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# Genetic considerations relevant to intracranial hemorrhage and brain arteriovenous malformations

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## Summary

Brain arteriovenous malformations (AVMs) cause intracranial hemorrhage (ICH), especially in young adults. Molecular characterization of lesional tissue provides evidence for involvement of both angiogenic and inflammatory pathways, but the pathogenesis remains obscure and medical therapy is lacking. Abnormal expression patterns have been observed for proteins related to angiogenesis (e.g., vascular endothelial growth factor, angiopoietin-2, matrix metalloproteinase-9), and inflammation (e.g., interleukin-6 [IL-6] and myeloperoxidase). Macrophage and neutrophil invasion have also been observed in the absence of prior ICH. Candidate gene association studies have identified a number of germline variants associated with clinical ICH course and AVM susceptibility. A single nucleotide polymorphism (SNP) in activin receptor-like kinase-1 (ALK-1) is associated with AVM susceptibility, and SNPs in IL-6, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and apolipoprotein-E (APOE) are associated with AVM rupture. These observations suggest that even without a complete understanding of the determinants of AVM development, the recent discoveries of downstream derangements in vascular function and integrity may offer potential targets for therapy development. Further, biomarkers can now be established for assessing ICH risk. These data will generate hypotheses that can be tested mechanistically in model systems, including surrogate phenotypes, such as vascular dysplasia and/or models recapitulating the clinical syndrome of recurrent spontaneous ICH.

*Keywords:* Angiogenesis; inflammation; vascular malformations.

## Introduction

Brain arteriovenous malformations (AVMs) represent a relatively infrequent but important source of neurological morbidity in relatively young adults [4]. Brain AVMs have a population prevalence of 10 to 18 per 100,000 adults [3, 7], and a new detection rate of approximately 1.3 per 100,000 person-years [58]. The basic morphology is that of a vascular mass, called the nidus, that directly shunts blood between the arterial and venous circulations without a true capillary bed. There is usually high flow through the feeding arteries, nidus, and draining veins. The nidus is a complex tangle of abnormal, dilated channels, not clearly artery or vein, with intervening gliosis.

Seizures, mass effect, and headache are causes of associated morbidity, but prevention of new or recurrent intracranial hemorrhage (ICH) is the primary rationale to treat AVMs, usually with some combination of surgical resection, embolization, and stereotactic radiotherapy. The risk of spontaneous ICH has been estimated in retrospective and prospective observational studies to range approximately from 2 to 4% per year [31]. Other than non-specific control of symptomatology, such as headache and seizures, primary medical therapy is lacking.

## Etiology and pathogenesis

The genesis of AVMs has been enigmatic. Unlike the association of antecedent head trauma or other injuries with the pathogenesis of dural arteriovenous fistulae (DAVF), environmental risk factors for AVMs are lacking. There is remarkably little evidence for the common assertion that AVMs are congenital lesions arising during the fourth to eighth week of embryonic development,

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considering the widespread use of prenatal ultrasound (Vein of Galen lesions are not true AVMs). Further, there have been multiple reports of AVMs that grow or regress, including de novo AVM formation [19]. Inciting event(s) might include the sequelae of even relatively modest injury from an otherwise unremarkable episode of trauma, infection, inflammation, irradiation, or compression. In susceptible individuals, one might posit some degree of localized venous hypertension [34, 68] from microvascular thrombosis, perhaps associated with a state of relative thrombophilia [56]. The scarce data available on longitudinal assessment of AVM growth suggests that approximately 50% of cases display interval growth [25]. Consistent with growth is the many-fold greater endothelial proliferation rate (Ki-67) in AVM surgical specimens, compared to control brain [25].

## Characterization of lesional tissue

Available evidence points toward an active angiogenic and inflammatory lesion rather than a static congenital anomaly. A conceptual, speculative synthesis of these observations is shown in Fig. 1. Our group and others [27] have shown that a prominent feature of the AVM phenotype is relative overexpression of vascular endothelial growth factor-A (VEGF-A), at both the mRNA and protein level. VEGF may contribute to the hemorrhagic tendency of AVMs, extrapolating from animal models [35]. Other upstream factors that may contribute to AVM formation might include Homeobox genes, such as excess pro-angiogenic Hox D3 or deficient anti-angiogenic Hox A5 [12]. The vascular phenotype of AVM tissue may be explained, in part, by inadequate recruitment of peri-endothelial support structure, which is mediated by angiopoietins and TIE-2 signaling. For example, angiopoietin-2 (Ang-2), which allows loosening of cell-to-cell contacts, is over-expressed in the perivascular region in AVM vascular channels [24].

A key downstream consequence of VEGF and Ang-2 activity, contributing to the angiogenic phenotype, is matrix metalloproteinase (MMP) expression. MMP-9 expression in particular appears to be orders of magnitude higher in AVM than control tissue [13, 26], with levels of naturally-occurring MMP inhibitors, TIMP-1 and TIMP-3, also higher, but to a lesser degree. Additional inflammatory markers that are over-expressed include myeloperoxidase (MPO) and interleukin (IL)-6, both of which are highly correlated with MMP-9 [13, 14]. MMP-9 expression is correlated with the lipocalin-MMP-9 complex, suggesting neutrophils as a major source. In a subset of unruptured, non-embolized AVMs, neutrophils (MPO), macrophages/microglia (CD68), T-lymphocytes (CD3), and B-lymphocytes (CD20) were clearly evident in the vascular wall and intervening stroma of AVM tissue, whereas T- and B-lymphocytes were rarely observed [15].

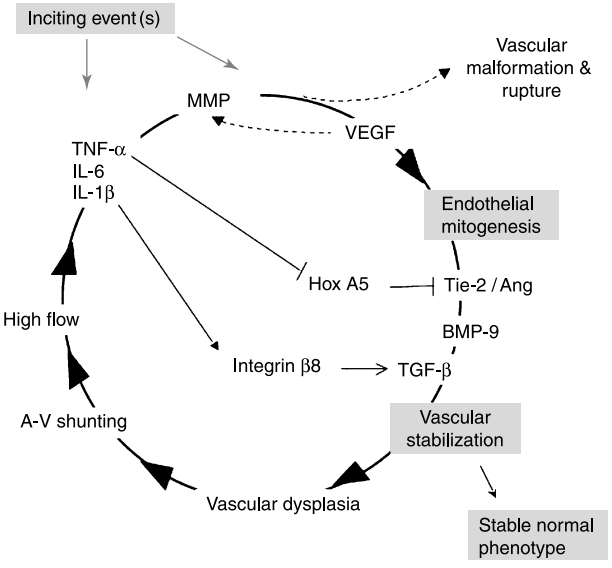


Fig. 1. Speculative synthesis of experimental observations relevant to AVM pathogenesis is presented in a simplified, conceptual fashion. After an inciting event or events, inflammatory or angiogenic activity (MMP, VEGF) initiates microvascular growth and remodeling, which are stabilized through interplay of pathways that include TIE-2/ANG and ALK-1/ENG. TGF-$\beta$ signaling occurs primarily through ALK-5 in smooth muscle and BMP-9 signaling through ALK-1 in the endothelium (see Fig. 2). Normal vessels stabilize, but a region that represents an incipient AVM undergoes a dysplastic response. Arteriovenous (A–V) shunting and high flow rates synergize with the dysplastic response and involve classical inflammatory signals, causing a vicious cycle in a localized area destined to become the AVM nidus. Eventually, the human disease phenotype results. Genetic variation can influence any and all of the pathways

## Genetic considerations relevant to AVMs

The majority of brain AVMs are sporadic; however, there is some evidence supporting a familial component to the AVM phenotype and there is evidence that genetic variation is relevant to the study of the disease. A simplified summary of relevant pathways is shown in Fig. 2.

## Mendelian disease

To date, the most significant candidate genes/pathways for brain AVM pathogenesis have come from Mendelian disorders that exhibit AVMs as part of their clinical phenotype. AVMs are highly prevalent in patients with

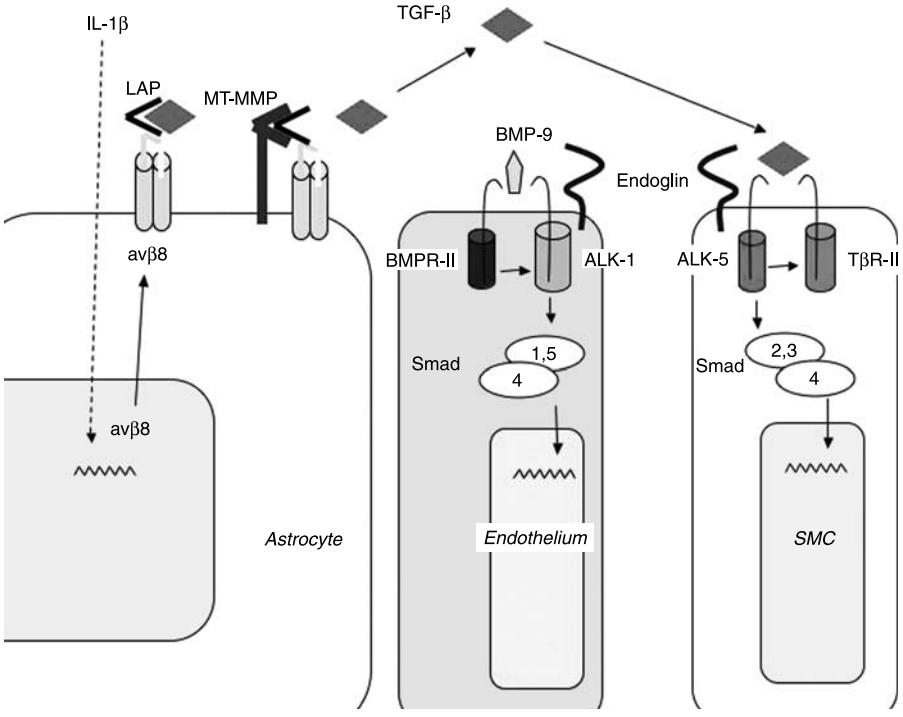


Fig. 2. Signaling pathways and speculative synthesis of signaling pathways. The $\alpha v\beta 8$ gene, which has an IL-1$\beta$ responsive region in human and mouse $\beta 8$ promoters, is critical for liberation of TGF-$\beta$ from LAP. MMP-9 activity and inflammation are associated with IL-1$\beta$, TNF-$\alpha$, and IL-6. TGF-$\beta$ signaling proceeds through the ALK-5 receptor expressed primarily on vascular smooth muscle. Endothelial cells express primarily ALK-1, which signals via the BMP-9 ligand. The ALK-1 signal is required for EC maturation, which when abrogated, leads to inappropriate EC migration and proliferation. ENG is an accessory receptor that can modulate both TGF-$\beta$ and ALK-1 signaling. ALK-1 and ALK-5 signal via distinct SMAD effector pathways converge on the common co-effector, SMAD4, in order to effect gene expression

hereditary hemorrhagic telangiectasia (HHT), an autosomal dominant disorder of mucocutaneous fragility and AVMs in various organs, including the brain. The 2 main subtypes of HHT (HHT-1 and -2) are caused by loss-of-function mutations in 2 genes [38] originally implicated in tumor growth factor (TGF)-$\beta$ signaling pathways (Fig. 1). The first is endoglin (ENG), which codes for an accessory protein of TGF-$\beta$ receptor complexes. The second is activin-like kinase-1 (ALK-1 or ACVLR1), which codes for a transmembrane kinase also thought to participate in TGF-$\beta$ signaling.

Two recent reports show that ALK-1 may instead signal through bone morphogenetic protein (BMP)-9 and that ENG can potentiate the signal, suggesting that BMP-9 may represent a physiologically relevant *endothelial* signaling pathway for HHT pathogenesis [17, 52]. Endothelial cell (EC)-specific ablation of the murine ALK-1 gene causes AVM formation during development, whereas mice harboring an EC-specific knockout of ALK-5 (the type I TGF-$\beta$ receptor) or TbRII (the type II TGF-$\beta$ receptor) show neither AVM formation nor any other perturbation in vascular morphogenesis [42]. A third candidate for AVM pathogenesis is the SMAD4 gene, encoding the downstream effector for both TGF-$\beta$ and BMP signaling. This gene is mutated in a combined syndrome of juvenile polyposis and HHT [21]. Two additional independent loci, termed HHT-3 and HHT-4, have recently been described [5, 16], but the genes underlying these less-common forms of HHT have yet to be identified.

In HHT, defects in either ENG or ALK-1 may affect a common pathway. By inference, this common pathway, which includes BMP-9, is also implicated in sporadic brain AVM development. A potential *mechanism* for the role of this pathway in AVM pathogenesis would include the requirement of ALK-1 for EC maturation [18, 32]. Disruption of this signaling pathway by mutation or possibly through physiological perturbation would result in a block in the maturation process, leading to inappropriate EC migration and proliferation. This suggests that aberrant EC migration and proliferation may be one of the earliest events in the development of an AVM.

The pathways involved in TGF-$\beta$ signaling are complex and interconnected [59]. In addition to direct effects

of abnormal ENG and ALK-1 signaling that result in abnormal angiogenic function [60], insufficient ENG may affect local hemodynamics through its interaction with nitric oxide signaling [61]. A loss of local microvascular flow regulation may in and of itself lead to the development of arteriovenous shunts, as predicted by computational modeling studies [46].

As a class, the inherited AVMs in HHT have some distinguishing morphological features, but are generally similar to the sporadic lesions and cannot be distinguished individually on the basis of their angioarchitecture [37, 40]. Brain AVMs are approximately 10 times more common in HHT-1/ENG (~20%) than HHT-2/ALK-1 (~2%) patients [6, 36, 50]. Compared to sporadic lesions, presence of an ENG or ALK-1 mutation results in an approximate 1000- or 100-fold increased risk, respectively, of developing a brain AVM. The greatly elevated risk of brain AVM development in the Mendelian disorders raises the possibility that germline *sequence variants* of these and other genes may likewise pose a significant risk for *sporadic* brain AVM development.

Because the population prevalence of HHT is roughly 1/10,000, and approximately 10% of all HHT cases harbor brain AVMs [36], this would yield a population prevalence of HHT-related AVMs of 1/100,000. Therefore, given the total AVM population prevalence of 10 to 18 per 100,000 adults [3, 7], the fraction of HHT-AVMs in large referral series should be approximately 5% to 10%. Interestingly, HHT accounts for less than 1% of the University of California San Francisco (UCSF) [31] and Columbia [39] AVM databases (unpublished data), suggesting that systematic underestimation of undiagnosed HHT may occur in the large referral cohorts.

## Familial aggregation

Although rare, familial cases of AVM outside the context of HHT have been reported in the literature [30, 63]. A recent review article examined all case reports and identified 53 patients without HHT in 25 families with AVMs, mostly of first-degree relationships (79%) [63]. While no clear pattern of inheritance emerged from the pedigrees, the clinical characteristics of familial AVM patients did not differ significantly from sporadic AVM, except for a younger age at diagnosis. In addition, linkage and association analysis of 6 Japanese families, each with 2 affected relatives, was recently reported [30]. Linkage analysis revealed 7 candidate regions, with the strongest signal at chromosome 6q25 (LOD = 1.88; $p = 0.002$) under a dominant genetic model. However, no association was observed with markers in the candidate linkage regions likely due to the small sample size.

Further evidence for a genetic component to AVMs comes from considering the excess risk of disease in relatives compared to the general population. A commonly used familial aggregation measure is the recurrence risk ratio, lambda ($\lambda$), defined as the risk of disease in relatives of an individual with disease ($K_{\text{relative}}$), divided by the population prevalence of the disease ($K$) [47]. This measure can be calculated for various relatives, such as siblings, and provides a quantitative measure of the genetic contribution to disease. Any $\lambda$ value equal to 1.0 indicates no evidence for a genetic influence, whereas higher $\lambda$ values suggest a greater genetic component to the pathogenesis of disease. For example, complex diseases have $\lambda_{\text{sibling}}$ values ranging from 2.0 to 5.0 for ischemic stroke [41], 58 for ankylosing spondylitis [11], and 215 for autism [48].

The lack of published population-based studies of AVM with family history information makes it difficult to assess familial aggregation. However, we estimated $\lambda_{\text{sibling}}$ for the recently reported linkage and association study of AVM by Inoue *et al.* [30], which included cases from a region of Japan thought to have a high prevalence of AVM. Five of 31 cases (assuming 26 cases included in the association analysis had no family history) had affected siblings, yielding a sibling recurrence risk ($K_s$) of 16%. Given a population risk of AVM ($K$) of 18 per 100,000 (0.018%) [3], the excess sibling risk ($\lambda_{\text{sibling}} = K_{\text{sibling}}/K$) is estimated to be 889 (16%/0.018%). As a sensitivity analysis, we calculated $\lambda_{\text{sibling}}$ varying the recurrence risk to siblings from 0.01% to 50% and assuming a population prevalence of AVM of 0.018%. A sibling recurrence risk even as low as 0.05% would yield a $\lambda_{\text{sibling}}$ of 2.78, which would still support a genetic contribution to the disease.

Taken together, there is modest evidence supporting familial aggregation for the AVM phenotype, although definitive proof is lacking. The high relative risk to siblings suggests a significant genetic influence, although this could also be the result of random chance, shared environmental factors, shared genetic factors, or any combination of these. The challenge is identifying enough families with imaging-confirmed AVM cases and genetic data to perform classical genetic studies.

## An alternative genetic mechanism for sporadic AVMs

An alternative hypothesis for sporadic AVM pathogenesis would posit that the relevant genes/pathways are disrupted by *somatic*, rather than germline mutations. This genetic mechanism would parallel that found for venous malformations, where germline TIE-2 mutations are found in autosomal dominant families with venous malformations [9, 64], but somatic mutations are found in *tissue* isolated from sporadic venous malformations [8, 65]. The somatic mutation mechanism might also explain the rarity of families with AVMs outside the context of HHT. The occasional but rare familial occurrence of the usually sporadic AVM is similar to that found with other vascular traits such as Klippel-Trenaunay syndrome. This pattern has been termed paradominant inheritance and invokes a crucial role for somatic mutation as its underlying mechanism [22, 23]. In the paradominant model, heterozygous individuals for a germline mutation are phenotypically normal, but zygotes that are homozygous for the mutation die during early embryogenesis. Thus, the mutation rarely manifests as familial inheritance of a trait, but instead is usually ''silently'' transmitted through many generations. However, the trait becomes manifest in an individual when a somatic mutation occurs at a later stage during embryogenesis, giving rise to a mutant cell population being either homozygous or hemizygous for the mutation. This clone of mutant cells has bypassed the developmental block, and these cells can now seed the development of the vascular anomaly. This intriguing hypothesis has yet to be explored for sporadic AVMs.

Another consideration is the way one construes the nature of an ''inherited disease''. Even if the mechanism of AVM initiation – as yet unknown – is a structural or mechanical insult, which is not in itself a heritable trait, the subsequent growth and behavior of the lesion can still be influenced by genetic variation in mechanistic pathways important in vascular biology. For example, there are multiple genetic loci that control VEGF-induced angiogenesis [49, 53]. Genetic influences on AVM pathobiology may therefore be evaluated in a case-control study design comparing affected patients to normal controls, or in cross-sectional or longitudinal cohort designs to investigate genetic influences on clinical course, such as propensity to rupture.

## Candidate gene studies in AVM patients

We have pursued 2 general classes of candidates for examination: (a) genes in pathways found to be upregulated in lesional tissue, i.e., inflammatory or angiogenic genes; and (b) genes mutated in Mendelian disorders affecting the cerebral circulation, e.g., HHT. Such genes provide a starting point for hypothesis generation for both clinical studies and laboratory experiments. Common polymorphisms may subtly alter protein function or expression, resulting in phenotypes relevant to the human disease. For example, abnormal vascular development has been observed in murine models with insufficient ALK-1 [57, 62] and ENG [51]. Importantly, adenoviral-mediated VEGF gene transfer in ENG-deficient mice causes enhancement of vascular abnormalities, suggesting a synergism between TGF-$\beta$ and VEGF signaling pathways in development of abnormal or ''dysplastic'' vessels [66]. Structural integrity may also be influenced by upstream influences on TGF-$\beta$ signaling. For example, of interest is the interaction of astrocytic integrin $\alpha$V$\beta$8 and its role in TGF-$\beta$ transport; its abrogation results in vascular instability leading to developmental ICH [10]. Preliminary data suggest decreased $\alpha$V$\beta$8 expression in resected AVM tissue (unpublished data).

We recently provided the first description of a common genetic variant associated with the sporadic disease: an intronic variant of ALK-1 (IVS3 -35 A>G) was present at a higher frequency in AVM cases compared to healthy controls [44]. This association was independently replicated [54, 55]. Preliminary data suggest that this single nucleotide polymorphism (SNP) is associated with alternative splicing (unpublished data). Other SNPs we have found to be associated with AVM susceptibility include common promoter polymorphisms in IL-1$\beta$ (IL-1$\beta$ -31 T>C and -511 C>T) [29].

There are also genetic influences on clinical course of AVM rupture resulting in ICH in 3 settings: presentation with ICH [28, 43], new ICH after diagnosis [1, 45], and ICH after treatment [2]. We found that the GG genotype of the IL-6 (IL-6 -174 G>C) promoter polymorphism was associated with clinical presentation of ICH [43]. The high-risk IL-6 -174 GG genotype was also associated with the highest IL-6 mRNA and protein levels in AVM tissue [14]. We have not yet identified any associations of sporadic AVM with polymorphisms in genes coding for important angiogenesis-related proteins, such as VEGF, TIE-2, or the angiopoietins.

We have further explored use of genotype to predict new ICH in the natural course after presentation, but before any treatment had been initiated [1, 45]. We found that the A allele of the TNF-$\alpha$ -238 G>A promoter SNP was associated with new hemorrhage in the

natural course of a sample of 280 AVM cases. Adjusting for initial presentation with hemorrhage, age, and race/ethnicity, resulted in an adjusted hazards ratio (HR) of 4.0 (95% CI = 1.3–12.3; $p = 0.015$) [1].

Additionally, the apolipoprotein (APOE) $\varepsilon 2$, but not APOE $\varepsilon 4$ allele, was associated with new hemorrhage ($n = 284$) in the natural course, with an adjusted HR of 5.1 (95% CI = 1.5–17.7; $p = 0.01$) [45]. When examined together in a multivariate model, both the APOE $\varepsilon 2$ and TNF-$\alpha$ -238 A alleles were independent predictors of ICH risk [45]. The TNF-$\alpha$ and APOE results are exciting, because they represent the first description of a genotype associated with increased natural history hemorrhagic risk in AVM patients. Newer evidence also associates IL-1$\beta$ with increased risk of new ICH [28]. In addition to their association with spontaneous ICH in the natural, untreated course, both APOE $\varepsilon 2$ and TNF-$\alpha$ -238 A alleles appear to confer greater risk for post-radiosurgery and post-surgical hemorrhage [2].

All of these genetic association results require replication and larger sample sizes, considerable challenges for a rare disease such as AVM. The largest cohorts to date have been assembled from clinical series. Although the large clinical series have not directly studied genetics, there may be indirect evidence of a genetic influence, in that race/ethnic background appears to affect spontaneous bleeding rate [31]. This association could be explained by genetic, socio-economic, and environmental factors, or a complex combination of all three. However, no specific factors have been identified in case series, with the possible exception of essential hypertension [33].

## Conclusions

Considering the tissue expression data together with the genetic studies, the available data are consistent with the hypothesis that angiogenic and inflammatory processes – including ENG and ALK-1 signaling pathways – contribute to AVM pathogenesis and clinical course. Although the data do not prove that such activity is causative, involvement of these pathways appears highly plausible. Replication studies are needed for the genetic association findings and animal models are needed for mechanistic studies.

A prevailing view is that AVM pathophysiology is governed to a large extent by chronic hemodynamic derangements [20, 67] imposed on a relatively fixed congenital lesion. Our findings raise the possibility that angiogenic and inflammatory pathways can either synergize with underlying defects or hemodynamic injury to result in the clinical phenotype and behavior. Further, it may be the case that the angiogenic and inflammatory components are actually the driving causal force in disease initiation and progression, perhaps in conjunction with as yet undetermined environmental influences. Progress in elucidating these pathways and mechanisms not only offer promise for developing innovative, safer treatments for the disease, but also may provide insights into the vascular failure seen in other hemorrhagic brain disorders.

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