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Citation for published version:

Leblanc, GG, Golanov, E, Awad, IA, Young, WL, Biology of Vascular Malformations of the Brain NINDS Workshop Collaborators & Al-Shahi Salman, R 2009, 'Biology of vascular malformations of the brain' Stroke; a journal of cerebral circulation, vol 40, no. 12, pp. e694-702. DOI: 10.1161/STROKEAHA.109.563692

Digital Object Identifier (DOI):

[10.1161/STROKEAHA.109.563692](https://doi.org/10.1161/STROKEAHA.109.563692)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Stroke; a journal of cerebral circulation

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Published in final edited form as:

Stroke. 2009 December ; 40(12): e694–e702. doi:10.1161/STROKEAHA.109.563692.

BIOLOGY OF VASCULAR MALFORMATIONS OF THE BRAIN

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Biology of Vascular Malformations of the Brain NINDS Workshop Collaborators **

Abstract

Background and Purpose—This review discusses recent research on the genetic, molecular, cellular, and developmental mechanisms underlying the etiology of vascular malformations of the brain (VMBs), including cerebral cavernous malformation (CCM), sporadic brain arteriovenous malformation (AVM), and the AVMs of hereditary hemorrhagic telangiectasia (HHT).

Summary of Review—The identification of gene mutations and genetic risk factors associated with CCM, HHT, and sporadic AVM has enabled the development of animal models for these diseases and provided new insights into their etiology. All of the genes associated with VMBs to date have known or plausible roles in angiogenesis and vascular remodeling. Recent work suggests that the angiogenic process most severely disrupted by VMB gene mutation is that of vascular stabilization, the process whereby vascular endothelial cells form capillary tubes, strengthen their intercellular junctions, and recruit smooth muscle cells to the vessel wall. In addition, there is now good evidence that in some cases CCM lesion formation involves a genetic two-hit mechanism, in which a germline mutation in one copy of a CCM gene is followed by a somatic mutation in the other copy. There is also increasing evidence that environmental second hits can produce lesions when there is a mutation to a single allele of a VMB gene.

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Conclusions—Recent findings begin to explain how mutations in VMB genes render vessels vulnerable to rupture when challenged with other inauspicious genetic or environmental factors, and have suggested candidate therapeutics. Understanding of the cellular mechanisms of VMB formation and progression in humans has lagged behind that in animal models. New knowledge of lesion biology will spur new translational work. Several well-established clinical and genetic database efforts are already in place, and further progress will be facilitated by collaborative expansion and standardization of these.

Keywords

vascular malformations; arteriovenous malformation; cerebral hemorrhage; angiogenesis, physiologic; genetics

Vascular malformations of the brain (VMBs) are relatively common lesions that cause serious neurological disability or death in a significant proportion of individuals bearing them. The most common VMBs are arteriovenous malformations (AVMs) and cerebral cavernous malformations (CCMs), with detection rates of approximately 1.1 and 0.6/100,000 adults per year, respectively.¹ These lesions can occur either sporadically or in the context of genetic syndromes (the primary one associated with cerebral AVM is hereditary hemorrhagic telangiectasia, or HHT). Neurological symptoms associated with VMBs include hemorrhagic stroke due to lesion rupture, epilepsy, and focal neurological defects.

The last few years have seen considerable progress in unraveling the etiology of VMBs. The identification of gene mutations and genetic risk factors associated with CCM, HHT, and sporadic AVM has enabled the development of animal models for these diseases. Research also gained momentum from new findings on mechanisms of developmental angiogenesis and interactions among cells of the neurovascular unit. This review focuses on the biology of CCM and AVM pathogenesis, but there are conceptually similar issues that apply to related brain vascular anomalies including leptomeningeal angiomas of Sturge-Weber Syndrome, dural arteriovenous fistula, capillary malformations, and the mixed types that are sometimes encountered. Discovery and dissemination of new knowledge concerning basic lesion biology can spur new translational research, leading to novel biomarkers for risk stratification and prognostication and novel treatments. Importantly, understanding these pathological states may also provide fresh insights into the basic biology of the cerebral vasculature.

Cellular and Molecular Interactions Controlling Angiogenesis

The development of the vasculature occurs in two stages: vasculogenesis (*de novo* blood vessel formation during embryogenesis) and angiogenesis (the growth of new blood vessels from pre-existing ones). Vasculogenesis of the cerebral vasculature occurs outside the brain, with the formation of the perineural plexus. Capillaries sprout from this plexus and penetrate the neural tube in a characteristic spatiotemporal pattern.² Subsequent growth of the cerebral vasculature occurs entirely by angiogenesis, the first phase of which involves vascular endothelial cell proliferation and migration. A key mediator of these processes is vascular endothelial growth factor (VEGF), which is produced by developing neuroectodermal cells and their neural and glial progeny. In response to hypoxia,³ VEGF also up-regulates capillary permeability, and developing capillaries are characterized by relatively high permeability and low levels of inter-endothelial junctional proteins.^{4,5}

The next phase of angiogenesis is vascular stabilization, during which endothelial cells form capillary tubes, strengthen their intercellular junctions, and recruit smooth muscle cells to their walls. Vascular stabilization involves reciprocal interactions between endothelial cells and pericytes, the precursors of vascular smooth muscle cells. Brain pericytes arise from mesoderm

and neural crest,⁶ and accompany capillary sprouts as they penetrate the brain.⁷ Pericyte differentiation and production of extracellular matrix is thought to be triggered by endothelial platelet-derived growth factor-B (PDGF-B) and TGF- β 1.⁸⁻¹⁰ As pericytes differentiate, they act back on the vascular endothelium to suppress capillary sprouting, stimulate wall growth, and promote intercellular junction formation and cell-matrix adhesion.¹⁰ These actions are mediated in part through angiopoietin-1; other mediators include tissue inhibitors of metalloproteinases (TIMPs)¹¹ and ephrin-B2.¹² Loss of pericytes (in PDGF-B deficient mice, for example) leads to vessel dilation, endothelial cell hyperplasia, and microaneurysm.⁹

Brain angiogenesis subsides after birth, but can be reactivated in response to physiological stimuli including exercise,¹³ sensory enrichment,¹⁴ chronic hypoxia,¹⁵ shear stress¹⁶ and certain hormones.¹⁷⁻¹⁸ Dramatic, local up-regulation of angiogenesis also occurs in response to pathological conditions such as tumor, stroke, or trauma.^{3,19} Adult angiogenesis is regulated by some of the same factors (e.g. VEGF and angiopoietins) that regulate developmental angiogenesis, but is also likely to involve unique mechanisms. Capillary sprouting in adulthood requires reactivation of quiescent endothelium and breakdown of previously stabilized vessel walls, and often occurs in the context of inflammation. For example, recent work indicates that endothelial sprouting is induced by different Notch pathway genes during development and inflammation.²⁰

Angiogenesis and VMB Formation

Cellular pathology and natural history of VMBs

VMBs form where capillary endothelium normally lies, at the interface between arterial and venous endothelium, where capillary endothelium normally lies. A CCM is a cluster of dilated, capillary caverns that are low-flow and may contain thrombi (Figure 1a). An AVM is a mass of arteries and veins that appear to fuse without intervening capillaries and form a network of direct, high-flow arteriovenous shunts (Figure 1b). The generally accepted histopathological conception of an AVM is that the nidus lacks a true capillary bed.²¹ However, the existence of dilated perinidal capillaries has long been appreciated, and recent studies suggest that these form a complex system that communicates directly with the nidus.²²

A common assumption has been that VMBs arise during embryonic development, but there is little direct evidence to support this idea. The mean age of presentation is about 34 years for CCM²³ and 40 years for AVM.²⁴ In addition, there is now clear evidence that active growth and *de novo* formation of CCMs and AVMs can occur.²⁵⁻²⁸ At the cellular level, the first step in the formation of both lesion types may be capillary dysplasia.^{29,30} In CCM, observations in mouse suggest that multiple cavernous capillaries can sprout from the initial lesion.³¹ In AVM, it is possible that perinidal capillaries fuse to become part of the nidus.²²

VMB genes and angiogenesis

Most of the VMB genes and genetic risk factors discovered so far (Table 1) have demonstrated roles in vasculogenesis, angiogenesis, and vascular remodeling (formation or regression of vessels within a pre-existing vascular bed). An ongoing problem has been to understand which of these roles are most relevant to lesion formation. An additional conundrum arises from the focal nature of CCM and AVM lesions. Inherited CCM and AVM syndromes result from loss of function of one copy of the relevant gene in *all* of the cells that normally express the gene (e.g., all endothelial cells in the case, for example, of *CCM1*), but lesions are seen only in discrete locations and not throughout the vasculature. This observation has led investigators to propose a genetic “two-hit” mechanism for VMB lesion formation, in which an inherited mutation in one copy of a VMB gene is followed by a somatic mutation in the second copy.³² Alternatively, the second “hit” could be environmental, in the form of a localized

physiological or pathological perturbation. These models and the evidence for them are discussed in more detail in subsequent sections.

CCM—Three CCM genes (*CCM1*, *CCM2*, and *CCM3*) have been identified to date. All three proteins are expressed in vascular endothelium³³ and can bind into a single complex that associates with cytoskeletal and interendothelial junction proteins and components of certain signal transduction pathways (Table 1, Figure 2).³⁴ The activity of the CCM protein complex may be regulated by the transmembrane receptor heart of glass, which is also expressed in vascular endothelium.³⁵ The ligand for this receptor is currently unknown.

Mice and zebrafish with complete loss of *ccm1* or *ccm2* function (e.g., by gene knockout) show profound disruption of vascular development, with severe and progressive dilation of major vessels followed by embryonic death.^{36,37} Mice with loss of a single copy of *ccm2* develop vascular lesions similar to those seen in human CCM.³⁸ Recent work in mouse and zebrafish CCM models suggest that lesions result from defects in endothelial cytoskeletal dynamics and cell-cell adhesion.^{36,37} For example, mouse endothelial cells in which *ccm2* is knocked out *in vitro* show reduced inter-cellular contacts and barrier function, together with failure of the actin redistribution that normally accompanies capillary tube formation. These deficits arise in part from loss of Ccm2 interactions with RhoA, a GTPase that regulates the cytoskeleton.

Ccm2^{+/-} mice (i.e., mice in which a single copy of the *ccm2* gene is disrupted) show greater dermal vascular permeability in response to VEGF treatment *in vivo* than wild type mice. Vascular permeability in *ccm2*^{+/-} and wild type mice did not differ under normal conditions, however. This observation is consistent with a two-hit model of CCM lesion development in which endothelial cells deficient in *ccm2* protein function normally unless challenged with an environmental perturbation. In addition, enhanced vascular permeability may predispose to hemorrhage, a consistent pathological feature of CCM lesions. Interestingly, the *in vivo* dermal hyper-permeability seen in *ccm2*^{+/-} mice was reversed by the statin simvastatin (which inhibits RhoA isoprenylation and association with cell membranes), pointing to a possible CCM therapy.³⁷

All three CCM genes are expressed in CNS neurons and glia as well as in vascular endothelium.^{33,38} However, studies in mice and zebrafish mutants have shown that *ccm1* and *ccm2* are specifically required in endothelial cells for normal vascular development, and that selective knockout of *ccm2* function in neuroglial cells does not lead to obvious cerebrovascular defects.^{36,37,39} Finally, studies in zebrafish have shown that identical cardiovascular phenotypes result from knockdown of *ccm1*, *ccm2*, or *ccm3*, thus providing further evidence that the three genes operate in a single functional pathway.^{36,40}

AVM—HHT types 1 and 2 result from loss-of-function mutations in one copy of the *Endoglin* (*ENG*) and *Activin-like kinase receptor 1* (*ACVRL1*; *ALK-1*) genes, respectively. *ALK-1* variants may also be associated with risk for sporadic AVM.⁴¹ Mutations in a third gene, SMAD 4, were recently described in some cases of combined juvenile polyposis and HHT syndrome.⁴² Alk-1, endoglin, and Smad4 all are components of TGF- β super-family signaling pathways (Table 1, Figure 3).⁴³ Mice in which both copies of any of these genes are knocked out die as embryos. However, mice with mutations in just one copy of *eng*^{44,45} or *Alk-1*⁴⁶ reproduce features of human HHT, including telangiectases and hemorrhage with unpredictable age of onset, severity, and location. Importantly, some of these mice spontaneously develop vascular dysplasias reminiscent of large vessel AVMs.^{47,48}

How do reduced levels of Alk-1 or endoglin lead to vascular lesions? During development, both proteins are expressed most prominently in vascular endothelium.^{49,50} Both are down-regulated in adulthood, but re-induced during vessel repair.^{43,49,51,52} Alk-1 and endoglin have

multiple actions on developing blood vessels, but their functions in vascular stabilization seem most central to disease etiology. Alk-1 or endoglin knock-out mice establish a primary capillary plexus, but fail to recruit pericytes and form a secondary plexus.^{53,54} Mice with mutations in just one copy of either gene establish relatively normal mature vasculature, but have small vessel malformations and loss of vascular smooth muscle.^{45,46} Defects in these mice resemble those in human HHT, in which dissociation of vascular smooth muscle cells is an early step,³⁰ and sporadic brain AVM, where there is reduced pericyte coverage of the perinidal capillaries.⁵⁵

While Alk-1 and endoglin deficiencies manifest first histologically as defects of smooth muscle development, the disease process probably begins in the vascular endothelium. Alk-1, endoglin, and Smad4 all are expressed in vascular endothelium, and Alk-1 and Smad4 are specifically required there for smooth muscle recruitment.^{48,56} Thus, the primary defect in HHT may be an impaired endothelium-specific TGF- β signaling pathway, with resulting smooth muscle defects. (Endoglin, however, is expressed not only in vascular endothelium but also in vascular smooth muscle and monocytes, and may be required there as well.)

Another potential cause of HHT lesions involves endothelial NO synthase (eNOS). Endothelial cells of *eng*^{+/-} mice exhibit dysregulated eNOS activity, leading to superoxide generation and impaired myogenic responses.⁵⁷ eNOS malfunction could contribute to the small vessel dilation seen at early stages of HHT, and superoxide may cause local endothelial damage and initiate capillary wall breakdown.

An unanswered question in AVM biology concerns the identity of the physiological ligand/s for Alk-1 in vascular endothelial cells. This ligand was previously believed to be TGF- β 1, which is expressed in developing vascular endothelium and known to regulate endothelial cell proliferation and migration. However, one study has indicated that deletion of TGF- β 2 (the ligand-binding TGF- β receptor) in endothelial cells does not affect vascular development, and raised the possibility that the physiological ligand for Alk-1 is BMP9 or BMP10.⁴⁸ Another mystery concerns the ten-fold difference in prevalence of brain AVMs in HHT1 (i.e., *ENG* deficiency) versus HHT2 (*ALK-1*), which have approximate penetrances of 10% and 1%, respectively.

VMBs and vascular patterning

Another angiogenic process in which VMB genes have been implicated is arteriovenous specification.⁵⁸ The arteries of mice with reduced levels of *Ccm1*, Alk-1, or endoglin show wall thinning and dilation, fewer smooth muscle cells, and loss of artery-specific markers such as ephrinB2, suggesting either loss of arteriovenous identity or transformation toward a more venous phenotype. Recently, overexpression of Notch-4 (a gene involved in arteriovenous specification) in vascular endothelium of developing mouse brain caused cerebral vascular dysplasias resembling AVMs.⁵⁹ However, studies of arteriovenous specification have so far focused on vessels outside the CNS; virtually nothing is known about how this process works in the brain. Another unexplored area concerns the development of vascular phenotypes that are specific to the brain or brain subregions. For example, two genes have been identified in zebrafish (*β pix* and *pak2a*) whose mutation causes failure of vascular wall stabilization and hemorrhage only in the head region.^{60, 61} Better understanding of genes specific to the CNS vasculature could help illuminate VMB etiology, and explain (for example) the different frequencies of brain AVMs in HHT1 and HHT2.

Instigators and Abettors of VMB Formation and Progression

Genetic and environmental second hits

The focal nature of VMBs has suggested that lesion formation may require not only a mutation in one copy of a given VMB gene within a particular group of cells but also a “second hit” that triggers the disease process in the region of the lesion. This second hit could be genetic, in the form of a somatic mutation to the second copy of that gene, or a mutation in another gene acting in the same cellular pathway. The possibility of a genetic two-hit mechanism in CCM is supported by discoveries of coexistence of germline (i.e., inherited) and somatic mutations in lesion tissue from subsets of patients with all three forms of inherited CCMs.^{62–64} In addition, it has been observed that CCM protein is lost from some but not all cells of CCM lesions.⁶⁵ Finally, mice with mutations in one copy of the *cml1* gene do not normally develop lesions, but will do so if they also lack the tumor suppressor gene *p53* (a genetic background known to increase the rate of somatic mutations).⁶⁶ It is not known how prevalent somatic mutations are in CCM, or if a genetic two-hit mechanism also operates in HHT or sporadic CCM or AVM.

Environmental factors, including angiogenic factors and inflammatory cytokines, could also act as second hits. For AVMs, hemodynamic factors such as high flow rates and the resultant high endothelial shear stress might also promote lesion formation and/or progression, either through direct mechanical effects on vessel walls, up-regulation of angiogenic factors, or triggering inflammation.^{67–69} Hemodynamic factors are probably less important for CCM, because the lesions do not conduct high flows. Direct evidence that environmental second hits can initiate AVM lesion formation comes from studies of mice with mutations in one copy of either the *eng* and *Alk-1* gene. Focal over-expression of VEGF greatly enhances the development of dysplasia in these mice,^{70, 71} and this experimental dysplasia can be further exacerbated by regional increases in tissue perfusion.⁷¹

Angiogenic factors

VMBs exhibit activated angiogenesis, including upregulated expression of VEGF and other angiogenic factors^{72,73} and increased endothelial cell proliferation.^{74,75} Thus, it is possible VMBs escape the normal controls down-regulating angiogenesis in adulthood, and that lesion growth occurs through an autocrine positive feedback loop. Angiogenic factor production by VMBs could also have adverse consequences for other cells of the neurovascular unit. For example, abnormal growth factor secretion by VMBs could cause inappropriate stimulation of neuronal or glial proliferation; indeed, there is evidence for the latter.⁷⁶ In addition, VEGF and other angiogenic factors increase BBB permeability, which could in turn predispose vascular walls to rupture.

Inflammation

VMBs are also sites of active inflammation. Robust B and plasma cell infiltration and oligoclonal IgG immune responses have been demonstrated in CCMs.⁷⁷ Neutrophils, macrophages, and inflammatory markers are seen in AVMs.⁷⁸ Inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and some interleukins (ILs), are potent stimulators of both angiogenesis and BBB breakdown and could contribute to lesion progression and rupture. Consistent with this idea, polymorphisms in the TNF- α ,⁷⁹ IL-1 β 80 and IL-681 genes are risk factors for intracerebral hemorrhage in sporadic AVM. Another link between inflammation and angiogenesis lies with endoglin, which is expressed in activated circulating monocytes. In post-ischemic heart, activated monocytes migrate to the infarct site, differentiate into endothelial cells and contribute to vascular repair, but monocytes from HHT1 (*ENG*^{+/-}) patients show reduced capacity to so.⁵¹

Breakdown of the BBB

BBB breakdown occurs in CCMs.^{82,83} Since loss of inter-endothelial contact and barrier function is also observed in *ccm2* mutant mice,³⁷ BBB breakdown may be a primary defect in CCM. Alternatively, BBB breakdown in CCMs could be a symptom of activated angiogenesis and/or local inflammation. The BBB barrier has not been extensively studied in AVMs, but most intranidal vessels are of a caliber such that the capillary-level barrier would not be expected. Clinically, unless there is secondary injury from local mass effect or hemorrhage, imaging contrast agents do not pass into the extravascular space, either in the nidus or in surrounding parenchyma.

New Insights and Mysteries Remaining

The past few years have shed considerable light on cellular mechanisms of VMB etiology. With regard to the roles of CCM and HHT disease genes in developmental angiogenesis, it is now clear that both sets of genes are required specifically in endothelium, and can act within common functional pathways. Several lines of evidence point to a critical requirement for these genes in vessel wall stabilization and smooth muscle recruitment, and may eventually help explain how mutations in these genes render vessels vulnerable to rupture when challenged with other inauspicious genetic or environmental factors. Finally, there is now evidence of a genetic two-hit mechanism operating in some CCM cases, as is demonstration that environmental second hits can produce vascular lesions in mice bearing mutations in a single copy of a VMB gene.

Much still remains to be learned. Given that some (perhaps most) VMBs arise post-natally and into adulthood, we need to understand better how VMB genes function in adult angiogenesis and vascular repair, and how their expression is affected by environmental perturbations. We also need to learn if mechanisms of lesion formation in sporadic VMB are the same as in familial forms. Other genes contributing to VMB syndromes should be identified -- a quest that can be pursued not only in human populations but also in *C. elegans*, *Drosophila*, or zebrafish.

With regard to experimental tools, more sophisticated animal models of VMB syndromes are being developed via conditional knockout, but currently available ones still have limitations: lesion frequency is low in heterozygous mutant mice and the available model systems do not precisely phenocopy either the angioarchitecture or the natural history of the human disease, especially with regard to intracranial hemorrhage. Better models might be generated by additional age- and cell type-specific knockouts, the use of different genetic backgrounds, or the identification of environmental triggers of lesion formation that can be applied easily and uniformly.

Finally, studies in humans have lagged behind those in animal models with regard to description of VMB etiology. Studies in human lesion tissue have been few, and would be stimulated by the establishment of tissue repositories and standardized collection procedures. We still have no clear understanding of when lesion formation is initiated, how it typically progress, or how progression is impacted by environmental risk factors. A critical step toward more sophisticated natural history studies and additional genetic studies will be the establishment of large cohorts of patients and databases of clinical information.

Translational implications

Translational advances will include describing the biology underlying certain clinical behaviors, for example hemorrhagic risk. Although neither CCM nor AVM has a model that has been developed to the point of actually testing such questions, refinement of animal models

that mimic disease natural history may make this possible. Studies in current animal models suggest specific therapeutic targets for slowing VMB formation and progression. For example, the reversal by simvastatin of some of the vascular defects caused by loss of *ccm2* function in mice raises the possibility of initiating clinical trials of statins for CCM. Observations that VMBs are sites of active angiogenesis and inflammation suggest the potential utility of anti-angiogenic or anti-inflammatory agents. MMP inhibitors, for example, have both anti-angiogenic and anti-inflammatory activities, and a Phase I clinical trial of minocycline and doxycycline (broad spectrum MMP inhibitors) has just seen successful completion.⁸⁴

A key goal for translational research is development of biomarkers that can identify subgroups most likely to benefit from a specific intervention, and whose disease progression is likely to be sufficiently dire and predictable to justify therapeutic risks and side effects. Such efforts can be aided in several ways. First, further development of consensus statements for standardized collection and reporting of clinical information are needed, which are important tools to foster cohesive translational and clinical research.⁸⁵ Second, there are number of ongoing registry efforts for vascular malformations.^{24,86,87,94} The establishment of regional and international clinical research consortia can help provide critical intellectual mass and sufficient sample sizes to make progress. For example, the first NIH-funded randomized trial for VMB management was recently initiated to answer questions regarding optimal management of patients with unruptured BAVM (ARUBA; <http://clinicaltrials.gov/ct/show/NCT00389181>). Because VMB syndromes are rare diseases, a particularly important aspect of establishing functional clinical consortia is that they can provide the infrastructure necessary to enable swift initiation of clinical trials when potential therapeutics are identified

Acknowledgments

This review is based in part on discussions at a workshop entitled "Biology of Vascular Malformations of the Brain" that was held by the National Institutes of Neurological Disorders and Stroke in Washington, DC, March 13–14, 2008. An agenda, participant list, and report of recommendations can be found at the workshop website (http://www.ninds.nih.gov/news_and_events/proceedings/index.htm) The workshop was supported in part by funds from the Angioma Alliance, the HHT Foundation International, Sturge-Weber Foundation and The Aneurysm and AVM Foundation (TAAF).

REFERENCES

1. Al-Shahi R, Bhattacharya JJ, Currie DG, Papanastassiou V, Ritchie V, Roberts RC, Sellar RJ, Warlow CP. Prospective, population-based detection of intracranial vascular malformations in adults: The scottish intracranial vascular malformation study (sivms). *Stroke* 2003;34:1163–1169. [PubMed: 12702837]
2. Plate KH. Mechanisms of angiogenesis in the brain. *J Neuropathol Exp Neurol* 1999;58:313–320. [PubMed: 10218626]
3. Greenberg DA, Jin K. From angiogenesis to neuropathology. *Nature* 2005;438:954–959. [PubMed: 16355213]
4. Saunders NR, Knott GW, Dziegielewska KM. Barriers in the immature brain. *Cell Mol Neurobiol* 2000;20:29–40. [PubMed: 10690500]
5. Vorbrodt AW, Dobrogowska DH, Tarnawski M. Immunogold study of interendothelial junction-associated and glucose transporter proteins during postnatal maturation of the mouse blood-brain barrier. *J Neurocytol* 2001;30:705–716. [PubMed: 12118158]
6. Etchevers HC, Vincent C, Le Douarin NM, Couly GF. The cephalic neural crest provides pericytes and smooth muscle cells to all blood vessels of the face and forebrain. *Development* 2001;128:1059–1068. [PubMed: 11245571]
7. Bauer HC, Bauer H, Lametschwandtner A, Amberger A, Ruiz P, Steiner M. Neovascularization and the appearance of morphological characteristics of the blood-brain barrier in the embryonic mouse central nervous system. *Brain Res Dev Brain Res* 1993;75:269–278.

8. Hirschi KK, Rohovsky SA, D'Amore PA. Pdgf, tgf-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10t1/2 cells and their differentiation to a smooth muscle fate. *J Cell Biol* 1998;141:805–814. [PubMed: 9566978]
9. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of pdgf-b and pdgfr-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 1999;126:3047–3055. [PubMed: 10375497]
10. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res* 2005;97:512–523. [PubMed: 16166562]
11. Saunders WB, Bohnsack BL, Fasse JB, Anthis NJ, Bayless KJ, Hirschi KK, Davis GE. Coregulation of vascular tube stabilization by endothelial cell timp-2 and pericyte timp-3. *J Cell Biol* 2006;175:179–191. [PubMed: 17030988]
12. Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, Lindblom P, Shani M, Zicha D, Adams RH. Ephrin-b2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell* 2006;124:161–173. [PubMed: 16413489]
13. Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc Natl Acad Sci U S A* 1990;87:5568–5572. [PubMed: 1695380]
14. Whitaker VR, Cui L, Miller S, Yu SP, Wei L. Whisker stimulation enhances angiogenesis in the barrel cortex following focal ischemia in mice. *J Cereb Blood Flow Metab* 2007;27:57–68. [PubMed: 16670699]
15. Xu K, Lamanna JC. Chronic hypoxia and the cerebral circulation. *J Appl Physiol* 2006;100:725–730. [PubMed: 16421279]
16. Li S, Chen BP, Azuma N, Hu YL, Wu SZ, Sumpio BE, Shyy JY, Chien S. Distinct roles for the small gtpases cdc42 and rho in endothelial responses to shear stress. *J Clin Invest* 1999;103:1141–1150. [PubMed: 10207166]
17. Louissaint A Jr, Rao S, Leventhal C, Goldman SA. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron* 2002;34:945–960. [PubMed: 12086642]
18. Ardelt AA, McCullough LD, Korach KS, Wang MM, Munzenmaier DH, Hurn PD. Estradiol regulates angiopoietin-1 mRNA expression through estrogen receptor-alpha in a rodent experimental stroke model. *Stroke* 2005;36:337–341. [PubMed: 15637314]
19. Reiss Y, Machein MR, Plate KH. The role of angiopoietins during angiogenesis in gliomas. *Brain Pathol* 2005;15:311–317. [PubMed: 16389943]
20. Sainson RC, Johnston DA, Chu HC, Holderfield MT, Nakatsu MN, Crampton SP, Davis J, Conn E, Hughes CC. Tnf primes endothelial cells for angiogenic sprouting by inducing a tip cell phenotype. *Blood* 2008;111:4997–5007. [PubMed: 18337563]
21. McCormick, W. Pathology of vascular malformations of the brain. In: Wilson, C.; Stein, B., editors. *Pathology of vascular malformations of the brain*. Baltimore: Williams & Wilkins; 1984. p. 44-63.
22. Sato S, Kodama N, Sasaki T, Matsumoto M, Ishikawa T. Perinidal dilated capillary networks in cerebral arteriovenous malformations. *Neurosurgery* 2004;54:163–168. discussion 168–170. [PubMed: 14683554]
23. Robinson JR, Awad IA, Little JR. Natural history of the cavernous angioma. *J Neurosurg* 1991;75:709–714. [PubMed: 1919692]
24. Kim H, Sidney S, McCulloch CE, Poon KY, Singh V, Johnston SC, Ko NU, Achrol AS, Lawton MT, Higashida RT, Young WL. Racial/ethnic differences in longitudinal risk of intracranial hemorrhage in brain arteriovenous malformation patients. *Stroke* 2007;38:2430–2437. [PubMed: 17673729]
25. Minakawa T, Tanaka R, Koike T, Takeuchi S, Sasaki O. Angiographic follow-up study of cerebral arteriovenous malformations with reference to their enlargement and regression. *Neurosurgery* 1989;24:68–74. [PubMed: 2927601]
26. Clatterbuck RE, Moriarity JL, Elmaci I, Lee RR, Breiter SN, Rigamonti D. Dynamic nature of cavernous malformations: A prospective magnetic resonance imaging study with volumetric analysis. *Neurosurg* 2000;93:981–986.
27. Zabramski JM, Wascher TM, Spetzler RF, Johnson B, Golfinos J, Drayer BP, Brown B, Rigamonti D, Brown G. The natural history of familial cavernous malformations: Results of an ongoing study. *J Neurosurg* 1994;80:422–432. [PubMed: 8113854]

28. Du R, Hashimoto T, Tihan T, Young WL, Perry V, Lawton MT. Growth and regression of arteriovenous malformations in a patient with hereditary hemorrhagic telangiectasia. Case report. *J Neurosurg* 2007;106:470–477. [PubMed: 17367071]
29. Rigamonti D, Johnson PC, Spetzler RF, Hadley MN, Drayer BP. Cavernous malformations and capillary telangiectasia: A spectrum within a single pathological entity. *Neurosurgery* 1991;28:60–64. [PubMed: 1994283]
30. Braverman IM, Keh A, Jacobson BS. Ultrastructure and three-dimensional organization of the telangiectases of hereditary hemorrhagic telangiectasia. *J Invest Dermatol* 1990;95:422–427. [PubMed: 2212727]
31. Shenkar R, Venkatasubramanian PN, Wyrwicz AM, Zhao JC, Shi C, Akers A, Marchuk DA, Awad IA. Advanced magnetic resonance imaging of cerebral cavernous malformations: Part ii. Imaging of lesions in murine models. *Neurosurgery* 2008;63:790–797. discussion 797–798. [PubMed: 18981891]
32. Marchuk DA, Srinivasan S, Squire TL, Zawistowski JS. Vascular morphogenesis: Tales of two syndromes. *Hum Mol Genet* 2003;12(Spec No 1):R97–R112. [PubMed: 12668602]
33. Petit N, Blecon A, Denier C, Tournier-Lasserre E. Patterns of expression of the three cerebral cavernous malformation (ccm) genes during embryonic and postnatal brain development. *Gene Expr Patterns* 2006;6:495–503. [PubMed: 16455310]
34. Hilder TL, Malone MH, Bencharit S, Colicelli J, Haystead TA, Johnson GL, Wu CC. Proteomic identification of the cerebral cavernous malformation signaling complex. *J Proteome Res* 2007;6:4343–4355. [PubMed: 17900104]
35. Kleaveland B, Zheng X, Liu JJ, Blum Y, Tung JJ, Zou Z, Chen M, Guo L, Lu MM, Zhou D, Kitajewski J, Affolter M, Ginsberg MH, Kahn ML. Regulation of cardiovascular development and integrity by the heart of glass-cerebral cavernous malformation protein pathway. *Nat Med* 2009;15:169–176. [PubMed: 19151727]
36. Hogan BM, Bussmann J, Wolburg H, Schulte-Merker S. Ccm1 cell autonomously regulates endothelial cellular morphogenesis and vascular tubulogenesis in zebrafish. *Hum Mol Genet* 2008;17:2424–2432. [PubMed: 18469344]
37. Whitehead KJ, Chan AC, Navankasattusas S, Koh W, London NR, Ling J, Mayo AH, Drakos SG, Marchuk DA, Davis GE, Li DY. The cerebral cavernous malformation signaling pathway promotes vascular integrity via rho gtpases. *Nat Med*. 2009
38. Plummer NW, Squire TL, Srinivasan S, Huang E, Zawistowski JS, Matsunami H, Hale LP, Marchuk DA. Neuronal expression of the ccm2 gene in a new mouse model of cerebral cavernous malformations. *Mamm Genome* 2006;17:119–128. [PubMed: 16465592]
39. Boulday G, Blecon A, Petit N, Chareyre F, Garcia LA, Niwa-Kawakita M, Giovannini M, Tournier-Lasserre E. Tissue-specific conditional ccm2 knockout mice establish the essential role of endothelial ccm2 in angiogenesis: Implications for human cerebral cavernous malformations. *Dis Model Mech* 2009;2:168–177. [PubMed: 19259391]
40. Voss K, Stahl S, Hogan BM, Reinders J, Schleider E, Schulte-Merker S, Felbor U. Functional analyses of human and zebrafish 18-amino acid in-frame deletion pave the way for domain mapping of the cerebral cavernous malformation 3 protein. *Hum Mutat* 2009;30:1003–1011. [PubMed: 19370760]
41. Pawlikowska L, Tran MN, Achrol AS, Ha C, Burchard E, Choudhry S, Zaroff J, Lawton MT, Castro R, McCulloch CE, Marchuk D, Kwok PY, Young WL. Polymorphisms in transforming growth factor-beta-related genes *alk1* and *eng* are associated with sporadic brain arteriovenous malformations. *Stroke* 2005;36:2278–2280. [PubMed: 16179574]
42. Gallione CJ, Richards JA, Letteboer TG, Rushlow D, Prigoda NL, Leedom TP, Ganguly A, Castells A, Ploos van Amstel JK, Westermann CJ, Pyeritz RE, Marchuk DA. *Smad4* mutations found in unselected hht patients. *J Med Genet* 2006;43:793–797. [PubMed: 16613914]
43. ten Dijke P, Goumans MJ, Pardali E. Endoglin in angiogenesis and vascular diseases. *Angiogenesis* 2008;11:79–89. [PubMed: 18283546]
44. Bourdeau A, Dumont DJ, Letarte M. A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest* 1999;104:1343–1351. [PubMed: 10562296]

45. Torsney E, Charlton R, Diamond AG, Burn J, Soames JV, Arthur HM. Mouse model for hereditary hemorrhagic telangiectasia has a generalized vascular abnormality. *Circulation* 2003;107:1653–1657. [PubMed: 12668501]
46. Srinivasan S, Hanes MA, Dickens T, Porteous ME, Oh SP, Hale LP, Marchuk DA. A mouse model for hereditary hemorrhagic telangiectasia (hht) type 2. *Hum Mol Genet* 2003;12:473–482. [PubMed: 12588795]
47. Satomi J, Mount RJ, Toporsian M, Paterson AD, Wallace MC, Harrison RV, Letarte M. Cerebral vascular abnormalities in a murine model of hereditary hemorrhagic telangiectasia. *Stroke* 2003;34:783–789. [PubMed: 12624308]
48. Park SO, Lee YJ, Seki T, Hong KH, Fliess N, Jiang Z, Park A, Wu X, Kaartinen V, Roman BL, Oh SP. Alk5- and tgfr2-independent role of alk1 in the pathogenesis of hereditary hemorrhagic telangiectasia type 2. *Blood* 2008;111:633–642. [PubMed: 17911384]
49. Seki T, Yun J, Oh SP. Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterIALIZATION and vascular remodeling. *Circ Res* 2003;93:682–689. [PubMed: 12970115]
50. Jonker L, Arthur HM. Endoglin expression in early development is associated with vasculogenesis and angiogenesis. *Mech Dev* 2002;110:193–196. [PubMed: 11744382]
51. van Laake LW, van den Driesche S, Post S, Feijen A, Jansen MA, Driessens MH, Mager JJ, Snijder RJ, Westermann CJ, Doevendans PA, van Echteld CJ, ten Dijke P, Arthur HM, Goumans MJ, Lebrin F, Mummery CL. Endoglin has a crucial role in blood cell-mediated vascular repair. *Circulation* 2006;114:2288–2297. [PubMed: 17088457]
52. Ma X, Labinaz M, Goldstein J, Miller H, Keon WJ, Letarte M, O'Brien E. Endoglin is overexpressed after arterial injury and is required for transforming growth factor-beta-induced inhibition of smooth muscle cell migration. *Arterioscler Thromb Vasc Biol* 2000;20:2546–2552. [PubMed: 11116051]
53. Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP. Defective angiogenesis in mice lacking endoglin. *Science* 1999;284:1534–1537. [PubMed: 10348742]
54. Urness LD, Sorensen LK, Li DY. Arteriovenous malformations in mice lacking activin receptor-like kinase-1. *Nat Genet* 2000;26:328–331. [PubMed: 11062473]
55. Tu J, Stoodley MA, Morgan MK, Storer KP. Ultrastructure of perinidal capillaries in cerebral arteriovenous malformations. *Neurosurgery* 2006;58:961–970. discussion 961–970. [PubMed: 16639333]
56. Lan Y, Liu B, Yao H, Li F, Weng T, Yang G, Li W, Cheng X, Mao N, Yang X. Essential role of endothelial smad4 in vascular remodeling and integrity. *Mol Cell Biol* 2007;27:7683–7692. [PubMed: 17724086]
57. Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH, Husain M, Letarte M. A role for endoglin in coupling enos activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. *Circ Res* 2005;96:684–692. [PubMed: 15718503]
58. Sorensen LK, Brooke BS, Li DY, Urness LD. Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific tgfbeta coreceptor. *Dev Biol* 2003;261:235–250. [PubMed: 12941632]
59. Murphy PA, Lam MT, Wu X, Kim TN, Vartanian SM, Bollen AW, Carlson TR, Wang RA. Endothelial notch4 signaling induces hallmarks of brain arteriovenous malformations in mice. *Proc Natl Acad Sci U S A* 2008;105:10901–10906. [PubMed: 18667694]
60. Liu J, Fraser SD, Faloon PW, Rollins EL, Vom Berg J, Starovic-Subota O, Laliberte AL, Chen JN, Serluca FC, Childs SJ. A betapix pak2a signaling pathway regulates cerebral vascular stability in zebrafish. *Proc Natl Acad Sci U S A* 2007;104:13990–13995. [PubMed: 17573532]
61. Buchner DA, Su F, Yamaoka JS, Kamei M, Shavit JA, Barthel LK, McGee B, Amigo JD, Kim S, Hanosh AW, Jagadeeswaran P, Goldman D, Lawson ND, Raymond PA, Weinstein BM, Ginsburg D, Lyons SE. Pak2a mutations cause cerebral hemorrhage in redhead zebrafish. *Proc Natl Acad Sci U S A* 2007;104:13996–14001. [PubMed: 17715297]
62. Gault J, Shenkar R, Recksiek P, Awad IA. Biallelic somatic and germ line *CCM1* truncating mutations in a cerebral cavernous malformation lesion. *Stroke* 2005;36:872–874. [PubMed: 15718512]
63. Akers AL, Johnson E, Steinberg GK, Zabramski JM, Marchuk DA. Biallelic somatic and germline mutations in cerebral cavernous malformations (*Ccm*): Evidence for a two-hit mechanism of *ccm* pathogenesis. *Hum Mol Genet*. 2008

64. Gault J, Awad IA, Recksiek P, Shenkar R, Breeze R, Handler M, Kleinschmidt-DeMasters BK. Cerebral cavernous malformations: Somatic mutations in vascular endothelial cells. *Neurosurgery* 2009;65:138–144. discussion 144–135. [PubMed: 19574835]
65. Pagenstecher A, Stahl S, Sure U, Felbor U. A two-hit mechanism causes cerebral cavernous malformations: Complete inactivation of *CCM1*, *CCM2* or *CCM3* in affected endothelial cells. *Hum Mol Genet.* 2008
66. Plummer NW, Gallione CJ, Srinivasan S, Zawistowski JS, Louis DN, Marchuk DA. Loss of p53 sensitizes mice with a mutation in *ccm1* (*krit1*) to development of cerebral vascular malformations. *Am J Pathol* 2004;165:1509–1518. [PubMed: 15509522]
67. Hashimoto T, Meng H, Young WL. Intracranial aneurysms: Links among inflammation, hemodynamics and vascular remodeling. *Neurol Res* 2006;28:372–380. [PubMed: 16759441]
68. Zhu Y, Lawton MT, Du R, Shwe Y, Chen Y, Shen F, Young WL, Yang GY. Expression of hypoxia-inducible factor-1 and vascular endothelial growth factor in response to venous hypertension. *Neurosurgery* 2006;59:687–696. discussion 687–696. [PubMed: 16955051]
69. Goetsch W, Gryczka C, Korff T, Ernst E, Goetsch C, Seebach J, Schnittler HJ, Augustin HG, Morawietz H. Flow-dependent regulation of angiopoietin-2. *J Cell Physiol* 2008;214:491–503. [PubMed: 17960565]
70. Xu B, Wu YQ, Huey M, Arthur HM, Marchuk DA, Hashimoto T, Young WL, Yang GY. Vascular endothelial growth factor induces abnormal microvasculature in the endoglin heterozygous mouse brain. *J Cereb Blood Flow Metab* 2004;24:237–244. [PubMed: 14747750]
71. Hao Q, Su H, Marchuk DA, Rola R, Wang Y, Liu W, Young WL, Yang GY. Increased tissue perfusion promotes capillary dysplasia in the alk1-deficient mouse brain following vegf stimulation. *Am J Physiol Heart Circ Physiol* 2008;295:H2250–H2256. [PubMed: 18835925]
72. Hashimoto T, Wu Y, Lawton MT, Yang GY, Barbaro NM, Young WL. Coexpression of angiogenic factors in brain arteriovenous malformations. *Neurosurgery* 2005;56:1058–1065. [PubMed: 15854255]
73. Gault J, Sarin H, Awadallah NA, Shenkar R, Awad IA. Pathobiology of human cerebrovascular malformations: Basic mechanisms and clinical relevance. *Neurosurgery* 2004;55:1–16. discussion 16–17. [PubMed: 15214969]
74. Hashimoto T, Mesa-Tejada R, Quick CM, Bollen AW, Joshi S, Pile-Spellman J, Lawton MT, Young WL. Evidence of increased endothelial cell turnover in brain arteriovenous malformations. *Neurosurgery* 2001;49:124–131. discussion 131–122. [PubMed: 11440433]
75. Sure U, Butz N, Schlegel J, Siegel AM, Wakat JP, Mennel HD, Bien S, Bertalanffy H. Endothelial proliferation, neoangiogenesis, and potential de novo generation of cerebrovascular malformations. *J Neurosurg* 2001;94:972–977. [PubMed: 11409527]
76. Ha Y, Kim TS, Yoon DH, Cho YE, Huh SG, Lee KC. Reinduced expression of developmental proteins (nestin, small heat shock protein) in and around cerebral arteriovenous malformations. *Clin Neuropathol* 2003;22:252–261. [PubMed: 14531551]
77. Shi C, Shenkar R, Batjer HH, Check JJ, Awad IA. Oligoclonal immune response in cerebral cavernous malformations. Laboratory investigation. *J Neurosurg* 2007;107:1023–1026. [PubMed: 17977276]
78. Chen Y, Zhu W, Bollen AW, Lawton MT, Barbaro NM, Dowd CF, Hashimoto T, Yang GY, Young WL. Evidence of inflammatory cell involvement in brain arteriovenous malformations. *Neurosurgery* 2008;62:1340–1349. discussion 1349–1350. [PubMed: 18825001]
79. Achrol AS, Pawlikowska L, McCulloch CE, Poon KY, Ha C, Zaroff JG, Johnston SC, Lee C, Lawton MT, Sidney S, Marchuk DA, Kwok PY, Young WL. Tumor necrosis factor-alpha-238g>a promoter polymorphism is associated with increased risk of new hemorrhage in the natural course of patients with brain arteriovenous malformations. *Stroke* 2006;37:231–234. [PubMed: 16322490]
80. Kim H, Hysi PG, Pawlikowska L, Poon A, Burchard EG, Zaroff JG, Sidney S, Ko NU, Achrol AS, Lawton MT, McCulloch CE, Kwok PY, Young WL. Common variants in interleukin-1-beta gene are associated with intracranial hemorrhage and susceptibility to brain arteriovenous malformation. *Cerebrovasc Dis* 2008;27:176–182. [PubMed: 19092239]
81. Pawlikowska L, Tran MN, Achrol AS, McCulloch CE, Ha C, Lind DL, Hashimoto T, Zaroff J, Lawton MT, Marchuk DA, Kwok PY, Young WL. Polymorphisms in genes involved in inflammatory and

- angiogenic pathways and the risk of hemorrhagic presentation of brain arteriovenous malformations. *Stroke* 2004;35:2294–2300. [PubMed: 15331795]
82. Clatterbuck RE, Eberhart CG, Crain BJ, Rigamonti D. Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations. *J Neurol Neurosurg Psychiatry* 2001;71:188–192. [PubMed: 11459890]
 83. Tu J, Stoodley MA, Morgan MK, Storer KP. Ultrastructural characteristics of hemorrhagic, nonhemorrhagic, and recurrent cavernous malformations. *J Neurosurg* 2005;103:903–909. [PubMed: 16304995]
 84. Frenzel T, Lee CZ, Kim H, Quinnine NJ, Hashimoto T, Lawton MT, Guglielmo BJ, McCulloch CE, Young WL. Feasibility of minocycline and doxycycline use as potential vasculostatic therapy for brain vascular malformations: Pilot study of adverse events and tolerance. *Cerebrovasc Dis* 2008;25:157–163. [PubMed: 18212521]
 85. Al-Shahi Salman R, Berg MJ, Morrison L, Awad IA. Hemorrhage from cavernous malformations of the brain: Definition and reporting standards. Angioma alliance scientific advisory board. *Stroke* 2008;39:3222–3230. [PubMed: 18974380]
 86. van Beijnum J, Lovelock CE, Cordonnier C, Rothwell PM, Klijn CJ, Al-Shahi Salman R. Outcome after spontaneous and arteriovenous malformation-related intracerebral haemorrhage: Population-based studies. *Brain* 2009;132:537–543. [PubMed: 19042932]
 87. Stapf C, Mast H, Sciacca RR, Berenstein A, Nelson PK, Gobin YP, Pile-Spellman J, Mohr JP. The new york islands avm study: Design, study progress, and initial results. *Stroke* 2003;34:e29–e33. [PubMed: 12690217]
 88. Glading A, Han J, Stockton RA, Ginsberg MH. Krit-1/ccm1 is a rap1 effector that regulates endothelial cell cell junctions. *J Cell Biol* 2007;179:247–254. [PubMed: 17954608]
 89. Uhlik MT, Abell AN, Johnson NL, Sun W, Cuevas BD, Lobel-Rice KE, Horne EA, Dell'Acqua ML, Johnson GL. Rac-mekk3-mkk3 scaffolding for p38 mapk activation during hyperosmotic shock. *Nat Cell Biol* 2003;5:1104–1110. [PubMed: 14634666]
 90. Crose LE, Hilder TL, Sciaky N, Johnson GL. Cerebral cavernous malformation 2 protein promotes smad ubiquitin regulatory factor 1-mediated rhoa degradation in endothelial cells. *J Biol Chem* 2009;284:13301–13305. [PubMed: 19318350]
 91. Ma X, Zhao H, Shan J, Long F, Chen Y, Chen Y, Zhang Y, Han X, Ma D. Pcd10 interacts with ste20-related kinase mst4 to promote cell growth and transformation via modulation of the erk pathway. *Mol Biol Cell* 2007;18:1965–1978. [PubMed: 17360971]
 92. Goumans MJ, Liu Z, ten Dijke P. Tgf-beta signaling in vascular biology and dysfunction. *Cell Res* 2009;19:116–127. [PubMed: 19114994]
 93. Schmierer B, Hill CS. Tgfbeta-smad signal transduction: Molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* 2007;8:970–982. [PubMed: 18000526]
 94. Wedderburn CJ, van Beijnum J, Bhattacharya JJ, Counsell CE, Papanastassiou V, Ritchie V, Roberts RC, Sellar RJ, Warlow CP, Al-Shahi, Salman R. Outcome after interventional or conservative management of unruptured brain arteriovenous malformations: a prospective, population-based cohort study. *Lancet Neurol* 2008;7:223–230. [PubMed: 18243054]

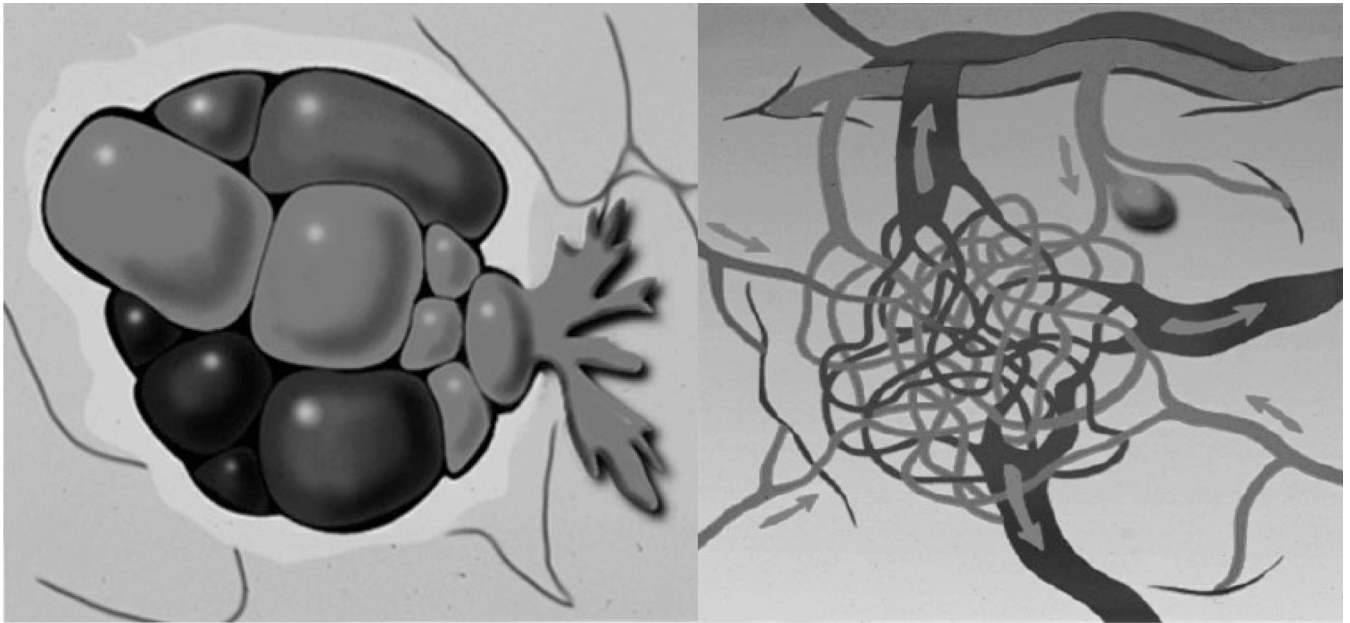


Figure 1. Schematic illustrations of (a) CCM and (b) AVM angioarchitecture

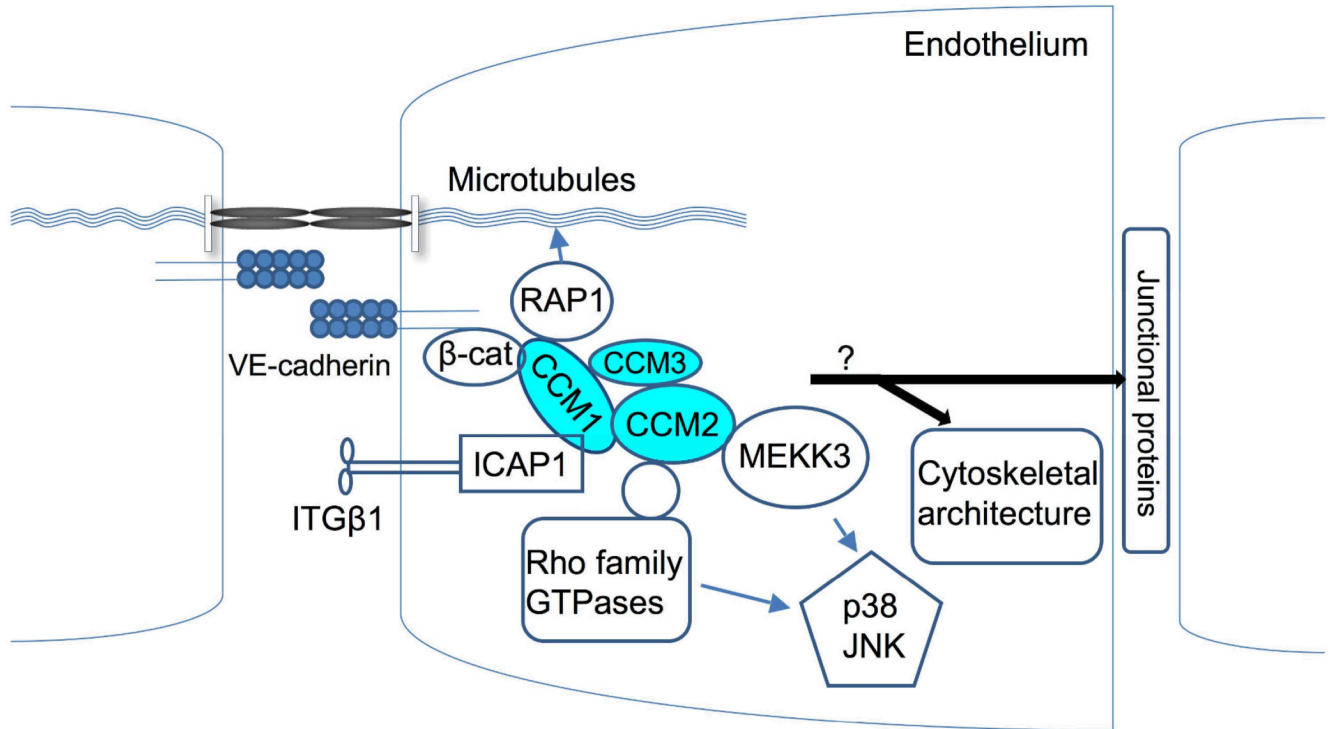


Figure 2. One possible model of genes and pathways involved in CCM signaling

CCM proteins form a molecular complex which interacts closely with cytoskeletal proteins and modulates inter-endothelial cell junctions. Mutations in one copy of a CCM gene may predispose to vascular permeability, which in turn may result in vascular leakage and vulnerability to form dysmorphic vessels. Somatic mutations in the same genetic pathway, immune responses, or altered capillary permeability after radiation injury all might act as “second hits” favoring CCM genesis or maintenance. Abbreviations: β-cat, β-catenin; CCM, cerebral cavernous malformation; ICAP-1, integrin cytoplasmic domain- associated protein-1; ITGβ1, integrin β1; JNK, JUN NH₂-terminal kinase; MEKK3, mitogen-activated protein kinase kinase kinase 3; RAP-1, Ras-proximate-1.

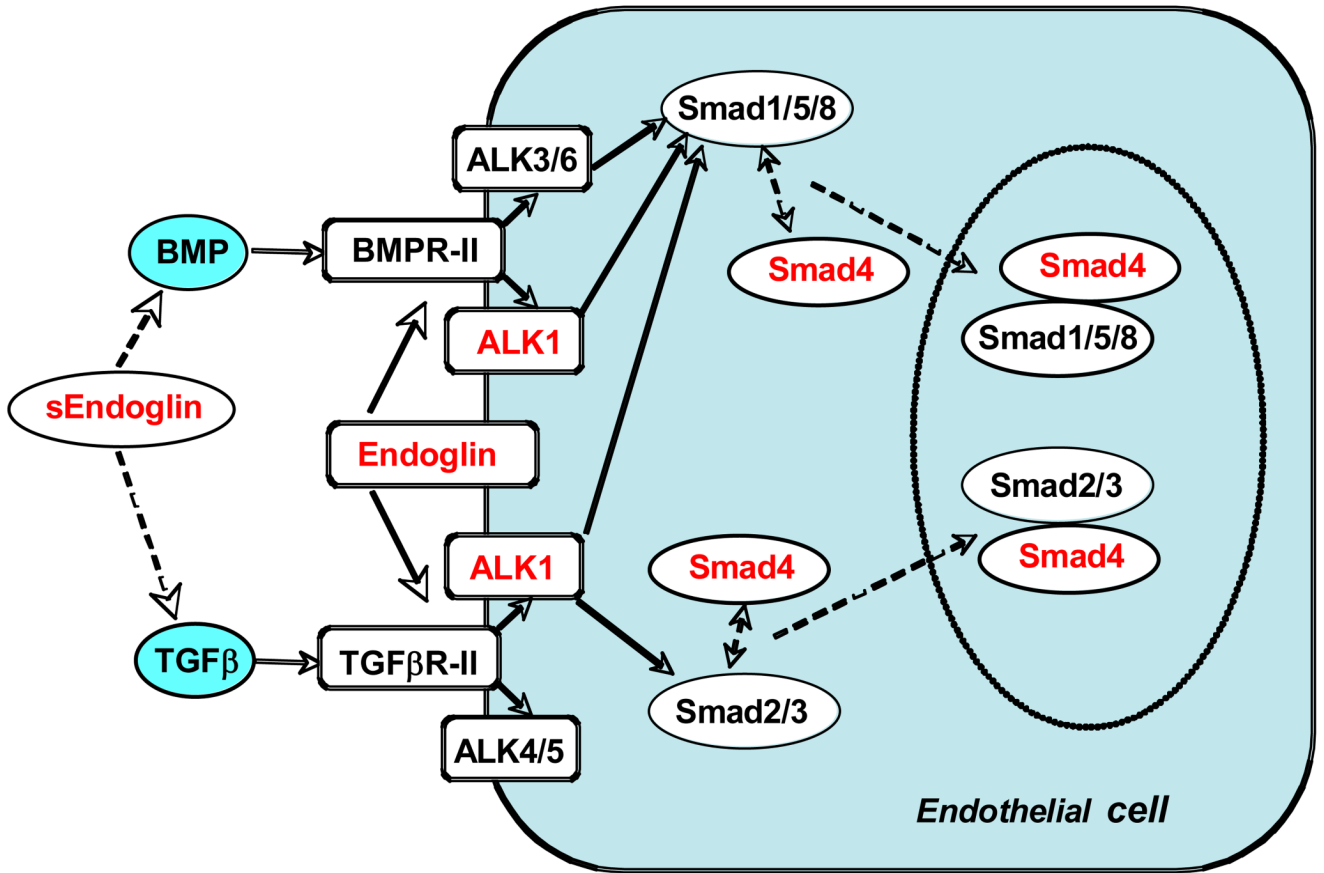


Figure 3. One possible model of genes and pathways involved in AVM pathogenesis

The genes associated AVM formation (shown in red) lie in the TGF β superfamily signaling pathway. TGF β and BMPs bind to TGF β R-II and BMPRII, respectively. Upon ligand binding, the type II receptors phosphorylate associated type I receptors (ALK1, 3, 4, 5, or 6). The ALKs in turn recruit and phosphorylate receptor-regulated Smads (Smads 1, 5, and 8 or Smads 2 and 3). The receptor-regulated Smads form complexes with the common mediator, Smad 4, and translocate to the nucleus to participate in regulating the transcription of target genes. Endoglin is a TGF- β co-receptor that can modulates signaling through either the TGF- β or BMP membrane-bound signaling complexes. Endoglin also exists in a soluble, extracellular form (sEndoglin) that can bind and sequester BMPs or TGF- β s. Mutations of the genes shown in red may lead to dysregulation of processes required for blood vessel wall stabilization. In susceptible patients, formation of an AVM may then result from a defective repair response to an otherwise minor insult. Abbreviations: ALK, activin-like kinase receptor; BMP, bone morphogenetic protein; BMP-R-II, BMP receptor type II; sEndoglin, soluble Endoglin; TGF β , transforming growth factor β ; TGF β -R-II, TGF β receptor type II.

Table 1

Genes underlying hereditary forms of cerebrovascular malformations.

| Gene | Alternative name/s | Known or putative cellular functions | References |
|-----------------|--|--|------------|
| <i>CCM 1</i> | <i>Krit 1</i> | Binds β -catenin, stabilizes interendothelial junctions associated with actin stress fibers | 88 |
| <i>CCM 2</i> | <i>Malcavernin; osmosensing scaffold for MEKK3 (OSM)</i> | Cellular responses to osmotic stress; modulates MAP kinase and RhoA GTPase signaling | 89, 90 |
| <i>CCM 3</i> | <i>Programmed cell death 10 (PDCD10)</i> | Cell proliferation and transformation (cancer cell lines); modulates extracellular signal-regulated kinase (ERK) | 91 |
| <i>Endoglin</i> | <i>HHT1</i> | TGF β superfamily co-receptor; modulates signaling by TGF β type II receptor, Alk-1 and Alk-5 | 43 |
| <i>Alk-1</i> | <i>Activin A receptor type II-like 1 (ACVRL-1); HHT2</i> | TGF β type I receptor | 92 |
| <i>SMAD4</i> | <i>SMAD family member 4</i> | Common downstream mediator of multiple TGF β superfamily signaling pathways | 92, 93 |