Vascular Anomalies: From a Clinicohistologic to a Genetic Framework

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Background: Vascular anomalies currently are classified according to their clinical and histological characteristics. Recent advances in molecular genetics have enabled the identification of somatic mutations in most types of vascular anomalies. The purpose of this study was to collate information regarding the genetic basis of vascular anomalies.
Methods: The PubMed literature was reviewed for all citations that identified a mutation in a vascular anomaly between 1994 and 2017. Search terms included "vascular anomaly," "mutation," "gene," "hemangioma," "pyogenic granuloma," "kaposiform hemangioendothelioma," "capillary malformation," "venous malformation," lymphatic malformation," "arteriovenous malformation," and "syndrome." Articles that identified both germline and somatic mutations in vascular anomalies were analyzed. Mutations were categorized by type (germline or somatic), gene, signaling pathway, and cell(s) enriched for the mutation.
Results: The majority of vascular anomalies had associated mutations that commonly affected tyrosine kinase receptor signaling through the RAS or PIK3CA pathways. Mutations in *PIK3CA* and G-protein–coupled receptors were most frequently identified. Specific types of vascular anomalies usually were associated with a single gene. However, mutations in the same gene occasionally were found in different vascular lesions, and some anomalies had a mutation in more than one gene. Mutations were most commonly enriched in endothelial cells.
Conclusions: Identification of somatic mutations in vascular anomalies is changing the paradigm by which lesions are diagnosed and understood. Mutations and their pathways are providing potential targets for the development of novel pharmacotherapy. In the future, vascular anomalies will be managed based on clinical characteristics and molecular pathophysiology. (*Plast. Reconstr. Surg.* 141: 709e, 2018.)

Vascular anomalies currently are classified by their clinical and histologic characteristics into two broad categories: tumors or malformations (Table 1).[1] Vascular tumors have proliferating endothelium, whereas vascular malformations are structural anomalies. Since the proposal of the clinicohistologic classification in 1982 by Mulliken and Glowacki (Level of Evidence III),[2] minimal insight into the pathophysiology of vascular anomalies has been achieved and pharmacotherapy for many lesions does not exist. Beginning in 1994, the genetic basis of familial forms of vascular anomalies began to be identified. Because almost all lesions are sporadic, the cause of most vascular anomalies has remained unknown. The development of next-generation sequencing has enabled the identification of low-level somatic mosaic mutations in sporadic lesions; in 2011, this technology was applied to vascular anomalies.[3] As a result, over the past few years, mutations associated with many lesions in the field has expanded significantly. The purpose of this article was to evaluate the field of vascular anomalies based on new information regarding the molecular basis of these lesions.

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Received for publication May 21, 2017; accepted November 3, 2017.
Presented at the 58th Annual Meeting of the New England Society of Plastic & Reconstructive Surgeons, in North Falmouth, Massachusetts, June 2 through 4, 2017.

DOI: 10.1097/PRS.0000000000004294

Disclosures: *The authors have no financial interest to declare in relation to the content of this article.*

Table 1. Current Classification of Vascular Anomalies*

	Malformations			
Tumors	**Simple**	**Combined**	**Major Named Vessels**	**Associated with Other Anomalies**
Benign Infantile hemangioma Congenital hemangioma Pyogenic granuloma Infantile myofibroma Enzinger hemangioma Intermediate Kaposiform hemangioendothelioma Tufted angioma Malignant Angiosarcoma Epithelioid hemangioendothelioma	Capillary Cutis marmorata telangiectatica congenita Fading stain Lymphatic Macrocystic Microcystic Generalized Gorham-Stout Primary lymphedema Venous Cutaneomucosal Glomuvenous Cerebral cavernous Blue rubber bleb nevus Fibro adipose Verrucous Arteriovenous Capillary malformation—arteriovenous malformation Hereditary hemorrhagic telangiectasia	Capillary-venous Capillary-lymphatic Capillary-arteriovenous Lymphatic-venous Capillary-lymphatic-venous Capillary-lymphatic-arteriovenous Capillary-venous-arteriovenous Capillary-lymphatic-venous-arteriovenous	Aneurysm Atresia Ectasia Stenosis	CLOVES Klippel-Trenaunay Megalencephaly-capillary malformation Maffucci Parkes Weber Proteus *PTEN* hamartoma Sturge-Weber

CLOVES, congenital, lipomatous overgrowth, vascular malformations, epidermal nevi, and scoliosis/skeletal/spinal anomalies; *PTEN*, phosphatase and tensin homolog.
*Adapted from Wassef M, Blei F, Adams D, et al.; ISSVA Board and Scientific Committee. Vascular anomalies classification: Recommendations from the International Society for the Study of Vascular Anomalies. *Pediatrics* 2015;136:e203–e214.

METHODS

The PubMed literature was reviewed for all citations that identified a mutation in a vascular anomaly between 1994 and 2017. Search terms included "vascular anomaly," "mutation," "gene," "hemangioma," "pyogenic granuloma," "kaposiform hemangioendothelioma," "capillary malformation," "venous malformation," lymphatic malformation," "arteriovenous malformation," and "syndrome." Articles were analyzed that identified either somatic or germline mutations. A somatic mutation is a randomly acquired alteration to the genetic sequence of a cell any time after fertilization that can be passed to the affected cell's progeny but not to the organism's offspring. A germline mutation is an alteration to the genetic sequence that occurs in gametes and can be inherited by the patient's children.

Mutations were categorized by type (germline or somatic), gene, signaling pathway, and cell(s) enriched for the mutation. Mutant allele frequency associated with somatic mutations were recorded when possible. An allele is one of two alternatives of a gene found at the same location along a chromosome that codes for a specific protein. Because each cell has two alleles (one inherited from each parent), it is generally assumed that a random somatic mutation would affect only one of the alleles. The mutant allele frequency is calculated by measuring the number of mutant alleles and dividing them by the total number of alleles present (mutant alleles/mutant alleles plus wild-type alleles). The higher the mutant allele frequency, the greater the number of affected cells in the lesion.

RESULTS

Forty-three publications identifying mutations in vascular anomalies were identified. Mutations most commonly involved tyrosine kinase receptor signaling through the RAS or PIK3CA pathway (Fig. 1). Mutations in the tyrosine kinase receptors *TIE2*, *PDGF*, and *ENG* were associated with venous malformation, infantile myofibroma, and hereditary hemorrhagic telangiectasia, respectively. Mutations affecting *RAS* were found in pyogenic granuloma and capillary malformation–arteriovenous malformation. Pyogenic granuloma and venous malformation

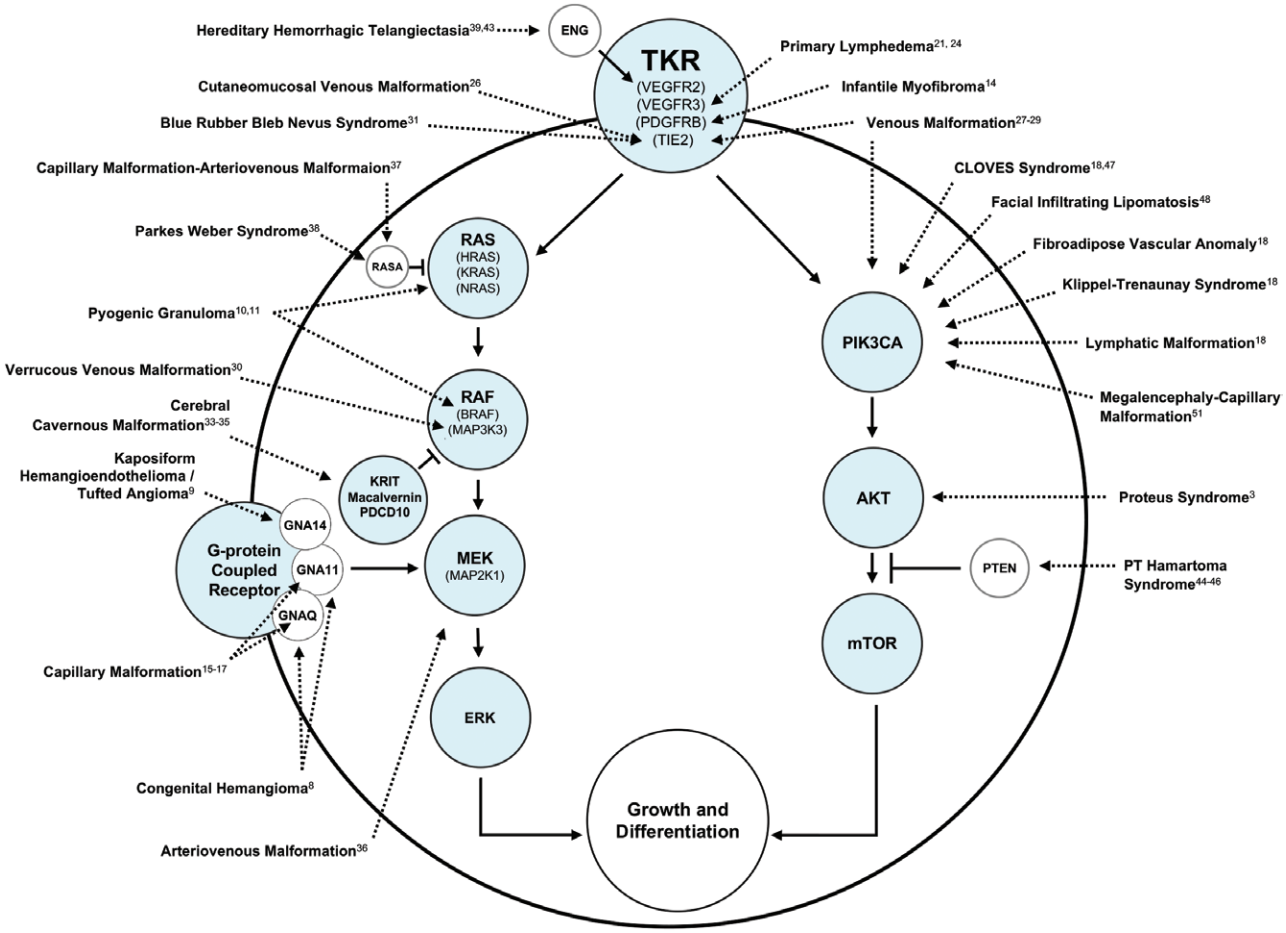

Fig. 1. Mutations in vascular anomalies most commonly affect genes associated with tyrosine kinase signaling through the RAS or PIK3CA pathway.

were associated with mutations affecting *RAF*. Alterations affecting *MEK*, including G-protein–coupled receptors, were noted in arteriovenous malformation, capillary malformation, congenital hemangioma, and kaposiform hemangioendothelioma. *PIK3CA* mutations were identified in lymphatic malformation, venous malformation, fibroadipose vascular anomaly, and overgrowth syndromes [congenital lipomatosis, overgrowth, vascular malformations, epidermal nevi, and skeletal anomalies (CLOVES), Klippel-Trenaunay, megalencephaly-capillary malformation]. Mutations affecting *AKT* were found in Proteus syndrome and *PTEN* hamartoma-tumor syndrome. Most vascular anomalies were associated with a single gene. However, mutations in the same gene occasionally were noted in different types of vascular lesions, and some anomalies had a mutation in more than one gene. Mutations were most commonly enriched in endothelial cells.

DISCUSSION

Current Clinicohistologic Framework

Most vascular anomalies are identified by history and physical examination. Imaging can aid the diagnosis and histopathology rarely is necessary. Although lesions usually can be identified clinically, significant heterogeneity and response to treatment exist. Successful management of a vascular anomaly is based on an accurate diagnosis. Tumors have dividing endothelium and thus may be treated with pharmacotherapy. In contrast, vascular malformations have minimal cellular turnover and have not shown efficacy to drugs (an exception is lymphatic malformation, which can respond to oral sirolimus).[4] Management of vascular malformations includes resection, laser, sclerotherapy, and/or embolization. Many vascular malformations are unable to be cured, and the goal of treatment is to control the lesion. Progression and recurrence after intervention are common. Consequently, drug development

is important to potentially cause the anomaly to regress and/or to prevent its growth and recurrence after treatment. A limitation of the clinicohistologic classification is that it does not incorporate the molecular basis of vascular anomalies. For example, venous malformations can be caused by mutations in eight different genes.

Genetic Basis of Vascular Anomalies

Infantile Hemangioma

Infantile hemangioma is the most common tumor of infancy and has a unique growth cycle: it enlarges rapidly during the first few months of life and then regresses in early childhood. A mutation has yet to be identified. Explanations that have been proposed for its etiopathology include placental, stem cell, and follicle-stimulating hormone hypotheses.[5–7]

Congenital Hemangioma

Two types of congenital hemangiomas exist: rapidly involuting congenital hemangioma and noninvoluting congenital hemangioma. Unlike infantile hemangioma, these lesions are fully formed at birth. Mutations in *GNAQ* or *GNA11* have been found in 12 of 16 specimens; the mutant allele frequency is 1 to 11 percent.[8] *GNAQ* and *GNA11* mutations are present in both rapidly involuting congenital hemangioma and noninvoluting congenital hemangioma specimens. Partially involuting congenital hemangioma is a rare subtype of rapidly involuting congenital hemangioma that does not involute fully and persists as a noninvoluting congenital hemangioma–type lesion; its somatic mutation has not been identified.[9]

Kaposiform Hemangioendothelioma

Kaposiform hemangioendothelioma is a vascular neoplasm that usually is present at birth, enlarges during infancy, and then partially involutes. Most patients are treated with systemic pharmacotherapy for associated Kasabach-Merritt phenomenon (i.e., thrombocytopenia, petechiae, and bleeding) or to minimize fibrosis. A mutation in *GNA14* was found in one of three kaposiform hemangioendothelioma specimens (mutant allele frequency, 10 percent) and in one of four closely related tufted angiomas.[10]

Pyogenic Granuloma

Pyogenic granuloma is a postnatal lesion with a mean age of onset of 6 years. It averages 6 mm in diameter and often bleeds. Mutations in *KRAS*, *NRAS*, or *HRAS* have been identified in six of 42 specimens in one study (mutant allele frequency, 16 to 40 percent).[11] Another investigation of 25 pyogenic granulomas found mutations in *BRAF* in four specimens and *KRAS* in one specimen.[12] Pyogenic granulomas that arise from capillary malformations show mutations in both *GNAQ* and *BRAF* in seven of 10 specimens.[12]

Rare Vascular Tumors

Angiosarcoma is a malignant lesion that rarely affects children; mutations in *PTPRB* and *PLCG* have been found in 10 of 39 and three of 34 specimens, respectively.[13] Epithelioid hemangioendothelioma is a malignant endothelial tumor with variable clinical behavior. Less than 10 percent involve the pediatric population. Lesions usually are multifocal and may be stable, slowly enlarge, or rapidly progress and metastasize. A *WWTR1-CAMTA1* gene fusion has been identified in 17 of 17 samples.[14] Infantile myofibroma is a fibrous tumor of early childhood that may be solitary, multifocal, or generalized. Solitary lesions can regress, whereas multifocal or generalized disease affecting the viscera can be life-threatening. The lesion is associated with mutations in *PDGFRB* in seven of 16 specimens.[15]

Capillary Malformation

Capillary malformation is the most common type of vascular malformation. Lesions are present at birth and affect the skin. The pink stain darkens over time and patients can develop soft-tissue and bony hypertrophy underneath the integument. Mutations in *GNAQ* have been found in 45 of 52 patients with sporadic or syndromic (Sturge-Weber syndrome) capillary malformation (mutant allele frequency, 1 to 22 percent).[16,17] A mutation in *GNA11* has been identified in the extremities of three of eight specimens with a diffuse capillary malformation including overgrowth (mutant allele frequency, 0.3 to 5.0 percent).[18] *GNAQ* mutations in capillary malformations are enriched in endothelial cells (3 to 43 percent), compared with pericytes (0 percent), stromal cells (0.4 to 11 percent), or hematopoietic cells (0.3 percent).[17]

Lymphatic Malformation

Lymphatic malformations are cystic structures that can be macrocystic, microcystic, or combined. Mutations in *PIK3CA* have been found in 16 of 17 specimens (mutant allele frequency, 0.8 to 10 percent).[19] Primary lymphedema also is a type of vascular malformation that can be hereditary. Only 8 percent of sporadic and 36 percent of familial cases have an identifiable mutation (e.g., *VEGFR3/FLT-4*, *FOXC2*, *SOX18*, *CCBE1*).[20–26]

Venous Malformation

Venous malformations usually are sporadic, but can be familial. Cutaneomucosal autosomal dominant venous malformations were found to be caused by *TIE2* mutations (61 of 61 samples).[27] Mutations in *TIE2* then were identified in sporadic venous malformations (80 of 130 specimens; mutant allele frequency, 4 to 48 percent).[28,29] *PIK3CA* mutations also are found in sporadic venous malformations in 27 of 130 specimens (mutant allele frequency, 1 to 18 percent); lesions usually involve the subcutis.[30] Verrucous venous malformations are hyperkeratotic anomalies typically affecting the skin of an extremity; mutations in *MAP3K3* have been identified in six of 10 specimens (mutant allele frequency, 6 to 19 percent).[31] Blue rubber bleb nevus syndrome is a nonhereditary condition with multifocal venous malformations in the skin, soft tissue, and gastrointestinal tract; a *TIE2* mutation has been found in 32 of 35 samples.[32] Fibroadipose vascular anomaly is a lesion that previously was thought to be an intramuscular venous malformation but exhibits more fibroadipose tissue and smaller, nonspongiform vessels. Mutations in *PIK3CA* were identified in four of eight samples (mutant allele frequency, 5 to 20 percent).[19] Glomuvenous malformation is an autosomal dominant condition that usually has multiple small lesions caused by mutations in *Glomulin* in six of seven families.[33] Cerebral cavernous malformation can be sporadic or autosomal dominant with anomalies usually in the brain; approximately 10 percent of patients have hyperkeratotic skin lesions. Cerebral cavernous malformation results from mutations in *CCM1/KRIT1* (12 of 20 samples),[34] *CCM2/malcavernin* (29 of 35 samples),[35] or *CCM3/PDCD10* (eight of 20 samples).[36]

Arteriovenous Malformation

Arteriovenous malformations are abnormal connections between arteries and veins without a normal capillary bed through either a nidus or a fistula. Arteriovenous malformations are associated with mutations in the *MAP2K1* gene in 16 of 25 specimens (mutant allele frequency, 1 to 13 percent).[37] *MAP2K1* mutations in arteriovenous malformations are exclusive to endothelial cells (mutant allele frequency, 31 to 53 percent).[37] Capillary malformation–arteriovenous malformation is an autosomal dominant disease characterized by multifocal cutaneous lesions and a one-third risk of having an arteriovenous malformation, including Parkes Weber syndrome (an overgrown extremity with a diffuse arteriovenous malformation). Capillary malformation–arteriovenous malformation is caused by a mutation in *RASA1* (six of 17 families)[38]; 13 of 16 patients with Parkes Weber syndrome exhibited an *RASA1* mutation.[39] Hereditary hemorrhagic telangiectasia is an autosomal dominant disease that exhibits epistaxis, mucocutaneous telangiectasias, and/or visceral arteriovenous malformations (i.e., pulmonary, cerebral, hepatic, gastrointestinal). Mutations in endoglin (*ENG*) or activin receptor–like kinase-1 gene (*ACVRL1/ALK1*) are responsible for almost all cases.[40,41] *SMAD4* (associated with juvenile polyposis) or *GDF2* mutations affect 2 percent of patients.[42,43] Recently, endoglin has been shown to affect VEGFR2 signaling.[44] *PTEN*-associated vascular anomaly is present in approximately 50 percent of patients with the autosomal dominant *PTEN* hamartoma-tumor syndrome.[45–47] Lesions can be multiple, are often intramuscular, and have excess adipose tissue and disproportionate dilatation of draining veins.

Overgrowth Syndromes Associated with Vascular Anomalies

Vascular anomalies are major components of several types of syndromes that cause enlargement of soft tissue and/or bone. Proteus syndrome (i.e., progressive, asymmetrical overgrowth of the skeleton, cerebriform nevi of the hands or feet, epidermal nevi, vascular malformations, cerebral anomalies, skull hyperostosis, and megaspondylodysplasia) is caused by a mutation in *AKT1* (26 of 29 patients; mutant allele frequency, 2 to 39 percent).[3] CLOVES syndrome was found to have a *PIK3CA* mutation in 36 of 38 patients (mutant allele frequency, 1 to 32 percent).[19,48] Individuals with isolated facial infiltrating lipomatosis also exhibit *PIK3CA* mutations in the subcutaneous adipose (six of six samples; mutant allele frequency, 9 to 31 percent).[49] The mutation is present throughout all structures of the face and is enriched in nonendothelial cells (mutant allele frequency, 28 to 49 percent) compared with endothelial cells (mutant allele frequency, 1 to 5 percent).[50] Klippel-Trenaunay syndrome is a capillary-lymphatic-venous malformation of an extremity causing overgrowth; *PIK3CA* mutations have been found in 19 of 21 patients (mutant allele frequency, 3 to 12 percent).[19] Maffucci syndrome is characterized by multiple enchondromas and soft-tissue venous malformations. The condition is caused by a somatic mutation in isocitrate dehydrogenase in 37 of 40 individuals; 98 percent have an isocitrate dehydrogenase 1 mutation and 2 percent exhibit an isocitrate dehydrogenase 2 error.[51] Megalencephaly-capillary malformation

commonly causes neurologic abnormalities, and patients typically have a capillary malformation involving the upper lip, trunk, and/or extremities; patients have mutations in *PIK3CA*.[52]

Evolving Genetic Framework

Most mutations found in vascular anomalies involve tyrosine kinase receptor signaling through the RAS or PIK3CA pathway; the function of some mutations remains unknown. Mutations in the same gene can be associated with different lesions. Although *PIK3CA* mutations result in relatively similar types of vascular malformations, *GNAQ/GNA11/GNA14* mutations are found in both tumors and malformations. It is unclear how mutations in the same gene can lead to different phenotypes, but it may be attributable to the location of the mutation in the gene, cell type(s) affected, and/or stage of development when the error occurred. RAS and PIK3CA signaling has broad effects on cell proliferation, differentiation, and survival.[53,54] Consequently, mutations can result in both tumors and malformations. For example, germline mutations in *MAP2K1* cause cardiofaciocutaneous syndrome, which includes malformations,[55] whereas different somatic *MAP2K1* mutations result in arteriovenous malformation[37] or cancers (e.g., melanoma, lung, hematopoietic).[56–58]

Occasionally, lesions reviewed in our vascular anomalies center have an unclear diagnosis. We now have the capability of testing specimens with droplet digital polymerase chain reaction against known vascular anomaly mutations. Mutation discovery in vascular anomalies also has allowed the formulation of genotype-phenotype relationships. For example, venous malformations have been classified as one entity, but it is now recognized that different mutations are associated with unique phenotypes: cutaneous venous malformation (*TIE2*), subcutaneous venous malformation (*PIK3CA*), hyperkeratotic venous malformation (*MAP3K3*), and painful venous malformation (*glomulin*). Similarly, arteriovenous malformations can be caused by mutations in *MAP2K1* (most common), *PTEN* (more adipose), or *RASA1* (Parkes Weber syndrome). Molecular findings also have confirmed clinical observations that certain lesions were closely related (e.g., verrucous "hemangioma" and venous malformation).[30]

Targeted treatments based on the specific pathway that is affected for the anomaly can be developed, similar to focused therapy of malignant neoplasms based on their mutation.[59] For example, vascular anomalies caused by *PIK3CA* mutations have been grouped under the umbrella term PIK3CA-related overgrowth spectrum and are no longer viewed as independent diseases.[60] Instead, they are considered a spectrum of conditions caused by the same mutation. Similarly, capillary malformation, congenital hemangioma, and kaposiform hemangioendothelioma may be affiliated under the category of G-protein receptor mutations affecting the closely related *GNAQ/GNA11/GNA14* genes (e.g., GNA-vascular anomaly).

The genetic framework of vascular anomalies is still evolving rapidly (Table 2). Somatic mutations are not found in all patient specimens subjected to whole-exome sequencing or droplet digital polymerase chain reaction. Possible explanations include the following: (1) the mutant allele frequency is below the detection limit of the assay; (2) a mutation is present in another part of the gene; (3) the mutation is in a different gene. Other mutations causing vascular anomalies will continue to be discovered, likely affecting components of the same signaling pathway. The cost of mutation identification is inexpensive if the mutation is already known using droplet digital polymerase chain reaction (approximately $4 per sample); the expense of mutation discovery using whole-exome sequencing continues to decrease (approximately $2700 per sample). Further investigation will show the specific cell type(s) harboring the mutation, and how the mutation affects cell biology. Understanding the mutations associated with vascular anomalies is the first step to developing targeted pharmacotherapy for these lesions. In the future, the framework by which vascular anomalies are diagnosed and managed will become more comprehensive by incorporating genetic findings with clinical information.

CONCLUSIONS

Next-generation sequencing has allowed the recent discovery of somatic mosaic mutations in vascular anomalies. Most mutations affect the RAS or PIK3CA tyrosine kinase signaling pathway. Identification of the molecular basis of vascular anomalies has facilitated the identification of lesions with an equivocal clinicohistologic diagnosis, and enabled genotype-phenotype correlations. Further investigation will determine how specific mutations affect the etiopathogenesis of the vascular anomaly. Pharmacotherapy targeting altered signaling pathways may prove efficacious in the future.

Table 2. Molecular Framework of Vascular Anomalies*

TKR		RAS		RAF		MEK		PIK3CA		AKT		Other	
Gene	Anomaly	Gene	Anomaly	Gene	Anomaly	Gene	Anomaly	Gene	Anomaly	Gene	Anomaly	Gene	Anomaly
TIE2	VM CMVM BRBNS	*HRAS*	PG	*BRAF*	PG	*MAP2K1*	AVM	*PIK3CA*	LM CLOVES KTS	*AKT1*	PS	*ACVRL SMAD4* *GDF2 SMAD4*	HHT
PDGF	IM	*KRAS*	PG	*MAP3K3*	VVM	*GNAQ*	CM CH		FIL FAVA	*PTEN*	PTENAVA	*Glomulin* *PTPRB* *PLCG*	GVM AS
ENG	HHT	*NRAS*	PG	*KRIT* *Macabermin*	CCM	*GNA11*	CM CH		MCAP VM			*WWTR1-CAMTA1*	EHE
		RASA	CM-AVM PWS	*PDCD10*		*GNA14*	KHE TA					*IDH1* *IDH2*	MS

VN, venous malformation; CMVM, cutaneomucosal venous malformation; BRBNS, blue rubber bleb nevus syndrome; PG, pyogenic granuloma; AVM, arteriovenous malformation; LM, lymphatic malformation; KTS, Klippel-Trenaunay syndrome; FIL, facial infiltrating lipomatosis; FAVA, fibroadipose vascular anomaly; MCAP, megalencephaly-capillary malformation; PS, proteus syndrome; HHT, hereditary hemorrhagic telangiectasia; GVM, glomuvenous malformation; IM, infantile myofibroma; VVM, verrucous venous malformation; CM, capillary malformation; CH, congenital hemangioma; AS, angiosarcoma; CCM, cerebral cavernous malformation; EHE, epithelioid hemangioendothelioma; CM-AVM, capillary malformation–arteriovenous malformation; PWS, Parkes Weber syndrome; KHE, kaposiform hemangioendothelioma; TA, tufted angioma; MS, Maffucci syndrome.
*The major column headings represent principal signaling pathway components. Mutations listed below are either subtypes of the heading or affect its signaling pathway (see Fig. 1).

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ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Awards NICHD-081004 (to A.K.G.), NICHD-082606 (to A.K.G.), and NHLBI-127030 (to A.K.G.), and the Translational Research Program Boston Children's Hospital (to A.K.G.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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