Published in final edited form as:

Acta Neurochir Suppl. 2008 ; 105: 199–206.

Genetic Considerations Relevant to Intracranial Hemorrhage and Brain Arteriovenous Malformations

Helen Kim, PhD^{1,5}, Douglas A. Marchuk, PhD⁴, Ludmila Pawlikowska, PhD¹, Yongmei Chen, MD, PhD¹, Hua Su, MD¹, GY Yang, MD, PhD^{1,2}, and William L. Young, MD^{1,2,3}

¹Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA 94110, USA

²Department of Neurological Surgery, University of California, San Francisco, CA 94110, USA

³Department of Neurology, University of California, San Francisco, CA 94110, USA

⁴Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC 27710, USA

⁵Department of Institute for Human Genetics, University of California, San Francisco, CA 94110, USA

SUMMARY

Brain arteriovenous malformations (AVM) 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 remain obscure and medical therapy is lacking. Abnormal expression patterns have been observed for proteins related to angiogenesis (e.g., VEGF, Angiopoietin-2, MMP-9), and inflammation (e.g., IL-6 and MPO). Macrophage and neutrophil invasion has 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 *ALK-1* is associated with AVM susceptibility, and SNPs in *IL-6*, *TNF-α* and *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. Finally, 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

Brain arteriovenous malformations (AVM) represent a relatively infrequent but important source of neurological morbidity in relatively young adults [4]. Brain AVMs have a population prevalence of 10–18 per 100,000 adults [3,7], and a new detection rate of ~1.3 per 100,000 person-years [58]. The basic morphology is 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 2–4% per year (see reference [31]). Other than non-specific control of symptomatology, e.g., 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 4th to 8th week of embryonic development, considering the high utilization 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 (see reference [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 [26]. Consistent with growth is the many-fold higher endothelial proliferation rate (Ki-67) in AVM surgical specimens, compared to control brain [26].

Characterization of lesional tissue

Available evidence points towards an active angiogenic and inflammatory lesion rather than a static congenital anomaly. A conceptual, speculative synthesis of these observations is shown in Figure 1. Our group and others (see reference [25]) have shown that a prominent feature of the AVM phenotype is relative overexpression of 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 [14]. 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 overexpressed 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 MMP expression. MMP-9 expression in particular appears to be orders of magnitude higher in AVM than control tissue [12,27], with levels of naturally occurring MMP inhibitors, TIMP-1 and TIMP-3, also higher, but to a lesser degree. Additional inflammatory markers that are overexpressed include myeloperoxidase (MPO) and IL-6, both of which are highly correlated with MMP-9 [12,13]. 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].

Genetic considerations relevant to AVMs

The majority of brain AVMs is 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 Figure 2.

Mendelian disease

To date, the most significant candidate genes / pathways for brain AVM pathogenesis have come from Mendelian disorders which exhibit AVMs as part of their clinical phenotype. AVMs are highly prevalent in patients with hereditary hemorrhagic telangiectasia (HHT), an autosomal dominant disorder of mucocutaneous fragility and AVMs in various organs, including the brain. The two main subtypes of HHT (HHT1 and 2) are caused by loss-of-function mutations in two genes [38] originally implicated in TGF- β signaling pathways (Figure 1). The first is endoglin (*ENG*), which codes for an accessory protein of TGF- β 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- β signaling.

Two recent reports show that ALK-1 may instead signal through BMP-9 and that endoglin can potentiate the signal, suggesting that BMP-9 may represent a physiologically relevant *endothelial* signaling pathway for HHT pathogenesis [17,52]. Endothelial cell-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- β receptor) or TbrII (the type II TGF- β 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- β and BMP signaling. This gene is mutated in a combined syndrome of juvenile polyposis and HHT [21]. Two additional independent loci, termed HHT3 and HHT4, 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 endothelial cell 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- β signaling are complex and interconnected.[59]. In addition to direct effects of abnormal endoglin and ALK-1 signaling that result in abnormal angiogenic function,[60] insufficient endoglin 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 ten times more common in HHT1/*ENG* (~20%) than HHT2/*ALK-1* (~2%) patients [6,36,50]. Compared to sporadic lesions, presence of an *ENG* or *ALK-1* mutation results in approximately a 1,000 and 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 10–18 per 100,000 adults [3,7], the fraction of HHT-AVMs in large referral series should be approximately 5–10%. Interestingly, HHT accounts for less than 1% of the 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 six Japanese families, each with two 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, λ (lambda), defined as the risk of disease in relatives of an individual with disease (K_{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 λ value equal to 1.0 indicates no evidence for a genetic influence, whereas higher λ values suggest a greater genetic component to the pathogenesis of disease. For example, complex diseases have λ_{sibling} values ranging from 2.0 – 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 λ_{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 λ_{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 λ_{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 for 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 two general classes of candidates to examine: (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 endoglin-deficient mice causes enhancement of vascular abnormalities, suggesting a synergism between TGF- β and VEGF signaling pathways in development of abnormal or “dysplastic” vessels [66]. Structural integrity may also be influenced by upstream influences on TGF- β signaling. For example, of interest is the interaction of astrocytic integrin $\alpha V\beta 8$ and its role in TGF- β 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* (IVS3-35A>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 interleukin-1 beta (*IL-1β* -31 T>C and >511 C>T) [29].

There are also genetic influences on clinical course of AVM rupture resulting in intracranial hemorrhage (ICH) in three settings: presentation with ICH [28,45], new ICH after diagnosis [2,43], and ICH after treatment [1]. We found that the GG genotype of the interleukin-6 (*IL-6* -174G>C) promoter polymorphism was associated with clinical *presentation of ICH* [45]. The high risk *IL-6* -174 GG genotype was also associated with the highest *IL-6* mRNA and protein levels in AVM tissue [13]. 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 [2,43]. We found that the A allele of the *TNF-α*-238G>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) [2].

Additionally, the Apolipoprotein (*APOE*) ε2, but not *APOE* ε4 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) [43]. When examined together in a multivariate model, both the *APOE* ε2 and *TNF-α*-238 A alleles were independent predictors of ICH risk [43]. The *TNF-α* and *APOE* results are exciting, because they represented the first description of a genotype associated with increased natural history hemorrhagic risk in AVM patients. Newer evidence also associates *IL-1β* with increased risk of new ICH [28]. In addition to their association with spontaneous ICH in the natural, untreated course, both *APOE* ε2 and *TNF-α*-238 A alleles appear to confer greater risk for post-radiosurgery and post-surgical hemorrhage [1].

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, socioeconomic, 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 endoglin 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.

REFERENCES

1. Achrol AS, Kim H, Pawlikowska L, Poon KY, Ko NU, McCulloch CE, Zaroff JG, Johnston SC, McDermott MW, Lawton MT, Kwok PY, Young WL. Association of tumor necrosis factor- α -238G>A and Apolipoprotein E2 polymorphisms with intracranial hemorrhage after brain arteriovenous malformation treatment. *Neurosurgery* 2007;61:731–739. [PubMed: 17986934] discussion 740
2. Achrol AS, Pawlikowska L, McCulloch CE, Poon KY, Ha C, Zaroff JG, Johnston SC, Lee C, Lawton MT, Sidney S, Marchuk D, Kwok PY, Young WL. Tumor necrosis factor- α -238G>A promoter polymorphism is associated with increased risk of new hemorrhage in the natural course of patients with brain arteriovenous malformations. *Stroke* 2006;37:231–234. [PubMed: 16322490]
3. Al-Shahi R, Fang JS, Lewis SC, Warlow CP. Prevalence of adults with brain arteriovenous malformations: a community based study in Scotland using capture-recapture analysis. *J Neurol Neurosurg Psychiatry* 2002;73:547–551. [PubMed: 12397149]
4. Arteriovenous Malformation Study Group. Arteriovenous malformations of the brain in adults. *N Engl J Med* 1999;340:1812–1818. [PubMed: 10362826]
5. Bayrak-Toydemir P, McDonald J, Akarsu N, Toydemir RM, Calderon F, Tuncali T, Tang W, Miller F, Mao R. A fourth locus for hereditary hemorrhagic telangiectasia maps to chromosome 7. *Am J Med Genet A* 2006;140:2155–2162. [PubMed: 16969873]
6. Bayrak-Toydemir P, McDonald J, Markewitz B, Lewin S, Miller F, Chou LS, Gedge F, Tang W, Coon H, Mao R. Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia: mutations and manifestations. *Am J Med Genet A* 2006;140:463–470. [PubMed: 16470787]
7. Berman MF, Sclaccia RR, Pile-Spellman J, Stapf C, Connolly ES Jr, Mohr JP, Young WL. The epidemiology of brain arteriovenous malformations. *Neurosurgery* 2000;47:389–396. [PubMed: 10942012]discussion 397
8. Brouillard P, Viskula M. Genetic causes of vascular malformations. *Hum Mol Genet* 2007;16(Spec No 2):R140–R149. [PubMed: 17670762]
9. Calvert JT, Riney TJ, Kontos CD, Cha EH, Prieto VG, Shea CR, Berg JN, Nevin NC, Simpson SA, Pasyk KA, Speer MC, Peters KG, Marchuk DA. Allelic and locus heterogeneity in inherited venous malformations. *Hum Mol Genet* 1999;8:1279–1289. [PubMed: 10369874]
10. Cambier S, Gline S, Mu D, Collins R, Araya J, Dolganov G, Einheber S, Boudreau N, Nishimura SL. Integrin $\alpha(v)\beta 8$ -mediated activation of transforming growth factor- β by perivascular astrocytes: an angiogenic control switch. *Am J Pathol* 2005;166:1883–1894. [PubMed: 15920172]
11. Carter N, Williamson L, Kennedy LG, Brown MA, Wordsworth BP. Susceptibility to ankylosing spondylitis. *Rheumatology (Oxford)* 2000;39:445. [PubMed: 10817782]
12. Chen Y, Fan Y, Poon KY, Achrol AS, Lawton MT, Zhu Y, McCulloch CE, Hashimoto T, Lee C, Barbaro NM, Bollen AW, Yang GY, Young WL. MMP-9 expression is associated with leukocytic but not endothelial markers in brain arteriovenous malformations. *Front Biosci* 2006;11:3121–3128. [PubMed: 16720380]
13. Chen Y, Pawlikowska L, Yao JS, Shen F, Zhai W, Achrol AS, Lawton MT, Kwok PY, Yang GY, Young WL. Interleukin-6 involvement in brain arteriovenous malformations. *Ann Neurol* 2006;59:72–80. [PubMed: 16278864]
14. Chen Y, Xu B, Arderiu G, Hashimoto T, Young WL, Boudreau NJ, Yang GY. Retroviral delivery of homeobox d3 gene induces cerebral angiogenesis in mice. *J Cereb Blood Flow Metab* 2004;24:1280–1287. [PubMed: 15545924]
15. Chen Y, Zhu W, Bollen AW, Lawton MT, Barbaro NM, Dowd CF, Hashimoto T, Yang GY, Young WL. Evidence for inflammatory cell involvement in brain arteriovenous malformations. *Neurosurgery*. 2008(in press)
16. Cole SG, Begbie ME, Wallace GM, Shovlin CL. A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. *J Med Genet* 2005;42:577–582. [PubMed: 15994879]

17. David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) endothelial cells. *Blood* 2007;109:1953–1961. [PubMed: 17068149]
18. David L, Mallet C, Vailhe B, Lamouille S, Feige JJ, Bailly S. Activin receptor-like kinase 1 inhibits human microvascular endothelial cell migration: potential roles for JNK and ERK. *J Cell Physiol* 2007;213:484–489. [PubMed: 17620321]
19. Du R, Hashimoto T, Tihan T, Young WL, Perry V, Lawton MT. Growth and regression of an arteriovenous malformation in a patient with hereditary hemorrhagic telangiectasia: case report. *J Neurosurg* 2007;106:470–477. [PubMed: 17367071]
20. Duong DH, Young WL, Vang MC, Sciacca RR, Mast H, Koennecke HC, Hartmann A, Joshi S, Mohr JP, Pile-Spellman J. Feeding artery pressure and venous drainage pattern are primary determinants of hemorrhage from cerebral arteriovenous malformations. *Stroke* 1998;29:1167–1176. [PubMed: 9626290]
21. Gallione CJ, Richards JA, Letteboer TG, Rushlow D, Prigoda NL, Leedom TP, Ganguly A, Castells A, Ploos van Amstel JK, Westermann CJ, Pyeritz RE, Marchuk DA. SMAD4 Mutations found in unselected HHT patients. *J Med Genet* 2006;43:793–797. [PubMed: 16613914]
22. Happle R. Paradoxical inheritance: a possible explanation for Becker's pigmented hairy nevus. *Eur J Dermatol* 1992;2:39–40.
23. Happle R. Klippel-Trenaunay syndrome: is it a paradoxical trait? *Br J Dermatol* 1993;128:465–466. [PubMed: 8388238]
24. Hashimoto T, Lam T, Boudreau NJ, Bollen AW, Lawton MT, Young WL. Abnormal balance in the angiotensin-converting enzyme system in human brain arteriovenous malformations. *Circ Res* 2001;89:111–113. [PubMed: 11463715]
25. Hashimoto T, Lawton MT, Wen G, Yang GY, Chaly T Jr, Stewart CL, Dressman HK, Barbaro NM, Marchuk DA, Young WL. Gene microarray analysis of human brain arteriovenous malformations. *Neurosurgery* 2004;54:410–423. [PubMed: 14744289]discussion 423–415
26. Hashimoto T, Mesa-Tejada R, Quick CM, Bollen AW, Joshi S, Pile-Spellman J, Lawton MT, Young WL. Evidence of increased endothelial cell turnover in brain arteriovenous malformations. *Neurosurgery* 2001;49:124–131. [PubMed: 11440433]discussion 131–122
27. Hashimoto T, Wen G, Lawton MT, Boudreau NJ, Bollen AW, Yang GY, Barbaro NM, Higashida RT, Dowd CF, Halbach VV, Young WL. Abnormal expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in brain arteriovenous malformations. *Stroke* 2003;34:925–931. [PubMed: 12649522]
28. Hysi PG, Kim H, Pawlikowska L, McCulloch CE, Zaroff JG, Marchuk DA, Lawton MT, Kwok PY, Young WL. 57th Annual Meeting of the American Society of Human Genetics. San Diego, CA: 2007. Association of interleukin-1 beta (IL1B) gene and risk of intracranial hemorrhage in brain arteriovenous malformation patients (Abstract #2538).
29. Hysi PG, Kim H, Pawlikowska L, McCulloch CE, Zaroff JG, Sidney S, Burchard EG, Marchuk DA, Lawton MT, Kwok PY, Young WL. Association of interleukin-1 beta (IL1B) gene and brain arteriovenous malformation in Caucasians (Abstract). *Stroke* 2007;38:456.
30. Inoue S, Liu W, Inoue K, Mineharu Y, Takenaka K, Yamakawa H, Abe M, Jafar JJ, Herzig R, Koizumi A. Combination of linkage and association studies for brain arteriovenous malformation. *Stroke* 2007;38:1368–1370. [PubMed: 17322094]
31. Kim H, Sidney S, McCulloch CE, Poon KY, Singh V, Johnston SC, Ko NU, Achrol AS, Lawton MT, Higashida RT, Young WL. Racial/ethnic differences in longitudinal risk of intracranial hemorrhage in brain arteriovenous malformation patients. *Stroke* 2007;38:2430–2437. [PubMed: 17673729]
32. Lamouille S, Mallet C, Feige JJ, Bailly S. Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis. *Blood* 2002;100:4495–4501. [PubMed: 12453878]
33. Langer DJ, Lasner TM, Hurst RW, Flamm ES, Zager EL, King JT Jr. Hypertension, small size, and deep venous drainage are associated with risk of hemorrhagic presentation of cerebral arteriovenous malformations. *Neurosurgery* 1998;42:481–486. [PubMed: 9526981]discussion 487–489
34. Lawton MT, Jacobowitz R, Spetzler RF. Redefined role of angiogenesis in the pathogenesis of dural arteriovenous malformations. *J Neurosurg* 1997;87:267–274. [PubMed: 9254092]

35. Lee CZ, Xue Z, Zhu Y, Yang GY, Young WL. Matrix metalloproteinase-9 inhibition attenuates vascular endothelial growth factor-induced intracranial hemorrhage. *Stroke* 2007;38:2563–2568. [PubMed: 17673717]
36. Letteboer TG, Mager JJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel JK, Westermann CJ. Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. *J Med Genet* 2006;43:371–377. [PubMed: 16155196]
37. Maher CO, Piepgras DG, Brown RD Jr, Friedman JA, Pollock BE. Cerebrovascular manifestations in 321 cases of hereditary hemorrhagic telangiectasia. *Stroke* 2001;32:877–882. [PubMed: 11283386]
38. Marchuk DA, Srinivasan S, Squire TL, Zawistowski JS. Vascular morphogenesis: tales of two syndromes. *Hum Mol Genet* 2003;12:R97–R112. [PubMed: 12668602]
39. Mast H, Young WL, Koennecke HC, Sciacca RR, Osipov A, Pile-Spellman J, Hacein-Bey L, Duong H, Stein BM, Mohr JP. Risk of spontaneous haemorrhage after diagnosis of cerebral arteriovenous malformation. *Lancet* 1997;350:1065–1068. [PubMed: 10213548]
40. Matsubara S, Mandzia JL, ter Brugge K, Willinsky RA, Faughnan ME, Manzia JL. Angiographic and clinical characteristics of patients with cerebral arteriovenous malformations associated with hereditary hemorrhagic telangiectasia. *AJNR Am J Neuroradiol* 2000;21:1016–1020. [PubMed: 10871005]
41. Meschia JF, Brown RD Jr, Brott TG, Chukwudelunzu FE, Hardy J, Rich SS. The Siblings With Ischemic Stroke Study (SWISS) protocol. *BMC Med Genet* 2002;3:1. [PubMed: 11882254]
42. Park SO, Lee YJ, Seki T, Hong KH, Fliess N, Jiang Z, Park A, Wu X, Kaartinen V, Roman BL, Oh SP. ALK5- and TGFBR2-independent role of ALK1 in the pathogenesis of hereditary hemorrhagic telangiectasia type 2 (HHT2). *Blood* 2008;111:633–642. [PubMed: 17911384]
43. Pawlikowska L, Poon KY, Achrol AS, McCulloch CE, Ha C, Lum K, Zaroff J, Ko NU, Johnston SC, Sidney S, Marchuk DA, Lawton MT, Kwok PY, Young WL. Apolipoprotein E epsilon2 is associated with new hemorrhage risk in brain arteriovenous malformation. *Neurosurgery* 2006;58:838–843. [PubMed: 16639317]discussion 838-843
44. Pawlikowska L, Tran MN, Achrol AS, Ha C, Burchard EG, Choudhry S, Zaroff J, Lawton MT, Castro RA, McCulloch CE, Marchuk DA, Kwok PY, Young WL. Polymorphisms in transforming growth factor-B-related genes ALK1 and ENG are associated with sporadic brain arteriovenous malformations. *Stroke* 2005;36:2278–2280. [PubMed: 16179574]
45. Pawlikowska L, Tran MN, Achrol AS, McCulloch CE, Ha C, Lind DL, Hashimoto T, Zaroff J, Lawton MT, Marchuk DA, Kwok PY, Young WL. Polymorphisms in genes involved in inflammatory and angiogenic pathways and the risk of hemorrhagic presentation of brain arteriovenous malformations. *Stroke* 2004;35:2294–2300. [PubMed: 15331795]
46. Quick CM, Hashimoto T, Young WL. Lack of flow regulation may explain the development of arteriovenous malformations. *Neurol Res* 2001;23:641–644. [PubMed: 11547934]
47. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. *Am J Hum Genet* 1990;46:222–228. [PubMed: 2301392]
48. Ritvo ER, Jorde LB, Mason-Brothers A, Freeman BJ, Pingree C, Jones MB, McMahon WM, Petersen PB, Jenson WR, Mo A. The UCLA-University of Utah epidemiologic survey of autism: recurrence risk estimates and genetic counseling. *Am J Psychiatry* 1989;146:1032–1036. [PubMed: 2750975]
49. Rogers MS, D'Amato RJ. The effect of genetic diversity on angiogenesis. *Exp Cell Res* 2006;312:561–574. [PubMed: 16321383]
50. Sabba C, Pasculli G, Lenato GM, Suppressa P, Lastella P, Memeo M, Dicuonzo F, Guanti G. Hereditary hemorrhagic telangiectasia: clinical features in ENG and ALK1 mutation carriers. *J Thromb Haemost* 2007;5:1149–1157. [PubMed: 17388964]
51. Satomi J, Mount RJ, Toporsian M, Paterson AD, Wallace MC, Harrison RV, Letarte M. Cerebral vascular abnormalities in a murine model of hereditary hemorrhagic telangiectasia. *Stroke* 2003;34:783–789. [PubMed: 12624308]
52. Scharpfenecker M, van Dinther M, Liu Z, van Bezooijen RL, Zhao Q, Pukac L, Lowik CW, Ten Dijke P. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci* 2007;120:964–972. [PubMed: 17311849]

53. Shaked Y, Bertolini F, Man S, Rogers MS, Cervi D, Foutz T, Rawn K, Voskas D, Dumont DJ, Ben-David Y, Lawler J, Henkin J, Huber J, Hicklin DJ, D'Amato RJ, Kerbel RS. Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell* 2005;7:101–111. [PubMed: 15652753]
54. Simon M, Franke D, Ludwig M, Aliashkevich AF, Koster G, Oldenburg J, Bostrom A, Ziegler A, Schramm J. Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous malformations of the central nervous system. *J Neurosurg* 2006;104:945–949. [PubMed: 16776339]
55. Simon M, Schramm J, Ludwig M, Ziegler A. Response to letter by Young et al, "Arteriovenous malformation". *J Neurosurg* 2007;106:732–733.
56. Singh V, Smith WS, Lawton MT, Halbach VV, Young WL. Thrombophilic mutation as a new high-risk feature in DAVF patients (Abstract). *Ann Neurol* 2006;60:S30.
57. Srinivasan S, Hanes MA, Dickens T, Porteous ME, Oh SP, Hale LP, Marchuk DA. A mouse model for hereditary hemorrhagic telangiectasia (HHT) type 2. *Hum Mol Genet* 2003;12:473–482. [PubMed: 12588795]
58. Stapf C, Mast H, Sciacca RR, Berenstein A, Nelson PK, Gobin YP, Pile-Spellman J, Mohr JP. The New York Islands AVM Study: design, study progress, and initial results. *Stroke* 2003;34:e29–e33. [PubMed: 12690217]
59. ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 2007;8:857–869. [PubMed: 17895899]
60. Thomas B, Eyries M, Montagne K, Martin S, Agrapart M, Simerman-Francois R, Letarte M, Soubrier F. Altered endothelial gene expression associated with hereditary haemorrhagic telangiectasia. *Eur J Clin Invest* 2007;37:580–588. [PubMed: 17576210]
61. Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH, Husain M, Letarte M. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. *Circ Res* 2005;96:684–692. [PubMed: 15718503]
62. Urness LD, Sorensen LK, Li DY. Arteriovenous malformations in mice lacking activin receptor-like kinase-1. *Nat Genet* 2000;26:328–331. [PubMed: 11062473]
63. van Beijnum J, van der Worp HB, Schippers HM, van Nieuwenhuizen O, Kappelle LJ, Rinkel GJ, Berkelbach van der Sprenkel JW, Klijn CJ. Familial occurrence of brain arteriovenous malformations: a systematic review. *J Neurol Neurosurg Psychiatry* 2007;78:1213–1217. [PubMed: 17259353]
64. Viskula M, Boon LM, Carraway KL 3rd, Calvert JT, Diamonti AJ, Goumnerov B, Pasyk KA, Marchuk DA, Warman ML, Cantley LC, Mulliken JB, Olsen BR. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. *Cell* 1996;87:1181–1190. [PubMed: 8980225]
65. Wouters, V.; Limaye, N.; Uebelhoer, M.; Mulliken, JB.; Boon, LM.; Viskula, M. American Society of Human Genetics 57th Annual Meeting. San Diego, CA: 2007. Sporadic venous malformation is caused by somatic mutations in TIE2 (Abstract).
66. Xu B, Wu YQ, Huey M, Arthur HM, Marchuk DA, Hashimoto T, Young WL, Yang GY. Vascular endothelial growth factor induces abnormal microvasculature in the endoglin heterozygous mouse brain. *J Cereb Blood Flow Metab* 2004;24:237–244. [PubMed: 14747750]
67. Young WL, Kader A, Ornstein E, Baker KZ, Ostapovich ND, Pile-Spellman J, Fogarty-Mack P, Stein BM. Cerebral hyperemia after arteriovenous malformation resection is related to "breakthrough" complications but not to feeding artery pressure. Columbia University AVM Study Project. *Neurosurgery* 1996;38:1085–1093. [PubMed: 8727137]discussion 1093-1085
68. Zhu Y, Lawton MT, Du R, Shwe Y, Chen Y, Shen F, Young WL, Yang GY. Expression of hypoxia-inducible factor-1 and vascular endothelial growth factor in response to venous hypertension. *Neurosurgery* 2006;59:687–696. [PubMed: 16955051]discussion 687-696

Figure 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 / Endoglin. TGF- β signaling occurs primarily through ALK-5 in smooth muscle and BMP-9 signaling through ALK-1 in the endothelium (see Figure 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.

Figure 2. Signaling pathways

Speculative synthesis of signaling pathways. The $\alpha v\beta 8$ gene, which has an IL-1 β responsive region in human and mouse $\beta 8$ promoters, is critical to liberate TGF- β from LAP. MMP-9 activity and inflammation are associated with IL-1 β , TNF- α and IL-6. TGF- β 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. Endoglin is an accessory receptor that can modulate both TGF- β and ALK-1 signaling. ALK-1 and ALK-5 signal via distinct SMAD effector pathways that converge on the common co-effector, SMAD4, in order to effect gene expression.