3CA* or *AKT1* pathogenic variants can also be detected [94].
- Proteus syndrome (PLS; OMIM 176920) also results from overactivation of AKT, but due to direct somatic *AKT1* mutations leading to gain-of-function [80]. Complementing the human phenotype, hyperactivation of AKT causes skin hyperplasia in transgenic mice [81].

98.8 Therapeutic Impact of a Detailed Genetic Analysis

Many drugs targeting the mentioned signaling pathways are available from the oncological field, inhibiting specific proteins belonging to the PI3K-AKT-mTOR or RAS-RAF-MEK cascades. Repurposing these agents in the vascular malformation setting allowed a drastic improvement in the outcome of patients. Genetic analysis is of course the key to identify correctly the patients who may benefit or not from targeted therapies (Fig. 98.6).

The mTOR inhibitor sirolimus is now a standard, yet off label, treatment in slow-flow vascular malformations, based on the fact that many of these malformations harbor a sustained activation in the PI3K-AKT-mTOR axis. Different small prospective trials have shown a high efficacy rate of sirolimus [10, 95, 96]. A prospective multicentric single-arm phase III trial (VASE) is ongoing to confirm the efficacy of sirolimus in pediatric and adult patients with various slow-flow vascular malformations (EudraCT number: 2015-001703-32). All patients are treated with rapamycin for 2 years. The preliminary results confirmed an efficacy rate of exceeding 80%,

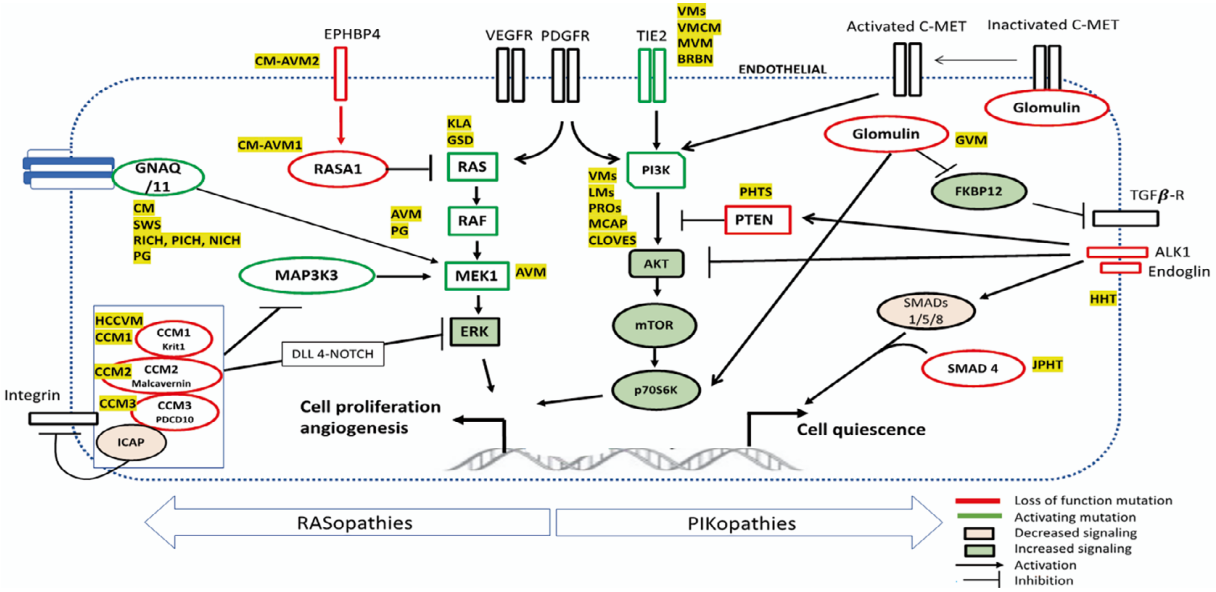

Fig. 98.6 Signaling pathways involved in vascular malformation pathogenesis AKT protein kinase B, ALK activin receptor-like kinase, AVM arteriovenous malformation, BRBN blue rubber bleb nevus syndrome, CCM cerebral cavernous malformation, CLOVES congenital lipomatous overgrowth with vascular anomalies epidermal nevi and scoliosis, CM capillary malformation, CM-AVM capillary malformation-arteriovenous malformation, DLL 4-NOTCH delat-like canonical notch ligand, EC endothelial cell, EphB4 ephrin B4, FKBP12 FK506 binding protein 12, GNAQ G protein subunit alpha Q, GSD Gorham–Stout Disease, GVM glomuvenous malformation, HCCVM hyperkeratotic cutaneous capillary-venous malformations, HHT hereditary hemorrhagic telangiectasia, ICAP integrin cytoplasmic-associated protein, JPHT juvenile polyposis/HHT syndrome, KLA Kaposiform lymphangiomatosis, KRIT Krev interaction trapped, KTS Klippel–Trenaunay syndrome, LM lymphatic malformation, MAP3K3 mitogen-activated protein kinase kinase kinase 3, MCAP megalencephaly–capillary malformation syndrome, MVM multifocal venous malformation, NICH noninvoluting congenital hemangioma, PDCD programmed cell death 10, PG pyogenic granuloma, PHTS PTEN hamartoma tumor syndrome, PIK3CA phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, PROS PIK3CA-related overgrowth spectrum, PTEN phosphatase and tensin homolog, RASA RAS p21 protein activator, RICH rapidly involuting congenital hemangioma, SMAD mothers against decapentaplegic homolog, TGF transforming growth factor, VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor, VM venous malformation, VMCM inherited cutaneomucosal venous malformation, VVM verrucous venous malformations

improving pain, functional limitation and quality of life (Seront E et al, JCI Insight 2023).

A PIK3CA inhibitor, BYL719 or Alpelisib, was tested on 19 patients with PROS, also showing good outcomes [97]. The efficacy of alpelisib in non-syndromic vascular malformations (with or without *PIK3CA* mutation) remains however unknown. Similarly, the Pan-AKT inhibitor ARQ 092 (Miransertib) showed promising results on a cohort of six patients with PIK3CA-Related Overgrowth Syndrome [98].

Different MEK inhibitors are currently evaluated in RASopathies; trametinib has been evaluated in a prospective phase II trial enrolling patients with AVMs and showed promising results (TRAMAV trial, EudraCT number: 2019-003573-26; Coulie et al, in preparation). Antiangiogenic agents are also available, including bevacizumab and thalidomide, both inhibiting VEGF. These two agents have shown efficacy in HHT, and thalidomide showed highly interesting results in 18 AVM patients [99]. Once again, identification of the target by genetic analysis should be performed in a routine practice in order to offer an optimal management to patients.

98.9 Summary and Key Points

- Discovery of genes associated with vascular malformations and determining their functions have helped in understanding the signaling pathways that are affected during development and regrowth after therapy of vascular malformations. Knowledge of the underlying mechanisms enables generation of animal models and aids in identification of therapeutic targets for more effective treatment of vascular lesions.
- Inherited vascular malformations are complex. Simply possessing a germline genetic mutation is not necessarily sufficient for a patient to form a lesion. A somatic second-hit resulting in complete localized loss of the gene or accentuation of the primary gain-of-function mutation is needed, as demonstrated in VMCM, GVM, CM-AVM, and CCM.
- Certain vascular anomalies, particularly lymphedema, are caused by several genes that likely play a role in the same functional pathway, and yet do not fit in one linear signaling pathway.
- The more common sporadic cases are explained by somatic/mosaic mutations, as suggested by our « somatic-basis-hypothesis » [5, 86]. Sensitive targeted next-generation sequencing and droplet PCR are ideal techniques to identify them.
- The PI3K/AKT pathway is involved in several syndromic, combined, and pure malformations. Inhibitors of this pathway may be promising therapeutic options, especially for difficult-to-treat cases. Clinical trials are needed to demonstrate efficacy and safety.

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