Biology of vascular malformations of the brain

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BIOLOGY OF VASCULAR MALFORMATIONS OF THE BRAIN

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

Abstract

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

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

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

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

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

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

Cellular and Molecular Interactions Controlling Angiogenesis

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

The next phase of angiogenesis is vascular stabilization, during which endothelial cells form capillary tubes, strengthen their intercellular junctions, and recruit smooth muscle cells to their walls. Vascular stabilization involves reciprocal interactions between endothelial cells and pericytes, the precursors of vascular smooth muscle cells. Brain pericytes arise from mesoderm...
and neural crest, and accompany capillary sprouts as they penetrate the brain. Pericyte differentiation and production of extracellular matrix is thought to be triggered by endothelial platelet-derived growth factor-B (PDGF-B) and TGF-β. As pericytes differentiate, they act back on the vascular endothelium to suppress capillary sprouting, stimulate wall growth, and promote intercellular junction formation and cell-matrix adhesion. These actions are mediated in part through angiopoietin-1; other mediators include tissue inhibitors of metalloproteinases (TIMPs) and ephrin-B2. Loss of pericytes (in PDGF-B deficient mice, for example) leads to vessel dilation, endothelial cell hyperplasia, and microaneurysm.

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

**Angiogenesis and VMB Formation**

### Cellular pathology and natural history of VMBs

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

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

### VMB genes and angiogenesis

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

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

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

All three CCM genes are expressed in CNS neurons and glia as well as in vascular endothelium. However, studies in mice and zebrafish mutants have shown that ccm1 and ccm2 are specifically required in endothelial cells for normal vascular development, and that selective knockout of ccm2 function in neuroglial cells does not lead to obvious cerebrovascular defects.

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

How do reduced levels of Alk-1 or endoglin lead to vascular lesions? During development, both proteins are expressed most prominently in vascular endothelium. Both are down-regulated in adulthood, but re-induced during vessel repair.
multiple actions on developing blood vessels, but their functions in vascular stabilization seem most central to disease etiology. Alk-1 or endoglin knock-out mice establish a primary capillary plexus, but fail to recruit pericytes and form a secondary plexus.\textsuperscript{53,54} Mice with mutations in just one copy of either gene establish relatively normal mature vasculature, but have small vessel malformations and loss of vascular smooth muscle.\textsuperscript{45,46} Defects in these mice resemble those in human HHT, in which dissociation of vascular smooth muscle cells is an early step, and sporadic brain AVM, where there is reduced pericyte coverage of the perinidal capillaries.\textsuperscript{55}

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

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

An unanswered question in AVM biology concerns the identity of the physiological ligand/s for Alk-1 in vascular endothelial cells. This ligand was previously believed to be TGF-\(\beta\)1, which is expressed in developing vascular endothelium and known to regulate endothelial cell proliferation and migration. However, one study has indicated that deletion of TGF-\(\beta\)R2 (the ligand-binding TGF-\(\beta\) receptor) in endothelial cells does not affect vascular development, and raised the possibility that the physiological ligand for Alk-1 is BMP9 or BMP10.\textsuperscript{48} Another mystery concerns the ten-fold difference in prevalence of brain AVMs in HHT1 (i.e., ENG deficiency) versus HHT2 (ALK-1), which have approximate penetrances of 10% and 1%, respectively.

**VMBs and vascular patterning**

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

Genetic and environmental second hits

The focal nature of VMBs has suggested that lesion formation may require not only a mutation in one copy of a given VMB gene within a particular group of cells but also a “second hit” that triggers the disease process in the region of the lesion. This second hit could be genetic, in the form of a somatic mutation to the second copy of that gene, or a mutation in another gene acting in the same cellular pathway. The possibility of a genetic two-hit mechanism in CCM is supported by discoveries of coexistence of germline (i.e., inherited) and somatic mutations in lesion tissue from subsets of patients with all three forms of inherited CCMs. In addition, it has been observed that CCM protein is lost from some but not all cells of CCM lesions. Finally, mice with mutations in one copy of the ccm1 gene do not normally develop lesions, but will do so if they also lack the tumor suppressor gene p53 (a genetic background known to increase the rate of somatic mutations). It is not known how prevalent somatic mutations are in CCM, or if a genetic two-hit mechanism also operates in HHT or sporadic CCM or AVM.

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

Angiogenic factors

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

Inflammation

VMBs are also sites of active inflammation. Robust B and plasma cell infiltration and oligoclonal IgG immune responses have been demonstrated in CCMs. Neutrophils, macrophages, and inflammatory markers are seen in AVMs. Inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and some interleukins (ILs), are potent stimulators of both angiogenesis and BBB breakdown and could contribute to lesion progression and rupture. Consistent with this idea, polymorphisms in the TNF-α, IL-1β80 and IL-681 genes are risk factors for intracerebral hemorrhage in sporadic AVM. Another link between inflammation and angiogenesis lies with endoglin, which is expressed in activated circulating monocytes. In post-ischemic heart, activated monocytes migrate to the infarct site, differentiate into endothelial cells and contribute to vascular repair, but monocytes from HHT1 (ENG+) patients show reduced capacity to do.
Breakdown of the BBB

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

New Insights and Mysteries Remaining

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

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

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

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

Translational implications

Translational advances will include describing the biology underlying certain clinical behaviors, for example hemorrhagic risk. Although neither CCM nor AVM has a model that has been developed to the point of actually testing such questions, refinement of animal models
that mimic disease natural history may make this possible. Studies in current animal models suggest specific therapeutic targets for slowing VMB formation and progression. For example, the reversal by simstatin of some of the vascular defects caused by loss of *ccm2* function in mice raises the possibility of initiating clinical trials of statins for CCM. Observations that VMBs are sites of active angiogenesis and inflammation suggest the potential utility of anti-angiogenic or anti-inflammatory agents. MMP inhibitors, for example, have both anti-angiogenic and anti-inflammatory activities, and a Phase I clinical trial of minocycline and doxycycline (broad spectrum MMP inhibitors) has just seen successful completion.84

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

Acknowledgments

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Figure 1. Schematic illustrations of (a) CCM and (b) AVM angioarchitecture
Figure 2. One possible model of genes and pathways involved in CCM signaling
CCM proteins form a molecular complex which interacts closely with cytoskeletal proteins and modulates inter-endothelial cell junctions. Mutations in one copy of a CCM gene may predispose to vascular permeability, which in turn may result in vascular leakage and vulnerability to form dysmorphic vessels. Somatic mutations in the same genetic pathway, immune responses, or altered capillary permeability after radiation injury all might act as “second hits” favoring CCM genesis or maintenance. Abbreviations: β-cat, β-catenin; CCM, cerebral cavernous malformation; ICAP-1, integrin cytoplasmic domain- associated protein-1; ITGβ1, integrin β1; JNK, JUN NH₂-terminal kinase; MEKK3, mitogen-activated protein kinase kinase kinase 3; RAP-1, Ras-proximate-1.
Figure 3. One possible model of genes and pathways involved in AVM pathogenesis
The genes associated AVM formation (shown in red) lie in the TGFβ superfamily signaling pathway. TGFβ and BMPs bind to TGFβRII and BMPRII, respectively. Upon ligand binding, the type II receptors phosphorylate associated type I receptors (ALK1, 3, 4, 5, or 6). The ALKs in turn recruit and phosphorylate receptor-regulated Smads (Smads 1, 5, and 8 or Smads 2 and 3). The receptor-regulated Smads form complexes with the common mediator, Smad 4, and translocate to the nucleus to participate in regulating the transcription of target genes. Endoglin is a TGF-β co-receptor that can modulates signaling through either the TGF-β or BMP membrane-bound signaling complexes. Endoglin also exists in a soluble, extracellular form (sEndoglin) that can bind and sequester BMPs or TGF-βs. Mutations of the genes shown in red may lead to dysregulation of processes required for blood vessel wall stabilization. In susceptible patients, formation of an AVM may then result from a defective repair response to an otherwise minor insult. Abbreviations: ALK, activin-like kinase receptor; BMP, bone morphogenetic protein; BMP-RII, BMP receptor type II; sEndoglin, soluble Endoglin; TGF β, transforming growth factor β; TGF β-RII, TGF β receptor type II.
### Table 1

Genes underlying hereditary forms of cerebrovascular malformations.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alternative name/s</th>
<th>Known or putative cellular functions</th>
<th>References</th>
</tr>
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<tr>
<td>CCM 1</td>
<td>Krit 1</td>
<td>Binds β-catenin, stabilizes interendothelial junctions associated with actin stress fibers</td>
<td>88</td>
</tr>
<tr>
<td>CCM 2</td>
<td>Malcavernin; osmosensing scaffold for MEKK3 (OSM)</td>
<td>Cellular responses to osmotic stress; modulates MAP kinase and RhoA GTPase signaling</td>
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<td>Programmed cell death 10 (PDCD10)</td>
<td>Cell proliferation and transformation (cancer cell lines); modulates extracellular signal-regulated kinase (ERK)</td>
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<td>Endoglin</td>
<td>HHT1</td>
<td>TGFβ superfamily co-receptor; modulates signaling by TGFβ type II receptor, Alk-1 and Alk-5</td>
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<td>Activin A receptor type II-like 1 (ACVRL-1); HHT2</td>
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