Neurological Sciences (2019) 40:243–251
https://doi.org/10.1007/s10072-018-3674-x

REVIEW ARTICLE

Vis-à-vis: a focus on genetic features of cerebral cavernous malformations and brain arteriovenous malformations pathogenesis

Concetta Scimone [1,2] · Luigi Donato [1,2] · Silvia Marino [3] · Concetta Alafaci [1] · Rosalia D'Angelo [1] · Antonina Sidoti [1,2]

Received: 7 September 2018 / Accepted: 1 December 2018 / Published online: 6 December 2018

Abstract

Cerebrovascular malformations include a wide range of blood vessel disorders affecting brain vasculature. Neuroimaging differential diagnosis can result unspecific due to similar phenotypes of lesions and their deep localization. Next-generation sequencing (NGS) platforms simultaneously analyze several hundreds of genes and can be applied for molecular distinction of different phenotypes within the same disorder's macro-area. We discuss about the main criticisms regarding molecular bases of cerebral cavernous malformations (CCM) and brain arteriovenous malformations (AVM), highlighting both common pathogenic aspects and genetic differences leading to lesion development. Many recent studies performed on human CCM and AVM tissues aim to detect genetic markers to better understand molecular bases and pathogenic mechanism, particularly for sporadic cases. Several genes involved in angiogenesis show different expression patterns between CCM and AVM, and these could represent a valid starting point to project a NGS panel to apply for differential cerebrovascular malformation diagnosis.

Keywords Cerebral cavernous malformations · Arteriovenous malformations · Genetics · Differential molecular diagnosis

Introduction

Cerebrovascular malformations include a wide spectrum of intracranial blood vessel disorders, involving arterial wall, capillary bed, and venous and lymphatic systems. Their main associated risks are intracerebral hemorrhage (ICH), seizures, and focal neurological deficits. All cerebrovascular malformations arise due to impairment in embryonal vasculogenesis and subsequent angiogenesis, as result of both genetic and environmental factors. Cerebral cavernous malformations (CCM, OMIM #116860) and arteriovenous malformations (AVM, OMIM #108010) are the most frequent, affecting more than 3% of the population [1]. Venous developmental anomalies (VDAs) are rare conditions usually associated with CCM and probably represent a progression from the only pathological event. More rarely, capillary malformations congenital (CMC) can be also observed and, in many cases, they co-exist with AVM [2]. Each lesion shows peculiar neuro-radiological features; however, cases of uncertain diagnosis are not rare. Reasons that cannot make discernible the different malformations may be their deep localization, small dimensions, or presence of mixed lesions. Cerebrovascular malformations may occur sporadically or inherited causing familial syndromes. All familial forms show an autosomal dominant inheritance pattern, while genetic factors involved in pathogenesis of sporadic cases are still not well elucidated. Main clinical features and genetic bases of both CCM and AVM are here reviewed in order to compare their pathogenic mechanisms. The possibility to highlight the specific molecular markers can be exploit for differential genetic diagnosis that we think could be supportive at clinicians, neurologists, neurosurgeons, and neuroradiologists in all those cases of controversial diagnosis.

✉ Rosalia D'Angelo
rdangelo@unime.it

[1] Department of Biomedical and Dental Science and Morphological and Functional Images, University of Messina, Consolare Valeria street 1, 98125 Messina, Italy

[2] Department of Vanguard Medicine and Therapies, Biomolecular Strategies and Neuroscience, I.E.ME.S.T, Michele Miraglia Street 20, 90139 Palermo, Italy

[3] IRCSS Centro Neurolesi "Bonino-Pulejo", c.da Casazza, Messina, Italy

Cerebral cavernous malformations

General features

CCM affects brain capillaries appearing like mulberries. Lesions are formed by enlarged and tangled vessels. The single layer of endothelial cells is very disorganized as consequence of pericyte absence and of defective tight and adherens junctions. This results in an increased capillary permeability with high risk of ICH. Together with seizures, ICH is the most frequent clinical manifestation. Other symptoms include headaches, vertigo, and focal neurological deficits related to lesions localization. Worldwide, CCM incidence ranges between 0.1 and 0.5%, although these data are underestimated due to incomplete penetrance. Only about 50–75% of patients show clinical manifestations, and age of symptoms onset can be very variable [3]. Recently, we reported a case of a man, affected by a sporadic form of CCM, who became symptomatic at the age of 80 despite that he was harbored more than 70 brain lesions [4]. However, although sporadic cases usually arise from third decades of life, they show a single lesion. Multiple cavernomas are typical of familial forms, and they are often observed since childhood. Familial CCM is linked to the three loci *KRIT1/CCM1* (Entrez Gene: 889; 7q11.2–21), *MGC4607/CCM2* (Entrez Gene: 83605; 7p13), and *PDCD10/CCM3* (Entrez Gene: 11235; 3q26.1) [5], and their mutation detection rates range about 60%, 20%, and 15%, respectively. No germ-line mutations were detected in about 10% of patients affected by familial forms or sporadic with multiple lesions, hypothesizing probable involvement of other genes in disease development [6]. Clinical penetrance is estimated to be about 88% for *KRIT1*, 100% for *CCM2*, and 63% for *PDCD10* [7]. Lesions can affect also peripheral organs. Cutaneous vascular malformations are present in about 9% of patients harboring mutations in *KRIT1* [8]. Patients linked to *PDCD10* locus, instead, show a more early, severe, and aggressive phenotype, usually characterized by recurrent ICH [9]. Conversely, patients affected by sporadic forms may often have CCM lesions coexistent with VDA [10]. Neuro-radiological diagnosis of CCM is performed by magnetic resonance imaging with T-2 gradient-echo and susceptibility-weighted imaging [11, 12]. Guidelines for CCM patients management were recently published by the American Angioma Alliance [13].

Molecular bases of CCM

Molecular mechanisms that lead to CCM development are not yet fully known. However, familial and sporadic forms have different pathogenic models. Several studies are highlighting roles of the three CCM genes in angiogenesis [14]. *KRIT1* encodes for *Krev interaction trapped 1* (KRIT1) protein containing at N-terminus three NPxY/F motifs and four ankyrin repeat domains, and a FERM domain at C-terminus. KRIT1 binds integrin cytoplasmic-associated protein-1 (ICAP1α) [15]. ICAP1α, through the same domain, can interact with both KRIT1 and integrins leading, in this case, to the activation of β-integrin signaling and angiogenesis trigging. In this contest, KRIT1 acts as a modulator of ICAP1α activity competing with β-integrin binding site [16]. Impairment of cell junctions is also a feature of affected vessels, and KRIT1 contributes to cell-cell adhesion by interacting with heart of glass (HEG) receptor. This interaction guides KRIT1 localization at cell-cell junctions, and it is mediated by Rap1a [17]. KRIT1 maintains adherens junctions' integrity by binding β-catenin and promoting its interaction with vascular endothelial-cadherin. Moreover, β-catenin, bound to KRIT1, is unable to move into the nucleus to promote *Wnt* and vascular endothelial growth factor (VEGF) pathway gene expression. Beta-catenin also activates transforming growth factor beta (TGF-β) and bone morphogenetic proteins (BMPs) signaling, involved in endothelial-to-mesenchymal transition (EndMT) [18]. EndMT includes several events that lead endothelial cells to express mesenchymal and stem-cell-like phenotype, as loss of cell-cell junctions and increased migratory capacity, and is a key event in CCM development. KRIT1 loss of function leads to EndMT due to a TGF-β and BMP6 increased expression [19], mediated by both β-catenin and Notch signaling. Notch3 activity is inhibited by KRIT1 deficiency, leading to increased level of KLF4 and, consequently, of BMP6 in endothelial cells, and to an altered control of angiogenetic process in pericytes. In pericytes, endothelial-Notch3 signaling results in expression of proteins involved in maintaining of capillaries architecture, like platelet-derived growth factors (PDGF) family members [20]. This paracrine communication points out the close interconnection existing between endothelial and glial cells in the central nervous system (CNS). KRIT1 also interacts with the other two CCM proteins, malcavernin, and programmed cell death 10, encoded by *CCM2* and *PDCD10*, respectively. They form a ternary complex that localizes at intracellular side of plasma membrane and contributes to junction integrity maintenance [21]. In this ternary complex, malcavernin acts as bridge allowing interaction between KRIT1 and PDCD10. In its structure, malcavernin contains a phosphotyrosine binding domain by which binds KRIT1, short LD motifs and a C-terminal harmonin–homology domain [22]. Malcavernin was identified as a scaffold protein that regulates p38 MAPK pathway interacting with MEKK3, in response to osmotic stress [23]. This interaction results in MEKK3 inhibition. Increased phosphorylation of ERK5 and expression of RhoA result by *CCM2* deficiency, and promote cell survival and stress fibers formation. In physiological conditions, malcavernin encourages RhoA degradation promoting its bond with SMURF1 [24]. Recently, atrial natriuretic peptide was described as inhibitor of RhoA activity by phosphorylation of myosin light chain,

and particularly, this event is allowed by p21-activated kinase 4 (PAK4) and malcavernin, resulting in enhancement of blood-brain barrier properties [25]. Expression of the *CCM2* paralog, named *CCM2L*, was reported in endothelial cells during angiogenesis, and it seems to be involved in cardiovascular development. Although CCM2L gene functions are still not understood, its overexpression was observed in *CCM2* knockout mice, suggesting its possibility to replace the deficit of malcavernin [26]. PDCD10, the third protein involved in CCM pathogenesis, is also known as *TFAR15* (TF-1 cell apoptosis-related gene 15) for its pro-apoptotic activity, mediated by caspase-3 [27]. It contains an N-terminal dimerization domain and a C-terminal Focal Adhesion Targeting-Homology domain for interactions with malcavernin and paxillin [28]. Many controversial hypotheses regard the mechanism by which PDCD10 regulates apoptosis. In physiological conditions, PDCD10 localizes in cytoplasm where it acts as anti-apoptotic protecting endothelial cells by PDPK-1/AKT pathway [29]. Increased apoptosis, instead, results from the PDCD10 down-regulation, probably related to its association with the protein phosphatase 2A (PP2A). Also, depletion of *PDCD10* leads to EndMT due to over-expression of β-catenin and TGF-β/BMP pathway amplification [19]. Moreover, *PDCD10* regulates angiopoietin 2 (ANGPT-2) exocytosis by endothelial cells towards UNC13B and VAMP-3; increased *ANGPT-2* expression due to loss of *PDCD10* causes destabilization of endothelial cell junctions, enlarged lumen formation, and endothelial cell-pericyte dissociation [30]. At embryo stage, CCM lesions can arise due to aberrant apoptosis that leads to an imbalance between endothelial and glial cells at neurovascular unit (NVU). *PDCD10* knock-down in endothelial cells determines both a gain in proliferation and survival of astrocytes and a remodeling of capillaries that take a CCM-like phenotype [31]. Moreover, PDCD10 forms a complex with the three Germinal Centre Kinase III (GCKIII) proteins, Mst4, STK24, and STK25, involved in regulation of cell cycle, cytoskeleton, and Golgi apparatus. Under stress conditions, cellular survival/apoptosis switch is related to differential interaction between PDCD10 and the different proteins of GCKIII. Reactive oxygen species, instead, seem to be related with severity of CCM, and their degradation is compromised by the decreased *SOD2* expression, observed after *KRTI1* depletion [32]. Moreover, together with GCKIII, striatins, PP2A, and PP2C, PDCD10 forms STRIPAK (STRiatin-interacting phosphatase and kinase) complex, which functions contribute to maintaining of cell polarity [33]. The three CCM proteins, then, show a dualism in their functions; they act in synergy by forming a ternary complex involved in stabilization and maintaining of adherens junctions and focal adhesions, and, at the same time, each of them takes part to different molecular pathways. However, all these signaling converge on angiogenesis regulation through endothelial architecture maintaining both in physiological and under oxidative stress conditions. Patients affected by familial forms with no germline or somatic CCM genes mutations were reported [34]. Therefore, involvement of still undiscovered causative genes or alterations in normal expression patterns of the three CCM genes are the hypotheses being considered. Epigenetic phenomena or presence of variants at CCM gene promoters is also considered in pathogenesis of sporadic lesions, since more than 96% of affected harbors no mutations in CCM genes. In this contest, we previously reported protective role of two SNPs at *PDCD10*/*SERPINI1* bidirectional promoter in CCM development [35]. Down-expression of phosphatase and tensin homolog (PTEN) gene was reported in endothelial cell cultures derived from CCM [36], and D allele of angiotensin-converting enzyme (ACE) was considered a potential risk factor [37]. In vitro studies performed on endothelial tissues isolated from sporadic CCM lesions have shown an increased *VEGF* and *VEGFR2* expression in CCM-derived endothelia. *VEGFR2*, regulated by PDCD10 [38], promotes cell proliferation, migration, and sprouting, contrary to *VEGFR1* that has antagonist effects and resulted down-regulated in the same study. CCM genes are ubiquitously expressed overall during early stage of development; however, lesions affect mainly CNS also in patient carrying germ-line mutations. Based on this evidence, deficits of paracrine communication between NVU cells are the key event that can trigger CCM development. Moreover, hypothesis of involvement of modifier genes may explain the not uncommon variable expressivity of CCM also between consanguineous or patients carrying the same causative mutations.

Arteriovenous malformations

General features

AVMs are lesions affecting brain vasculature, characterized by the presence of mature vessel wall elements and the direct transition from arteries to veins, without a capillaries bed. Involved vessels are dilated and tangled and form a nidus which rupture represents the main severe outcome. ICH appears in about 50% of affected and is the most frequent clinical manifestation, occurring often at young age. Other symptoms include seizures, psychiatric disorders, dizziness, neurological deficits, and headaches [39]. Rupture risk is also increased by failed dissipation of arterial pressure that, in addition, leads to hypertrophy of muscular layer, hyalinization and vessels dilatation. Particularly, deficit of smooth muscle cells (SMCs), as well as the increased collagen III/I ratio, impairs vessel response to hemodynamic stress [40]. Close to the nidus, feeding arteries show the same features of normal mature vessels, but draining veins appear arterialized with segmental loss of the internal elastic membrane; pericytes are also reduced. Lesions can be focal, with no cerebral parenchyma

involvement, or diffused that show trapped brain portions into the vessels glomerulus. Fluorangiography is performed for neuro-radiological confirmation and allows to discriminate three types of AVM lesions: micro AVMs (< 1 cm), regular AVMs (< 3 cm), and arteriovenous fistulas with no nidus. AVM frequency is estimated to be 0.6% with no sex differences and usually occur sporadically [41]. However, they can be included in the hereditary autosomal dominant Rendu-Osler-Weber syndrome, also known as hereditary hemorrhagic telangiectasia (HHT, OMIM #187300) affecting multi-organ blood vessels. HHT is characterized by co-existence of both telangiectasies and AVM. Telangiectasies are dilatations involving post-capillary venules of mucosaand skin, while AVMs appear at solid organ as the brain, lung, or liver. HHT has an average incidence of 1/8000 despite this value that may be higher in specific geographical area due to founder mutations [42]. Clinical diagnosis is based on four Curaçao criteria that include (i) the presence of spontaneous and recurrent epistaxis, (ii) mucocutaneoustelangiectasias, (iii) visceral AVM, and (iv) certain HHT diagnosis in a first degree relative, based on the same criteria [43]. Only few cases of familial brain AVM without telangiectasia were reported in literature [44].

Molecular bases of AVM/HHT

Sporadic AVM shows the same features of hereditary ones usually observed in HHT syndromes. Genetic bases of sporadic AVM are still unknown; however, in vitro and in vivo studies showed alterations at several pathways controlling angiogenesis and angio-architecture maintenance. Moreover, single nucleotide polymorphisms (SNPs) at genes involved in arterial and venous differentiation and in inflammatory response may be considered susceptibility factors predisposing to an increased risk of developing lesions [45]. AVM arises due to impaired arteries or vein differentiation during early angiogenesis consequent to imbalanced Ephrin proteins, particularly EFNB2 and EPHB4. Loss of vein differentiation results from the *EFNB2* over-expression following Hedgehog (Hh)-VEGF-Notch signaling activation [46]. Alteration of arterial and venous marker profiles in affected vessels derives from impaired Notch signal and, particularly, venous markers FLT4 and EPHB4 are expressed in arteries, following Notch down-regulation due to *ACVRL1* ablation [47]. Together with VEGF pathway, also *PDGF-B* contributes to correct angiogenesis recruiting pericytes that maintain integrity of capillary endothelium [48]. However, also increased level of PDGF-B was detected in some human AVM tissues. Besides molecules involved in vascular maintenance, also proteins involved in extra-cellular matrix (ECM) remodeling can cause angiogenesis impairment as well as metalloproteinases (MMPs). *MMP-9* appears over-expressed in AVM as consequence of VEGF-ANGPT-2 signaling perturbation [49]. In addition, pro-inflammatory cytokines act promoting MMP expression [50]. SNPs at ILs genes are considered susceptibility genetic factors that can increase lesion development risk following exposure to environmental condition as chronic hypoxia, hormonal fluctuation, and hemodynamic stress. Prolonged exposure to risk factors may continuously promote vascular remodeling leading to sporadic AVM development at adulthood, probably due to induction of epigenetic modifications. Hypoxic condition, especially, stimulate hypoxia-inducible factor 1 (HIF-1) and, then, NF-κB–VEGF pathway [51]. Germ-line mutations at four genes, instead, cause familial HHT. Six different HHT forms were reported, and they differ for causative gene, phenotype, expressivity, and severity. HHT1 and HHT2 arise due to mutations at Endoglin (*ENG*, Entrez Gene: 2022; 9q34.11) and activing A receptor type II-like kinase 1 (*ACVRL1*, also named *ALK1*, Entrez Gene: 94; 12q13.13) genes, respectively. HHT3 and HHT4 are clinically defined, but genetic causes are still unknown. Mutations at *GDF2* (also known as BMP9, Entrez Gene: 2658; 10q11.22) lead to a HHT-like phenotype called HHT5. HHT may co-exist with juvenile polyposis (JPHT, OMIM #175050) following mutations at *SMAD4* (Entrez Gene: 4089; 18q21.2) [52]. All these genes are involved in TGF-β transduction pathway; particularly, *ENG* and *ACVRL1* encode for TGF-β type III and type I receptors, and their signals are involved in regulation of both activation and resolution of angiogenetic phases [53]. Down-stream cascades include modulation of cell cycle, differentiation and apoptosis. In HHT, AVMs show imbalanced phases with higher predominance of activation one causing ECM remodeling, sprouting, and increased vessel permeability. In this cascade, BMP9 is a ligand of Endoglin and ACVRL1, and SMAD4 is the last target gene that forms a heterotrimeric complex with SMAD2 and SMAD3 translocating into the nucleus and binding AP1 sites, with consequent activation of target gene expression [54]. Surprisingly, a novel missense mutation in *ACVRL1* was recently described in three consanguineous patients affected by familial brain AVM without HHT [55]. Finally, mutations in *RASA1* (Entrez Gene: 5921; 5q14.3) and *EPHB4* (Entrez Gene: 2050; 7q22.1) [56] were detected in patients affected by mixed capillary malformations (CM)-AVM (CMAVM, OMIM #608354), recently described syndrome in which multiple capillary malformations affect the skin, face, and brain becoming symptomatic already during the first age of life [57].

CCM vs AVM

CCM and AVM are two well-distinguished vascular dysfunctions involving overall brain capillaries and resulting from impaired vasculogenesis, angiogenesis, and maintaining of endothelial cells properties. Vessels homeostasis results from balanced pro-angiogenetic and anti-angiogenetic signaling. At

CNS, specifically, correct vascular development derives from a dense communication network between different NVU cell types. Pericytes secrete TGF-β and Sonichedgehog (Shh) protein, acting in a paracrine manner on endothelial cells; shh is essential at embryo stage for correct neural tube, somites, and limb development, while in adults controls stem cells proliferations [58]. Both CCM and AVM lack of pericytes. This may explain as mutations in glial cells can lead to endothelial disfunctions, emphasizing the reason why germ-line mutation carriers often develop vascular lesions only at CNS. Both lesions types exhibit common pathogenic molecular mechanisms, especially regarding VEGF signaling. Particularly, mutations at genes involved in TGF-β/BMP signaling lead to AVM development as well as the same pathway causes EndMT, a key event in CCM progression. Several studies focused on comparison of pathological tissues isolated from both lesions in order to establish differential expression patterns of angiogenetic markers. The main difference between CCM and AVM concerns the presence of mature vessel wall, only observed in AVM. In this context, a dysfunction of vascular smooth muscle cells (VSMC) was evaluated. VSMCs show different markers related to the differentiation grade; Uranishi et al. [59] studied expression of α-Smooth muscle actin (*α-SMA*), myosin heavy chain 1 and 2 (*SM1*, *SM2*), and smoothelin (*SMTN*) in both pathological tissues showing similar expression profiles, and particularly, they reported no differences in α-SMA levels, and decreased levels of SM and SMNT in CCM and AVM, respectively. Myosin heavy chain is usually expressed in the more differentiate VSMC contractile phenotype, and its absence in CCM cultures indicates the absence of mature wall elements; *SMNT* is down-expressed in AVM indicating a loss of contractile property peculiar of these lesions. Another study [60] highlighted differential expression pattern of the most angiogenetic factors in CCM and AVM, showing higher levels of both VEGF and PDGF in these lesions compared to normal endothelial tissues. Particularly, AVM specimens were characterized by a more increased expression of *PDGF-A*, while *VEGF*, *PDGF-B*, and *PDGFR-β* were more expressed in CCM tissues. These data suggest that *PDGF-B* can contribute to CCM progression by stimulating endothelial proliferation towards Ras pathway, while, in AVM high levels of *PDGF-A*, involved in vessel remodeling, result from hemodynamic stress. Likewise, integrins have different expression profiles and αvβ1 integrin is more represented in AVM tissues, while αvβ3 and αvβ5 are expressed mostly in CCMs. These observations are in accordance with the different maturation grade of endothelial cells in these lesions; αvβ1 integrin is physiologically expressed in both early and in maturation phases and its higher level in AVM indicates the presence of mature wall elements. Conversely, major αvβ3 and αvβ5 integrin expression detected in CCM indicates the persistence of early angiogenetic stimuli [61]. All these data suggest that, globally, CCM and AVM arise due to angiogenetic process imbalance; however, in the first case, immature vessel wall results from altered early angiogenesis signaling while AVM forms following response to hemodynamic stress by mature endothelium. Phenotype severity may be influenced by additional modifier genetic factors including SNPs at pro-inflammatory chemokines and immune response proteins. As previously described for AVM, also in CCM, polymorphisms at *IL-4*, *IL-6R*, *CD14*, and *MSR1* genes are liked with more aggressive lesions; likewise, *TGFBR2* rs9823731 is associated with development of a large number of big lesion, with an increased ICH risk due to endothelial cell activation [62].

Co-existence of CCM and AVM lesions was rarely described but hypothesis of a common pathogenic mechanism has not yet been proven. However, an immunostaining performed on both specimens dissected from the same patient showed a comparable expression profile for *VEGF*, *FGF*, and laminin, while different expression patterns were detected for *α-SMA* and collagen IV, confirming the intense angiogenetic activity of endothelium [63]. Mixed CCM–AVM lesions were also reported, although patients have not undergone genetic analysis [64]. Table 1 summarizes the main genes linked to both familial and sporadic cerebrovascular malformations here examined. Cerebrovascular malformations are usually considered monogenic conditions, and their molecular diagnosis is based on mutational analysis performed on causative genes. However, potential pathogenic role of several SNPs remains controversial. Considering our patients cohort, among familial CCM cases, about 35% shows no mutations at the three CCM loci and whole exome sequencing (WES) is underway. About AVMs, WES analysis allowed to detect 11 novel candidate genes and data are being validated. NGS technology may represent the most valid approach both during research phase and for diagnostic application. Genetic classification was already proposed for several monogenic diseases [65] as retinal dystrophies and neurodegenerative disorders, also based on small cohorts of patients. About cerebrovascular malformations, their incidence is underestimated due to incomplete penetrance and phenotype's spectra are often coincident, so WES of large number of samples is required to better standardize diagnostic criteria based on genotypes and for select further causative genes. Therefore, targeted panel for research application could result incomplete due to high number of genes involved in vasculogenesis and angiogenetic pathway. About molecular diagnosis, the main controversy is related to assignment of clinical significance at SNPs leading to missense substitution that show a higher frequency in patient cohort. Valid examples are rs11542682 and rs11552377 affecting KRTI1 and CCM2 genes, respectively, that recently were included in Human Genome Mutation Database (HGMD®) as causative of CCM. Since NGS development, its major limitation was related to false results inherent copy number variants (CNV) detection that, although

Table 1 Genes and risk factors associated to the main classes of cerebrovascular malformations

Malformation syndrome			Causative loci	Susceptibility genetic factors	Pathway
AVM	Associated to familial HHT	HHT1	ENG		TGF-β/BMPs HIF-VEGF PDGFB/PDGFβ2 signaling
		HHT2	ACVRL1		
		HHT3	Not detected		
		HHT4	Not detected		
		HHT5	GDF2		
		JP-HHT	SMAD4		
	Sporadic			IL6, IL8, IL1B, TNFA	Inflammation TGF-β/BMPs VEGF-ANG-2 signaling
CCM	Sporadic			PTEN, IL-6, IL-6R, IL-4, TGFBR2, ANGPT2	VEGF-Notch Inflammation
	Familial		KRIT1 CCM2 PDCD10	ACE	β-Integrins signaling β-Catenin/Wnt signaling Notch3-PDGFβ2 TGF-β/BMPs p38 MAPK cascade TrkA signaling Caspase 3- mediated apoptosis VEGFR2 signaling
CM-AVM			RASA1 EPHB4		MAPK, NGF-TrkA Hh-VEGF-Notch
CMC			GNAQ		Cell adhesion/integrins signaling FAK signaling

For AVM and CCM, the two different etiologies (sporadic and familial) are considered. Together with causative loci (third column) and genetic susceptibility factors (fourth column), pathways in which they are involved were considered (fifth column). Details are discussed in the text

rarely, also can contribute to cerebrovascular malformations [66]. However, recently, more approaches were proposed and resulted efficient to solve reliability concerning CNV [67].

Moreover, another critical point is related to patients affected by sporadic forms and negative for germ-line causative mutations. Difficulty of their genetic diagnosis management is related to further two different aspects as somatic mutations and alterations in angiogenetic-related genes expression, as revealed by studies performed on lesions specimens. Therefore, parallel to mutational analysis, also a panel for promoter methylation status should be validated.

The rapid increase of genomic data available could be a very precious resource both for researchers and for clinicians. In the era of Omics sciences, a tight collaboration between neurologists, neuro-radiologists, neurosurgeons, and geneticists can result both in a more accurate diagnosis and in the possibility to personize the therapy. This modern approach is beginning to apply also in other complex and heterogeneous diseases as muscular dystrophies [68].

Conclusions

Cerebrovascular malformations represent an important cause of ICH and usually occur due to imbalanced proliferation/apoptosis of endothelial cells. Here, we reviewed the main differences and analogies between the most diffuse congenital vascular malformation, CCM and AVM. To date, therapeutic strategies for their treatment include inhibitors of key angiogenesis regulator such as RhoA or ROCK and VEGF, for CCM and AVM, respectively. However, several pathogenic mechanisms still have to be discovered. Surgical treatment can be also considered for removal of lesions that not affect critical cerebral areas, as brain stem or pontine region. Although these strategies allow to improve patients' outcome, knowledge today available lack of genotype-phenotype correlations data. Patients' classification in relation to their allelic settings could be useful to better manage prevention and prognosis, as recidivism risk, also for CCM and AVM sporadic cases. About CCM patient management, we previously reported prognostic validity of several CCM gene SNPs resulted associated to an increased probability of disabling outcome [69, 70] as well as ACVRL1 haplotypes resulted protective in AVM development [71].

Therefore, a more clear classification criterion based on molecular genetic tests could help neuro-radiologists in differential diagnosis, especially for lesions easily misunderstood. In this contest, development of a customized panel for next-generation sequencing analysis including causative/associate genes for cerebrovascular malformations could represent the diagnostic gold standard.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

The author declares that the manuscript has not been submitted to more than one journal for simultaneous consideration and has not been published previously.

All the authors read and approved the final version of the manuscript.

Human and animal rights The manuscript is a collection of literature data and does not involve animals or human participants.

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Gault J, Sarin H, Awadallah NA, Shenkar R, Awad IA (2004) Pathobiology of human cerebrovascular malformations: basic mechanisms and clinical relevance. Neurosurgery 55:1–16
2. Català A, Roé E, Vikkula M, Baselga E (2013) Capillary malformation-arteriovenous malformation syndrome: a report of 2 cases, diagnostic criteria, and management. Actas Dermosifiliogr 104:710–713
3. Brugulat-Serrat A, Rojas S, Bargalló N, Conesa G, Minguillón C, Fauria K, Gramunt N, Molinuevo JL, Gispert JD (2017) Incidental findings on brain MRI of cognitively normal first-degree descendants of patients with Alzheimer's disease: a cross-sectional analysis from the ALFA (Alzheimer and families) project. BMJ Open 7: e013215
4. Scimone C, Bramanti P, Alafaci C, Granata F, Piva F, Rinaldi C, Donato L, Greco F, Sidoti A, D'Angelo R (2017) Update on novel CCM gene mutations in patients with cerebral cavernous malformations. J Mol Neurosci 61:189–198
5. Spiegler S, Rath M, Paperlein C, Felbor U (2018) Cerebral cavernous malformations: an update on prevalence, molecular genetic analyses, and genetic counselling. Mol Syndromol 9:60–69
6. Riant F, Bergametti F, Ayrignac X, Boulday G, Tournier-Lasserve E (2010) Recent insights into cerebral cavernous malformations: the molecular genetics of CCM. FEBS J 277:1070–1075
7. Haasdijk RA, Cheng C, Maat-Kievit AJ, Duckers HJ (2012) Cerebral cavernous malformations: from molecular pathogenesis to genetic counselling and clinical management. Eur J Hum Genet 20:134–140
8. Sirvente J, Enjolras O, Wassef M, Tournier-Lasserve E, Labauge P (2009) Frequency and phenotypes of cutaneous vascular malformations in a consecutive series of 417 patients with familial cerebral cavernous malformations. J Eur Acad Dermatol Venereol 23:1066–1072
9. Shenkar R, Shi C, Rebeiz T, Stockton RA, McDonald DA, Mikati AG, Zhang L, Austin C, Akers AL, Gallione CJ, Rorrer A, Gunel M, Min W, De Souza JM, Lee C, Marchuk DA, Awad IA (2015) Exceptional aggressiveness of cerebral cavernous malformation disease associated with PDCD10 mutations. Genet Med 17:188–196
10. Brinjikji W, El-Masri AE, Wald JT, Flemming KD, Lanzino G (2017) Prevalence of cerebral cavernous malformations associated with developmental venous anomalies increases with age. Childs Nerv Syst 33:1539–1543
11. De Souza JM, Domingue RC, Cruz LC Jr, Domingues FS, Iasbeck T, Gasparetto EL (2008) Susceptibility-weighted imaging for the evaluation of patients with familial cerebral cavernous malformations: a comparison with t2-weighted fast spin-echo and gradient-echo sequences. AJNR Am J Neuroradiol 29:154–158
12. Nagpal K, Prakash S (2018) An unusual presentation of superficial siderosis with focal dystonia and 'Giant panda morphology' on MRI: atypical clinicoradiological amalgam. Neurol Sci 1–4. https://doi.org/10.1007/s10072-018-3650-5
13. Akers A, Al-Shahi Salman R, Awad IA, Dahlem K, Flemming K, Hart B, Kim H, Jusue-Torres I, Kondziolka D, Lee C, Morrison L, Rigamonti D, Rebeiz T, Tournier-Lasserve E, Waggoner D, Whitehead K (2017) Synopsis of guidelines for the clinical management of cerebral cavernous malformations: consensus recommendations based on systematic literature review by the angioma alliance scientific advisory board clinical experts panel. Neurosurgery 80:665–680
14. Hauptman JS, Moftakhar P, Dadour A, Malkasian D, Martin NA (2010) Advances in the biology of cerebral cavernous malformations. SurgNeurolInt 1:63
15. Zawistowski JS, Serebriiskii IG, Lee MF, Golemis EA, Marchuk DA (2002) KRIT1 association with the integrin-binding protein ICAP-1: a new direction in the elucidation of cerebral cavernous malformations (CCM1) pathogenesis. Hum Mol Genet 11:389–396
16. Faurobert E, Rome C, Lisowska J, Manet-Dupé S, Boulday G, Malbouyres M, Balland M, Bouin AP, Kéramidas M, Bouvard D, Coll JL, Ruggiero F, Tournier-Lasserve E, Albiges-Rizo C (2013) CCM1-ICAP-1 complex controls β1 integrin-dependent endothelial contractility and fibronectin remodelling. J Cell Biol 202:545–561
17. Glading AJ, Ginsberg MH (2010) Rap1 and its effector KRIT1/CCM1 regulate beta-catenin signalling. Dis Model Mech 3:73–83
18. Bravi L, Malinverno M, Pisati F, Rudini N, Cuttano R, Pallini R, Martini M, Larocca LM, Locatelli M, Levi V, Bertani GA, Dejana E, Lampugnani MG (2016) Endothelial cells lining sporadic cerebral cavernous malformation cavernomas under goendothelial-to-mesenchymal transition. Stroke 47:886–890
19. Cuttano R, Rudini N, Bravi L, Corada M, Giampietro C, Papa E, Morini MF, Maddaluno L, Baeyens N, Adams RH, Jain MK, Owens GK, Schwartz M, Lampugnani MG, Dejana E (2016) KLF4 is a key determinant in the development and progression of cerebral cavernous malformations. EMBO Mol Med 8:6–24
20. Schulz GB, Wieland E, Wüstehube-Lausch J, Boulday G, Moll I, Tournier-Lasserve E, Fischer A (2015) Cerebral cavernous Malformation-1 protein controls DLL4-Notch3 signaling between the endothelium and Pericytes. Stroke 46:1337–1343
21. Draheim KM, Fisher OS, Boggon TJ, Calderwood DA (2014) Cerebral cavernous malformation proteins at a glance. J Cell Sci 127:701–707
22. Fisher OS, Zhang R, Li X, Murphy JW, Demeler B, Boggon TJ (2013) Structural studies of cerebral cavernous malformations 2 (CCM2) reveal a folded helical domain at its C-terminus. FEBS Lett 587:272–277
23. Fong B, Watson PH, Watson AJ (2007) Mouse preimplantation embryo responses to culture medium osmolarity include increased expression of CCM2 and p38 MAPK activation. BMC Dev Biol 7: 2
24. Crose LE, Hilder TL, Sciaky N, Johnson GL (2009) Cerebral cavernous malformation 2 protein promotes smad ubiquitin regulatory factor 1-mediated RhoA degradation in endothelial cells. J Biol Chem 284:13301–13305
25. Miura K, Nojiri T, Akitake Y, Ando K, Fukuhara S, Zenitani M, Kimura T, Hino J, Miyazato M, Hosoda H, Kangawa K (2017) CCM2 and PAK4 act downstream of atrial natriuretic peptide signaling to promote cell spreading. Biochem J 474:1897–1918

26. Rosen JN, Sogah VM, Ye LY, Mably JD (2013) Ccm2-like is required for cardiovascular development as a novel component of the Heg-CCM pathway. Dev Biol 76:74–85
27. Chen L, Tanriover G, Yano H, Friedlander R, Louvi A, Gunel M (2009) Apoptotic functions of PDCD10/CCM3, the gene mutated in cerebral cavernous malformation 3. Stroke 40:1474–1481
28. Li X, Zhang R, Zhang H, He Y, Ji W, Min W, Boggon TJ (2010) Crystal structure of CCM3, a cerebral cavernous malformation protein critical for vascular integrity. J Biol Chem 285:24099–24107
29. Schleider E, Stahl S, Wüstehube J, Walter U, Fischer A, Felbor U (2011) Evidence for anti-angiogenic and pro-survival functions of the cerebral cavernous malformation protein 3. Neurogenetics 12: 83–86
30. Jenny Zhou H, Qin L, Zhang H, Tang W, Ji W, He Y, Liang X, Wang Z, Yuan Q, Vortmeyer A, Toomre D, Fuh G, Yan M, Kluger MS, Wu D, Min W (2016) Endothelial exocytosis of angiopoietin-2 resulting from CCM3 deficiency contributes to cerebral cavernous malformation. Nat Med 22:1033–1042
31. Louvi A, Chen L, Two AM, Zhang H, Min W, Günel M (2011) Loss of cerebral cavernous malformation 3 (Ccm3) in neuroglia leads to CCM and vascular pathology. Proc Natl Acad Sci U S A 108:3737–3742
32. Jung KH, Han DM, Jeong SG, Choi MR, Chai YG, Cho GW (2015) Proteomic analysis reveals KRIT1 as a modulator for the antioxidant effects of valproic acid in human bone-marrow mesenchymal stromal cells. Drug Chem Toxicol 38:286–292
33. Goudreault M, D'Ambrosio LM, Kean MJ, Mullin MJ, Larsen BG, Sanchez A, Chaudhry S, Chen GI, Sicheri F, Nesvizhskii AI, Aebersold R, Raught B, Gingras AC (2009) A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. Mol Cell Proteomics 8: 157–171
34. D'Angelo R, Marini V, Rinaldi C, Origone P, Dorcaratto A, Avolio M, Goitre L, Forni M, Capra V, Alafaci C, Mareni C, Garrè C, Bramanti P, Sidoti A, Retta SF, Amato A (2011) Mutation analysis of CCM1, CCM2 and CCM3 genes in a cohort of Italian patients with cerebral cavernous malformation. Brain Pathol 21:360
35. Scimone C, Bramanti P, Ruggeri A, Donato L, Alafaci C, Crisafulli C, Mucciardi M, Rinaldi C, Sidoti A, D'Angelo R (2016) CCM3/SERPINI1 bidirectional promoter variants in patients with cerebral cavernous malformations: a molecular and functional study. BMC Med Genet 17:74
36. Kar S, Samii A, Bertalanffy H (2015) PTEN/PI3K/Akt/VEGF signaling and the cross talk to KRIT1, CCM2, and PDCD10 proteins in cerebral cavernous malformations. Neurosurg Rev 38:229–236
37. Altas M, Bayrak OF, Cerci A, Isik N, Celik M, Culha M, Sahin F, Elmaci I (2010) Angiotensin-converting enzyme insertion/deletion gene polymorphism in patients with familial multiple cerebral cavernous malformations. J Clin Neurosci 17:1034–1037
38. Zhu Y, Wu Q, Fass M, Xu JF, You C, Müller O, , Sandalcioglu IE, Zhang JM, Sure U (2011) In vitro characterization of the angiogenic phenotype and genotype of the endothelia derived from sporadic cerebral cavernous malformations. Neurosurgery 69:722–732
39. Chen IH, Lin YH, Wu MN, Lai CL, Liou LM (2018) Cerebral arteriovenous malformation presenting as isolated bilateral pupil-sparing oculomotor, pseudoabducens palsy, and hemiataxia. NeurolSci 39:1289–1290
40. Zhang R, Zhu W, Su H (2016) Vascular integrity in the pathogenesis of brain arteriovenous malformation. ActaNeurochirSuppl 121: 29–35
41. Thomas JM, Surendran S, Abraham M, Rajavelu A, Kartha CC (2016) Genetic and epigenetic mechanisms in the development of arteriovenous malformations in the brain. Clin Epigenetics 8:78
42. Brinjikji W, Iyer VN, Wood CP, Lanzino G (2017) Prevalence and characteristics of brain arteriovenous malformations in hereditary hemorrhagic telangiectasia: a systematic review and meta-analysis. J Neurosurg 127:302–310
43. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H (2000) Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 91:66–67
44. van Beijnum J, van der Worp HB, Schippers HM, van Nieuwenhuizen O, Kappelle LJ, Rinkel GJ, Berkelbach van der Sprenkel JW, Klijn CJ (2007) Familial occurrence of brain arteriovenous malformations: a systematic review. J Neurol Neurosurg Psychiatry 78:1213–1217
45. Komiyama M (2016) Pathogenesis of brain arteriovenous malformations. Neurol Med Chir (Tokyo) 56:317–325
46. Shi S, Sun J, Meng Q, Yu Y, Huang H, Ma T, Yang Z, Liu X, Yang J, Shen Z (2018) Sonic hedgehog promotes endothelial differentiation of bone marrow mesenchymal stem cells via VEGF-D. J Thorac Dis 10:5476–5488
47. Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, Weinstein BM (2001) Notch signaling is required for arterial-venous differentiation during embryonic vascular development. Development 128:3675–3683
48. Hashimoto T, Wu Y, Lawton MT, Yang GY, Barbaro NM, Young WL (2005) Co-expression of angiogenic factors in brain arteriovenous malformations. Neurosurgery 56:1058–1065
49. Hashimoto T, Wen G, Lawton MT, Boudreau NJ, Bollen AW, Yang GY, Barbaro NM, Higashida RT, Dowd CF, Halbach VV, Young WL, University of California, San Francisco BAVM Study Group (2003) Abnormal expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in brain arteriovenous malformations. Stroke 34:925–9231
50. Mouchtouris N, Jabbour PM, Starke RM, Hasan DM, Zanaty M, Theofanis T, Ding D, Tjoumakaris SI, Dumont AS, Ghobrial GM, Kung D, Rosenwasser RH, Chalouhi N (2015) Biology of cerebral arteriovenous malformations with a focus on inflammation. J Cereb Blood Flow Metab 35:167–175
51. Ng I, Tan WL, Ng PY, Lim J (2005) Hypoxia inducible factor-1alpha and expression of vascular endothelial growth factor and its receptors in cerebral arteriovenous malformations. J Clin Neurosci 12:794–799
52. McDonald J, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P (2015) Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. Front Genet 6:1
53. Blanco FJ, Santibanez JF, Guerrero-Esteo M, Langa C, Vary CP, Bernabeu C (2005) Interaction and functional interplay between endoglin and ALK-1, two components of the endothelial transforming growth factor-beta receptor complex. J Cell Physiol 204:574–584
54. Tillet E, Bailly S (2015) Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. Front Genet 5:456
55. Yılmaz B, Toktaş ZO, Akakın A, Işık S, Bilguvar K, Kılıç T, Günel M (2017) Familial occurrence of brain arteriovenous malformation: a novel ACVRL1 mutation detected by whole exome sequencing. J Neurosurg 126:1879–1883
56. Yu J, Streicher JL, Medne L, Krantz ID, Yan AC (2017) EPHB4 mutation implicated in capillary malformation-arteriovenous malformation syndrome: a case report. PediatrDermatol 34:e227–e230
57. Lapinski PE, Doosti A, Salato V, North P, Burrows PE, King PD (2017) Somatic second hit mutation of RASA1 in vascular endothelial cells in capillary malformation-arteriovenous malformation. Eur J Med Genet S1769–7212:30256–30262
58. Luissint AC, Artus C, Glacial F, Ganeshamoorthy K, Couraud PO (2012) Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids Barriers CNS 9:23

59. Uranishi R, Baev NI, Kim JH, Awad IA (2001) Vascular smooth muscle cell differentiation in human cerebral vascular malformations. Neurosurgery 49:671–679
60. Yildirim O, Bicer A, Ozkan A, Kurtkaya O, Cirakoglu B, Kilic T (2010) Expression of platelet-derived growth factor ligand and receptor in cerebral arteriovenous and cavernous malformations. J Clin Neurosci 17:1557–1562
61. Seker A, Yildirim O, Kurtkaya O, Sav A, Günel M, Pamir NM, Kiliç T (2006) Expression of integrins in cerebral arteriovenous and cavernous malformations. Neurosurgery 58:159–168
62. Choquet H, Pawlikowska L, Nelson J, CE MC, Akers A, Baca B, Khan Y, Hart B, Morrison L, Kim H, Brain Vascular Malformation Consortium (BVMC) Study (2014) Polymorphisms in inflammatory and immune response genes associated with cerebral cavernous malformation type 1 severity. Cerebrovasc Dis 38:433–440
63. Kamiyama H, Nishimura S, Kaimori M, Watanabe M, Furuno Y, Saito A, Nishijima M (2010) Cavernous angioma associated with arteriovenous malformation of the brain. Neurol Med 50:131–134
64. Awad IA, Robinson JR Jr, Mohanty S, Estes ML (1993) Mixed vascular malformations of the brain: clinical and pathogenetic considerations. Neurosurgery 33:179-188
65. Gan D, Li M, Wu J, Sun X, Tian G (2017) Analysis of genetic mutations in a cohort of hereditary optic neuropathy in Shanghai, China. J Ophthalmol 2017:6186052
66. Bendjilali N, Kim H, Weinsheimer S, Guo DE, Kwok PY, Zaroff JG, Sidney S, Lawton MT, McCulloch CE, Koeleman BP, Klijn CJ, Young WL, Pawlikowska L (2013) A genome-wide investigation of copy number variation in patients with sporadic brain arteriovenous malformation. PLoS One 8:e71434
67. Ellingford JM, Horn B, Campbell C, Arno G, Barton S, Tate C, Bhaskar S, Sergouniotis PI, Taylor RL, Carss KJ, Raymond LFL, Michaelides M, Ramsden SC, Webster AR, Black GCM (2018) Assessment of the incorporation of CNV surveillance into gene panel next-generation sequencing testing for inherited retinal diseases. J Med Genet 55:114–121
68. Gawlik KI (2018) At the crossroads of clinical and preclinical research for muscular dystrophy-are we closer to effective treatment for patients? Int J MolSci 19:E1490
69. D'Angelo R, Scimone C, Rinaldi C, Trimarchi G, Italiano D, Bramanti P, Amato A, Sidoti A (2012) CCM2 gene polymorphisms in Italian sporadic patients with cerebral cavernous malformation: a case-control study. Int J Mol Med 29:1113–1120
70. Rinaldi C, Bramanti P, Scimone C, Donato L, Alafaci C, D'Angelo R, Sidoti A (2017) Relevance of CCM gene polymorphisms for clinical management of sporadic cerebral cavernous malformations. J Neurol Sci 380:31–37
71. Ge M, Du C, Li Z, Liu Y, Xu S, Zhang L, Pang Q (2017) Association of ACVRL1 genetic polymorphisms with arteriovenous malformations: a case-control study and meta-analysis. World Neurosurg 108:690–697