Molecular Basis of Vascular Birthmarks

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ABSTRACT

Vascular anomalies affect up to 10% of newborns, largely because of the high incidence of hemangioma of infancy. Vascular anomalies also frequently occur in adults; there is high prevalence of capillary malformations (0.3%). These cutaneous stains often cause psychosocial problems related to their visibility. Venous malformations occur in the skin and in internal organs and may cause destruction. Primary lymphedema causes lifelong morbidity, and arteriovenous malformations, in addition to causing distortion, obstruction, and pain, can be life endangering. The pathophysiology of these anomalies has stayed largely unknown, but genetic studies have revealed clues to their etiology. Genetic defects cause hereditary types of venous malformation, cutaneous and mucosal (VMCM); glomuvenous malformation (GVM); primary congenital lymphedema (Milroy disease); lymphedema-distichiasis syndrome; hypotrichosis-lymphedema-telangiectasia (HLT) syndrome; hereditary hemorrhagic telangiectasia (HHT); cerebral cavernous malformation (CCM); and a newly recognized disorder, capillary malformation–arteriovenous malformation (CM-AVM). These seminal discoveries have not only permitted a more precise clinical classification and diagnosis (a prerequisite for corrective measures for prevention, treatment, and follow-up) but also pointed the way to the identification of factors that play an important role in vasculogenesis or angiogenesis, or both.

KEYWORDS: Vascular anomaly, hemangioma, genetic, angiogenesis, mutation

Vascular birthmarks occur in about 40% of neonates, with a variety of manifestations, including macular stains, hemangiomas, malformations, and telangiectasias.[1] The classification of these lesions has been an evolving process, which in 1996 saw the International Society for the Study of Vascular Anomalies (ISSVA) approve a system based on clinical, radiological, histopathological, and hemodynamic characteristics. Two major categories exist for these vascular anomalies, namely tumors and malformations.[2–5] With regard to tumors, the most common lesion is the infantile hemangioma, which is rapidly growing during the neonatal period, involutes during childhood, and does not appear in adulthood.[6] Vascular malformations, on the other hand, represent malformed vessels without endothelial cell proliferation; these lesions are present at birth, do not regress spontaneously, and may either be stable or expand.

Vascular malformations may be further subdivided into fast-flow and slow-flow categories. These lesions may also be localized or diffuse, occurring in any body part including the viscera, and although some are inconsequential, some may cause cosmetic or functional disabilities. The majority of lesions may be diagnosed clinically; however, radiological imaging may be required to delineate the lesion and tailor therapy.[7,8] Studies of the molecular basis of vascular anomalies offer new hopes for treatment.[9]

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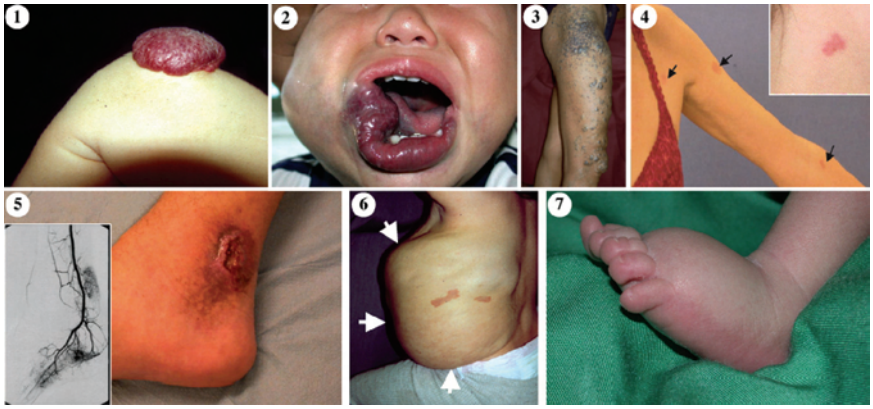

Figure 1 Vascular anomalies. (1) Hemangioma of infancy (HOI); (2) venous malformation (VM); (3) glomuvenous malformation (GVM); (4) capillary malformation of CM-AVM; (5) arteriovenous malformation of CM-AVM; (6) lymphatic malformation (LM); (7) lymphedema.

Some vascular birthmarks have been seen to display inheritance patterns in families, and it has been observed that the majority occur in an autosomal dominant manner. This means that the majority of inherited vascular malformations occur with equal frequency in males and females, with a 50% risk of children inheriting the mutant allele. Because reduced penetrance has been observed, it would seem that other factors, for example, an environmental one, may play a role in their development.

VASCULAR MALFORMATIONS

Fast-Flow Vascular Malformations

ARTERIOVENOUS MALFORMATION

Most fast-flow cutaneous vascular malformations are arteriovenous malformations (AVMs). Purely arterial malformations, such as arterial aneurysms, rarely occur in the skin, and cutaneous arterial fistulas are commonly the result of trauma. AVMs are fast-flow lesions that are composed of an epicenter or nidus of feeding arteries, dilated veins, and arteriovenous shunts of different sizes. Present at birth, they may not become evident until later life, but they never regress spontaneously.

AVM may initially be diagnosed as a hemangioma or port-wine stain, but puberty and trauma can trigger their growth, when fast flow becomes clinically evident. As well as the development of purple discoloration and a mass, other signs include warmth, thrill, and bruit. The most unpredictable of vascular malformations, these lesions often enlarge with time, causing local destruction and cardiac complications, which may necessitate intervention.

CAPILLARY MALFORMATION—ARTERIOVENOUS MALFORMATION

During the work with families with inherited capillary malformation, it was observed that six families manifesting atypical capillary malformations associated with fast-flow lesions (Fig. 1), either AVM, arteriovenous fistula, or Parkes-Weber syndrome, had *RASA1* gene mutations on chromosome 5q13.3[10,11] (Table 1). This condition, named capillary malformation–arteriovenous malformation or CM-AVM, displays a phenotypic variability that may be explained by the involvement of p120-RasGAP in signaling for various growth factor receptors that control proliferation, migration, and survival of several cell types, including vascular endothelial cells.

Slow-Flow Vascular Malformations

VENOUS MALFORMATION

Venous malformations (VMs) are malformations that affect the venous aspect of the vascular network (Fig. 1) (Table 1). They constitute the most common cause for consultation at centers specializing in vascular anomalies because of their propensity for cosmetic disfigurement, bleeding, pain, and functional compromise. Although these may be localized or extensive, single or multiple, they are most commonly located on the head and neck. Present at birth, they may not become evident until later in life.

Composed of ectatic venous channels within the dermis, these lesions have a characteristic blue coloration. Histologically, the distended vein-like channels are lined by flat, nonproliferative endothelial cells, surrounded by reduced numbers of supportive mural vascular smooth muscle cells (vSMCs).[12] VMs enlarge with exercise and dependence, the deformation of involved tissues slowly worsening with time. Unlike AVMs, VMs have no associated warmth, thrill, or bruit. Deformity and functional deficits may necessitate treatment in the form of sclerotherapy and possibly surgical resection.

MUCOCUTANEOUS VENOUS MALFORMATION

VMs are mostly sporadic in occurrence, but some familial cases (1 to 2% of VMs) have been reported.[12–16] Linkage analysis identified a locus on chromosome

Table 1 Etiology of Vascular Anomalies

Vascular Anomaly	Chromosome	Gene	Protein Function
Arteriovenous malformation (AVM)	—	—	—
Capillary malformation–arteriovenous malformation (CM-AVM)	5q13	*RASA1*	RasGTPase
Venous malformation (VM)	—	—	—
Mucocutaneous venous malformation (VMCM)	9p22	*TIE2*	Tyrosine kinase receptor in vascular endothelial cells (angiopoietin receptor)
Glomuvenous malformation (GVM)	1p22	Glomulin (*GLMN*)	Unknown function: HGF/TGF-β pathways?
Lymphatic malformation (LM)	—	—	—
Primary congenital lymphedema (PCL)	5q35	*VEGFR3*	Endothelial cell tyrosine kinase receptor, important for lymphangiogenesis
Lymphedema-distichiasis (LD)	16q24	*FOXC2*	Transcription factor, regulates PDGFB
Hypotrichosis-lymphedema-telangiectasia (HLT)	20q13	*SOX18*	Transcription factor
Hennekam syndrome	—	—	—
Aagenaes syndrome	15q	—	—
OLEDAID	Xq28	*NEMO*	NF-κB modulator
Capillary malformation (CM)	—	—	—
Cerebral cavernous malformation (CCM1)	7q11	*KRIT1*	Intracellular signaling?
Cerebral cavernous malformation (CCM2)	7p15	*CCM2*	Intracellular signaling?
Cerebral cavernous malformation (CCM3)	3q25	*PDCD10*	Intracellular signaling?
HCCVM	7q11	*KRIT1*	Intracellular signaling?
Hereditary hemorrhagic telangiectasia (HHT1)	9q34.1	*ENG*	TGF-β coreceptor
Hereditary hemorrhagic telangiectasia (HHT2)	12p11	*ACVRL1*	TGF-β coreceptor
Ataxia-telangiectasia (AT)	11q22	*ATM*	DNA repair and/or cell cycle control
Hemangioma of infancy	—	—	—

9p21,[13] with further revelation of a mutation in the angiopoietin receptor, TIE2/TEK[12] (Table 1). This mutation, an arginine849-to-tryptophan substitution (R849W), leads to altered intracellular signaling[17] and altered smooth muscle cell recruitment.[18] In addition, TIE2/TEK has been shown to be a chemotactic factor for vascular endothelial cells[19,20] and plays a role in cell adhesion.[21] In murine models, a lack of TIE2/TEK leads to altered primary capillary plexus maturation and compromised mural cell composition.[22]

GLOMUVENOUS MALFORMATION

Glomuvenous malformations (GVMs) are clinically similar to VMs but are harder and painful to palpation; have a nodular, cobblestone appearance; and do not empty by compression[23] (Fig. 1). The plaque-like variant of the young may easily be misdiagnosed.[24] Histologically, these lesions differ from VMs in that VMs have low numbers of normal vSMCs, whereas GVMs have variable numbers of pathognomic "glomus cells," which have a round to polygonal shape and centrally located nucleus.[13,25] Their cell structure and stains for markers of SMC lineage lead one to believe that these abnormal mural cells in GVMs are poorly differentiated vSMCs.[26,27]

These lesions are clearly, perhaps even always, hereditary,[28,29] with an autosomal dominant manner.[30–32] A locus, VMGLOM, on chromosome 1p21-p22 was found by molecular genetic linkage analysis.[29] The identification of the mutated gene, glomulin, was revealed by positional cloning,[33] yet the precise function of glomulin remains elusive (Table 1). The mutations cause premature termination codons in the glomulin gene, and in one lesion a second hit was observed on the allele without the inherited mutation. Thus, GVMs may follow a paradominant mode of inheritance. A dysregulation of transforming growth factor β (TGF-β) signaling[34] may be involved, as TGF-β is vital for vSMC differentiation,[35] glomulin may bind to TGF-β receptors, and abnormal signaling could explain the occurrence of glomus cells in GVM.

LYMPHATIC MALFORMATION

Lymphatic malformation (LM) consists of vesicles containing lymphatic fluid, affecting not only the soft tissues but also underlying bone (Fig. 1). Characteristic translucent bumps may be noted under normal-appearing skin, and these lesions may expand in the presence of inflammation or intralesional bleeding. LM usually involves a large area of skin, underlying soft tissue, and bone, with resultant difficulty in effective treatment. Infection may require the use of intravenous antibiotics, and, as with VM, sclerotherapy and surgery are the mainstays of definitive therapy. The etiology is unknown (Table 1).

LYMPHEDEMA

Primary lymphedema, which usually affects the lower extremities, can be uni- or bilateral and is often part of a syndrome, such as Turner or Noonan syndrome (Fig. 1). The nonsyndromic types are divided into congenital (Milroy lymphedema) and late-onset (Meige lymphedema) categories. Although congenital lymphedema is evident at birth and Meige lymphedema occurs around puberty, in both cases a causative gene has been identified.

Congenital Lymphedema or Milroy Disease A genetic cause for this disease began with the observation of this condition inherited in an autosomal dominant manner in several families.[36–41] Linkage analysis led to the identification of a chromosomal locus, 5q34–35, linked to the phenotype.[37] Further analysis showed that mutations in receptor 3 for vascular endothelial growth factor (VEGFR3) are responsible for the malfunctioning lymphatic system[38,41] (Table 1). Additional work with expression analyses and knockout murine models has shown that VEGFR3 expression is confined to the lymphatic endothelium of adults and that a lack of VEGFR3 leads to defective embryonic vascular development.[42,43]

Late-Onset Lymphedema or Meige's Lymphedema The late-onset variant represents 80% of patients with lymphedema, of whom 35% are estimated to have a familial predisposition.[44,45] Of particular interest in this variant of lymphedema are the families with distichiasis, abnormal growth of eyelashes, seen to be inherited in an autosomal dominant manner. Genetic analysis revealed a chromosomal locus, 16q24.3, linked to this disorder,[40] and further work revealed a mutation in the *FOXC2* gene, which encodes a forkhead family transcription factor.[46] This mutation is also responsible for creating a premature termination codon and therefore loss of function.[46–48] Further work with regard to the variation in expressivity of the *FOXC2* gene mutations may explain why lymphedema is seen to be associated with conditions such as distichiasis, cleft palate, and cardiac septal defects (Table 1).

Hypotrichosis-Lymphedema-Telangiectasia Three families with severe hypotrichosis associated with variable-onset lymphedema and limited cutaneous telangiectasias have been reported. Using the ragged mouse caused by mutations in the murine Sox18 transcription factor as a model, the *SOX18* gene was found to carry either recessive or dominant mutations in these three families[49] (Table 1).

Hennekam Syndrome Unlike the majority of lesions with an autosomal dominant inheritance pattern, this autosomal recessive syndrome is manifested by intestinal LMs with a resultant protein-losing enteropathy and hypoalbuminemia as well as lymphedema of the lower and upper extremities, mental retardation, and dysmorphic facial features. Craniosynostosis; vesicourethral, rectal, and renal abnormalities; and cardiac defects have also been seen in this condition.

Aagenaes Syndrome or Hereditary Cholestasis with Lymphedema This syndrome, which was first seen in southwest Norway and occurs in families with consanguinity, has an autosomal recessive inheritance pattern. In addition to malabsorption, growth retardation, rickets, cholestatic jaundice, and hepatomegaly, severe lymphedema of the lower extremities is observed. Extending throughout the entire lower extremity and developing in childhood, the lymphedema is complicated by multiple infections, and little is of benefit apart from elastic bandaging.

Osteoporosis, Lymphedema, Anhidrotic Ectodermal Dysplasia, and Immunodeficiency Syndrome This rare condition, described in two case reports, is the result of a premature termination codon mutation of nuclear factor κB essential modulator (NEMO). In this condition, lymphedema develops early in childhood.

CAPILLARY MALFORMATION

Capillary malformation (CM) or port-wine stain is a malformation consisting of thin-walled capillary channels, present at birth and persisting throughout life. Above all a cosmetic problem, these lesions may be an indicator of a more complex vascular disorder. CMs are the most common vascular malformation, occurring in up to 0.3% of neonates.[50] Given their high incidence, these lesions have been thought to be nonhereditary, but recent familial patterns have shown some CMs to occur in an autosomal dominant manner.[51,52] It may be that the familial variant is a specific subtype of common CM.

CMs may occur on the face, trunk, or extremities and tend to grow in proportion to the rest of the body. Over time, these lesions tend to show hyperplastic skin changes, which clinically may mimic AVMs. The red staining of CMs may be treated with a pulsed dye laser, although the eventual skin thickening may require surgical correction.[53–58]

Histological analysis shows that there are both an increased number of small vessels in the immediate subepidermal area and an increased size of vessels in the deeper subepidermal layer.[59,60] Nonetheless, vascular walls have a normal composition as they stain for both type IV collagen and fibronectin.

CEREBRAL CAVERNOUS MALFORMATION

Cerebral cavernous malformation or cerebral capillary malformation (CCM) is a vascular malformation particularly common to the brain, although the eye may also be

affected, with headaches, epilepsy, and bleeding possible.[61] These lesions are composed of thin-walled capillary-like vessels that may show thickened fibrotic walls, hence the capillary and cavernous descriptions.[62]

CCM has also been seen to be inherited in an autosomal dominant manner, with linkage analysis identifying three chromosomal loci: 7q21-q22, 7p15-p13, and 3q25.2–27[63,64] (Table 1). Further work has identified the mutated gene related to 7q21-q22 as *KRIT1* (Krev1 Interaction Trapped 1),[65] the 7p15-p13 as malcavernin, and 3q25.2–27 as *PDCD10*. The exact function of these genes is unclear, although a loss of function in each is likely; however, a defect in cell adhesion, apoptosis, and endothelial cell proliferation has been suggested.[66]

Hyperkeratotic Cutaneous Capillary Venous Malformation Hyperkeratotic cutaneous capillary venous malformation (HCCVM) is a rare vascular malformation sometimes seen in association with CCM.[67,68] In one such family, a mutation similar to the ones seen in families with only CCM was seen in the *KRIT1* gene[67] (Table 1). HCCVM involves malformations of both capillaries and venules with a characteristic dark red coloration and hyperkeratosis. It is unclear whether HCCVM may occur in association with CCM caused by any of the three known genes.

TELANGIECTASIAS

Hereditary Hemorrhagic Telangiectasia or Rendu-Osler-Weber Syndrome Hereditary hemorrhagic telangiectasia (HHT) was the first vascular malformation for which genetic investigation was able to reveal the underlying cause of the disease. HHT, which is inherited as an autosomal dominant disorder, was shown using linkage analysis to be related to two chromosomal loci, one in 9q34.1 and the other in 12p11–14[69–72] (Table 1). Positional candidate gene screening revealed mutations in two genes coding for TGF-β receptors: endoglin (ENG) and activin receptor–like tyrosine kinase 1 (ACVRL1).[73,74] These mutations have been shown to create premature termination codons that induce loss of function.[75] The loss of function in endoglin (chromosome 9q34.1) has been classified as HHT-1, whereas loss of function with regard to chromosome 12q11–14 has been named HHT-2. The histological defect characteristic of HHT is a localized disappearance of peripheral capillaries that results in direct anastomoses between arterioles and venules, especially evident in the skin.

Ataxia-Telangiectasia (Louis-Bar Syndrome) Ataxia-telangiectasia is a disorder inherited in an autosomal recessive manner with complex neurovascular manifestations and multiple telangiectasias. These develop around the age of 3 years and are observed most commonly near the canthus as well as the face, neck, and dorsum of the hand and foot. Cerebellar ataxia begins during the second year of life, manifested by dysarthric speech, choreoathetosis, myoclonic jerks, and impaired intelligence. This syndrome is the result of mutations of the ATM gene, localized to chromosome 11q22–23[76] (Table 1).

VASCULAR TUMORS

Hemangioma of Infancy

Infantile hemangioma is the most common benign tumor of infancy, affecting about 10% of Caucasian neonates within the first year of life (Fig. 1). These lesions occur more frequently in preterm infants weighing less than 1000 g[77] but less frequently in Japanese (0.8%), African-Americans (1.4%), and Asians (3%).[78,79]

Hemangiomas develop over a life cycle of three phases. The proliferating phase until the age of 6 to 12 months sees a rapid increase in size of the tumor, followed by an involuting phase in which spontaneous regression begins. Finally, by the age of 5 to 7 years, the involuted phase sees disappearance of the hemangioma.

Most hemangiomas involute without complications and do not require treatment. Some lesions with ulceration may respond to simple wound dressings, corticosteroid therapy, pulsed dye laser, or surgery. Tissue-endangering lesions, such as the nasal tip hemangioma, or life-threatening lesions, such as those with cardiac complications, may respond to corticosteroids, interferon, vincristine, or surgery. Some pedunculated lesions, where a fibrofatty residuum will eventually occur, may respond well to early surgical resection.

In contrast to findings for certain vascular malformations, there is no clear evidence that there may be an inheritance pattern for hemangiomas. Various theories of their cause have been proposed, such as viral (e.g., papillomavirus or human herpes virus 8),[80,81] abnormal hormonal influence,[82] or embryonic sequestration of unipotent angioblastic cells,[83] but none adequately explains the pathogenesis or the spontaneous regression.

More recent work demonstrated that hemangioma endothelial cells are clonal, unlike surrounding fibroblast cells, and that these endothelial cells migrate and proliferate three times more than normal cells and are not inhibited by endostatin, an otherwise powerful antiangiogenic factor.[84] It is thought that a somatic mutation in a gene related to angiogenesis may have occurred in these endothelial cells, leading to clonal transformation.[85]

Another interesting theory of the origin of hemangiomas is that there is immunophenotypic similarity with placental tissue, the glucose transporter protein

GLUT-1 antigen[86,87] suggesting the possibility that hemangiomas may be caused by the embolization of placental cells. A final theory relies on the observation that many angiogenic factors are overexpressed in proliferating hemangiomas, suggesting that local hypoxia as an initiating factor may be involved.[88–90]

CONCLUSION

Having been able to observe inheritance patterns in families with several different types of vascular anomalies has allowed molecular techniques to be used to identify the underlying genetic mutations. Many of these mutant molecules are not surprisingly involved in vasculogenesis and angiogenesis and relate to a loss of function. This work may as yet be in its infancy but will be vital not only to help understand the biology of these lesions but also to direct therapy for their treatment.

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