Hereditary Vascular Anomalies
New Insights Into Their Pathogenesis
J.-C. Tille, M.S. Pepper

Abstract—Increased understanding of the mechanisms of angiogenesis and lymphangiogenesis has provided a glimpse at some of the molecules involved in the pathophysiology of hemangiomas and vascular malformations. This review focuses on recent advances in our understanding of the mechanisms of angiogenesis/lymphangiogenesis and the differentiation of arterial, venous, and lymphatic vessels. We integrate this knowledge with new data obtained from genetic studies in humans, which have revealed a number of heretofore-unsuspected candidates involved in the development of familial vascular anomalies. We present a common infantile vascular tumor, hemangioma, and then focus on hereditary familial vascular and lymphatic malformations. We also summarize transgenic mouse models for some of these malformations. It seems reasonable to believe that novel therapeutic strategies will soon emerge for the treatment of hemangiomas and vascular malformations. (Arterioscler Thromb Vasc Biol. 2004;24:1578-1590.)

Key Words: vascular malformation ■ angiogenesis ■ lymphangiogenesis

The cardiovascular system is the first functional organ system to form in the body. In humans, development of the circulatory system starts in the third week of embryonic life. The cardiovascular system consists of the heart, blood vessels (arteries, capillaries, and veins), and lymphatic vessels.

Development of the Blood Vascular and Lymphatic Systems
During embryogenesis, development of the blood vascular system occurs via 2 processes, vasculogenesis and angiogenesis. Vasculogenesis involves the de novo differentiation of endothelial cells from mesoderm-derived precursor cells, called hemangioblasts. Hemangioblasts aggregate to form primary blood islands in which the inner cells differentiate into hematopoietic stem cells and the outer cells differentiate into endothelial cell precursors, called angioblasts.1 Angioblasts then reorganize to form capillary-like tubes that constitute the primary vascular plexus. Once the primary vascular plexus is formed, new capillaries form from pre-existing vessels in a process called angiogenesis. The primary capillary network is remodeled into a functional structure containing large-caliber vessels for low-resistance rapid flow and small capillaries optimized for diffusion. This remodeling occurs by regression, sprouting, splitting, or fusion of pre-existing vessels. In the primary capillary plexus, endothelial cells start to differentiate into arterial and venous types.2

Stabilization of the forming vasculature occurs when periendothelial cells such as smooth muscle cells and pericytes are recruited to the vessel wall. Periendothelial cells stabilize nascent vessels by inhibiting endothelial proliferation and migration, and by stimulating production of extracellular matrix (ECM) and the formation of a basement membrane. They thereby provide homeostatic control and protect new endothelial-lined vessels against rupture or regression. Results from several studies indicate that the angiopoietin/Tie, the PDGF-B/PDGFR-β, and the transforming growth factor (TGF)-β1/TGF-βR ligand–tyrosine kinase receptor systems regulate endothelial cell-pericyte/vascular smooth muscle cell (VSMC) interactions.3

The development of the human lymphatic vascular system begins in the sixth to seventh week of embryonic life, nearly 1 month after the development of the first blood vessels. New lymphatic capillaries sprout from primary lymph sacs in a centrifugal manner, whereas the lymph sacs themselves are derived from veins. Lymphangioblasts have also been identified, which differentiate in situ from mesenchyme into lymphatic endothelial cells. These cells can be recruited into developing lymphatic vessels.4 A combination of the 2 mechanisms is likely to occur during embryonic lymphatic development, in which sprouting lymphatic vessels anastomose with lymphatics that have differentiated from lymphangioblasts.5

Gene Families Involved in Angiogenesis and Lymphangiogenesis
Angiogenesis and lymphangiogenesis are tightly regulated by growth factors, intercellular and cell-ECM signaling mecha-
VEGFs form a family of secreted glycoproteins and, at present, 4 members of the VEGF family have been identified: VEGF-A, VEGF-B, VEGF-C, and VEGF-D. All VEGFs have unique as well as overlapping patterns of expression and binding to the VEGF tyrosine kinase receptors (VEGFRs) VEGF-1, VEGF-2, and VEGF-3.

VEGF-A is one of the most important regulators of angiogenesis in vivo. There are multiple VEGF-A isoforms, the most abundant in humans being polypeptides of 121, 165, and 189 amino acids. All VEGF-A isoforms bind to VEGFR-1 and VEGFR-2. VEGF-A is critical for the earliest stages of vasculogenesis, because blood islands, endothelial cells, and major vessels fail to develop in VEGF-A knockout embryos. The deletion of even a single VEGF-A allele is embryonic lethal, demonstrating a remarkably strict dosage effect during embryonic development.

VEGF-B exists as 2 alternatively spliced forms, VEGF-B165 and VEGF-B189. Targeted inactivation of VEGF-B affects cardiac conduction as well as inflammatory cell recruitment in a murine arthritis model but does not affect embryonic vascular development.

VEGF-C and VEGF-D are produced as preproproteins with long N-terminal and C-terminal propeptides. Initial proteolytic cleavage of the C-segment, by a protein convertase, produces a form of 30 kDa with intermediate affinity for VEGFR-3. A second proteolytic step, mediated by plasmin, is required to generate the fully processed 21 kDa form, which binds with high affinity to both VEGFR-2 and VEGFR-3. Overexpression of VEGF-C and VEGF-D in transgenic mice induces the formation of hyperplastic lymphatic vessels. Conversely, inhibition of VEGF-C and/or VEGF-D by overexpression of a soluble form of VEGF-3 in the skin of transgenic mice leads to inhibition of lymphatic vessel growth. Targeted inactivation of both VEGF-C alleles results in prenatal death: endothelial cells commit to the lymphatic lineage but do not sprout from veins. This results in fluid accumulation in tissues. Heterozygous mice are viable but develop cutaneous lymphedema because of hypoplasia of the lymphatic system.

VEGFRs dimerize and undergo transphosphorylation on ligand binding. Targeted inactivation of the VEGFR-1 gene results in increased hemangioblast commitment leading to overgrowth of endothelial-like cells and disorganization of blood vessels. Deletion of only the intracellular domain of VEGF-1 is compatible with normal vascular development but impairs tumor angiogenesis. The latter effect is consistent with recent evidence suggesting that during adult life, VEGF-1 signaling plays a role in pathological angiogenesis by mobilizing endothelial progenitor cells from the bone marrow.

VEGFR-2 is first expressed in hemangiogenic lateral plate mesoderm but later becomes restricted to blood islands. Targeted disruption of the VEGFR-2 gene results in the failure of blood island and embryonic vessel formation, thus leading to embryonic lethality. VEGFR-2 is considered to be the main signal-transducing VEGFR for angiogenesis. Activation of VEGFR-2 stimulates endothelial cell proliferation, migration, and survival, as well as blood vessel permeability.

VEGFR-3 is initially expressed in all embryonic vasculature. However, during development its expression becomes restricted to lymphatic vessels and a subset of fenestrated
capillaries. VEGFR-3 is re-expressed in blood vessels during pathological angiogenesis. VEGFR-3–deficient mouse embryos die at mid-gestation as a result of defective remodeling of the primary vascular network, with resultant cardiovascular failure, before lymphatic vessels start to develop. Conditional knockouts will be required to fully understand the role of VEGFR-3 during lymphatic development.

**Angiopoietins and the Tie-2 Receptor**

The Tie receptor tyrosine kinase family consist of 2 members, Tie-1 and Tie-2, which are predominantly expressed by vascular endothelial cells. Several angiopoietin (Ang) family members, including Ang-1 to Ang-4, have been identified as ligands for Tie-2. Tie-1 ligands have thus far not been reported. Whereas Ang-1 and Ang-4 activate Tie-2, Ang-2 and Ang-3 appear to function as specific antagonists that inhibit Ang-1–mediated Tie-2 signaling.

In mouse embryos lacking Tie-2, vasculogenesis occurs normally. However, endothelial cells assemble into an immature vascular network lacking proper hierarchical organization into large and small vessels as well as periendothelial cells. Tie-2 therefore appears to control vascular remodeling, including the capacity of endothelial cells to recruit perivascular cells that are necessary to stabilize vessel structure. In embryos lacking Tie-1, endothelial cell integrity is compromised, leading to edema, hemorrhage, and death.

In the embryo, Ang-1 is expressed in the mesenchyme and smooth muscle cells surrounding the developing vasculature. Deletion of Ang-1 results in an angiogenic defect very similar to that seen in mice lacking Tie-2 or overexpressing Ang-2, including the absence of perivascular cells. Recent data suggest that Ang-2 has a role in lymphatic development. Deletion of Ang-2 in mice results in disorganization and hypoplasia of intestinal and dermal lymphatic capillaries leading to death at age 2 weeks. In addition, a second phenotype has been shown in which there is failure of hyaloid vessel regression in the eye and as well as failure of retinal vessels to sprout from the central retinal artery.

**TGF-β and Receptors**

The TGF-β signaling pathways play an important role in vasculogenesis and angiogenesis. During mouse embryogenesis, TGF-β1 is expressed in many tissues, including endothelial and hematopoietic precursor cells. Targeted inactivation of TGF-β1 in mice results in mid-gestation lethality in half of homozygotes and approximately one quarter of heterozygotes. The primary cause of death appears to be a defect in the yolk sac vasculature and hematopoietic system. Although initial differentiation of mesodermal precursors into endothelial cells occurs normally, subsequent differentiation into capillary-like tubes leads to the formation of vessels with reduced wall integrity. TGF-β receptor II-deficient mice demonstrate a similar mutant phenotype suggesting that TGF-β signaling is essential for maintenance of vessel wall integrity.

**PDGF-B and Receptor**

During development of the blood vascular system, PDGF-B is expressed in endothelial cells of arteries and angiogenic vessel sprouts, whereas its receptor PDGFR-β is expressed in the perivascular cells of arteries, arterioles, and capillaries. Newly formed vessels signal to the surrounding mesenchyme to induce the expression of PDGFR-β in periendothelial progenitors. These cells respond to PDGF-B secreted by endothelial cells by proliferating and migrating along sprouting capillaries. Targeted inactivation of PDGF-B or PDGFR-β results in loss of pericytes, resulting in dilated blood capillaries with increased numbers of endothelial cells and a fragile vessel wall, which in turn results in lethal hemorrhage in late embryogenesis.

**Notch and Jagged**

Members of the Notch and Jagged families are membrane-associated molecules, which require cell–cell contact for activation. The Notch family comprises a number of transmembrane proteins that interact as regulators of cell fate decisions. Notch signaling is activated after binding by either of its known ligands, Delta or Jagged. Delta1, Jagged1, and Jagged2 directly interact with Notch3, with the latter 2 being expressed exclusively by arterial endothelial cells. Notch appears to be involved in mesenchymal–endothelial cell signaling that stabilizes the vasculature; it has also been implicated in arteriovenous differentiation. Mutations of Jagged1 affect blood vessels and lead to Alagille syndrome.

**Integrins**

Adhesive interactions between endothelium and the ECM are mediated by integrins, a family of cell surface receptors that bind to collagens, vitronectin, laminins, and fibronectin. Integrins are heterodimeric molecules composed of α and β subunits. Integrins not only function as ECM adhesion molecules but also transduce biochemical signals into the cell. The potential for cross-talk between integrins and receptor tyrosine kinases exists as a result of the physical interaction between these 2 classes of proteins, which form macromolecular complexes on the cell surface. Thus, αvβ3 integrin was immunoprecipitated with VEGFR-2, whereas β1 integrin can associate with VEGFR-3. α9 integrin forms dimers only with β1 integrin chains, and targeted inactivation of α9 leads to chylothorax in newborn mice and leads to death in the first 2 weeks of postnatal life. Thus, α9β1 integrin appears to be required for normal development of the thoracic lymphatic system. Targeted inactivation of αV and β8 integrins leads to embryonic lethality with vascular defects in the placenta, brain, and gastrointestinal tract.

**Vascular Anomalies**

According to current classifications of vascular anomalies, 2 major groups can be identified: hemangiomas and vascular malformations (Table 1). Hemangiomas are present at birth, proliferate, and then involute. However, vascular malformations are present at birth or develop later, but they do not involute.

**Hemangiomas**

Hemangiomas occur in 10% to 12% of newborns. Familial forms account for 10% of all hemangiomas, which means that only 1% of hemangiomas can be considered as familial. Female infants are 3– to 4-times more likely to develop hemangiomas than males. Classically, complete resolution of
hemangiomas occurs in >50% of children by age 5 years and in >70% by age 7, with continued improvement in the remaining children up to ages 10 to 12 years. Glut-1 is purportedly expressed by endothelial cells during all phases in most hemangiomas with the exception of noninvoluting and rapidly involuting congenital hemangiomas (noninvoluting congenital hemangioma and rapidly involuting hemangioma, respectively). Glut-1 may therefore be a useful marker that allows distinction of hemangiomas from the new entities noninvoluting congenital hemangioma and rapidly involuting hemangioma.

The Proliferative Phase
The hallmark of a growing hemangioma is endothelial cell proliferation. Continued proliferation increases vessel diameter allowing for lumen formation and blood perfusion. The organizing endothelial tubes are covered by closely associated pericytes. Toward the end of the proliferative phase, hemangiomas mature and organize into lobules, separated by fibrous septae, each with its own blood supply and venous drainage.

During the phase of rapid growth, hemangiomas overexpress cytokines and other molecules known to play a role in angiogenesis. These include FGF-2, VEGF-A, and matrix metalloproteinases. With the exception of FGF-2, these factors decrease with the onset of involution and are not present in fully involuted lesions. FGF-2 remains elevated throughout early involution in both hemangiomatous tissue and in urine. In addition to a decrease in angiogenic factors, involution is characterized by high levels of angiogenic inhibitors and an increase in endothelial cell apoptosis.

The Involuting Phase
The transition from proliferation to involution is gradual and coincides with the appearance of mast cells and with the induction of tissue inhibitors of metalloproteinases. Involution is also characterized by a progressive deposition of fibro-fatty tissue, together with a decrease in the number of vascular channels. The remaining vessels become progressively ectatic and may persist as telangiectasia. Two proteins, mitochondrial cytochrome b and homer-2a, both of which are implicated in apoptosis, are specifically expressed during the involuting phase of hemangiomas; this indicates that apoptosis plays an important role during this phase.

Many theories have been advanced to explain the pathogenesis of hemangiomas. The most accepted appears to be that the primary defect is intrinsic to endothelial cells. A familial form has been described with an autosomal dominant trait and high penetrance. Whole-genome linkage studies in these families mapped a locus on chromosome 5q31–33, but the responsible gene has not yet been identified. Loss of heterozygosity on chromosome 5q has been found in 50% of sporadic hemangiomas, suggesting that a somatic mutation in this region may be associated with sporadic and familial hemangiomas. Cultured endothelial cells isolated from hemangiomas display a nonrandom pattern of X-chromosome inactivation, which was not observed in nonendothelial stromal cells isolated from the same lesions. These results demonstrate that clonality is restricted to endothelial cells within the lesion. Missense mutations in the kinase domains of both VEGFR-2 and VEGFR-3 were found in the hemangioma samples but not in adjacent normal skin. Furthermore, endothelial cells isolated from hemangiomas have a higher rate of proliferation and migration than normal endothelial cells. These results support the concept that hemangiomas might be caused by a somatic mutation in a single endothelial or progenitor cell that results in disruption of normal endothelial cell growth control in the resulting daughter cells.

Vascular Malformations
Vascular malformations are errors in morphogenesis that may affect any segment of the vascular tree, including arterial,

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**TABLE 1. Hereditary Vascular Anomalies*\(^\text{a}\)**

<table>
<thead>
<tr>
<th>Vascular Anomaly</th>
<th>Inheritance</th>
<th>Chromosome</th>
<th>Gene</th>
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</thead>
<tbody>
<tr>
<td>Hemangioma</td>
<td>AD</td>
<td>5q31–33</td>
<td>NK</td>
</tr>
<tr>
<td>Hereditary hemorrhagic telangiectasia 1</td>
<td>AD</td>
<td>9q33–34</td>
<td>Endoglin</td>
</tr>
<tr>
<td>Hereditary hemorrhagic telangiectasia 2</td>
<td>AD</td>
<td>12q11–14</td>
<td>ACVRL1</td>
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<td>AD</td>
<td>3p22</td>
<td>NK</td>
</tr>
<tr>
<td>CADASIL</td>
<td>AD</td>
<td>19p13.2–13.1</td>
<td>Notch3</td>
</tr>
<tr>
<td>Cutaneous venous malformation</td>
<td>AD</td>
<td>9p21–22</td>
<td>Tie-2</td>
</tr>
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<td>AD</td>
<td>1p21–22</td>
<td>Glomulin</td>
</tr>
<tr>
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<td>7q11.2–21</td>
<td>KRT1</td>
</tr>
<tr>
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<td>AD</td>
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</tr>
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<td>AD</td>
<td>3q25.2–27</td>
<td>NK</td>
</tr>
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<td>Capillary malformation 1</td>
<td>AD</td>
<td>5q13–15</td>
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<td>AD</td>
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<td>VEGFR-3</td>
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<td>Xq28</td>
<td>NEMO</td>
</tr>
<tr>
<td>Lymphedema-cholestatic</td>
<td>AR</td>
<td>15q</td>
<td>NK</td>
</tr>
</tbody>
</table>

*See text for references. AD indicates autosomal dominant; AR, autosomal recessive; NK, not known.
venous, capillary, and lymphatic vessels, or a combinations thereof.

**Hereditary Hemorrhagic Telangiectasia (OMIM 187300)**

Hereditary hemorrhagic telangiectasia (HHT), also known as Osler-Weber-Rendu disease, is an inherited disorder that leads to the development of arteriovenous malformations (AVMs) and telangiectasia, predominantly in the skin, lungs, liver, gastrointestinal tract, and brain.58

HHT is inherited in an autosomal-dominant manner and occurs with equal gender distribution.59 Penetrance is high with an age-dependent phenotype and variable onset. Nose-bleeds are usually the earliest sign of the disease, often occurring in childhood. Pulmonary AVMs become apparent from puberty. Mucocutaneous and gastrointestinal telangiectasias develop progressively with age and are present in nearly all patients by age 40 years.60

Genetic linkage studies identified a locus on chromosome 9q33–34 in some HHT families. The disease gene was subsequently recognized to be endoglin (ENG).61 A second HHT locus was mapped to chromosome 12q, and the mutated gene was identified as activin receptor-like kinase 1 (ACVRL1).62 Other families without defects in these 2 genes have been reported, suggesting that mutations in other genes may lead to the same phenotype.63,64

ENG and ACVRL1 are predominantly expressed by endothelial cells and encode transmembrane coreceptors of the TGF-β family. A wide body of experimental evidence suggests that TGF-β plays an important role in vascular remodeling and in the maintenance of vessel wall integrity. It modulates the activities of cytokines involved in endothelial cell proliferation and migration, it affects production or degradation of the ECM, and it regulates the interaction of endothelial and smooth muscle cells in the process of vascular remodeling and vessel wall maturation.65,66 TGF-β receptor mutations probably lead to HHT by altering these processes.

**HHT1: Endoglin**

A number of mutations in the ENG gene, including deletions, insertions, missense, and point mutations, have been detected in HHT1 families. Additional mutations are predicted in promoter or intronic regions.67 Elegant studies have revealed a 50% reduction in expression levels of ENG at the cell surface of human umbilical vein endothelial cells and monocytes from HHT1 patients carrying ENG mutations. Some mutations in the ENG gene result in proteins that are retained intracellularly, whereas in others, transcripts are undetectable.68,69 In vivo, the relative levels of ENG were reduced to 50%, indicating that only the normal allele is expressed by the endothelium.70 This strongly implicates haploinsufficiency in the pathogenesis of HHT1.

ENG knockout mice are embryonic lethal at E10.5 because of defective heart and vascular development. Although vasculogenesis occurs normally, there is an impairment in vascular remodeling, ie, VSMC differentiation and recruitment, leading to vessel enlargement and rupture.71 Heterozygous ENG mice develop clinical signs of HHT, including bleeding; this occurs from dilated cutaneous postcapillary venules, which have a disorganized media.72 Telangiectasia are also observed in liver, lung, brain, and the gastrointestinal tract. The abnormal vascular lesions arise predominantly in certain genetic backgrounds such as the 129/Ola strain and only affect a proportion of heterozygous mice.73 This reflects the clinical variability seen in the human disease and supports the possibility that additional modifying genetic or environmental factors contribute to the disease. Recently, it was suggested that the 129/Ola strain has lower plasma TGF-β1 levels than other strains. The presence of locally active VEGF-A appears to be required for the development of vascular anomalies.74 Thus, injection of VEGF-A into heterozygous ENG and wild-type mice led to the same increase in microvesSEL number. However, morphological abnormalities including enlarged, twisted, and spiraled vessels were only seen in heterozygous ENG mice.

**HHT2: ACVRL1**

Mutations have been described in the sequence encoding the extracellular, transmembrane, and kinase domains of ACVRL1, and include small deletions, insertions, nonsense, and missense mutations.67 Similar to ENG, functional ACVRL1 levels were reduced, suggesting haploinsufficiency as a potential mechanism for the disease.75 ACVRL1 knockout mice die at gestation day 10.5 and demonstrate defective vascular remodeling, ie, excessive dilatation of large vessels with reduced recruitment of VSMCs.76,77 Vascular abnormalities in ACVRL1−/− mice may also be caused by persistent activation of angiogenesis. Disruption of ACVRL1 in zebrafish results in a vasculature containing dilated vessels and an increase in endothelial cell number.78 ACVRL1 therefore seems to regulate the resolution phase of angiogenesis, characterized by the cessation of endothelial cell proliferation and VSMC recruitment.79 Some mice heterozygous for ACVRL1 develop a phenotype similar to that observed in HHT patients.80 This consists of mucocutaneous telangiectasia affecting the oral and gastrointestinal mucosa. In addition, AVMs are found in the lung, liver, brain, and spleen.

Since the identification of genes responsible for HHT, the search for genotype–phenotype correlations has intensified. No significant differences in phenotype have been observed between ENG and ACVRL1 mutations.81 This points to the contribution of additional causative factors in the HHT phenotype, such as female hormones during pregnancy or possibly a predisposing genetic background.82 A complete understanding of the exact role of ENG and ACVRL1 in TGF-β and VEGF-A signaling and in the pathogenesis of HHT lesions such as AVMs and telangiectasia is still required. Both mouse models mimicking this disease could help to achieve this goal and should be useful for testing new therapies.

**Cadasil (OMIM 125310)**

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (Cadasil) begins at approximately 35 to 45 years of age and manifests as recurrent brain infarcts leading to progressive dementia. The cardinal symptoms are migraine, mostly with aura, ischemic strokes, mood disorders, cognitive defects, and epilepsy.83,84 Males and females are equally affected.85
Pathologically, small arteries are affected, with thickening of arterial media and fragmentation and duplication of the internal elastic lamina. The pathognomonic ultrastructural feature of CADASIL is granular osmiophilic material deposited close to the membranes of smooth muscle cells of small and medium arterioles. Granular osmiophilic material deposits are found within the brain and also in other tissues, including peripheral nerves, skeletal muscle, intestine, liver, kidneys, and skin, indicating a systemic arteriopathy.86–88

A number of genetic studies linked CADASIL to chromosome 19q, and the gene involved was subsequently identified as Notch3.89 Notch3 contains an extracellular domain composed of 34 tandemly arranged epidermal growth factor-like repeats, followed by a transmembrane region, and an intracellular domain containing 6 ankyrin repeats and nuclear localization sequences.90

The majority of mutations identified in CADASIL are missense mutations (95%) with rare examples of splice site mutations and small in-frame deletions.91,92 All Notch3 mutations are located in the EGF-like repeat domain and result in a modification of the number of cysteine residues. Clinical diagnostic analysis of CADASIL revealed that 70% to 90% of patients have mutations in exons 3 or 4 of Notch3.93 Many different mutations have been identified within affected families or sporadic patients, but to date no genotype–phenotype correlations have been established.94

Cellular expression of different naturally occurring mutations in the Notch3 extracellular domain has demonstrated different domain requirements for Notch3 function.95–97 Some mutations result in reduced membrane expression because of an impairment in protein maturation in the Golgi apparatus, which in turn leads to intracellular aggregate formation. There is also an impairment in ligand-induced transcriptional activity. Other Notch3 mutations are normally expressed at the cell surface but do not bind or respond to ligand stimulation. Interestingly, most Notch3 mutations are expressed and respond to ligand stimulation like the wild-type receptor. This suggests that other as-yet-unidentified cellular mechanisms are affected.

A mouse model for CADASIL has been generated by overexpressing a mutated form of human Notch3, specifically in arterial smooth muscle cells.98 The first morphological changes appear at 12 months, with an increase in the intercellular space between smooth muscle and endothelial cells, similar to changes seen in CADASIL patients. These effects were found in cerebral, renal, carotid, femoral, and tail arteries. This study suggests that the disruption of VSMC anchorage may be one of the key events leading to vascular degeneration in CADASIL.

Recent work has demonstrated expression, albeit restricted, of Notch3 on VSMCs, indicating a possible role in their physiology. Activation of Notch3 signaling in VSMCs increases their survival and proliferation.99,100 This raises the question as to the effect of mutant Notch3 in VSMCs. Is this caused by haploinsufficiency? Could this be a dominant inhibition of Notch3 signaling? Does accumulation of the Notch3 extracellular domain lead to cytotoxicity? Additional studies are needed to further understand the normal function of Notch 3 and how disruption may lead to disease.

Multiple Cutaneous and Mucosal Venous Malformations (OMIM 600195)

Multiple cutaneous and mucosal venous malformations is a syndrome characterized by multiple venous malformations (VM) in the skin and bleeding of the gastrointestinal tract.101 Multiple cutaneous and mucosal venous malformations can be inherited in an autosomal-dominant manner.102 The lesions usually appear at birth, although in some cases they appear during childhood or later in life. VM are progressive and the degree of ectasia increases with age, albeit at a variable rate. The size can vary from capillary spongy blebs to cavernous lesions. A rapid expansion may be seen after trauma, after partial resection, or after hormonal modulation such as during pregnancy.103

Histopathologic examination of the affected blood vessels shows thin and irregular walls with a variable number of smooth muscle cells and an absent internal elastic membrane.47

Genetic linkage studies have implicated a region on chromosome 9p in 2 unrelated multiple cutaneous and mucosal venous malformations families.103 The disease gene was subsequently identified as Tie-2.102,104 Overexpression of the mutated Tie-2 protein in insect cells resulted in ligand-independent hyperphosphorylation of the receptor and in activation of STAT1, which was not observed with the wild-type receptor.105 Taken together, these observations suggest that abnormal vessel development in this syndrome is caused by a local uncoupling of endothelial smooth muscle cell signaling.102 It is important to note that some VM families do not show linkage to the Tie-2 locus, suggesting the existence of additional loci for inherited VM.

Glomuvenuous Malformation (OMIM 138000)

Glomuvenuous malformations (GVM) are a subtype of cutaneous venous malformation surrounded by one or a few layers of glomus cells. These lesions show no gender predominance and appear from birth to childhood. They are raised, blue–purple with a cobblestone surface, and are extremely painful on palpation.106 In contrast to common VM, GVM are rarely encountered in mucous membranes.107

GVM consist of irregular, dilated blood vessels surrounded by cuboidal epithelioid-like glomus cells.108 Immunohistochemically, glomus cells express α-smooth muscle actin and vimentin109 and have ultrastructural characteristics of smooth muscle cells; for this reason, they are considered to be variant smooth muscle cells.110

The few families so far identified with GVM have shown an autosomal-dominant pattern of inheritance with incomplete penetrance, estimated to be 70% by the age of 20 years.111 Affected individuals in these families often develop multiple lesions.112 Linkage analysis in unrelated families identified a locus on chromosome 1p21–22, which was termed VMGLOM.113,114 The affected gene was identified as glomulin.115 All mutations identified to date are deletions, insertions, or point mutations resulting in premature termination of the protein, which suggests that haploinsufficiency may contribute to lesion formation.115 However, because GVMs are localized, haploinsufficiency on its own is unlikely to be sufficient for lesion development, and additional genetic
factors are likely to be required for complete localized lack of glomulin. Brouillard et al screened GVM lesions for somatic mutations and identified a truncating mutation that was not present in genomic DNA. This suggests the presence of a de novo somatic mutation in GVM lesional DNA leading to local loss of functional glomulin.115

To date, little is known about glomulin function, despite the fact that glomulin transcripts are ubiquitously expressed in human tissues. Recently, analysis of glomulin expression in the mouse revealed that it is restricted to VSMCs.116 Further studies will hopefully elucidate the functional importance of glomulin in vessel remodeling or smooth muscle cell differentiation.

**Cerebral Cavernous Malformations (OMIM 116860)**

Cerebral cavernous malformations (CCM) are vascular lesions that may involve any part of the central nervous system. CCM can occur in sporadic or autosomal dominant forms and show incomplete penetrance. Sporadic cases usually consist of a single lesion. Familial CCM is characterized by multiple lesions whose number is positively correlated with patient age, thereby suggesting that the lesions are dynamic in nature.117,118 These patients typically present between the ages of 20 and 40 with intracranial hemorrhage, focal neurological deficits, seizures, or headaches.119

CCM are small, well-circumscribed, multilobulated vascular lesions composed of dilated sinusoidal vascular spaces lined by a single layer of endothelium. A layer of collagenous, fibronectin-rich matrix usually devoid of VSMCs surrounds the endothelium. There is no intervening brain parenchyma between the lobules of a lesion.120

Linkage studies have identified loci mapping to chromosome 7q21–22 (CCM1), 7p13–15 (CCM2), and 3q25.2 to 27 (CCM3) in autosomal-dominant CCM.121,122 CCM1 and CCM3 each account for 40% of all cases, with CCM2 found in the remaining 20%.123

Mutations have been localized to the KRIT1 gene in CCM1-linked families.124 All are putative loss-of-function mutations including frame shifts, nonsense, missense, and invariant splice site sequence mutations.124 A subset of patients with sporadic CCM lesions was linked to the CCM1 locus, suggesting that they may have inherited a de novo mutation in KRIT1.125 Somatic mutations in KRIT1 have been identified in 1 patient.126

KRIT1 protein contains 3 ankyrin repeats, a motif known to mediate protein–protein interactions,127 followed by a FERM domain, which mediates interactions between the actin cytoskeleton and the plasma membrane128 and 1 NPXY motif. It has been recently demonstrated that the NPXY domain of KRIT1 mediates interaction with the integrin cytoplasmic domain-associated protein 1 (icap1α), a protein involved in β1-integrin–dependent angiogenesis.129 This suggests that KRIT1 may be involved in modulating integrin–microtubule signaling or function in endothelial cells.

Interestingly, rare families with CCM1 also develop a cutaneous malformation called hyperkeratotic cutaneous capillary venous malformation (HCCVM).130 and mutations in KRIT1 have been identified in these families.131

The gene responsible for CCM2 was recently discovered and was named malcavernin132 or MGC4607.133 Expression studies indicate that malcavernin mRNA is expressed in most organs, including the brain. Bioinformatic analysis revealed that malcavernin contains a phosphotyrosine-binding (PTB) domain that is also present in icap1α. Most of the mutations identified thus far arise before or within the PTB domain. Two families have a small deletion including the first exon of the gene.132,133 Could there be an interaction or competition between icap1α and malcavernin for integrin cytoplasmic tail-binding and intracellular signal modification? The gene(s) responsible for CCM3 still await identification.

**Capillary Malformations (OMIM 163000)**

Capillary malformations (CM), also called port-wine stains or naevus flammeus, are present at birth in >90% of neonates and have an equal gender distribution.101 The lesions grow proportionately with the child and do not involute, unlike other vascular birthmarks such as salmon patches, which occur more frequently (40% of newborns).134 The majority of CM are located on head and neck skin, with 85% occurring in a unilateral distribution that follows a dermatome.135

Histologically, CM are characterized by ectatic papillary dermal capillaries and postcapillary venules. These ectatic vessels retain normal endothelial and smooth muscle cell morphology and maintain normal rates of turnover. CM lesions therefore represent vascular ectasia rather than a proliferative process.136 In addition, it has been shown that CM have defective cutaneous sympathetic innervation.137,138

Although CM are usually sporadic, families have been reported that inherit lesions in a dominant manner with incomplete penetrance.139,140 Linkage analysis identified a locus on chromosome 5q13–15, which has been termed CMC1.141,142 The causative gene for CMC1 was recently identified as RASA1, also called p120-RasGAP, a negative regulator of Ras.143 Some affected members have more complex vascular malformations comprising CM and AVM as in Parkes–Weber, Sturge–Weber, or Klippel–Trenaunay syndrome.144 Further investigation is needed to define whether the RASA1 mutation affects angiogenesis and/or neurogenesis.

**Tufted Angioma (OMIM 607859)**

Tufted angioma is a rare benign vascular lesion of unknown cause that predominantly affects children younger than age 5 years, although it may also occur in adulthood.144 There is no significant gender predilection. The lesions appear mainly on the neck, shoulders, and trunk, although other areas can be affected.145–147

Characteristically, the lesions appear histologically as a “cannonball” distribution of rounded nodules or tufts.148 These nodules contain capillary-sized vessels in the dermis with lymphatics present at the periphery.149 The lesions form and grow slowly and then remain stable in size; in some cases, regression has been reported.150,151

Although tufted angiomas are usually sporadic, 2 families have been reported in which the lesions segregate in a dominant manner with low penetrance.149,152
**Lymphedema**

Lymphatic vessels play a central role in maintaining interstitial fluid balance. Lymphedema is characterized by a chronic, disabling swelling of the extremities caused by insufficient lymphatic drainage. Lymphedema is divided into 2 categories: primary and secondary. Primary lymphedema can be present at birth, develop at puberty, or, more rarely, develop in adulthood. In hereditary lymphedema, lymphatic vessels can be either hypoplastic or hyperplastic, but are nonfunctional. In all forms of lymphedema, there is persistent accumulation of stagnant, protein-rich fluid within the interstitium. As a consequence, the affected area often shows increased tissue fibrosis, accumulation of adipose tissue, susceptibility to infections, and, infrequently, cancerous degeneration to lymphangiosarcoma.

**Type I Hereditary Lymphedema (OMIM 153100)**

Type I hereditary lymphedema (or Milroy disease) is an early-onset form of lymphedema. It is usually present either at birth or soon after birth and is inherited in an autosomal-dominant manner. In affected individuals, the initial superficial lymphatics of edematous areas are absent by microlymphography and are believed to be hypoplastic. However, in nonedematous skin, superficial lymphatics are observed.

Linkage analysis has enabled the identification of a locus on chromosome 5q35.3. The causative gene was subsequently identified as VEGFR-3. All known VEGFR-3 mutations result in amino acid substitutions in the catalytic domain of the receptor. In vitro experiments indicate that the mutant VEGFR-3 protein is not phosphorylated and can form heterodimers with wild-type VEGFR-3. However, cotransfection of both mutated and wild-type VEGFR-3 into cells leads to a 50% decrease in wild-type VEGFR-3 phosphorylation. However, mutant VEGFR-3 is more stable on the cell surface than the wild-type receptor. Thus, the mutant receptor may accumulate on the cell surface, leading to the formation of inactive receptor dimers. VEGFR-3 is required for endothelial cell migration, and mutation of the kinase domain reduced migration by 50%. A mouse model for type I hereditary lymphedema has been produced by chemical mutagenesis. Similar to the human disease, Chy mice are heterozygous for a germline inactivating mutation in the VEGF-R3 tyrosine kinase domain. Chy mice have hypoplasia of dermal but not visceral lymphatic vessels, accumulate subcutaneous fluid, and demonstrate paw swelling. Because mice carrying one functional VEGFR-3 allele appear normal whereas those carrying a tyrosine kinase-inactivating mutation have lymphedema, the phenotype is proposed to arise from a dominant-negative effect of the inactive receptor.

**Type II Hereditary Lymphedema (OMIM 153200)**

Type II hereditary lymphedema (or Meige disease) is a late-onset inherited autosomal-dominant disorder with reduced penetrance and variable phenotype. Associated features of type II lymphedema include distichiasis, ptosis, cleft palate, yellow nails, and congenital heart disease. The age of onset for type II lymphedema is at or after puberty; however, this is variable. Lymphoscintigraphy of affected areas shows abundant dilated lymphatic vessels and an impairment in lymphatic drainage.

Linkage analysis identified a locus on chromosome 16q24.3, where the causative gene was identified as FOXC2. Nonsense mutations as well as base-pair deletions or duplications were found that would produce premature stop codons and a truncated FOXC2 protein. Thus, haploinsufficiency may be responsible for this disorder, although a dominant-negative effect is also possible. Functional analysis of mutated FOXC2 protein has thus far not been reported.

FOXC2 encodes a member of the forkhead/winged-helix family of transcription factors. FOXC2 is expressed in the developing mesenchyme of the head, kidney, and bones, and appears to play a role in somite formation. It is not known whether FOXC2 is expressed in developing lymphatics or whether it plays a role in the development of the lymphatic system.

FOXC2-deficient mice die during embryogenesis and perinatally; this is because of abnormalities of the heart, aorta, palate, and skeleton. FOXC2 heterozygous mice display generalized hyperplasia of dermal lymphatic vessels and also have distichiasis, which closely mimics the human disease. Interestingly, paw swelling is only present in a small percentage of these mice. Lymphoscintigraphy shows lymph reflux apparently caused by incompetent lymphatic valves.

The broad phenotypic heterogeneity seen in families carrying FOXC2 mutations illustrates the developmental pleiotropy of this transcription factor. Phenotypic and mutation analysis may help to shed light on the functional domains of FOXC2 and the regulatory regions of this gene.

**Hypotrichosis-Lymphedema-Telangiectasia (OMIM 607823)**

Hypotrichosis-lymphedema-telangiectasia is a recently described syndrome in which lymphedema is present at birth or develops during infancy. This syndrome is inherited in a dominant and recessive manner. Lymphoscintigraphy has revealed the absence of lymph flow, thereby indicating abnormal lymphatic function. All known families exhibit mutations in the SOX18 gene, a classical transcription factor with modular DNA-binding proteins and a trans-activating domain. In the recessive form, missense mutations predictivate, whereas the dominant form is linked to mutations that cause a truncated SOX18 protein lacking its trans-activating domain.

Ragged mice are naturally occurring SOX18 mutants that have a phenotype similar to the human syndrome. Interestingly, as in humans, the mode of inheritance is either dominant or recessive, depending on the nature of the mutation. However, homozygous inactivation of the SOX18 gene leads to viable mice with a slight coat hair phenotype. This suggests that mutant SOX18 in ragged mice interferes with wild-type protein function in a dominant-negative manner.

**Ectodermal Dysplasia and Immunodeficiency With Osteoporosis and Neonatal Lymphedema (OMIM 300301)**

It has recently been suggested that a rare disorder named ectodermal dysplasia and immunodeficiency with osteoporo-
s and neonatal lymphedema, or OL-EDA-ID, is caused by a specific mutation in the stop codon of the nuclear factor-kappaB (NF-κB) essential modulator (NEMO) gene producing a protein product that is 27 amino acids longer than the native protein.\textsuperscript{178,179} NEMO encodes the regulatory gamma subunit of the inhibitor of the NF-κB kinase complex, which is critical for the activation of the NF-κB pathway.\textsuperscript{180} It was shown that NEMO mutations in OL-EDA-ID reduce NF-κB signaling by 50%, suggesting haploinsufficiency as a potential mechanism for this disease.\textsuperscript{178} Further studies are needed to clarify the importance of NF-κB signaling during lymphangiogenesis.

**Cholestasis–Lymphedema Syndrome (OMIM 214900)**

Cholestasis–lymphedema syndrome is an autosomal-recessive disorder.\textsuperscript{181} Cholestasis–lymphedema syndrome patients have severe neonatal cholestasis, and leg lymphedema develops during childhood.\textsuperscript{182} There is an equal gender distribution.\textsuperscript{183} Lymphoscintigraphy has revealed hypoplastic lymphatic vessels with back-flow and delayed emptying.\textsuperscript{184} A whole-genome screen was performed on affected families to identify candidate genes. An interval of 6.6-cM on chromosome 15q was identified, which might contain a gene involved in cholestasis–lymphedema syndrome.\textsuperscript{185}

### Summary and Future Research

Overt vascular malformations represent a minority of vascular diseases. Genetic and molecular studies in families with vascular malformations have implicated genes expressed in endothelial and VSMCs. These genes encode receptors, transcription factors, and proteins of unknown function. Subtle alterations in their function may also underlie some of the more common vascular diseases.

It is likely that genes identified in families are also involved in sporadic cases of vascular anomalies. Because most vascular malformations occur sporadically, it will be interesting to determine whether germline mutations arise de novo in these cases or whether somatic mutations are sufficient to cause the same phenotype. There is a prevailing tissue or organ predilection in those vascular malformations transmitted by germline mutation. Therefore, the double-hit mechanism is a likely possibility in hemangioma, GMV, and CCM1 (Table 2). It could also be that secondary somatic mutations affect other genes that interact with the disease-causing gene. Laser microdissection of different components of vascular malformations such as endothelial or VSMCs should permit a more precise detection of the cell types carrying somatic mutations in the genes implicated.

Gene mapping in families with vascular malformations will almost certainly enhance our understanding of angiogenesis, lymphangiogenesis, and probably also of neurogenesis. In many cases, the challenge is now to determine the function of the genes identified. This in turn will hopefully open the door to novel biological therapies as a complement to laser and surgical treatment. The further development of transgenic mouse models for many of these lesions should likewise be useful for developing novel strategies for preventing the growth and extension of existing vascular malformations.

### References

9. Siegfried G, Basak A, Cronjash JA, Benjannet S, Marcinkiewicz J, Chretien M, Seidah NG, Khaith AM. The secretory proprotein convertases furin,


60. McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Pepper MS. Transforming growth factor-


62. Torsney E, Charlton R, Diamond AG, Burn J, Soames JV, Arthur HM. \textit{Devel-}


64. Wallace GM, Shovlin CL. A hereditary haemorrhagic telangiectasia family with pulmonary involvement is linked to the known HHT genes, endoglin and ALK-1. \textit{Thorax.} 2000;55:685–690.

65. Pepper MS. Transforming growth factor-


76. Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donohoe PK, Li L, Miyazono K, ten Dijke P, Kim S, Li E. Activin receptor-like kinase 1 modulates transforming growth factor-


79. Ota T, Fujii M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, Miyazono K. Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor-


95. Arterioscler Thromb Vasc Biol. September 2004


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