PATHO BIOLOGY OF HUMAN CEREBROVASCULAR MALFORMATIONS: BASIC MECHANISMS AND CLINICAL RELEVANCE

CEREBROVASCULAR MALFORMATIONS AFFECT more than 3% of the population, exposing them to a lifetime risk of hemorrhagic stroke, seizures, and focal neurological deficits. Cerebral cavernous malformations (CCMs) exhibit an immature vessel wall, a brittle hemorrhagic tendency, and epileptogenesis, whereas arteriovenous malformations (AVMs) lack capillary beds and manifest apoplectic bleeding under high-flow conditions. There are also more benign venous anomalies, capillary malformations, and lesions with mixed and transitional features. Advances have been made toward understanding the natural history, radiological and pathological correlates, and clinical management. Yet, mechanisms of lesion genesis and clinical manifestations remain largely unknown, and the clinical behavior in individual patients is highly unpredictable. Lesion pathogenesis likely involves abnormal assembly or maintenance of blood vessels, resulting in dysmorphic vessel phenotypes. Familial CCM disease is in part caused by mutations in a cytoskeletal-related protein that is likely integral to interendothelial cell connectivity and maturation of the vascular wall. Rare familial forms of AVM disease have been correlated with two different transforming growth factor-β receptor components, possibly causing disturbance in signaling during vascular assembly. Relevance of these mechanisms to the more common and otherwise identical sporadic CCM and AVM lesions is being explored. In this report, basic mechanisms of vasculogenesis and angiogenesis and how they possibly relate to the common cerebrovascular malformation lesions are reviewed. Novel concepts are discussed related to the cellular, molecular, and genetic substrates in CCM and AVM as well as to how this knowledge can be applied to predict, explain, and possibly modify clinical disease manifestations.

KEY WORDS: Angiogenesis, Arteriovenous malformations, Cerebral cavernous malformations, Hereditary hemorrhagic telangiectasia, Vasculogenesis

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apeutic modification of these lesions. In this report, we review the spectrum of cerebrovascular malformations and the basic mechanisms of vasculogenesis and angiogenesis and how they possibly relate to the individual lesions. We also present novel concepts about the cellular, molecular, and genetic substrates in cerebrovascular malformations and possible roles in lesion genesis and progression.

SPECTRUM OF CEREBROVASCULAR MALFORMATION PHENOTYPES

The modern conceptualization of cerebrovascular malformations starts with the neurosurgical visions of Harvey Cushing and Walter Dandy (66). In retracing their original monographs, we gain insight into the difficulties of logical classification and choices of therapeutic interventions for these lesions. These initial challenges to neurosurgical pioneers paved the way for the ensuing technical and conceptual advances by the next generation of neurosurgeons and neuropathologists. The parallel revolution in diagnostic radiology and imaging allowed the premortem and, eventually, the preoperative visualization of arteriovenous malformations (AVMs) as well as the study of angiographically occult vascular malformations. With the advent of magnetic resonance imaging (MRI) in particular, the cerebral cavernous malformation (CCM) emerged as a distinct clinical-radiological-pathological entity among the spectrum of pathologically heterogeneous, angiographically occult vascular malformations (122).

Current lesion nomenclature of cerebrovascular malformations (Fig. 1) is based on classic histological descriptions by Russell and Rubinstein (20, 92, 127, 128). The AVMs exhibit mature vessel wall elements with direct communications between arteries and veins and a high-flow profile predisposing to vascular recruitment, arterialization of venous structures, and gliosis of intervening and adjacent brain tissue (Fig. 1, A and B). The AVMs are prone to apoplectic hemorrhage by rupture of nidal vessels or associated aneurysms or by venous outflow obstruction (20, 30, 92). By contrast, CCMs seem to grow by a process of vascular cavern proliferation in the setting of repetitive lesional hemorrhages and exhibit brittle vascular morphology devoid of mature vessel wall elements (122, 126) (Fig. 1, C and D). CCMs do not exhibit the high-flow features of AVMs and are less commonly associated with apoplectic hemorrhage (6, 37, 120).

A third and the most common form of cerebrovascular malformation is the venous malformation, also known as venous angioma or venous developmental anomaly, a lesion that rarely manifests clinical sequelae and typically only when associated with a CCM lesion (2, 7). Population lesion prevalence is estimated at 0.5% for CCMs (37, 92, 104, 120) and 0.6% for AVMs (92). Cerebrovascular malformation lesions are well visualized by MRI, and their radiological lesion appearance can be categorized as likely AVM, likely CCM, likely venous malformation, or mixed CCM-venous malformation (2). Lesions can be further classified by size, multiplicity, and location. In this review, we do not consider the dural arteriovenous fistulae, in which the nidus of arteriovenous shunting is in the pachymeninges rather than the cerebral parenchyma, or spinal vascular malformations, although related biological mechanisms are likely involved in these lesions.

Despite the apparently distinct clinical-radiological-pathological profiles of the various cerebrovascular malformations, some lesions exhibit histopathological findings of mixed or transitional features implying related elements of pathogenesis (2, 8, 92, 128). Many CCMs seem to arise in close proximity to venous malformations (2, 7). It is not known whether mixed vascular malformations with a CCM component occur in the setting of genetic predisposition to CCM. Even within apparently distinct clinical-radiological-pathological lesion categories, the clinical course of individual cases remains highly unpredictable. Biological mechanisms underlying differential and overlapping lesion phenotypes and associated clinical manifestations have not been elucidated.

The clinical manifestations of cerebrovascular malformations can be empirically defined, including clinical penetrance (in familial cases), age at clinical presentation, symptomatic presentation (incidental; headache or nonspecific symptoms; seizures; hemorrhage; focal neurological or other deficits; frequency and severity of epilepsy; frequency of overt hemorrhage).
rhage; and associated nonneurological manifestations, including skin lesions) (121).

**BIOLOGY OF VASCULOGENESIS AND ANGIOGENESIS**

A disturbance of vascular assembly or maintenance is a *sine qua non* condition of cerebrovascular malformation lesions, along with a pathological response to abnormal vascular phenotype. The interrelated processes of vasculogenesis and angiogenesis result in blood vessel formation. Vascular endothelial cells play a central role in both processes. The formation, maturation, and remodeling of blood vessels are influenced by paracrine signals, growth factors, and cell-cell adhesion molecules within the extracellular matrix milieu (9, 77, 103, 146).

**Biology of Vasculogenesis**

Vasculogenesis refers to the formation of primitive blood vessels inside the embryo (the primitive heart and the primary vascular plexus) and its surrounding membranes (the extraembryonic yolk sac). Hematopoietic and endothelial cells form primitive vessels by expansion, differentiation, and interconnection of mesoderm-derived stem cells, hemangioblasts, which aggregate and form blood islands (48, 107, 119). Endothelial cells fuse together to form the extraembryonic vascular network, which grows toward the embryo. The first intraembryonic angioblasts appear at the single somite stage, and interconnection with their extraembryonic counterparts is established at the two-somite stage (167). After circulation is initiated, vascular smooth muscle cells and pericytes are recruited to the endothelial tubes to form increasingly mature vessels.

**Biology of Angiogenesis**

Angiogenesis is the process of remodeling and expansion of preexisting vessels (the primary capillary plexus) formed during vasculogenesis and does not include the primary differentiation of mesoderm-derived precursors as in vasculogenesis. Unlike vasculogenesis, angiogenesis occurs in the embryo as well as in adults. Angiogenesis occurs during tissue growth, development, and repair as well as in diseases (e.g., tumor growth). Organs such as the brain and neuroectoderm that are derived from ectoderm-mesoderm are vascularized by angiogenesis (106). The angiogenesis process is initiated through endothelial cell activation and mediation of angiogenesis-promoting proteins, including extracellular proteolytic enzymes (membrane-associated matrix metalloproteinases) that degrade the basement membrane (Type IV collagen and laminin) surrounding the endothelial cell lining (95). Growth and remodeling rely on similar branching processes, which are in part directed by flow conditions. Branching occurs by splitting of the vessel lumen by intussusceptive microvascular growth (107) and by capillary sprouting through endothelial cell migration from preexisting vessels, proliferation, and tube formation (5, 107, 118). Remodeling also includes “pruning,” which occurs by endothelial cell division. Recent evidence has indicated that endothelial cells express specific molecular markers, ephrin-B2 and EphB4, identifying arterial or venous endothelial cell fate, respectively (163).

**Vasculogenesis- and Angiogenesis-associated Ligand-receptor Complexes and Signaling**

Vasculogenesis and angiogenesis are regulated by a balance of stimulatory and inhibitory paracrine signals that target endothelial cells and other vascular wall elements and act through ligands (growth factors and extracellular matrix molecules), receptors (growth factor and cell-matrix receptors), and signaling cascades that are activated through these interactions. Ligands are polypeptide regulatory factors that are released by living cells or the extracellular matrix (110). Advances in identifying molecules essential to angiogenesis have been the focus of cancer research, because angiogenesis is required for tumor growth and metastasis (48). Endothelial cells emerge from existing blood vessels into the organ (e.g., kidney, brain, limb buds) or tumor that secretes angiogenesis factors such as vascular endothelial growth factor (VEGF). The general roles of many of these growth factors have been partially worked out with in vitro, in vivo, and genetic studies and are summarized in *Table 1*. The specific roles of many of these growth factors in cerebral vessels in relation to cerebrovascular malformations is not clear, and it is possible that blood vessels are heterogeneous in their capacity to respond to different angiogenic factors (98).

**VEGF and Its Receptors and Splice Variants**

VEGF is an early positive regulator of vasculogenesis, acting as a mitogen with primary specificity for endothelial cells (42, 47, 161, 168). VEGF exists as five secreted isoforms produced by alternative splicing from a single gene (45). The isoforms have varying potencies for endothelial migration, proliferation, and differentiation (26, 97). All five VEGF isoforms bind vascular endothelial tyrosine kinase receptor FLT1 (fms-related tyrosine kinase 1, alias VEGFR1) and KDR (kinase insert domain receptor, alias FLK1 or VEGF-R2), inducing dimerization and triggering kinase activation and cytoplasmic signal transduction cascades related to vasculogenesis and angiogenesis control (3, 28, 45, 98). KDR is found on hemangioblasts and endothelial and hematopoietic precursor cells and is essential to yolk sac blood island formation in early vasculogenesis and hematopoiesis (38, 126, 139). FLT1 plays a role in late vasculogenesis. Loss of a single Vegf (VEGF human ortholog) allele is lethal to mice embryos between Days 11 and 12 of gestation because of angiogenesis and blood island formation defects, indicating that the dose of Vegf is critical to vasculogenesis and angiogenesis (27, 46, 60). Knockout of Flt1 (FLT1 human ortholog), Kdr (KDR human ortholog), or Vegf in mice impairs vasculogenesis and angiogenesis, and the mice die at 8.5 to 9 days of gestation (46, 135). Flt1 is important for endothelial tube formation, and Kdr is integral to the generation of hemangio-
Angiopoietins and Their Receptors

Angiopoietins and VEGF are involved in angiogenesis vascular remodeling. Angiopoietin 1 (ANGPT1) binding induces endothelial cell-specific tyrosine kinase (TEK, alias TIE2) receptor (130, 148), whereas angiopoietin 2 (ANGPT2) is a TEK antagonist/angiogenic antagonist (68). TEK receptors control recruitment of mesenchymal cells that encase endothelial tubes. Vilkku et al. (157) have shown that there is increased TEK autophosphorylation activity in venous malformations and arteriovenous malformations (12). Mice deficient in Angpt1 (ANGPT1 human ortholog) or Tek (TEK human ortholog) die in the embryonic stage on Day 10.5 and exhibit angiogenesis deficiencies, including insufficient maturation, remodeling, and stabilization of primitive vasculature, resulting from endothelial failure to adhere to supporting pericytes or smooth muscle cells (48, 132). Overexpression of Angpt in mice results in increased vascularization and nonleaky vessels (150). Knockout of Angpt2 (ANGPT2 human ortholog) or Tie2 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains [TIE] human ortholog) results in poor vessel integrity, edema, hemorrhage, and death between embryonic Day 12.5 and postnatal Day 1 (87). In addition to modulating Tek signaling, Angpt1 and Angpt2 can directly support integrin-mediated cell adhesion (25). TIE (alias TIE1) receptors in combination with ANGPT1 are critical for development of vascular polarity during angiogenesis (84), and targeted disruption results in mice lacking endothelial cell structural integrity (132). The TIE ligand has not yet been elucidated (52).

Transforming Growth Factor-β1

Within the transforming growth factor-β (TGFβ) superfamily of structurally related growth factors (90), TGFβ1 (alias TGFβ1) is an important epitheliomesenchymal signaling molecule directing proximate interactions in embryonic organ development, including vasculogenesis and angiogenesis. TGFβ1 dimers signal mesenchymal cells to differentiate into pericytes and smooth muscle cells while inhibiting endothelial cell proliferation. TGFβ1 acts through serine-threonine kinase receptor (Type I and Type II) complexes to regulate gene transcription through mothers against decapentaplegic homolog (MAD, alias SMAD) proteins. TGFβ1 binding to TGFBR1/TGFBR2 heterotetrameric receptor complexes is regulated in part by binding to accessory receptor proteins (betaglycan and endoglin). Mutations in the endoglin gene (ENG) or activin A receptor Type II-like 1 (ACVRL1, alias ALK1) result in hereditary hemorrhagic telangiectasia (HHT) Types I and II, respectively, also known as the disease of Osler-Weber-Rendu (Fig. 2). ACVRL1 encodes a TGFβ Type I receptor. Patients with HHT have increased susceptibility to cerebral

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<th>Ligand</th>
<th>Receptors/cell type</th>
<th>Vascular function</th>
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<tr>
<td>VEGF</td>
<td>FLT1, KDR, neuropilin/hemangioblasts, endothelial</td>
<td>Vasculogenesis initiation, endothelial tube formation</td>
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<td>ANGPT1</td>
<td>TEK, receptor/endothelial</td>
<td>Endothelial tube encasement, agonist, mesenchymal cell recruitment</td>
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<tr>
<td>ANGPT2</td>
<td>TEK, receptor/endothelial</td>
<td>Angiogenic antagonist, antagonist, vascular destabilization</td>
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<tr>
<td>TGFβ</td>
<td>TGFB1/R2 complex, including ACVRL1 and ENG/ mesenchymal, endothelial</td>
<td>Inhibits proliferation, promotes differentiation of endothelial and hematopoietic cells</td>
</tr>
<tr>
<td>FGF2 (bFGF)</td>
<td>FGFR1, FGFR2/endothelial, smooth muscle, many other tissues and cell types</td>
<td>Vasculogenesis initiation with VEGF, synergistically initiates angiogenesis, and vessel maintenance with VEGF and PDGF</td>
</tr>
<tr>
<td>PDGF</td>
<td>PDGFRα, PDGFRβ/endothelial</td>
<td>Synergistically initiates angiogenesis and vessel maintenance with VEGF and FGF2, oncogene</td>
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<tr>
<td>HGF</td>
<td>c-met/hepatocytes, endothelial, smooth muscle</td>
<td>Angiogenic, mesenchymal-epithelial/endothelial interactions</td>
</tr>
<tr>
<td>IL-8</td>
<td>IL-8RA and B/endothelial</td>
<td>Angiogenic endothelial, induced by VEGF and BCL2 signaling</td>
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*VEGF, vascular endothelial growth factor; ANGPT1, angiopoietin 1; ANGPT2, angiopoietin 2; TGFβ, transforming growth factor-β1; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor-β; HGF, hepatic growth factor; IL-8, interleukin-8; FLT1, fms-related tyrosine kinase 1; KDR, kinase insert domain receptor; TEK, endothelial cell-specific tyrosine kinase; TGFB1, transforming growth factor receptor-β1; ACVRL1, activin A receptor Type II-like 1; ENG, endoglin; FGFR1, fibroblast growth factor receptor 1; FGFR2, fibroblast growth factor receptor 2; PDGFRα, platelet-derived growth factor receptor A; PDGFRβ, platelet-derived growth factor receptor B; IL-8R, interleukin-8 receptor; BCL2, B-cell chronic lymphocytic leukemia/lymphoma 2.*
AVMs (51). TGFB1 binds TGFBR2, TGFBR1, and the signal can be propagated by phosphorylation of pathway-specific MAD proteins (MADH2, alias SMAD2; MADH3, alias SMAD3), which heterodimerize with the common mediator MADH4 (alias SMAD4) (Fig. 2). The activated MAD complex translocates to the nucleus and binds deoxyribonucleic acid (DNA) to regulate TGFB1-targeted gene transcription like VEGF (13), fibronectin (64), and the production and secretion of extracellular matrix proteins, including Type I collagen by vascular smooth muscle cells (78, 159).

Half of Tgfb1 (TGFB1 human ortholog) gene knockout mice die secondary to dysvasculogenesis from defective interactions between epithelial and mesenchymal cells (39, 93, 96). Eng (ENG human ortholog) (4, 18, 82) and Acrv1 (ACVRL1 human ortholog) (101, 154) knockout mice generally have normal vasculogenesis but die at midgestation from defective angiogenesis, including poor vascular smooth muscle development, and endothelial remodeling. Some mice heterozygous for Eng (4, 18, 82, 133) or Acrv1 (142) mutations exhibited vascular phenotypes similar to HHT, including brain AVM-like vascular lesions (for a review, see Marchuk et al. [88]).

Basic Fibroblast Growth Factor and Platelet-derived Growth Factor-β Polypeptide

Initiation of vasculogenesis is thought to involve basic fibroblast growth factor (FGF2, alias bFGF) and VEGF (100, 115). FGF2 works synergistically with platelet-derived growth factor-β polypeptide to promote angiogenesis and regulate extracellular matrix molecules (collagenase, proteinases, urokinase-plasminogen activator, and integrins) to form new capillary cord structures (65). FGF2 stimulates tumor angiogenesis (11). FGF2 is detected in the basal lamina of blood capillaries, primarily at sites of vessel branching and in the endothelium of the capillaries of some tumors (40, 49), and may have an autocrine mode of action (74, 131). Interleukin-8 is a chemotactic angiogenic cytokine of endothelial cells and is induced by VEGF and B-cell chronic lymphocytic leukemia/lymphoma 2 signaling (76, 99, 140). Interferon-γ-inducible protein 10 (IP-10) is a potent inhibitor of both interleukin-8 and FGF2-induced angiogenesis (144).

Hepatocyte Growth Factor

Hgf (hepatic growth factor human ortholog) is synthesized and secreted by vascular smooth muscle cells and targets hepatocytes and vascular endothelial cells as an angiogenic growth factor stimulating migration, protease production, invasion, proliferation, and differentiation into capillary-like tubes (56, 125). Expression patterns of Hgf and its receptor, Met, a proto-oncogene tyrosine kinase (94), suggest they are mediators of mesenchymal-epithelial/endothelial interactions in angiogenesis (125, 141).

Intercellular Junction, Cytoskeletal Adhesion, and Cell Differentiation Signaling

The processes of vasculogenesis and angiogenesis not only include the differentiation of cells but the morphogenesis of an intricate multicellular arrangement of cells. The cellular basis of the morphogenesis is differential cell affinity. Integrins, immunoglobulin superfamily members, cadherins, and selectins are cell-cell adhesion molecules found in blood vessels undergoing remodeling. These molecules, along with the extracellular matrix milieu, likely mediate endothelial cell migration into the perivascular space and assembly of new vasculature in conjunction with surrounding supportive cells. Integrins mediate VEGF signaling and cell adhesion (cell-cell and cell-cytoskeletal adhesion) (23). Vascular endothelial-cadherin complexes with KDR to suppress endothelial cell apoptosis and plays an important role in permeability, vascu-
logenesis, and vascular remodeling (44, 166). E-selectin plays a role in endothelial cell proliferation, migration, and capillary formation. Cell adhesion specificity occurs in response to paracrine signaling (growth factors) during vasculogenesis, angiogenesis, and vessel maintenance.

**Growth Factor and Receptor Expression in Cerebrovascular Malformations**

VEGF and FGF2 are expressed at high levels during embryonic development but normally are absent in adult cerebral vasculature. VEGF, FGF2, and TGFβ1 expression in cerebrovascular malformations is possibly induced by proliferation of new vessels, hemodynamic stress, ischemia, and/or hemorrhage (72, 126). VEGF is predominantly expressed in the subendothelial layer and media of vessels in AVMs (Fig. 3A) and in the intercavernous matrix and subependymal layer of some CCM caverns but not others in the same CCM lesion. FGF2 expression is detected in the media of AVM vessels and the subependymal layer and intercavernous matrix of CCMs (126). The proportion of FLT1 (Fig. 3B) and KDR-immunopositive vessels was significantly greater in AVMs and in CCMs compared with control brain (P < 0.05) (153). Increased TEK receptors were detected in AVMs and CCMs compared with control brain (not statistically significant), and TIE was detected in rare vessels of all lesion types as well as in the brain (153).

**ULTRASTRUCTURE AND EXPRESSION OF STRUCTURAL PROTEINS IN NORMAL CEREBRAL VASCULATURE AND CEREBROVASCULAR MALFORMATIONS**

Cerebral vasculature is unique because of the existence of the blood-brain barrier, blood-cerebrospinal fluid barrier, and a valveless venous system (111). Brain microvessels are composed of endothelial cells, pericytes that encase the endothelial tubes, and astroglial processes that ensheath more than 95% of the abluminal microvessel surface (1) and are thereby believed to influence barrier-specific endothelial differentiation (31). Endothelial cells are the principal anatomic sites of the blood-brain barrier (111) and are interconnected by complex interendothelial tight junctions (165). These anatomic features in conjunction with specific transport systems result in highly selective transport of water-soluble compounds across the barrier endothelium. The pericytes providing maintenance and modulatory functions to small capillaries have smooth muscle-like properties (55). Smooth muscle cells perform similar structural and contractile functions in larger vessels.

**Histopathology and Ultrastructure of Normal Vasculature**

Cerebral blood vessels (as well as peripheral blood vessels) are composed of a tunica intima, media, and adventitia (10). The tunica intima consists of a layer of endothelial cells lining the vessel interior surface resting on a basal lamina and a subendothelial layer consisting of loose connective tissue that may contain occasional smooth muscle cells. The basal lamina contains extracellular matrix proteins fibronectin, laminin, and Type IV collagen (10), which provide structural support by anchoring endothelial cells to the luminal surface (Fig. 4A) (123, 126). Generally, immature vessels early in angiogenesis consist of nonadherent proliferating endothelium in a fibronectin-rich matrix, whereas more mature vessels have an adherent nonproliferating endothelium and express laminin more uniformly with a paucity of fibronectin in the matrix (123).
The tunica media consists chiefly of concentric layers of helically arranged smooth muscle cells; interposed among smooth muscle cells are variable amounts of elastic and reticular fibers in addition to proteoglycans (10). The tunica media layer is rich in extracellular matrix protein, Type III collagen, and various vascular smooth muscle cell proteins, including α-smooth muscle actin, myosin heavy chain, and smoothelin, are expressed in this layer (126, 152). The adventitia consists principally of longitudinally oriented Type III collagen elastic fibers in addition to the vasa vasorum (blood supply to the arteries).

**Abnormal Angioarchitecture of Cerebrovascular Malformations**

CCMs are characterized by caverns that are filled with blood or thrombus, which are lined by a single layer of endothelial cells separated by a dysmorphic connective tissue matrix (collagen) (Fig. 4C) (33). The lining of CCM caverns lacks interendothelial cell tight junctions (Fig. 4D) and subendothelial support (33, 165). Endothelial tight junctions (Fig. 4B) are a component of the blood-brain barrier, and their disruption is consistent with an underlying abnormality in cytoskeletal structure. The CCM subendothelium at the level of the basal lamina (extracellular matrix) expresses fibronectin to a greater extent than normal brain vessels and AVMs. In contrast, laminin is underexpressed in the subendothelial layer of CCMs compared with AVMs and normal vessels. The CCMs express Type IV collagen within the subendothelial layer and Type III collagen focally in the perivascular tissue.

Most caverns express actin in the subendothelial layer (Fig. 5A). Only 20% of large caverns express myosin heavy chain in the subendothelial layer (Fig. 5B), and most small caverns do not express myosin heavy chain. Smoothelin expression has not been noted in CCMs (Fig. 5C) (152). The CCMs have characteristic hemosiderin deposits near the basal lamina (Fig. 4C) and lack astrocytic foot processes; the processes stop at the border of the lesion (33, 165).

AVMs reveal preserved features of mature vessel wall phenotype altered by high flow and hemodynamic stress, including arterial, nidal, and venous aneurysms. In contrast to CCM ultrastructure, AVM lesions maintain normal vessel wall and structural integrity with endothelial cell denudation (Fig. 4, E and F) (165). The AVMs exhibit intense laminin expression localized in and around the internal elastic lamina and fail to demonstrate significant fibronectin expression. Type IV collagen seems to be expressed intensely in the subendothelium at the level of the basal lamina, whereas Type III collagen is observed in the media and perivascular tissue. Actin and myosin heavy chain immunohistochemical stains demonstrate intense expression within the media; some fibrocytes of perivascular connective tissue seem to stain with actin but not with myosin heavy chain (Fig. 5, D and E). Smoothelin displays mild to moderate expression in the media of some large vessels of AVMs; overall, its expression, including that in arterialized veins, is low and significantly less than that observed in normal brain vessels (Fig. 5F) (152). This finding may reflect the disappearance of the contractile property in vascular smooth muscle cells of AVM vasculature resulting from hemodynamic stress of turbulent blood flow through these lesions (152, 165). Consistent with AVM vascular remodeling and instability, levels of matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases (TIMP-1, TIMP-3, and TIMP-4) are increased in the endothelial/periendothelial cell layer of cerebral AVMs compared with control vessels (62). MMPs and TIMPs regulate many biological processes, including angiogenesis, by degrading pericellular substrates (143).

Compared with CCMs and AVMs, venous malformations and capillary telangiectasias have a relatively indolent natural history. They rarely hemorrhage and are variants of normal venous drainage and capillary networks, respectively. Kilic et al. (72) described two venous malformations that expressed Type IV collagen and laminin within the endothelia and subendothelia but little to no Type III collagen, fibronectin, or actin and negligible expression of angiogenic factors VEGF,
FGF2, and TGFβ. These two venous malformations demonstrate structural properties similar to those of normal cerebral veins and are angiogenically dormant (72).

Cultured Cells from Cerebrovascular Malformations

Endothelial cells cultured from AVMs have reduced secretion of endothelin-1, a molecule involved in vascular cell phenotypes (117), and increased proliferation (164). Angiogenic receptor differences were not found on endothelial cells cultured from CCMs (9). Cultured cell lines from a lesion cannot be assumed to represent a pure or homogeneous clonal heritage, and there is a loss of characteristic phenotypic features, especially after multiple passages (9, 152), in cell cultures from AVMs and CCMs. Despite potential for phenotypic change in culture, cultured cell lines from lesions may retain differential features of receptor expression or signaling in response to specific antigens compared with nonlesional cells, especially in primary cultures before multiple passages (108, 117, 149, 164). Cell cultures are a promising resource for molecular investigation of somatic mutation. Endothelial cells from umbilical veins of neonates have been successfully assayed for endoglin expression, establishing a diagnosis of HHT Type 1 (35).

GENETICS OF CEREBROVASCULAR MALFORMATIONS

Considerable progress in understanding the genetics of cerebrovascular malformations has been made by focusing on familial forms of disease. Three cerebrovascular malformation genes have been identified using a positional cloning strategy that relies on linkage analysis to identify chromosomal regions that cosegregate with disease in families more than is expected by chance (Table 2). Polymorphic regions or markers spanning the chromosomes are used to trace the inheritance of each section of a chromosome in families with disease to determine the region that is linked to disease. A disease gene is identified once a mutation (a nucleotide change that disrupts the function of a gene) that cosegregates with disease in the family is discovered. Finding different mutations in the same gene in other families with the same disease provides additional evidence that the correct gene has been implicated. Mutations may result in disease through gain of function (i.e., increased permeability, affinity for substrate), loss of function caused by dominant negative interaction (i.e., structural, multimeric proteins), or a reduction in the synthesis of normal protein.

Cerebral Cavernous Malformations

Most patients (50–80%) with CCMs are apparently sporadic without a known family history of CCM (33, 37). Single CCM lesions may be found in roughly 75% of sporadic cases and 8 to 19% of familial cases (21, 33, 79). The presence of multiple lesions is indicative of familial forms of CCM.

Familial CCM is genetically heterogeneous, and families exhibit Mendelian (single gene) inheritance at three loci (CCM1, CCM2, and CCM3) contributing to approximately 40%, 20%, and 40% of non-Hispanic familial cases, respectively (34). All three genetically distinguishable types of CCM exhibit autosomal dominant inheritance and have different preliminary estimates of disease penetrance, defined by genetic inheritance of the linked region presumably containing a mutation and the manifestation of clinical symptoms (88, 100, and 63% in CCM1, CCM2, and CCM3, respectively) (34). It is likely that some clinically asymptomatic family members have a mutation in CCM1, CCM2, or CCM3 and harbor CCM lesions that have not bled to cause symptoms (21, 22). The CCM2 and CCM3 genes map to the 7p13–15 and 3q25.2–27 regions, respectively, and have yet to be identified (34).

There has been recognized clustering of cases in Hispanics of Mexican descent (57), all apparently related to the same founder mutation (Q455X) (129). Other clusters have been identified in other parts of the world, but it is not clear if these

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<td>CCM (CCM1)</td>
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<td>CCM (CCM2 and CCM3)</td>
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<td>AVM, HHT1 (ENG)</td>
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<td>AVM, HHT2 (ACVRL1)</td>
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<td>AVM, HHT3</td>
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a CCM, cerebral cavernous malformation; AVM, arteriovenous malformation; HHT, hereditary hemorrhagic telangiectasia; ENG, endoglin; AD, autosomal dominant; RAP1A, Ras-related protein 1A; TGFβ1, transforming growth factor-β1.

b see Note Added in Proof at end of text.
represent epidemiological variations in CCM prevalence or apparent clustering secondary to recognition and identification biases. No race has been shown to be immune from familial or sporadic CCM.

The CCM1 gene was positionally cloned by linkage, haplo-type, and mutation analyses mainly in CCM families with a Hispanic-American ancestral disease haplotype (57, 129) in the 7q11.2–21 region and a common mutation in CCM1 that encodes the CCM1 or Krev interaction trapped 1 protein (KRIT1) (41, 113, 129, 134). The CCM1 gene consists of 20 exons spanning 45,799 base pairs and maps to the 7q11.2–21 region. The start of translation seems to be in exon 5, and approximately 88 different germ line mutations distributed throughout the CCM1 gene have been described in association with CCM in many different races (32, 36, 80, 81, 129, 156). Mutations in CCM1 described to date all presumably result in CCM1 premature termination and loss of function. Germ line CCM1 mutations have been identified in apparently sporadic CCM cases that were caused by unrecognized familiality or spontaneous germ line mutations (81, 85). Two different somatic mutations in CCM1 were identified in DNA isolated from a lesion surgically excised from a patient without a family history of the disease and harboring a single CCM, supporting a two-hit hypothesis of lesion genesis (71, 75). In the two-hit model, a vascular cell with two mutations (either germ line or somatic), resulting in complete loss of functional CCM1 protein, clonally expands to form a CCM lesion. Presumably, the multiple CCM lesions found in familial cases result from the same germ line mutation found in every cell plus a different somatic mutation. Multiple lesions would not be the result of metastasis. Lesions are a mosaic of normal cells morphologically disrupted by abnormal cells with two hits (71). Familial CCM exhibits an autosomal dominant mode of inheritance but is likely recessive at the cellular level, and lesion genesis may require two hits to the same gene. Other mechanisms such as haploinsufficiency or trans-transheterozygous mutations (somatic mutations at CCM2 or CCM3 in addition to a CCM1 somatic mutation) are a possibility (53).

Since the identification of the CCM1 gene, investigations have focused on functional characterization of the CCM1 protein. Three functional domains have been predicted on the basis of sequence homology with known proteins and protein-protein analysis using yeast two-hybrid screening. The two-hybrid system is set up to detect transcription of a reporter gene either by colorimetric tests or selection for growth. Reporter gene transcription depends on association of a DNA-binding domain fused to a gene of interest and a transcriptional activation domain fused to many different genes that may interact with the gene of interest. Protein-protein interactions are identified when a yeast colony grows or turns blue, indicating that the fusion protein containing the gene of interest is interacting with one of the fusion proteins containing the activation domain and allowing transcription of the reporter gene. The NPXY motif in the amino terminal of CCM1 apparently binds integrin cytoplasmic domain-associated protein (ICAP1), suggesting that CCM1 is part of the integrin signaling pathway (specifically with β1-integrin complexes) and cell adhesion to other cells as well as to the extracellular matrix (134, 169, 170). At least 8 of 22 integrin heterodimers are expressed on angiogenic and quiescent vascular endothelial cells, including 5 with β1-integrin components (145). Integrins are activated by VEGF and FG2 signaling in angiogenesis (23, 145). Ankyrin repeats in the middle of the CCM1 protein are thought to be involved in protein-protein interaction. The FERM domain found in exons 14 to 18 of CCM1 has been found in proteins that link cytoplasmic proteins to transmembrane proteins. The carboxy-terminal of CCM1 interacts with Ras-related protein 1A (RAP1A, alias Krev1/rap1a), a member if the Ras-family of guanosine triphosphatases, with a yeast two-hybrid screen, suggesting a tie to the tumor suppression pathway possibly at the point where Ras becomes part of the integrin pathway (16, 134, 169). The CCM1-RAP1A interaction may not be biologically relevant, because the reciprocal bait-prey swapping with the yeast two-hybrid system does not show interaction (88). The CCM1 gene is transcribed as a 3.50-kilobase message in brain and additional tissues not known to be affected in CCM patients (heart and muscle) (43, 71, 134). With CCM1 antibodies, CCM1 colocalizes with β2-tubulin in endothelial cells in culture and is thought to interact with the cytoskeleton to determine cell shape through cell-cell and cell-matrix interactions (Fig. 2) (58).

**Arteriovenous Malformations**

The AVMs typically present as solitary lesions, except in the rare setting of HHT, also known as the disease of Osler-Weber-Rendu. Clinical presentations of patients with symptomatic cerebral AVMs associated with HHT are similar to those of patients with “sporadic AVMs” and include hemorhage, seizures, and/or progressive focal neurological deficits. The cumulative hemorrhagic risk of “sporadic” cerebral AVMs is 2 to 4% per lesion per year (102); the bleeding rate of cerebral AVMs associated with HHT is not known but is likely similar to that of sporadic AVMs. HHT is inherited as an autosomal dominant multisystemic vascular dysplasia with an estimated prevalence of 1 in 10,000 to 40,000 persons (59, 61, 67, 73). Cerebral AVMs are found in 10 to 25% of patients with HHT (116, 124, 162), and two of three genes are known to cause HHT disease: the ENG gene on chromosome 9q and the ACVR1 gene on chromosome 12q (162). Mutations in ENG result in HHT Type 1, which is associated with a higher prevalence of cerebral and pulmonary AVMs than HHT Type 2 (15, 35). HHT Type 2 disease tends to be milder with a later age of onset and results from mutations in ACVR1 (67, 91). The ENG and ACVR1 genes encode proteins in TGFβ1 receptor complexes. The ENG gene consists of 15 exons spanning 35 kilobases and encodes endoglin, a homodimeric membrane glycoprotein found in human vascular endothelium (Fig. 2) (91). The ACVR1 gene consists of 9 exons spanning less than 15 kilobases and encodes ACVR1, a Type 1 cell-surface receptor that is part of a heteromeric signaling complex for the TGFβ superfamily of ligands on endothelial cells; it contains serine-
threonine kinase subdomains, a glycine- and serine-rich region preceding the kinase domain, and the short C-terminus tail (14). Approximately 46 different mutations have been described in \textit{ENG} and 23 different mutations have been described in \textit{ACVRI}, resulting in protein truncation, loss of protein, or amino acid changes that have been identified in HHT patients. Investigation into various mutations suggests that the predominant mechanism of HHT disease results in a reduction of functional protein through haploinsufficiency of either \textit{ENG} or \textit{ACVRI} receptor subunits (35, 54, 105, 108, 109, 137). One \textit{ENG} mutation, \textit{\delta}GC frameshift, is able to form heterodimers with normal endoglin, which is thought to result in a reduction in functional protein (86). Theories concerning a focal nature of lesion formation include the two-hit model, local inflammation, endothelial cell injury, and hypoxia (88). Although there have been case reports of 19 families with at least two members affected with an AVM without HHT, mutations have not been sought in the \textit{ENG} or \textit{ACVRI} genes (63, 69).

Venous Malformations

The most common form of cerebrovascular malformation is the venous malformation, also known as venous angioma or venous developmental anomaly, a lesion that rarely manifests clinical sequelae and typically only when associated with a CCM lesion (2, 7). Approximately 3% of the general population harbors cerebral venous malformations (37, 92). Investigation of a dominantly inherited cutaneous venous malformation syndrome has clearly implicated the \textit{TEK} gene (alias \textit{TIE2}), an ANGPT1/ANGPT2 receptor tyrosine kinase (Fig. 2) involved in endothelial cell-smooth muscle cell communication in venous morphogenesis (157, 158), and a gene yet to be identified (24). Investigation of the \textit{TEK} mutations seems to result in ligand-independent hyperphosphorylation (increased and uncontrolled signaling) (24, 157). Increased and uncontrolled angiogenesis signaling may be a trigger of venous malformations in the central nervous system (88).

Genetic Modifiers and Multiple Gene Interactions

Substantial intrafamilial heterogeneity is found in CCM1, HHT Type 1, and HHT Type 2 diseases, supporting the hypothesis that clinical penetrance and severity of disease are dependent on additional genetic and environmental factors. It is likely that part of the clinical variability may be a result of differences in genes involved in the same disease pathways or other genes involved in the development or maintenance of cerebral vessels. The additional genetic determinants of clinical phenotype may include mutations that obviously disrupt a gene’s function or polymorphisms that are not sufficient to cause disease themselves. Obvious candidates for genetic modification are the genes implicated in heritable forms of cerebrovascular malformation disease. Genetic changes in the \textit{CCM1} gene may affect disease caused by either the \textit{CCM2} or \textit{CCM3} gene. Disease penetrance in mice with a single \textit{Eng} seems to depend on the mouse strain, suggesting a role of genetic modifiers (19). Characterization of the pathways involved in vasculogenesis and maintenance should identify additional candidates that may modify cerebrovascular malformation disease phenotypes. In addition to genes modulating lesion genesis, there may be other genes causing or predisposing to lesion growth, hemorrhage, or associated epilepsy. Identification of disease genes and modifiers of disease phenotypes should allow the integration of molecular genetics into predicting disease manifestations and outcome.

Differential Gene Expression in Cerebrovascular Malformations

Significant advances, including the prediction of some disease outcomes and the identification of new therapeutic strategies, have been made with the use of microarray technology (29, 114, 138, 155). Comparison of gene expression between two or more tissues allows identification of expression that is unique to a particular disease state. Differential gene expression may be related to genesis of disease or modifiers of disease manifestation. The expression profile of a disease state includes genes that may be targets for therapy.

Several components of the molecular pathways involved in causing and maintaining familial forms of cerebrovascular malformations have been identified. However, complex interactions are expected between proteins and other molecules that define the AVM and CCM lesions, necessitating the concurrent examination of pathways in more detail.

Differentially Expressed Vasculogenesis/Angiogenesis Genes

The expectation that some vasculogenesis, angiogenesis, and disease-related genes are differentially expressed in cerebrovascular malformations has been confirmed in part (136). Preliminary gene expression results identified 310 up-regulated and 558 down-regulated genes in AVMs and CCMs compared with superficial temporal arteries (STAs) \((P = 0.012)\), including differences in genes involved in growth factor signaling (decreased \textit{ANGPT1}; increased \textit{VEGF} [a trend], and increased \textit{ENG}, a TGFB receptor component), decreases in a cell adhesion gene (\textit{PECAM1}, alias CD31), decreases in an endothelium-specific gap junction (\textit{GJA4}), and decreases in extracellular matrix genes (\textit{LAMA3} [laminin], \textit{SMTN} [smoothelin]) (136). Increased protein expression measured by immunohistochemistry of \textit{VEGF} (123, 126, 152, 153) and decreased expression of \textit{LAMA3} and \textit{SMTN} (152) in AVMs and CCMs compared with STAs are consistent with results of transcript quantitation.

In addition, CCMs showed unique decreased expression of a \textit{VEGF} receptor (\textit{KDR}) and cell adhesion molecules involved in integrin signaling (\textit{ITGB3} [integrin \textit{\beta}3], \textit{ROCK1}) compared with AVMs and STAs. The AVMs showed specific differential gene expression of growth factor signaling molecules (increased \textit{FLT1} [a VEGF receptor] and decreased \textit{TIE} and \textit{TEK} [angiopoietin receptors]), decreased integrin signaling mole-
cules (ITGB5 [integrin β5], ITGA6, [integrin α6], and CTNNAL1, [α-catenin]), and decreased CCM1 gene expression (136). Previous studies have shown overexpression of endoglin proteins in sporadic and familial AVM lesions (17) consistent with up-regulated gene expression for ENG in AVMs. Some of these preliminary differential gene expression findings confirm immunohistochemistry results as mentioned previously; others need to be verified with alternate quantitative methods of transcripts and subsequent analyses of the proteins.

Differential Gene Expression and Novel Discoveries

Expression profiles unique to CCM or AVM lesions include candidate genes for lesion genesis and targeted intervention. Two genes responsible for inheritable CCM and mapping to 7p13–15 (CCM2) and 3q25.2–27 (CCM3) have yet to be identified. Differential gene expression of CCM lesion results implicate three candidates for CCM genesis: TAXI-binding protein 1 gene (decreased TAX1BP1), a tumor necrosis factor receptor-associated factor 6 interacting protein involved in interleukin-1 signaling (83), and a gene with unknown function (DFNA5) mapping to the CCM2 region. A voltage-gated K⁺ channel (decreased KCNAB1) that interacts with integrin (112) maps to the CCM3 region.

Additional expression profiles indicative of disease stage and prognosis might allow integration of molecular data into clinical decision making. The preliminary results of differential gene expression have revealed possible evidence of a unique immune response within the CCM lesions. Thirteen immunoglobulin genes and a unique allele of the major histocompatibility complex were markedly up-regulated in CCMs compared with AVMs and STAs. Progression of CCMs may be caused by inflammation resulting from an immune response, or hosts may have a unique immune predisposition.

In summary, genes involved in the growth, integrity, and maintenance of blood vessels and immune response may be important disease modifiers in CCMs and AVMs. These modifiers may affect the severity of the disease, including lesion size and age at clinical presentation, or may be associated with specific clinical manifestations, including hemorrhage or epilepsy. These candidate genetic modifiers should be examined systematically as individual genes and in related mechanistic and functional pathways in relation to specific categories of lesion genotype, phenotype, and clinical manifestations.

FUTURE RESEARCH DIRECTIONS AND CLINICAL RELEVANCE

Enhanced Disease Classification

The field of cerebrovascular malformations has come a long way since the early classification schemes based solely on descriptive pathoanatomy and empirical clinical observations. Imaging advances, notably MRI techniques and rigorous histopathological features, have allowed the definition of distinct phenotypes as well as endophenotypes representing clinically relevant disease states (2, 8, 21). Terms such as cryptic vascular malformations, angiographically occult vascular malformations, angiomatosus telangiectasias have been replaced by more precise lesion nomenclature with biological underpinning and clinical relevance. Familial CCMs and the AVMs of HHT are now correlated with specific gene loci. Imaging studies allow sensitive and specific lesion recognition and surveillance of disease progression (lesion genesis, progression, and clinical behavior). Special imaging sequences allow the recognition of mixed vascular malformations (including mixed CCM and venous malformations) and the occult precursors of clinically relevant lesions (gradient echo imaging of immature CCMs). Specific molecular markers are emerging for more sensitive and specific disease classification, as in CCM-1, CCM-2, or CCM-3 or HHT Type 1 or Type 2. These may carry clinical or prognostic significance, as with Hispanic Americans of Mexican descent likely carrying CCM1 disease and patients with HHT Type 1 more likely to harbor brain and pulmonary AVMs. Most sporadic CCMs and AVMs still elude specific molecular classification, but it is clear that these lesions form by mechanisms affecting the same pathways as familial lesions, as in the recent discovery of somatic CCM1 mutations in a case of solitary CCM with unaffected parents (no germ line mutation) (71). The future holds much promise of classification of most lesions for more accurate diagnosis and prognosis.

Screening and Genetic Counseling

These advances have already had an impact on practical clinical management. Multifocal CCM or AVM disease now calls for a thorough family history, and this, in turn, can uncover previously unrecognized or misdiagnosed affected patients. Cases with myelopathy of unknown cause and others misdiagnosed as multiple sclerosis have now been clearly shown to represent unrecognized CCMs because of familial clustering. The management of epilepsy in the setting of CCM is vastly different if lesions are solitary or multiple on gradient echo MRI (160). The presence of an associated venous anomaly calls for special surgical considerations aimed at preserving the venous angiomatosis (2). The likelihood of de novo lesion genesis or progression of preexisting lesions has imposed rational surveillance strategies to accompany symptomatic follow-up. The counseling of women of childbearing age about pregnancy with CCM or AVM, options of management before and during gestation, and screening regarding possibly affected offspring has removed much fear and replaced anxiety with truly informed decisions. In the case of HHT Type 1, assaying umbilical vein endothelial cells for endoglin expression can quickly establish whether the neonate carries the disease (35).

Prognostication and Prediction of Disease Behavior

With more accurate molecular classification, careful correlation of genotype and phenotype should emerge as well as the significance of surrogate endophenotypes in predicting disease behavior. Not all forms of disease are likely to carry
the same clinical associations or prognosis. The behavior of CCMs and AVMs remains highly unpredictable, and it is clear that host, environmental, and gene susceptibility factors affect disease severity and specific clinical manifestations. The definition of these factors should allow better screening for patients harboring a particular lesion who are more likely to bleed or develop intractable epilepsy. There may be specific factors predicting individual lesion progression, and clinicians should learn to seek these factors in individual patients.

Gene and Molecular Therapy
The modification of clinical phenotype is the ultimate goal of molecular medicine, and this is also true of vascular malformations. Gene or molecular therapy may soon be guided to the host with the aim of preventing lesion genesis or, more likely, to the lesions to make them less vulnerable to bleeding, more sensitive to radiotherapy, or less epileptogenic, or even to promote lesion involution altogether. Therapeutic vectors have been developed altering gene expression in endothelial cells (147, 151), and these could be delivered to lesions by endovascular or stereotactic routes.

NOTE ADDED IN PROOF
The CCM2 gene (MGC4607) has been identified recently by Liquori et al. (83a). Eight different mutations were described in nine families with CCM. The predicted CCM2 protein encodes a phosphotyrosine-binding domain similar to ICAP1α and may be part of the integrin signaling pathway.

REFERENCES
GAULT ET AL.


Data about the ultrastructure and the expression of structural proteins in normal and abnormal vascular tissues and in cell cultures are summarized. Finally, the genetics of cerebrovascular malformations and recent data about differential gene expression in these tissues are explained. Thus, the main trunks of pathobiology are considered. Of course, many details in special fields may be missing; however, it is a well-known problem to keep in frame with a review article. The authors solve this problem by presenting current data with reference to the recent literature, two-thirds of it published within the past 5 years.

There are still many unsolved problems associated with cerebrovascular malformations: e.g., the initial pathogenesis, development, and natural course are not entirely understood; genetic factors involved in their pathogenesis are only partially known; and disease screening with a biological marker is still debated. This summary of the data helps the neurosurgeon to form an impression of the state of the art and to develop new future projects in that field.

Gabriele Schackert
Dresden, Germany

Although the clinical manifestations of cerebrovascular malformations are well known, the pathogenesis and pathobiology of these lesions are largely to be elucidated. The authors extensively reviewed basic mechanisms of vasculogenesis and angiogenesis and their relation to cerebrovascular malformations. Although recent immunohistochemical and genetic studies have disclosed many important features of cerebrovascular malformations, these findings are insufficient to clarify the genesis and clinical behavior of the lesions. The immunohistochemical and gene expression analyses of surgical specimens show only temporary expression at a given time in the adult stage, and the mutations of several genes that relate to familial cerebrovascular malformations cannot be detected in sporadic types; we have no adequate animal models for these lesions, although gene-targeting mice are useful for the analysis of target molecules. There is still a long way to go to predict and modify the clinical manifestations of these cerebral lesions.

Kazuhiko Nozaki
Nobuo Hashimoto
Kyoto, Japan

This is an excellent and timely review article summarizing a considerable body of recently accumulated knowledge regarding the cellular, molecular, and genetic biology underlying the vasculogenesis, angiogenesis, and pathogenesis of cerebrovascular malformations. Gault et al. have been leaders in investigating these important issues, and as we have come to expect from this group, this review is insightful, thoughtful, and well referenced. The identification of several genes responsible for the development of cerebrovascular malformations in patients with familial cavernous malformation and hereditary hemorrhagic telangiectasia is exciting and has allowed for genetic screening. However, it is not yet clear precisely how these mutations lead to the formation of vascular malformations or which of the hundreds of differentially expressed genes in cerebrovascular malformations, including...
many vasculogenesis/angiogenesis genes, are most important in influencing the development or behavior of these lesions. It will also be interesting to determine whether sporadic cavernous malformations and AVMs, as well as cavernous malformations secondary to radiation injury, have genetic profiles similar to those of their familial counterparts. Although our burgeoning knowledge of the molecular and cellular mechanisms responsible for cerebrovascular malformations thus far has not been substantially translated into better prediction of the natural history or improved clinical treatment of these vascular lesions, there is great optimism that these clinically relevant advances will be made in the near future.

Gary K. Steinberg
Stanford, California

The authors have surveyed the complex interplay of genes, ligands, and receptors involved in regulating elements of the vascular wall, including the assembly, maintenance, and remodeling of vessels during development, growth, and formation of cerebrovascular malformations. The nomenclature of these species is unwieldy, perhaps because of the variety of approaches used to discover them, which include both population genetics and molecular analysis.

Although not addressed in this review, hemodynamic forces undoubtedly play an additional role in the genesis and progression of cerebrovascular malformations. For instance, shear stress and turbulent flow, characteristic of AVMs, are known to alter the expression of many genes in brain endothelium.

As the authors note, the insights divulged in this article and similar ones that will succeed it may lead to better prognostication of the clinical behavior of these lesions in individual patients. Ultimately, outcomes will be improved by gene therapy interventions that render them less deleterious by making them less likely to bleed or enlarge. Endovascular delivery is likely to play an important role in conveying therapeutic vectors into these vascular malformations.

Arun Paul Amar
New Haven, Connecticut

Gault et al. present a timely and well-written review of the current state of knowledge of the basic science of vasculogenesis and angiogenesis and relate it to our current understanding of the clinical science of cavernous malformations and arteriovenous malformations. This is an area in which basic science is advancing rapidly and will eventually affect the care of patients with cerebrovascular disease. The authors have made a needed contribution to the neurosurgical literature that should be appreciated by practicing cerebrovascular surgeons and interested residents alike.

The authors are recognized experts on vascular malformations, and this lends considerable authority to their discussion. Several ideas presented, however, are matters for scientific debate. The authors state that the known mutations in CCM1 result in protein truncation. This idea has been perpetuated in the cerebral cavernous malformation literature. To the best of our knowledge, no published data have demonstrated the production of endogenous truncated CCM1 protein. In fact, the most common fate of ribonucleic acid transcripts containing a premature termination codon is degradation through nonsense-mediated ribonucleic acid decay pathways. This process would lead to an absence of CCM1 protein and not to the presence of truncated protein.

As a second minor point, the authors state that CCM1 interacts with RAP1A. The experiments in which this interaction was defined involved only a partial CCM1 (KRIT1) clone (1). There is now evidence that this interaction with RAP1A might not be biologically relevant: similar experiments with full-length CCM1 failed to demonstrate this interaction (2). The best current evidence suggests that CCM1 interacts with ICAP1 and that the relevant signal transduction pathways are those related to integrin β1-mediated angiogenesis (2).