

Section 1

Development, anatomy, and physiology of arteriovenous malformations

Chapter

1

Development of the central nervous system vasculature and the pathogenesis of brain arteriovenous malformations

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Introduction

An arteriovenous malformation (AVM) is a spectacular freak of nature, a tangle of hemodynamic energy and red fury, throbbing and swirling in the sulci and gyri of the brain. There is nothing like it in the realm of brain pathology, at once so beautiful and so fearsome. Where do these lesions come from and how do they form? These questions have been without answers for as long as we have known about AVMs, and in our ignorance, we simply say that they are congenital. But are they really? Maybe they arise from miscues or miscommunications during embryogenesis when arteries and veins are in direct contact without intervening capillaries, and then persist after birth as AVMs rather than remodeling and maturing into normal circulatory architecture during vasculogenesis. Maybe they arise as a result of underlying genetic abnormalities that produce signaling errors and structural defects leading to arteriovenous pathology. Maybe AVMs are not congenital at all but acquired, as are dural arteriovenous fistulae after an injury where they are an abnormal response to that injury. The pathogenesis of brain AVMs remains a mystery, although tantalizing clues are emerging. This chapter will examine the development of the vasculature of the central nervous system (CNS), experimental AVM models, inflammation, and genetics in order to explore some of these ideas about AVM pathogenesis.

Vasculogenesis

Vessels exist to transmit nutrients to and remove waste from tissues. The arteries of the brain and spinal cord develop to supply the CNS during early embryonic stages in response to the metabolic needs of the neural tube. By the end of the third gestational week, the neuroectoderm differentiates into the neural plate, which itself folds longitudinally into a tube. Before the neural tube closes, nutrients and metabolites diffuse freely across the inner (ependymal) surface of the neural tissue from the amniotic fluid [1]. On the 23rd day of development in the human, the cephalic end of the neural tube (the anterior neuropore) closes to form the lamina terminalis (third ventricle anterior wall); the caudal neural tube will become the spinal cord [1]. After anterior neuropore closure, during the

prechoroidal stage, the neural tube is surrounded by *meninx primitiva*, a connective tissue derived from the neural crest that supplies nutrition by diffusion across the neural tube's outer (meningeal) surface (Fig. 1.1) [1]. In the choroidal phase, as the cerebral tissues grow and convolute, the meninx invaginates into the neural tube (ventricular lumen) to become the choroid plexus [1]. Consequently, metabolic exchange is possible across both ependymal and meningeal surfaces of the neural tissue. The locations of choroid plexus in relation to the thickening neural cortex dictate the morphology of the early afferent arterial tree to the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) [1,2].

As the cortical mantle continues to thicken and fold, the parenchymatous stage of cerebral vascularization consists of angiogenesis from the superficial anastomotic vascular network stimulated by the metabolic demands of the primitive brain tissue [1]. The neurovascular unit, a functional partnership of neural tissue and blood vessels, may arise during this period [3,4]. The basic arterial pattern laid down in the early embryonic period (Fig. 1.2) persists but is subsequently partially reorganized as a result of metabolic demands and concomitant hemodynamic changes of the later embryonic and fetal periods.

Development of craniocervical arteries: aortic arch and great vessels

The complex development of the craniocervical arteries can be broken down by embryonic stages and anatomical locations. Early embryonic development of the aortic arch and great vessels consists of formation and partial regression of undifferentiated plexiform paired vascular arches along the surface of the pharyngeal arches connecting the ventral aorta (aortic sac) with paired dorsal aortae (Table 1.1) [5]. The first pair of pharyngeal arches appears about day 22 and the concomitant first aortic vascular arches appear about day 24. The second pharyngeal arches appear by day 24 and, while the first pair of aortic arches regress, the second aortic arches appear by day 26. The third through sixth aortic arches have appeared by day 28 and 29. Blood flow to the brain is supplied mainly by the

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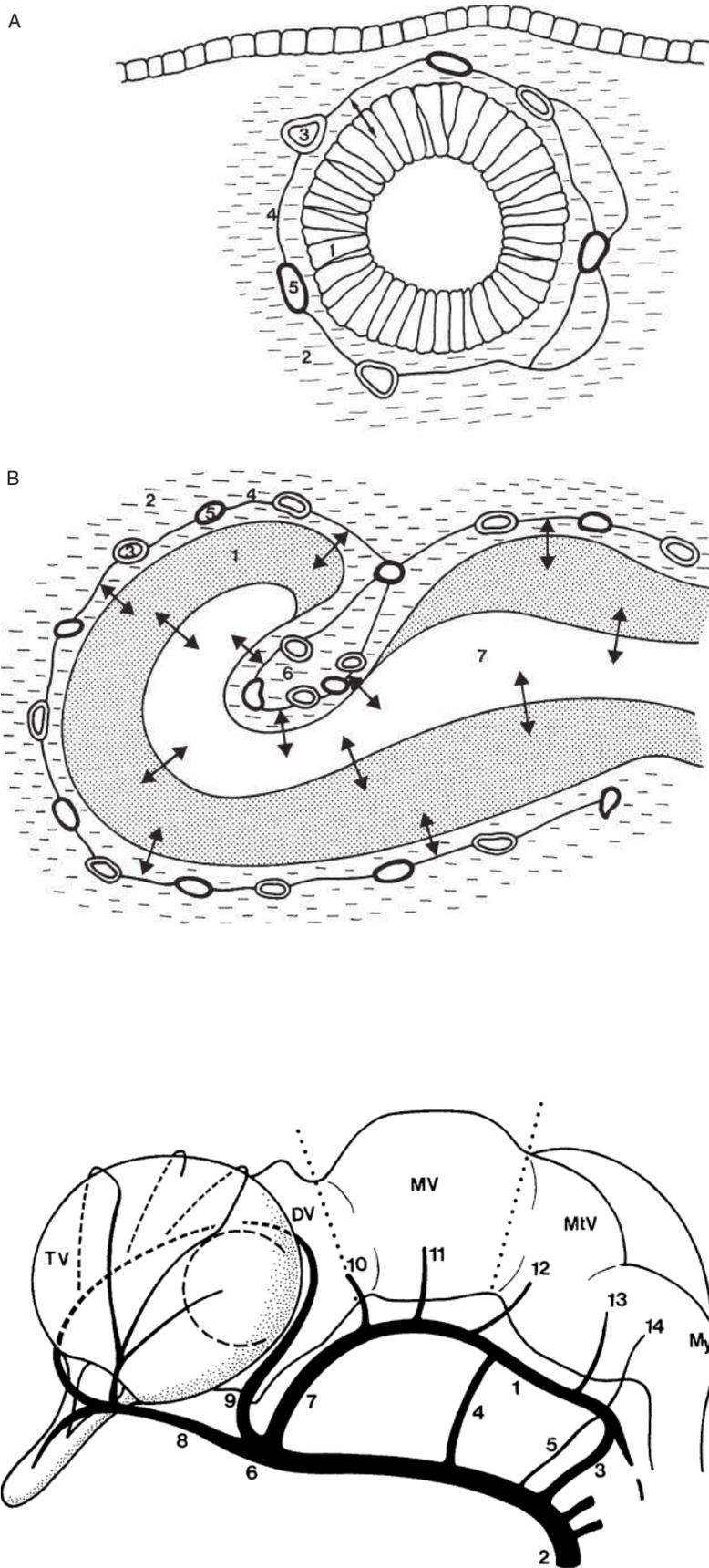


Fig. 1.1. Supply of nutrients to the neural tube. (A) After the neural tube (1) has closed, it is surrounded by the meninx primitiva (2), which contains arterial (3), capillary (4), and venous (5) channels. Metabolites diffuse from the capillary channels into the meninx and from there centripetally into the neural tissue (arrow). (B) As the neural tube thickens, centripetal diffusion cannot meet its metabolic demands. Invagination of the meninx primitiva into the ventricular lumen (choroid plexus; 6), allows exchange of metabolites between the capillaries of the meninx and the ventricular fluid (7), and between the ventricular fluid and the neural tissue via the ependymal surface. Metabolic exchanges across the external surface of the brain and spinal cord also persist as development continues.

Fig. 1.2. Development of the arterial vascularization of the brain. The longitudinal neural artery (1) of the ventral aspect of the rhombencephalon is supplied by branches of the primitive common carotid artery (2), the proatlantal artery (3) caudally, the trigeminal artery (4) and cranially by the hypoglossal artery (5). The longitudinal system of anastomoses between the cervical intersegmental arteries has not yet evolved into the vertebral arteries. More cranially, the primitive carotid artery ends as a rostral (6, olfactory artery) and a caudal (posterior communicating artery; 7) division. The anterior branch subdivides into the anterior cerebral (8) and future anterior choroidal (9) arteries, and both encircle the neck of the telencephalic vesicle (TV) and anastomose. Their lateral branches form the pericerebral arterial network of the hemispheres, including what is to become the middle cerebral artery. The posterior branch of the primitive carotid artery sends secondary branches toward the diencephalon (DV) (posterior choroidal arteries; 10), the mesencephalon (MV) (collicular arteries; 11), and the metencephalon (MtV) (superior cerebellar artery; 12). It connects with the longitudinal neural artery, thereby causing the trigeminal artery to regress, while the development of the vertebral artery supplies the caudal arterial system in place of the proatlantal artery, which then also regresses. 13, anterior inferior cerebellar artery; 14, posterior inferior cerebellar artery; MyV, myelencephalic vesicle.

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Table 1.1. Development of aortic arch, great vessels, and craniocervical arteries

Embryonic precursor	Primitive artery formed	Regression/remodeling	Becomes
Bulbus cordis	Truncus arteriosus	Aorticopulmonary septum forms	Ascending aorta, pulmonary trunk
Aortic sac	Ventral aorta	Persists	Ascending aorta, brachiocephalic trunk
Dorsal aortae (paired)	Carotid arteries (distal)	Right partially regresses (distal to SCA); left persists	Right: part of right SCA, right distal ICA, right ACA (from primitive ICA and olfactory artery), right MCA, right PComA (variable regression), right PCA (often annexed by basilar) Left: descending aorta, left distal ICA, left ACA (from primitive ICA and olfactory artery), left MCA, left PComA (variable regression), left PCA (often annexed by basilar) Fusion of midline plexus between ACAs: anterior communicating artery
Cervical plexi	Cervical intersegmental arteries	C1–C6 (proximal segments regress); C7	C1–C6: vertebral arteries C7: subclavian arteries
Dorsal plexi	Longitudinal neural arteries (paired)	Midline fusion	Basilar artery
<i>Aortic arches</i>			
I	Mandibular arteries	Involute	± distal ECA
II	Hyoid arteries	Mostly involute	Caroticotympanic arteries
	Stapedial arteries	Partially involute	Distal segments persist as middle meningeal artery and internal maxillary artery
III	Carotid arteries (proximal)	Persist	Common carotid arteries, proximal ICAs, ECA
IV	Right and left primitive aortic arches	Asymmetric remodeling	Right becomes proximal right SCA; left becomes definitive aortic arch
V	None		
VI	Ductus arteriosus	Asymmetric remodeling	Bilateral pulmonary arteries; ductus arteriosus

ACA, anterior cerebral artery; ECA, external carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery; SCA, subclavian artery; PCA, posterior cerebral artery; PComA, posterior communicating artery.
 Source: adapted with permission of Wolters Kluwer Health from Osborn, 1999 [5].

primitive carotid arteries whose proximal aspects (future definitive common carotid arteries [CCAs]) are derived from the ventral aorta and third aortic arches and whose distal aspects (future definitive internal carotid arteries [ICAs]) are derived from the paired dorsal aortae [5].

During the fifth week of embryonic development, the dorsal aortic segments between the third and fourth aortic arches regress (Fig. 1.3), leaving the ICAs supplied by the ventral aorta and third aortic arches. Around the same time, two vascular plexi – the longitudinal neural arteries – form dorsal to the third and fourth arches and supply the developing rhombencephalon. These arteries are supplied from below via cervical intersegmental arteries and also anastomose with the primitive carotid arteries via the primitive trigeminal, otic, hypoglossal, and proatlantal intersegmental arteries (some of which occasionally persist into adulthood as variant caroticobasilar anastomoses). The external carotid arteries also begin to develop in the fifth week, sprouting from the third aortic arches (CCAs) and possibly with contributions from the first and second arches.

During the sixth week, the caudal division of the ICA anastomoses with the longitudinal neural artery to become the

posterior communicating artery. Plexiform connections between the cervical intersegmental arteries fuse to form the vertebral arteries while the first six intersegmental arterial connections to the dorsal aortae regress. The subclavian arteries form from the seventh cervical intersegmental arteries.

In the seventh week, further remodeling of the primitive aortic arches occurs. The paired longitudinal neural arteries fuse in the midline to form the definitive basilar artery which itself anastomoses to the vertebral arteries. By the eighth week of development, the definitive adult configuration of the aortic arch and great vessels – including the ICAs and external carotid arteries arising from CCA trunks – has been achieved.

Development of craniocervical arteries: the circle of Willis and its branches

By embryonic week 6, the ICAs have divided into cranial and caudal divisions (Fig. 1.4). The cranial ICA divisions subsequently give rise to the primitive olfactory arteries, the anterior cerebral arteries, the anterior choroidal arteries, and the middle cerebral arteries. The anterior communicating

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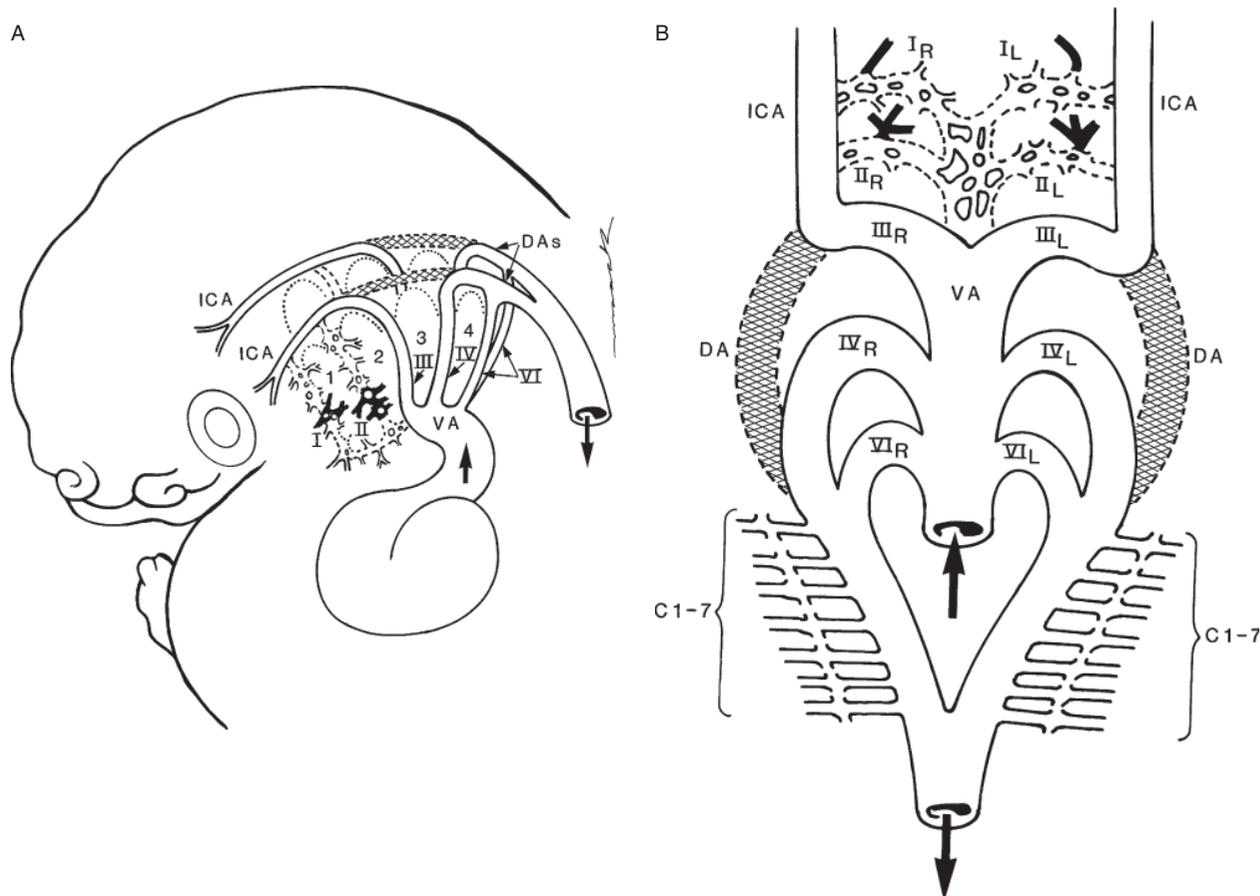


Fig. 1.3. Craniocerebral and aortocervical arterial development at approximately 4 weeks of gestation (6–8 mm crown–rump length). (A) Oblique three-dimensional anatomical sketch depicts the craniocerebral vasculature. The relationship between the embryonic pharyngeal pouches (1–4) and the developing aortic arches (I–VI) is shown. The third, fourth, and sixth arches (solid lines) have appeared. The plexiform first and second arches have regressed (dotted lines) except for some small remnants (solid black areas) that may persist as part of the future distal external carotid arteries. The primitive internal carotid arteries (ICAs) are formed from cephalad continuation of the dorsal aortae (DAs), whereas the common carotid arteries are derived from the ventral aorta (VA) and third arches. The DA segments between the third and fourth arches (cross-hatched areas) are still patent at this stage but will eventually regress completely. (B) This diagrammatic sketch (anteroposterior view) depicts the developing aortic arches (I–VI) and craniocervical vasculature. The plexiform first and second arches (dotted lines) have largely regressed, except for small remnants (solid black areas) that are annexed later by the future external carotid arteries. The third and fourth arches are prominent; the sixth arches are beginning to develop. The DA segments between the third and fourth arches (cross-hatched areas) are still present but will soon regress. Cephalad continuation of the right and left dorsal aortic segments from their junction with the third arches forms the primitive ICAs; their more proximal segments (i.e., the future common carotid arteries) are formed by the ventral aorta (VA) and third arches. The seven cervical intersegmental arteries are also depicted (C1–C7). Their midsegments are beginning to coalesce and will eventually form the definitive vertebral arteries. Direction of blood flow is indicated by large black arrows. (From Osborn 1999 [5], reprinted with permission of Wolters Kluwer Health.)

artery coalesces from a plexiform midline vascular network, connecting the two anterior cerebral arteries. Although initial branches of the middle cerebral arteries form in the embryonic period, the massive growth of the neocortex during fetal development results in a deepening sylvian fissure, an insula buried under opercula, and a highly convoluted mature middle cerebral artery architecture.

The caudal ICA divisions anastomose with the longitudinal neural arteries to become the posterior communicating arteries and proximal segments of the posterior cerebral arteries. After the longitudinal neural arteries fuse to become the basilar artery, the posterior communicating arteries regress to a variable degree, resulting in some individuals deriving supply to the distal posterior cerebral arteries primarily from the anterior (ICA) circulation and some primarily from the posterior (vertebrobasilar) circulation.

Development of cranial veins and sinuses

The cranial veins can be divided into several groups familiar in the mature human brain, including the superficial cortical veins, the deep subependymal veins, the posterior fossa veins, and the dural venous sinuses. They can also be divided based on evolutionary patterns in vertebrates into a dorsal venous system, a lateral–ventral venous system, and a ventricular venous system [6]. Development will be discussed in terms of the dural venous sinuses and the cerebral veins.

Analogous to, although more variable than, the development of the cerebral arteries, the dural venous sinuses arise from fusion of multiple plexi along the surfaces of developing brain. A primary head sinus arises from the primary dorsal hindbrain venous channel [5,7]. Anterior, middle, and posterior dural plexi drain their respective developing cerebral

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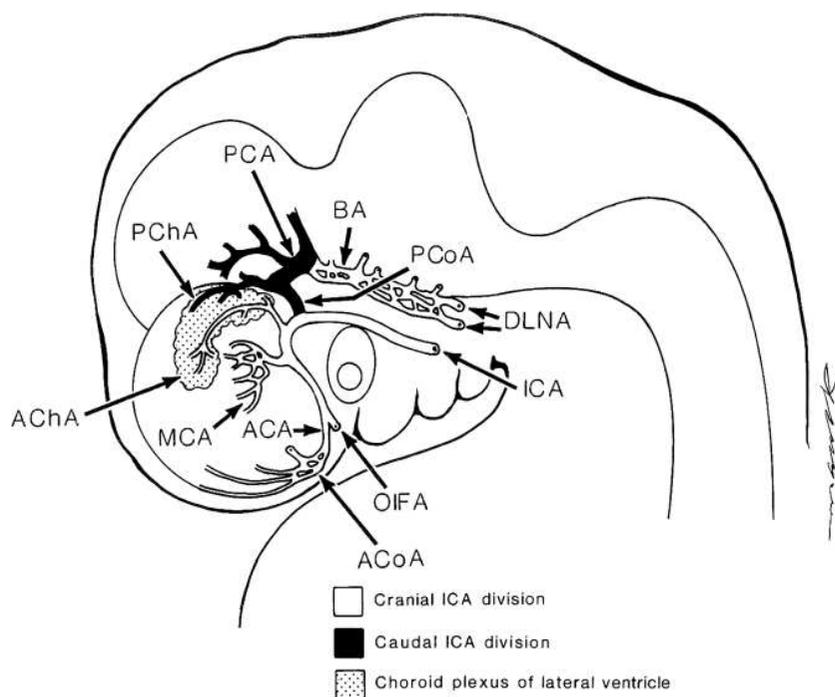


Fig. 1.4. Embryonic intracranial arteries at approximately 5 to 6 weeks of gestation. The primitive internal carotid arteries (ICAs) each have a cranial and a caudal division. The cranial divisions give rise to the primitive olfactory arteries (OIFA) as well as to the anterior cerebral arteries (ACAs), anterior choroidal arteries (AChA), and middle cerebral arteries (MCAs). The anterior communicating artery (ACoA) forms from coalescence of a midline plexiform network. The caudal divisions will become the posterior communicating arteries (PCoAs) and also supply the stems of the posterior cerebral arteries (PCAs). The basilar artery (BA) will be formed from fusion of the paired dorsal longitudinal neural arteries (DLNAs). (From Osborn 1999 [5], reprinted with permission of Wolters Kluwer Health.)

vesicles. By approximately eight weeks of embryonic development, paired primitive marginal sinuses extend from the anterior dural plexus along the sides of the anterior cerebral vesicle; these eventually fuse to form the superior sagittal sinus and the transverse sinuses (Fig. 1.5) [5]. The embryonic tentorial plexus gives rise to the straight sinus.

The median prosencephalic vein of Markowski drains the choroid plexus of the lateral ventricles by eight weeks, emptying into the falcine sinus, a midline dorsal interhemispheric plexus. As the basal ganglia and choroid plexus enlarge, the definitive internal cerebral veins develop and the median prosencephalic vein regresses, leaving its caudal remnant as the definitive vein of Galen connecting the internal cerebral veins to the straight sinus. It is thought that if the median prosencephalic vein persists as an outlet for deep venous drainage, a vein of Galen malformation results, along with concomitant atresia of the straight sinus and persistent falcine sinus. Many types of vascular malformations seen in postnatal life may have their origins in the primitive vascular plexus remodeling that normally occurs during embryogenesis, either as persistence of primitive connections during development or as aberrant activations of developmental genes later in life [5,8].

Pathogenesis and progression of brain arteriovenous malformations

The pathogenesis of brain AVM is not completely understood. Recent studies suggest that the initiation and progression of AVM require interplay among several factors, including (1) homozygous loss of function of causative genes in somatic endothelial cells, (2) angiogenic stimulation (response to injury), (3) participation of bone marrow-derived cells

(BMDCs), (4) alteration of monocyte/macrophage function, and (5) hemodynamic changes (Fig. 1.6).

Homozygous causative gene mutations

The genesis of sporadic brain AVM has been observed in a handful of reported patients. An important conceptual advance is that hereditary hemorrhagic telangiectasia (HHT) can serve as a familial form of the more common sporadic brain AVM disorder, similar to the homology between familial and sporadic cavernous malformations [9–12]. The two most prevalent causative genes for HHT are *ALK1* (encoding activin-like kinase) and *ENG* (encoding endoglin). The prevailing view is that HHT is caused by haploinsufficiency of one of its causative genes in somatic endothelial cells. However, inactivation of the remaining wild-type allele appears to have powerful effect, irrespective of the mechanism by which it is inactivated (e.g., loss of heterozygosity or loss of protein during inflammation) [13]. For example, the loss of a single allele of genes such as *Eng* or *Alk1* in animal models reproduces certain aspects of the human disease and is primarily found in older animals [14,15]. In contrast, loss of both alleles of any HHT-causative gene is embryonically lethal in mice [16,17], and conditional (tissue/time-specific) homozygous deletion of *Eng* [13] or *Alk1* [18,19] results in striking vascular malformations resembling the AVMs found in HHT. It has been shown that homozygous knockout of *Eng* in only around 1% of endothelial cells in mice resulted in a more severe cerebrovascular dysplasia after vascular endothelial growth factor (VEGF) stimulation than in *Eng*^{+/-} mice [20]. Moreover, analysis of human brain and lung AVMs in HHT indicates that haploinsufficiency of *ENG* is not sufficient to cause lesion development [21]. A tenable model explaining this phenotypic heterogeneity is that loss of function in the second allele locally or in

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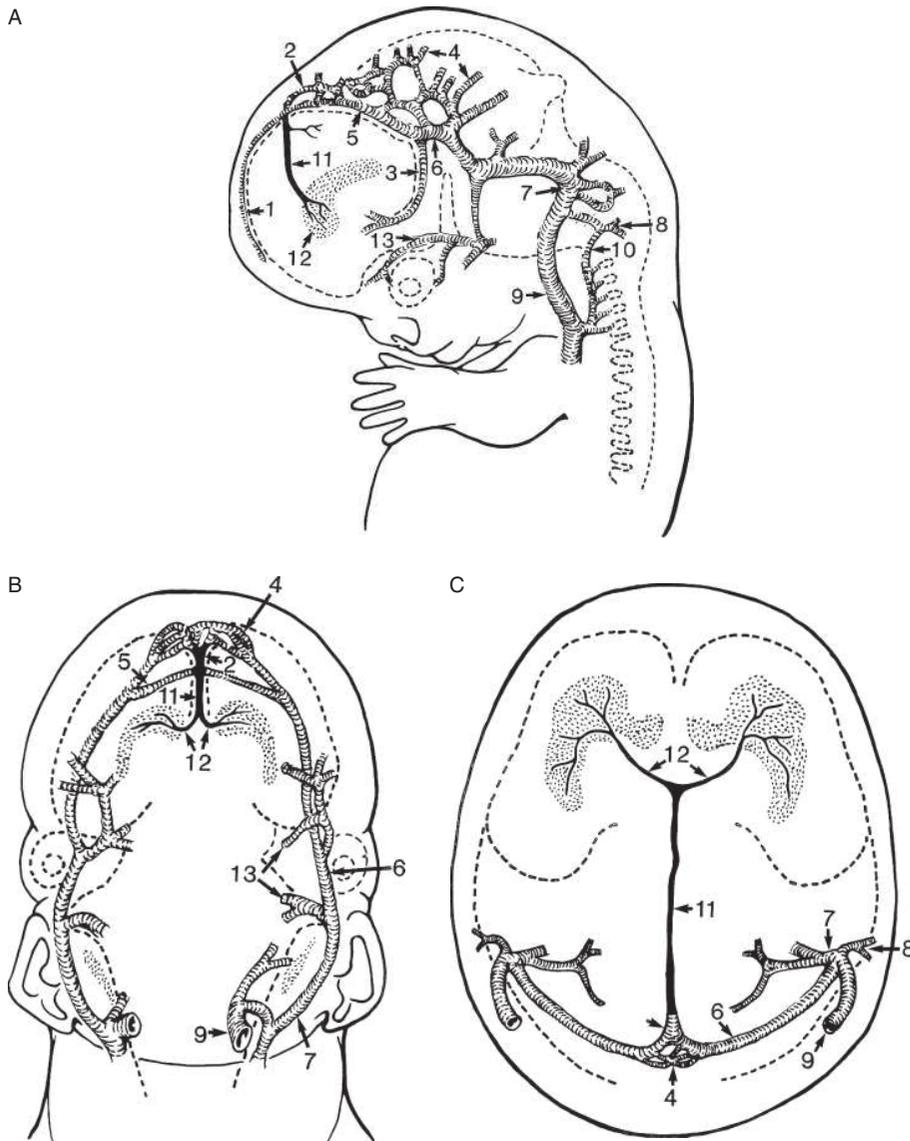


Fig. 1.5. Dural venous sinuses and cerebral veins at approximately 8 weeks of gestation. The choroid plexus is indicated by the dotted areas in the lateral (A), frontal (B), and axial (C) views. A single midline deep cerebral vein (the median prosencephalic vein of Markowski or primitive internal cerebral vein) drains the choroid plexi and is indicated by the solid black lines. (1) superior sagittal sinus; (2) primitive straight (falcine) sinus; (3) tentorial sinus; (4) tentorial plexi; (5) primitive marginal sinus; (6) primitive transverse sinus; (7) sigmoid sinus; (8) condylar, hypoglossal emissary veins; (9) internal jugular vein; (10) vertebral veins; (11) primitive internal cerebral vein (median prosencephalic vein); (12) choroid veins; (13) primitive supraorbital, maxillary veins. (From Osborn 1999 [5], reprinted with permission of Wolters Kluwer Health.)

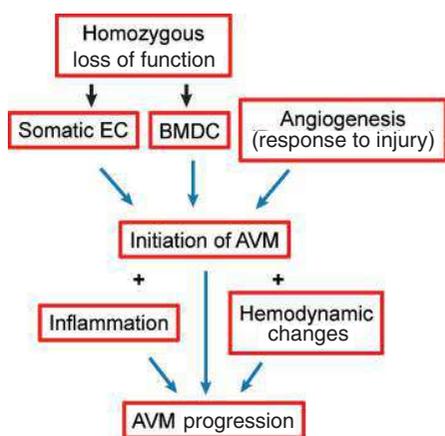


Fig. 1.6. Novel theories of arteriovenous malformations initiation and progression. AVM, arteriovenous malformations; EC, endothelial cell; BMDC, bone marrow-derived cells.

bone marrow-derived endothelial precursor cells contributes to the AVM phenotype. There is compelling proof-of-principle evidence that loss of function of the wild-type allele is relevant to vascular malformations, demonstrated for two related disorders: somatic mucocutaneous venous malformations [22] and cerebral cavernous malformations [23].

Angiogenesis and the “response to injury” hypothesis

The brain AVM lesional phenotype includes an active angiogenic and inflammatory component that is inconsistent with a static congenital anomaly [24]. A “response to injury” hypothesis may explain incomplete penetrance of brain AVM in patients with HHT. It is known that both *Eng*^{+/-} [25] and *Alk1*^{+/-} [14] adult mice develop vascular lesions in various organs, but spontaneous lesions in the brain are quite modest and only seen in older mice

[14,26]. However, more pronounced forms of cerebral microvascular dysplasia were induced using focal VEGF stimulation in *Eng*^{+/-} or *Alk1*^{+/-} mice [27–29].

Antenatal conditional deletion of *Alk1* causes arteriovenous fistula in neonatal brain and intracranial hemorrhage [19]. Conditional global *Alk1* deletion in adult mice induced AVM and hemorrhage in the lung and gastrointestinal tract, but not in the skin or brain. However, upon wounding, *Alk1*-deleted mice developed vascular dysplasia and direct arteriovenous connections around the skin wound, suggesting an abnormal response to injury. Direct arteriovenous connections have also been detected in the retina of *Eng*-deficient neonatal mice [13]. The combination of local angiogenic stimulation (Matrigel [a gelatinous protein mixture secreted by mouse sarcoma cells] plus VEGF/fibroblast growth factor) and *Eng* deletion led to gross venous enlargement [13]. Walker *et al.* described the brain AVM phenotype in mice with apparent arteriovenous shunting after focal VEGF stimulation in animals subjected to regional conditional *Alk1* deletion (Fig. 1.7) [30].

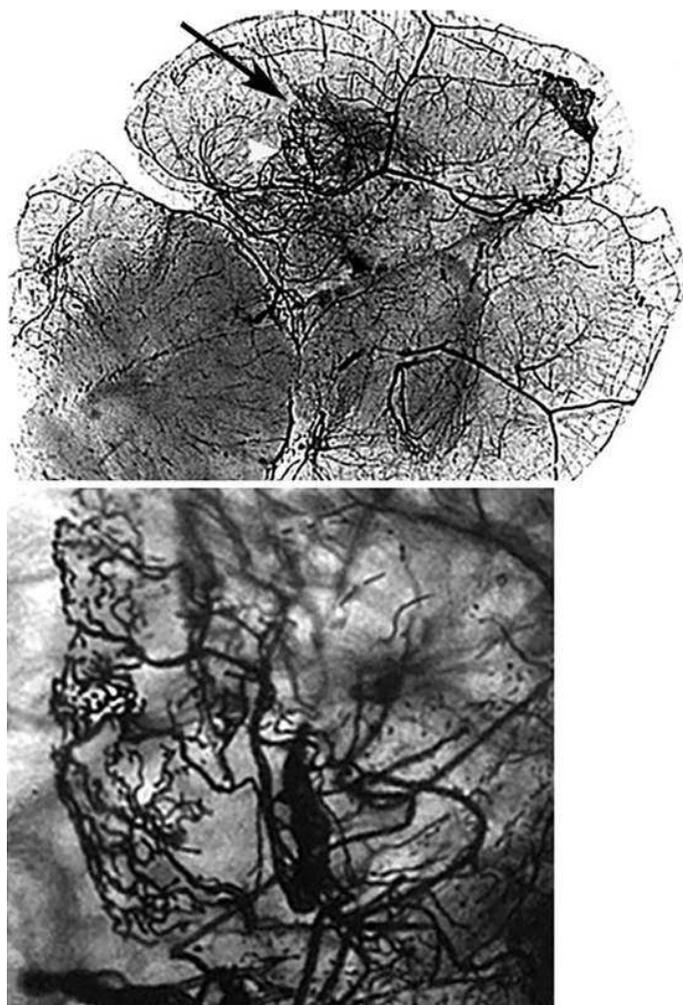


Fig. 1.7. Vessel casting showing arteriovenous malformations (AVM) in the brain angiogenic region. Top. Large tangled vessels resembling an AVM were detected in the brain angiogenic focus of an *Alk1*-deficient mouse (black arrow). Bottom. An enlarged image of angiogenic foci shows tangled dilated vessels. Scale bar, 100 μ m.

Taken together, both genetic manipulation and angiogenic stimulation seem to be required for AVM development. The angiogenic stimulus can be a minor injury, exogenous growth factor delivery, or high endogenous angiogenic factors in the brain of young and perinatal individuals.

Bone marrow-derived cells

Several studies support a pivotal role for BMDCs in AVM formation [31]. After VEGF stimulation in the brain, wild-type mice with *Eng*^{+/-} bone marrow developed a similar degree of dysplasia as somatically heterozygous *Eng*^{+/-} mice, suggesting that the loss of even one allele of a causative gene in BMDCs is sufficient to cause an abnormal vascular phenotype [31]. Similarly, *Eng*^{+/-} mice with wild-type bone marrow had fewer dysplastic vessels compared with *Eng*^{+/-} mice with *Eng*^{+/-} bone marrow. These data suggest that BMDCs contribute to AVM formation and indicate that the tendency to form AVMs might be rescued by transplantation of normal bone marrow.

So far, the cell type(s) in the bone marrow underlying AVM formation are unknown. There is evidence for two primary – probably complementary – BMDC types that serve as a locus for the phenomena: endothelial cells that incorporate into the angiogenic neovasculature [31,32] and monocytes/macrophages, which may provide critical repair function in response to injury [33–35] and/or provide guidance involving NOTCH signaling during angiogenesis [36,37].

The involvement of bone marrow-derived endothelial cells in focal angiogenesis has been shown in several conditions, such as tumor formation. Bone marrow-derived endothelial cells seed tumor vascular beds and regulate tumor angiogenesis [38,39]. Bone marrow-derived endothelial cells can incorporate into vessels in the brain angiogenic foci in mouse models [31,32,40]. In addition, endothelial precursor cells have been identified in vessels in adult human sporadic brain AVMs [41].

Inflammatory cells

Supporting evidence for myeloid cells playing a critical role in AVM progression includes (1) that most of the BMDCs that home to the brain angiogenic foci are CD68+ or CD45+ [31,40] and (2) that intraperitoneal administration of neutrophil neutralizing antibody reduces VEGF-mediated angiogenesis and matrix metalloproteinase (MMP)-9 activity [42]. Other experiments suggest that both neutrophils and macrophages are also relevant to large vessel remodeling [43].

Further, normal human monocytes rescue the impairment of *Eng*^{+/-} mice in repairing myocardial injury, whereas monocytes from patients with HHT fail to improve the myocardial repair [33,35]. The monocytes from patients with HHT type 1 migrate to stromal cell-derived factor 1 less effectively than normal monocytes, which is associated with an increase of CD26 expression [33,35]. These data suggest that the function of monocytes in vascular repair or remodeling is defective in patients with HHT, which could result in abnormal vascular remodeling and thus promote AVM progression.

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Hemodynamic changes in arteriovenous malformations

Vessels in an AVM are subjected to abnormally high flow rates. High vascular flow rates in *Alk1^{+/-}* mice induced by vasodilators (i.e. nicardipine or hydralazine) after focal VEGF stimulation increased the number of dysplastic vessels in the brain angiogenic foci [28].

Cerebral venous hypertension is a common symptom in brain AVMs [44]. Venous hypertension has been implicated in the formation of dural arteriovenous fistula [45,46] through a mechanism that involves the induction of angiogenesis [46]. In rats, non-ischemic levels of venous hypertension (15–23 mmHg) cause expression of hypoxia-inducible factor 1 α and its downstream signal VEGF [47]. Further, hypoxia-inducible factor 1 α , VEGF, stromal cell-derived factor 1 expression, neutrophils, macrophages, and MMP-9 activity all increase in the brains of the mice with venous hypertension. Capillary density in the parasagittal cortex also increases in the mouse venous hypertension model. These findings suggest that mild non-ischemic venous hypertension results in a pro-angiogenic state [48]. Consequently, venous hypertension could represent a kind of injury that triggers AVM development in subjects carrying mutant genes.

Inflammation in arteriovenous malformations formation and remodeling

A number of possibilities exist for the role of inflammation in the genesis and modulation of brain AVMs, apart from the fact that inflammatory infiltrates can be introduced into lesions through surgical manipulations, embolization, or secondary infectious processes [49–51]. The latter factors often confuse the interpretation of inflammatory cells such as lymphocytes and macrophages within AVM tissue specimens. In addition, hemorrhagic complications such as AVM rupture are associated with extravasation of hemosiderin and subsequent accumulation of macrophages and other reactive cells as a response to tissue damage. Therefore, the finding of inflammatory cells and cytokines within AVMs should be interpreted with caution.

However, even in the absence of obvious inciting processes, abnormal expression patterns of inflammatory mediators and cytokines, as well as influx of inflammatory cells into AVMs, have been observed by a number of investigators [52–56]. Studies on genetic and cytokine expression in AVMs suggest that inflammation is associated with AVM formation, progression, and rupture [24]. The typical findings in unruptured brain AVMs include perivascular inflammatory infiltrates (Fig. 1.8A) and intraparenchymal macrophages (Fig. 1.8B), occasionally associated with microscopic deposits of hemosiderin.

In humans and in animal models, AVMs have been associated with an increased inflammatory response [57–61]. Inflammatory markers are overexpressed in human AVMs, including myeloperoxidase and interleukin (IL)-6, both of which highly correlate with MMP-9 levels. Levels of MMP-9,

in turn, correlate with the expression of lipocalin–MMP-9 complex within AVMs, suggesting that neutrophils may play a role in their pathophysiology. Substantial numbers of neutrophils and macrophages occupy the perivascular spaces and the intervening stroma around even unruptured and non-embolized AVMs. While their presence does not indicate a causal association, inflammatory cells and cytokines may influence the formation and evolution of AVMs.

Remodeling of the vascular network in AVMs is facilitated by a number of proteases, which can enlarge the vascular elements in the nidus. This remodeling is partially mediated through VEGF activity and modulated by pro-angiogenic signals such as MMP. The MMPs maintain and remodel the extracellular matrix [62] and the MMPs, including MMP-9, are major components of neutrophilic tertiary granules and are also synthesized by monocytes and lymphocytes. There are significantly higher levels of MMP-9 in brain AVMs than in control tissue [53,63], but the source of this increase is unclear. Both production and activity of MMP-9 are stimulated during inflammation by the cytokines IL-8, IL-1 β , and IL-6. Matrix metalloproteinase-9 degrades key components of the cerebrovascular matrix including laminin, denatured collagen, and tight junction proteins such as zona occludens 1, leading to blood–brain barrier leakage and hemorrhage [64,65]. The MMP-9 signal colocalizes with myeloperoxidase and correlates with both myeloperoxidase and IL-6 levels, which suggests that the source of the MMP-9 may be the inflammatory cells in the environment [53].

Soluble ENG (extracellular domain) has been shown to contribute to another vascular disease, pre-eclampsia [66]. Soluble ENG is distinct from long- and short-form ENG, which have cytoplasmic tails of 47 and 14 amino acid residues, respectively [67]. Soluble ENG is also increased in brain AVMs [68]. It is not clear how soluble ENG is formed. A related type III transforming growth factor- β (TGF β) receptor, β -glycan, appears to be shed through a process that is mediated by MMP-1 [69]. Several different MMPs are also found in AVM nidus tissue [53,70,71], suggesting that similar mechanisms may contribute to the formation of soluble ENG or soluble ALK1 [66]. Tumor necrosis factor- α (TNF α) can induce the release of soluble ENG from normal placental villous explants [72]. Therefore, inflammatory proteins and cytokines in AVMs could cause shedding of soluble ENG and promote brain AVM instability. Another interesting observation is the increased levels of immunoglobulins within brain AVMs when compared with control brain [54].

Vascular inflammation is central to the pathogenesis of several vascular diseases, including intracranial aneurysm growth [73] and abdominal aortic aneurysm formation [74,75]. In addition to findings of vascular inflammation, associations between single nucleotide polymorphisms in genes encoding cytokines such as TNF α and increased brain AVM intracerebral hemorrhage risk have been described [76]. In addition, single nucleotide polymorphisms in the gene encoding IL-6 were also associated with a hemorrhagic clinical presentation in patients with brain AVM [52], and the highest risk *IL6* genotype (GG) was associated with the highest production of IL-6 in brain AVM tissue [71].

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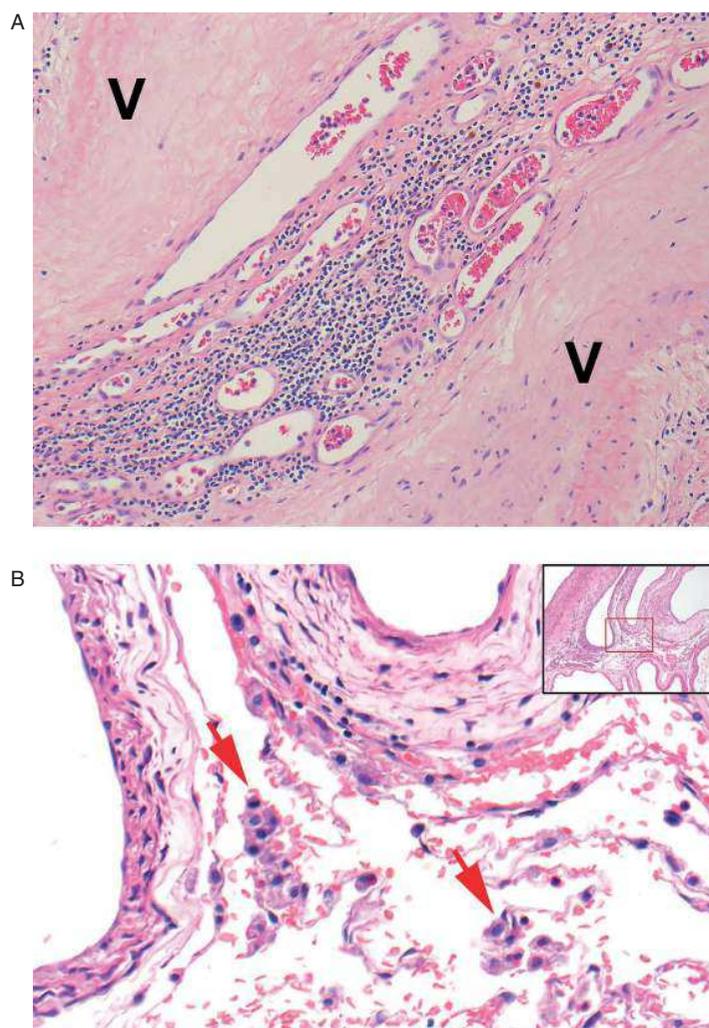


Fig. 1.8. Unruptured arteriovenous malformations without clinical or radiological evidence of hemorrhage. (A) Medium power magnification of perivascular lymphoplasmacytic infiltrate. The inflammatory cells are in the perivascular region between two large, abnormal vessels (V) along with numerous smaller capillaries (hematoxylin & eosin, original magnification $\times 200$). (B) High power magnification of perivascular macrophage aggregates. The inset demonstrates the region within the malformations at low power. The red square highlights the magnified area. Collections of macrophages (arrows) as well as scattered individual macrophages can be seen in the perivascular spaces (hematoxylin & eosin, original magnification $\times 400$; inset original magnification $\times 20$).

Hemodynamic stress can trigger vascular inflammation that initiates vascular remodeling and angiogenesis. High shear stress activates endothelial cells and upregulates leukocyte adhesion molecules, including intercellular adhesion molecule 1 and monocyte chemoattractant protein 1 [77–80]. Shear stress activates endothelial and smooth muscle cells and promotes their production and release of angiogenic factors and other cytokines critical for vascular remodeling [81,82]. Along with activated endothelial and smooth muscle cells, these inflammatory cells secrete proteinases, including MMPs and elastases [77], that digest the vascular wall and surrounding matrix to facilitate vascular remodeling and the release of angiogenic factors [83,84]. The MMPs and proinflammatory cytokines can interact to carry out both physiological and pathological vascular remodeling.

Clinical and experimental evidence suggests that AVMs undergo significant vascular remodeling and angiogenesis in adult life. The variable nature of AVMs, with respect to their growth, regression, and spontaneous hemorrhage [85], strongly suggests that AVMs are unstable blood vessels that must continuously remodel and repair in response to abnormally high flow conditions. A review of studies examining interval

angiography in 106 patients with mean follow-up periods of 8.4 years showed that over half of the AVMs increased in size and one-fifth decreased in size or vanished [86], suggesting active remodeling processes.

Histological studies further support the notion of active vascular remodeling and angiogenesis in AVMs. Endothelial cell proliferation rates measured using the Ki-67 index were higher in adult AVM specimens than the control brain (2.5 vs. 0.5%) [87]. Another study examining 37 AVMs and 5 control specimens found a seven-fold increase in non-nesting endothelial cells in AVMs compared with control brain [86], providing more evidence for active vascular remodeling and angiogenesis in AVMs. Underlying mechanisms for active vascular remodeling and angiogenesis in AVMs are under vigorous investigation [86,88–92].

An abnormal pattern of MMP-9 and tissue inhibitors of metalloproteinases has been found in AVM tissues [63]. There is markedly increased MMP-9 activity in AVMs compared with control brain samples and MMP-9 is found in the endothelial cell/peri-endothelial cell layer of AVMs. Along with endothelial and smooth muscle cells, inflammatory cells seem to be a major contributor to the abnormally high levels of MMP-9 in AVM

Section 1: Development, anatomy, and physiology of AVMs

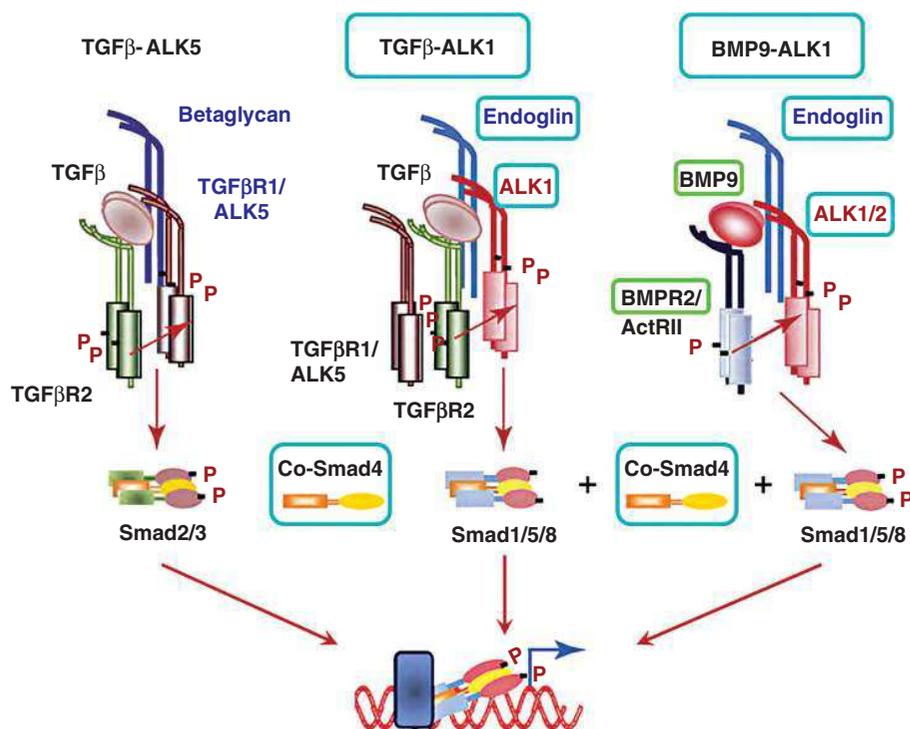


Fig. 1.9. A working model of transforming growth factor- β (TGF β) and bone morphogenetic protein 9 (BMP9) signaling relevant to arteriovenous malformations (AVM). TGF β signals through two pathways in endothelial cells, via the activin receptor-like kinases (ALK) 1 and 5. BMP9 signals via ALK1 and the BMP receptor type 2 (BMPR2). Endoglin is a membrane accessory protein required for efficient TGF β /ALK1 signaling. Genes linked to hereditary hemorrhagic telangiectasia are boxed in teal; other AVM syndrome genes within the pathway are boxed in green. (Adapted from Pardali E, Goumans MJ, ten Dijke P. Signaling by members of the TGF- β family in vascular morphogenesis and disease, *Trends Cell Biol* 2010;20: 556–67, with permission from Elsevier.)

tissue [53]. The increased MMP-9 activity can be expected to cause degradation of the vascular matrix, impairing structural stability of AVM vessels. Interestingly, higher levels of MMP-9 were associated with clinical characteristics that were linked to AVM hemorrhage [63]. There is interest in utilizing MMP inhibitors to stabilize fragile or inflamed blood vessels and prevent their rupture [93–96] and MMP-9 may serve as a pharmacological target to modify clinical behavior of AVMs [70].

Genetics, biomarkers, and implications for arteriovenous malformations management

Genetics of inherited syndromes

Studies of rare Mendelian forms of a disease often generate the first insights into understanding its molecular mechanisms. In the case of brain AVMs, insights into the pathogenesis and pathobiology have come from studies of HHT, a recessively inherited syndrome of mucocutaneous fragility and abnormal artery–vein connections, ranging in size from smaller telangiectases to larger AVMs [97,98]. In HHT, AVMs occur in brain and in visceral organs (lung, liver, gastrointestinal tract). There is significant morbidity and mortality associated with HHT, with the most serious manifestations related to severe bleeding or stroke, most commonly from complications of pulmonary AVM, or from brain AVM rupture. Sporadic AVMs and brain AVMs in HHT are morphologically and functionally similar; however, brain AVM multiplicity is much more likely in HHT than in sporadic AVM [99]. Over 85% of HHT is caused by heterozygous, presumed loss-of-function, mutations in three genes encoding proteins that function in the TGF β –bone morphogenetic protein (BMP)–9 signaling pathway: *ENG*, *ALK1*,

and *SMAD4* (Fig. 1.9) [97,100–105]. Mutations in two other genes encoding proteins in this pathway have been found in patients with HHT-like syndromes that feature lesions similar to AVMs: in *BMPR2* encoding a receptor [106] and in *BMP9*, encoding the ligand [107]. Taken together with mechanistic studies in mouse models, these findings suggest that it is BMP-9 signaling rather than canonical TGF β signaling that may be deranged in AVM lesions (Fig. 1.9).

Another pathway implicated in brain AVM pathobiology by studies of inherited syndromes is RAS/MAP kinase signaling. Capillary malformations–arteriovenous malformations syndrome, an autosomal dominant disorder featuring both cutaneous capillary malformations (“port-wine stain”) and arteriovenous fistulae or AVMs in organs including the brain, is caused by heterozygous inactivating mutations in *RASA1* [108,109]. These mutations have also been found in patients with spinal arteriovenous anomalies [110]. *RASA1* encodes p120-RasGTPase activating protein, a negative inhibitor of the Ras/MAP kinase signaling pathway, which mediates signaling from growth factor receptors and has effects on endothelial cell motility and apoptosis.

Genotype–phenotype correlations

Patients with HHT with different mutated genes show differences in AVM phenotypes. Both pulmonary AVMs [111–114] and brain AVMs [112–114] are more common among patients with *ENG* mutations than *ALK1* mutations; however, differences in brain AVM hemorrhagic presentation are not related to which gene is mutated [115]. Other genetic modifier effects could further alter HHT and AVM clinical manifestations; common polymorphisms in genes located in *TGF β* modifier