Published in final edited form as:

Trends Mol Med. 2019 April ; 25(4): 265–286. doi:10.1016/j.molmed.2019.01.009.

EphrinB2-EphB4-RASA1 signaling in human cerebrovascular development and disease

Xue Zeng^{1,3}, Ava Hunt², Sheng Chih Jin³, Daniel Duran², Jonathan Gaillard², and Kristopher T. Kahle^{2,4,5,*}

¹Department of Genetics, Yale School of Medicine, New Haven CT, USA.

²Department of Neurosurgery, Yale School of Medicine, New Haven CT, USA.

³Laboratory of Human Genetics and Genomics, The Rockefeller University, New York, NY, USA.

⁴Department of Pediatrics, Yale School of Medicine, New Haven CT, USA.

⁵Department of Cellular & Molecular Physiology, Yale School of Medicine, New Haven CT, USA.

Abstract

Recent whole exome sequencing studies in humans have provided novel insight into the importance of the ephrinB2-EphB4-RASA1 signaling axis in cerebrovascular development, corroborating and extending previous work in model systems. Here, we aim to review the human cerebrovascular phenotypes associated with ephrinB2-EphB4-RASA1 mutations, including those recently discovered in Vein of Galen malformation – the most common and severe brain arteriovenous malformation in neonates. We will also discuss emerging paradigms of the molecular and cellular pathophysiology of disease-causing ephrinB2-EphB4-RASA1 mutations, including the potential role of somatic mosaicism. These observations have potential diagnostic and therapeutic implications for patients with rare congenital cerebrovascular diseases and their families.

Keywords

Ephrin signaling; cerebrovascular disorders; vasculogenesis; arterio-venous malformation; Vein of Galen malformation; neurosurgery

*Correspondence:.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DECLARATION OF INTERESTS

None.

An Emerging Role of EphrinB2-EphB4-RASA1 Signaling Axis in Human Congenital Cerebrovascular Disorders

Normal vascular development includes *de novo* blood vessel formation from endothelial cells (ECs) (vasculogenesis) [1, 2], expansion of the network through vessel sprouting, splitting, and remodeling (angiogenesis) [3], and differentiation of these vessels into arteries, veins, and intervening capillaries (arterio-venous (A-V) specification) [4]. Studies in animal models have elucidated that these separate but overlapping mechanisms of vascular development are driven by a highly-regulated genetic program that involves the expression and function of multiple key signaling pathways (e.g., ephrin-Eph, Hedgehog, VEGF, TGF- β , Wnt, and Notch) and transcription factors (e.g., HEY and HES, SOX, and Forkhead factors) [4]. Among these genetic factors, animal models have demonstrated a critical role of ephrin-Eph signaling [2, 5-8].

Eph receptor tyrosine kinases (RTK) (EphA1 - EphA8, EphA10, EphB1 - EphB4, and EphB6) and their membrane tethered ligands, the ephrins (ephrinA1 - ephrinA5 and ephrinB1 - ephrinB3), play an important role in many developmental processes during embryogenesis, including axon guidance, tissue boundary formation, bone development, and vascular and lymphatic development [9, 10]. Ephrin-Eph signaling has a dizzying array of signaling modes and regulatory mechanisms (reviewed in [10, 11]), including **forward, reverse, parallel, and anti-parallel signaling** (see **Glossary**). Additionally, the strength of ephrin-Eph signaling through any one particular mode can be tuned by the abundance of the specific ephrin ligand cluster involved [12, 13], allowing for the dynamic regulation of multiple distinct molecular and cellular processes with a high precision.

Despite the high complexity of ephrin-Eph signaling, the development of the vasculature appears to be highly dependent on a specific ephrin ligand-receptor pair: ephrinB2 and EphB4 [10]. While mouse and zebrafish models have provided invaluable insights into the role of these ephrin-Eph signaling molecules, recent work in human patients employing whole exome sequencing (WES) techniques and unbiased genomic analytical strategies has corroborated and extended these findings, providing novel insights. Collectively, these efforts have identified mutations in *EFNB2*, *EPHB4*, and *RASA1* in both sporadic and Mendelian congenital cerebrovascular disorders (Box 1), including capillary malformation-arteriovenous malformation (CM-AVM) and Vein of Galen malformation (VOGM), indicating a conserved role of the signaling axis through vertebrates. Importantly, CM-AVM and VOGM are traditionally thought to be two distinct diseases with overlapped phenotypes, while genetic findings in human patients suggest a shared genetic mechanism underlying these two disorders, even though more comprehensive phenotyping is required to fully answer this question. Findings in human genetics have also provided candidates for genetic testing, potential targetable nodes for therapeutic intervention, and insights into the molecular mechanisms of other more common forms of cerebrovascular disorders (see Clinician's Corner). In this article, we will review the role of ephrinB2-EphB4-RASA1 signaling in vascular development (Figure 1, **Key Figure**), using data gleaned from animal models (Table 1), and more recently, WES studies in human patients with congenital cerebrovascular disease (Table 2).

EphrinB2

Human Diseases

Human ephrinB2 is a transmembrane protein encoded by the *EFNB2* gene. EphrinB2 has one transmembrane domain, a receptor-binding domain (RBD) on the extracellular side, and a PDZ domain on the intracellular side.

A microarray study of 116 **conotruncal heart defects** patients focusing on large (> 500kb), rare (< 1% in platform and ethnicity matched control groups) copy number variations identified 1 copy number gain at 13q33.3-33.3 harboring the entire *EFNB2* gene in 1 patient [14]. A 610-kb deletion encompassing *EFNB2* transmitted from asymptomatic mother was reported in 2 siblings with congenital heart disease (CHD) and mild developmental delay. The same group also reported a *de novo* missense mutation (c.239T>G, p.Met80Arg) in *EFNB2* in a patient with anal stenosis, hypoplastic left ventricle, and mild developmental delay [15]. Additionally, in a WES study of 55 unrelated VOGM probands, 1 deleterious missense mutation (c.830G>A, p.Arg277His) was reported in 1 proband. Unaffected parents and 2 healthy siblings of the proband were also genotyped for the c.830G>A *EFNB2* mutation. Interestingly, the mother, who is the only carrier of the risk allele among all unaffected family members, is also the only family member exhibiting cutaneous vascular abnormalities, suggesting incomplete penetrance and variable expressivity of the mutation [16]. Overall, despite several *EFNB2* mutations occasionally reported in various disorders discussed above, no clear association has been established statistically between *EFNB2* and human diseases.

Animal Studies

Studies in animal and cellular models have provided extensive evidence of a role for *EFNB2* in congenital cerebrovascular disorders. *Efnb2* knockout mouse model (ephrinB2^{tlacZ}) has normally occurred vasculogenesis, but angiogenesis was disrupted in the yolk sac, the internal carotid arterial branches were absent, angiogenesis of venous capillaries was defective in the head, and capillary ingrowth into the neural tube failed to occur [2]. In an *in vitro* sprouting assay, ephrinB2 ligand showed similar efficiency as angiopoietin-1 (Ang1) and VEGF when inducing capillary sprouting, indicating its stimulatory role in remodeling the developing vascular system [5]. Homozygous ephrinB2 knockin mice (ephrinB2^{C/C}) with its carboxyl terminal (C-terminal), which encodes the PDZ domain needed for its function as a receptor in ephrin-Eph reverse signaling, replaced by a hemagglutinin (HA) epitope tag showed defects in vasculogenesis and angiogenesis as well as mid-gestation lethality very similar to what has been observed in ephrinB2 null animals [17]. However, surprisingly, the ephrinB2 knockin mice (ephrinB2^{C-βgal/C-βgal}) with its C-terminal replaced by beta-galactosidase (βgal) instead of an HA tag were normal throughout gestation and exhibited none of the early vascular defects observed in ephrinB2^{C/C} animals [18]. Further investigation comparing the gross phenotype and cell surface expression of ephrinB2 in these two ephrinB2 knockin mouse models with a third C-terminal truncating ephrinB2 mouse model (ephrinB2^{T/T} [18]) generated by deleting the same C-terminal amino acid residues as ephrinB2^{C-βgal/C-βgal} but not conjugating with any other proteins revealed that improper localization of ephrinB2 rather than the deletion of

its C-terminal likely caused the vascular phenotype, indicating that it is likely not reverse signaling but rather ephrinB2 mediated forward signaling with EphB4 that is necessary for angiogenic remodeling. Human and mouse ephrinB2 proteins are overall highly homologous [19]. Understanding the function of each protein domain of ephrinB2 and the direction of the signaling they are mediating has provided invaluable guidance for accessing the pathogenicity of human mutations, it helps to (i) put identified mutations in context of the disease phenotype to evaluate whether they are disease-causing or benign and (ii) elucidate different pathways downstream of ephrinB2 reverse and forward signaling, respectively, underlying different human diseases.

In the vascular system, ephrinB2 is primarily expressed in (but not restricted to) arterial ECs. Conditional mouse knockout of ephrinB2 specifically in the endothelium and endocardium of the developing vasculature and heart (ephrinB2^{lacZ/loxP}; Tie2-Cre⁺) showed angiogenic remodeling defects [20] identical to those seen in global ephrinB2 knockout mice [20, 21], indicating that sufficient expression of ephrinB2 in endothelial and endocardial cells is required for angiogenesis and that the expression of ephrinB2 in surrounding mesenchymal cells and mural cells, including pericytes (PCs) and vascular smooth muscle cells (vSMCs), cannot compensate for the loss of ephrinB2 in vascular cells [20]. Furthermore, restoration of ephrinB2 expression specifically in vascular ECs of *Efnb2* null mice (*Efnb2*^{CR/GFP}; Tie2-Cre^{Tg/+}) was able to completely rescue defects in angiogenesis, suggesting the role of ephrinB2 for normal angiogenesis is restricted to vascular ECs [22], even though failure of rescuing cardiovascular defects in *Efnb2* null mice by ECs specific ephrinB2 restoration (*Efnb2*^{GFP/GFP}; T_g) possibly due to insufficient ephrinB2 level was also reported [23]. However, conditional mouse knockout of ephrinB2 in mural cells (*Efnb2*^{PC/vSMC}) resulted in perinatal lethality and caused edema and extensive hemorrhaging in the skin in addition to vascular defects in a range of organs [24] due to the scatter and loose attachment of PCs and vSMCs to the micro-vessels, suggesting a role of ephrinB2 in mediating interactions between mural cells as well as between PCs and the endothelium. Interestingly, while transgenic mice with a global overexpression of ephrinB2 (CAGp-ephrin-B2 Tg) exhibited abnormal segmental arrangement of intersomitic vessels, this was not observed in mice overexpressing ephrinB2 specifically in vascular ECs (Tie-2p-ephrin-B2 Tg) even though some do have intracerebral bleeding, suggesting that mediation of ECs by ephrin-Eph signaling can be altered by the ectopic expression of ephrinB2 [25]. These data collectively illustrate that precise regulation of the expression of ephrinB2 in specific time and space is absolutely essential for its proper function in vascular development. Both overexpression and loss of ephrinB2 expression break the balance of ephrin-Eph signaling and thus disrupt the biological processes it is regulating. Additionally, these mouse models provided important insights into the tissue specific functionality of ephrinB2, calling attention to its upstream regulators and downstream effectors in vascular ECs when studying cerebrovascular disorders.

EphB4

Human Diseases

Human EphB4 is a transmembrane RTK encoded by the *EPHB4* gene. On the extracellular side, EphB4 has a ligand-binding domain (LBD) that binds to the RBD of its ephrin ligand, a Cys-rich domain composed of a **sushi domain** and an epidermal growth factor (EGF)-like domain, and two fibronectin domains. Connected to the extracellular side by the transmembrane domain, the intracellular side of EphB4 encompasses a Tyrosine kinase domain, a sterile alpha motif, and a PDZ site.

Lymphatic angiogenesis starts from a certain subset of venous ECs that become responsive to lymphatic-inducing signals, which subsequently differentiate into the lymphatic lineage and form embryonic lymph sacs [3]. In two extended families with a history of *in utero* and neonatal death associated with hydrops fetalis resulting from a lymphatic abnormality, WES identified two distinct rare heterozygous missense mutations in the tyrosine kinase domain of *EPHB4* [26]. Both mutations caused the loss of phosphorylation activity of EphB4 *in vitro* and disrupted ephrinB2- dependent EphB4 activation in lymphatic ECs. All mutation carriers in these two families were either affected or presented with severe *in utero* swelling and/or atrial septal defect, suggesting highly variable expressivity of these mutations. Conditional knockout of *Ephb4* in mouse lymphatic vasculature (*Ephb4^{fl/fl}* Prox1-CreER^{T2} R26-mTmG) resulted in a high proportion of embryos with subcutaneous edema and/or blood filling in lymphatic vessels, recapitulating the phenotype of human patients [26]. Additionally, a splice site variant (c.2334 + 1G>C) in *EPHB4* was identified by WES in a four-generation family with central conducting lymphatic anomaly (CCLA) [27]. Knockdown of the *EPHB4* orthologue (*ephb4a*) with morpholino recapitulates human lymphatic phenotypes [27]. Of note, over-activation of mTORC1 was observed in both morpholino-treated zebrafish lysates and HEK293T cells with knock-in of the *EPHB4* mutation, and the zebrafish phenotype can be rescued by drugs inhibiting mTOR or RAS-MAPK signaling [27], suggesting PI3K/mTORC1 pathway downstream of ephrin-Eph signaling in vascular development. The identified human mutations accompanied by evidence from animal models strongly suggested a role of *EPHB4*, especially its downstream signaling through the PI3K/mTORC1 pathway mediated by its protein kinase domain, in human angiogenesis.

Like *EFNB2*, *EPHB4* is often selected as a candidate gene for CHD based on results from mouse models [6, 28], but to date, no clear association has been established from genetic studies in human patients. In a WES study of 182 individuals from 51 families with multiple members affected by CHD, one likely pathogenic missense mutation in *EPHB4* was found to co-segregate with the disease in 1 family with severe pulmonary stenosis, Tetralogy of Fallot, and ventricular septal defects [29].

CM-AVM is a congenital disorder characterized by atypical CM associated or not with AVM, arteriovenous fistula (AVF), or Parkes Weber syndrome [30]. CM-AVM in over 50% of the patients can be explained by mutations in *RASA1* (CM-AVM1, caused by *RASA1* mutation), a gene encoding p120RASGAP, which is a direct downstream effector of EphB4 [31]. Using genome wide linkage analysis in a large extended *RASA1* mutation negative

CM-AVM family, a peak on chromosome 7 harboring *EPHB4* was identified. Subsequent WES screen in 11 selected patients verified *EPHB4* as a disease associated gene and following targeted re-sequencing of *EPHB4* in 365 index individuals with either isolated multifocal CMs or CM-AVM identified 47 distinct predicted damaging mutations in 54 patients. Further investigation of clinical data of index cases and their relatives carrying the same *EPHB4* mutations revealed that *EPHB4* mutations are highly penetrant but have variable expressivity, with 93% of the carriers (n = 102) affected [31]. Of note, among 54 families carrying damaging mutations in *EPHB4*, 2 VOGM patients from the same family were reported, of whom 2 additional family members, 1 unaffected and 1 with isolated CMs, were also identified as carriers of the *EPHB4* mutation, further emphasizing the variable expressivity of *EPHB4* mutation. Additionally, 1 *de novo* mutation in *EPHB4* was reported in a 8-year-old boy affected by isolated multifocal CMs in a separate study [32]. These studies, even though mainly using targeted sequencing approaches, established *EPHB4* as a risk gene for CM-AVM (CM-AVM2, caused by *EPHB4* mutation).

Multiple recent studies have associated *EPHB4* with VOGM. WES in 19 unrelated VOGM patients identified *EPHB4*, with 1 *de novo* and 2 transmitted variants, as a candidate disease-causing gene. Targeted re-sequencing of *EPHB4* in 32 additional unrelated VOGM patients identified 2 additional predicted pathogenic missense mutations [33]. All 5 mutations identified were at different loci. Morpholino knockdown of *ephb4a* in zebrafish caused anomalies of the dorsal longitudinal vein, which is the orthologue of the median prosencephalic vein and the embryonic precursor of the vein of Galen, which was not observed in wild type nor ‘mismatch’ controls [33]. Additionally, using an unbiased burden test considering all genes captured in human genome (n = 18,989), an independent WES study of 55 unrelated VOGM probands identified *EPHB4*, with 4 distinct variants, as the only loss of function (LoF) intolerant gene (pLI 0.9) with genome-wide significant burden of rare damaging heterozygous mutations. Moreover, the Ephrin receptor signaling pathway was identified as the only over-represented pathway in cases but not controls using hypothesis-free pathway analysis, and genes (*EPHB4*, *RASA1*, *EPHA4*, *EPHA6*, *ITGB1*, *ITSN1*, and *NGEF*) contributing to the significance of the pathway map to a single experimentally supported interactome [16]. It is worth noting that in both studies, uncommon cutaneous CMs were reported in multiple VOGM probands as well as in *EPHB4* mutation carriers not diagnosed with VOGM, but not in non-carriers from the same family. These studies not only firmly established *EPHB4* as a bona fide risk gene for VOGM, but also highlighted the incomplete penetrance and variable expressivity of disease-associated mutations in families (see below).

Animal Studies

Among the 5 receptors (EphB1 - EphB4, and EphA4) of ephrinB2, EphB3 and EphB4 are the only 2 that are expressed in vascular ECs [5]. Of these, EphB4 is the only receptor that uniquely binds to ephrinB2 [34, 35]. While mouse knockout of *EphB3* did not result in a cardiovascular phenotype [5], *EphB4* knockout mice (EphB4^{taulacZ/taulacZ} [6]) lacking the signal peptide-encoding exon phenocopied ephrinB2 knockout mice (ephrinB2^{taulacZ/taulacZ} [2]) with defects in vascular development and embryonic lethality with very high penetrance [6] suggesting EphB4 as a specific functional partner for ephrinB2 in the vascular system

and also the redundant functionality of Eph receptors. Although mouse knockout of *EphB2* did not result in a cardiovascular phenotype, double knockout of *EphB2* and *EphB3* led to embryonic lethality as a result of defects in embryonic vascular remodeling with 30% penetrance [5], indicating a potentially redundant role of EphB3 shared with EphB4 even though its expression cannot compensate for the loss of EphB4 in vascular cells.

Compared to its ligand ephrinB2, which is expressed in not only ECs but also the surrounding mesenchymal and mural cells, expression of EphB4 is usually considered to be highly specific to vascular cells [20, 36]. Recently, male (but not female) knockout mice with a SMC-specific deletion of EphB4 were shown to have hypotension [37]. *In vivo* cellular molecular studies in vSMCs showed that small artery contractility seems to be regulated by both ephrinB2:EphB4 forward and reverse signaling. Even though it is not yet clear how sex hormones in concert with EphB4 are able to modulate vSMCs contractility, this is a starting point in understanding the role of ephrinB2:EphB4 signaling in blood pressure regulation. This study also brings attention to roles of *EPHB4* outside vasculogenesis, and could provide guidance for variant prioritization in human male patients with hypotension.

RASA1

Human Diseases

RASA1, also known as RAS p21 protein activator 1, is a GTPase activating protein (GAP) encoded by the *RASA1* gene. RASA1 interacts with **RAS** via its arginine finger motif in the GAP domain, thereby enhancing the intrinsic GTPase activity of RAS proteins and allowing for RAS inactivation [38, 39].

Since Eerola *et al* first characterized CM-AVM in 2003 [40], extensive reports have associated *RASA1* mutations with CM-AVM1 [30, 41-47], as well as with isolated CM [48-51] and AVM [52-54]. In 2 large scale cohort studies of CM-AVM, *RASA1* germline mutations were identified in ~70% of probands (44 and 68 out of 56 [41] and 100 [30] probands screened, respectively) with a high occurrence of *de novo* mutations (31.8% [41] and 26.5% [30], respectively), highlighting the high prevalence of *RASA1* mutation in CM-AVM cases as well as the importance of using a trio-family design to identify mutations with various inheritance models. Among all CM-AVM1 cases reported, 8 *RASA1* mutations were identified in VOGM, including two stop-gain mutations [16, 53, 55, 56] three missense variants [41, 56], and three frameshift deletions [30, 41]. However, when ascertaining for VOGM alone, only 1 *RASA1* mutation was identified in a total of 55 unrelated probands [16], suggesting a shared but distinct molecular mechanism underlying CM-AVM and VOGM.

Somatic second hits in affected tissues have long been proposed as a potential explanation for phenotypic variability and multifocality of the lesion associated with CM-AVM1. Importantly, mouse embryos mosaic for *Rasa1*^{-/-} cells exhibited localized vascular defects [57], suggesting **paradominant inheritance** in human CM-AVM [58]. A mosaic loss of chromosome 5q (including the entire *RASA1* gene) identified in DNA from a neurofibroma developed in the congenital Parkes Weber lesion was reported in a patient carrying

heterozygous germline splice site *RASA1* mutation (c.2603+5G>T), verified the somatic second hit model in CM-AVM for the first time [30]. More recently, a screen of *RASA1* mutation in endothelial cells derived from the biopsied CM lesions in 4 CM-AVM patients carrying germline *RASA1* mutation identified 1 somatically acquired LoF *RASA1* mutation in trans with the germline mutation in 1 of the patients [46], providing clear evidence of somatic mutation in relevant cell type contributing to the disease pathogenesis. One other somatic *RASA1* mutation in a CM-AVM1 patient was independently reported, even though whether the somatic hit is in *trans* or *cis* with the germline *RASA1* mutation was not tested [59]. These studies provide strong evidence of somatic second hit being a mechanism through which *RASA1* causes CM-AVM.

Animal Studies

RASA1 is a direct downstream effector of EphB4. Mutations disrupting the Tyrosine kinase domain of EphB4 identified in human VOGM patients abrogate the binding of *RASA1* in HEK293T cells [16]. Knockdown of *ephb4a* and *rasala* in zebrafish shared a similar phenotype of vascular deformities and a failure of caudal vascular plexus formation [8]. Interestingly, similar to the *ephb4a* knockdown zebrafish mentioned above, phenotypes of *efrib2a-ephb4a* and *rasala* knockdown zebrafish lines could also be rescued by chemical inhibitors of PI3K/mTORC1 [8], further emphasizing a role of the PI3K/mTORC1 pathway downstream of the ephrinB2-EphB4-*RASA1* signaling axis in vascular development. Consistent with the zebrafish model, the *RASA1* null mouse model (*Gap*^{-/-}) is embryonic lethal by E10.5 and exhibited severe blood vessel abnormalities [57], further emphasizing a crucial role of *RASA1* in vasculogenesis.

RASA1's arginine finger motif is required for its interaction with RAS but not for its RAS-independent functions [39]. *RASA1* knock-in mouse lacking the arginine finger (*Rasa*^{R780Q/R780Q}) exhibited embryonic lethality and severe vascular abnormalities similar to homozygous *Rasa* null mice [60], suggesting that dysregulation of RAS and downstream signaling is likely responsible for blood vessel abnormalities associated with *RASA1* mutations.

Similar to ephrinB2 and EphB4, *RASA1* also plays a crucial role in lymphatic vasculogenesis. An inducible *RASA1* adult mouse knockout (*RASA1*^{fl/fl}/U^b^{er2cre} [61]) showed extensive lymphatic vessel hyperplasia and leakage and early lethality caused by chylothorax [62-64]. Of note, even though very limited likely due to sample availability, lymphatic abnormalities have been reported in *RASA1* associated CM-AVM, particularly in patients with Parkes Weber syndrome [41, 63]. Taken together, these studies suggest that ephrinB2, EphB4, and *RASA1* function in the same pathway in the context of vasculogenesis. More attention should be paid to human mutations identified in the ephrinB2-EphB4-*RASA1* signaling axis as well as genes involved in ephrinB2:EphB4 forward signaling when studying cerebrovascular disorders.

Concluding Remarks and Future Perspectives

The ephrinB2-EphB4-*RASA1* signaling axis is essential for development of the vascular system. Recent successful application of WES and studies of large cohort have associated

mutations in *EFNB2*, *EPHB4*, and *RASA1* with multiple cerebrovascular disorders, corroborating the findings in model organisms. However, many key questions of their roles in human diseases remain unanswered (see Outstanding Questions).

CM-AVM and VOGM are thought to be two distinct disorders, with CM-AVM characterized by atypical CMs with or without AVM in variable body parts while VOGM being a type of cerebral AVM [16]. The fact that VOGM is infrequently reported as the AVM in CM-AVM patients, and CMs are quite often identified in patients with VOGM and their unaffected family members carrying the disease-causing mutation raises the question whether there is a shared genetic mechanism underlying these two diseases. Additionally, even though it is very difficult to estimate whether there is an enrichment of ephrinB2-EphB4-RASA1 mutations in cerebrovascular diseases compared to other vascular malformations given the incompleteness of the publicly available phenotype data, that Ephrin receptor signaling pathway was identified as the only over-represented pathway in VOGM cases but not controls [16] provided strong evidence of a role for ephrinB2-EphB4-RASA1 signaling axis in cerebrovascular development. Moreover, other than cardiac defects, cerebral manifestation is the most commonly described defect in animal models of ephrinB2 [2, 20, 25], EphB4 [6], and RASA1 [8]. Future study with thorough history and physical examination is needed to better understand the phenotypical overlap of different cerebrovascular disorders as well as between cerebral and other vascular defects in human, which will in turn aid the genetic study in understanding their shared mechanism and the prevalence of the risk genes.

The majority of the identified mutations in CM-AVM and VOGM are transmitted with incomplete penetrance and variable expressivity. Parents with mild cutaneous vascular lesions can have children with VOGM that is almost 100% lethal without surgical intervention.

The reason why one mutation can have such a large spectrum of manifestation is not yet completely clear. Two mechanisms can potentially explain some of the cases. First, some cases could have somatic second-hit during development, which would explain the localized nature and multifocality of the lesions, as well as the variable phenotype within families [41]. Somatic mosaicism has been verified in several vascular malformations [65, 66], including CM-AVM [30, 46]. Second mechanism would be that there are genetic modifiers that could interact and modify the expressivity of the disease-causing gene [67], or more than 1 gene with 1 defective allele (transcompound heterozygote) is needed for disease manifestation [58]. Future study with large **trio based cohort** and collection of tissue samples are needed to test these hypotheses.

Despite the large number of the CM-AVM cases explained by *RASA1* mutations, genetic causes for many patients remain unknown. As most of the published studies performed target resequencing on candidate genes, some additional patients could be explained if the unsolved cases were screened for genes that are more recently identified, such as *EPHB4* and *EFNB2*. Additionally, target sequencing limited the possibility of identifying novel disease-causing mutations. More unbiased genome-wide sequencing studies are in need to identify novel genes and better understand the genetic underpinnings of the disease.

Furthermore, mutations in the regulatory and intronic regions of the known disease-associated genes have not yet been examined. Future study using whole genome sequencing could potentially unveil the contribution of noncoding variants in known genes and even identify novel mutations in non-coding regions.

Genetic findings in ephrinB2-EphB4-RASA1 signaling axis genes also provided insights into potential diagnostic and therapeutic strategies for cerebrovascular disorders. Given the incomplete penetrance and variable expressivity, prenatal genetic screen in pregnant women with family history of cutaneous vascular lesions and/or other vascular malformations could help the early diagnosis and treatment design of the disease. Interestingly, the phenotype in *efnb2a-ephb4a* and *rasala* knockdown zebrafish could be rescued by chemical inhibitors of PI3K-TORC1, indicating that reduced ephrinB2-EphB4-RASA1 expression may cause excessive PI3K/mTORC1 pathway activity, which has also been observed in surgical tissue samples from patients with vascular anomalies associated with *RASA1* mutations [30]. This suggests therapy targeting PI3K-TORC1 signaling might be a viable therapeutic approach for cerebrovascular diseases associated with ephrinB2-EphB4-RASA1 mutations.

GLOSSARY

CCLA:

A type of complex lymphatic anomaly characterized by dilated lymphatic channels, lymphatic channel dysmotility and distal obstruction affecting lymphatic drainage [27].

Conotruncal heart defects

A group of congenital cardiac outflow tract anomalies that classically include tetralogy of Fallot, pulmonary atresia with ventricular septal defect, double-outlet right ventricle, double-outlet left ventricle, truncus arteriosus and transposition of the great arteries [75].

Trio based cohort:

Cohort composed of parent-offspring trios. This kind of pedigree enables the identification of *de novo*, recessive, and transmitted heterozygous mutations.

Ephrin-Eph forward, reverse, parallel, and anti-parallel signaling:

Eph receptors and ephrins function as classic receptors and ligands in ephrin:Eph forward signaling. However, the roles of Eph and ephrin proteins can be switched to ligands and receptors respectively in Eph:ephrin reverse signaling. When Eph receptors and ephrins are localized on the same cell surface, they can act as ligands for ephrins and Eph receptors respectively to activate signaling in the same direction (parallel signaling) or they can each act as receptors and ligands to activate signaling in opposite directions (anti-parallel signaling) [10, 11].

LoF mutation:

Mutations predicted *in silico* to cause frameshift, premature termination codon, stoploss, startloss, and/or affect canonical splice site.

Paradominant inheritance:

A form of non-mendelian inheritance where carriers of the heterozygous mutation are phenotypically normal until postzygotic loss of heterozygosity occurs at an early developmental stage that forms a mosaic patch affected by the disease [76].

RAS:

The RAS gene family encodes membrane-associated guanine-nucleotide binding proteins (p21), which cycle between an active guanosine-triphosphate (GTP) bound form and an inactive guanosine-diphosphate (GDP) bound form.

Sushi domain:

Also known as complement control protein (CCP) module. It is an evolutionarily conserved protein domain of about 60 amino acids in length that is characterized by a consensus sequence including 4 invariant cysteines, an almost invariant tryptophan and highly conserved prolines, glycines and hydrophobic residues [77].

REFERENCES

- [1]. Sabin FR, Preliminary note on the differentiation of angioblasts and the method by which they produce blood-vessels, blood-plasma and red blood-cells as seen in the living chick. 1917. *J Hematother Stem Cell Res*, 2002 11(1): p. 5–7. [PubMed: 11846999]
- [2]. Wang HU, Chen ZF, and Anderson DJ, Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph- B4. *Cell*, 1998 93(5): p. 741–53. [PubMed: 9630219]
- [3]. Eichmann A, et al., Vascular development: from precursor cells to branched arterial and venous networks. *Int J Dev Biol*, 2005 49(2–3): p. 259–67. [PubMed: 15906240]
- [4]. Fish JE and Wythe JD, The molecular regulation of arteriovenous specification and maintenance. *Dev Dyn*, 2015 244(3): p. 391–409. [PubMed: 25641373]
- [5]. Adams RH, et al., Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. *Genes Dev*, 1999 13(3): p. 295–306. [PubMed: 9990854]
- [6]. Gerety SS, et al., Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. *Mol Cell*, 1999 4(3): p. 40–314.
- [7]. Herbert SP, et al., Arterial-venous segregation by selective cell sprouting: an alternative mode of blood vessel formation. *Science*, 2009 326(5950): p. 294–8. [PubMed: 19815777]
- [8]. Kawasaki J, et al., RASA1 functions in EPHB4 signaling pathway to suppress endothelial mTORC1 activity. *J Clin Invest*, 2014 124(6): p. 2774–84. [PubMed: 24837431]
- [9]. Kuijper S, Turner CJ, and Adams RH, Regulation of angiogenesis by Eph-ephrin interactions. *Trends Cardiovasc Med*, 2007 17(5): p. 145–51. [PubMed: 17574121]
- [10]. Kania A and Klein R, Mechanisms of ephrin-Eph signalling in development, physiology and disease. *Nat Rev Mol Cell Biol*, 2016 17(4): p. 240–56. [PubMed: 26790531]
- [11]. Taylor H, Campbell J, and Nobes CD, Ephs and ephrins. *Curr Biol*, 2017 27(3): p. R90–R95. [PubMed: 28171762]
- [12]. Egea J, et al., Regulation of EphA 4 kinase activity is required for a subset of axon guidance decisions suggesting a key role for receptor clustering in Eph function. *Neuron*, 2005 47(4): p. 515–28. [PubMed: 16102535]
- [13]. Himanen JP, et al., Architecture of Eph receptor clusters. *Proc Natl Acad Sci U S A*, 2010 107(24): p. 10860–5. [PubMed: 20505120]
- [14]. Mak CCY, et al., De novo large rare copy-number variations contribute to conotruncal heart disease in Chinese patients. *NPJ Genom Med*, 2016 1: p. 16033. [PubMed: 29263819]
- [15]. Levy J, et al., EFN2 haploinsufficiency causes a syndromic neurodevelopmental disorder. *Clin Genet*, 2018 93(6): p. 1141–1147. [PubMed: 29508392]

- [16]. Duran D, et al., Mutations in Chromatin Modifier and Ephrin Signaling Genes in Vein of Galen Malformation. *Neuron*, 2018.
- [17]. Adams RH, et al., The cytoplasmic domain of the ligand ephrinB2 is required for vascular morphogenesis but not cranial neural crest migration. *Cell*, 2001 104(1): p. 57–69. [PubMed: 11163240]
- [18]. Cowan CA, et al., Ephrin-B2 reverse signaling is required for axon pathfinding and cardiac valve formation but not early vascular development. *Dev Biol*, 2004 271(2): p. 263–71. [PubMed: 15223333]
- [19]. Lisle JE, et al., Murine, but not human, ephrin-B2 can be efficiently cleaved by the serine protease kallikrein-4: implications for xenograft models of human prostate cancer. *Exp Cell Res*, 2015 333(1): p. 136–46. [PubMed: 25724897]
- [20]. Gerety SS and Anderson DJ, Cardiovascular ephrinB2 function is essential for embryonic angiogenesis. *Development*, 2002 129(6): p. 1397–410. [PubMed: 11880349]
- [21]. Dravis C, et al., Bidirectional signaling mediated by ephrin-B2 and EphB2 controls urorectal development. *Dev Biol*, 2004 271(2): p. 272–90. [PubMed: 15223334]
- [22]. Lewis AE, et al., Neural crest defects in ephrin-B2 mutant mice are non-autonomous and originate from defects in the vasculature. *Dev Biol*, 2015 406(2): p. 186–95. [PubMed: 26385750]
- [23]. Luxey M, et al., Generation of transgenic mice overexpressing EfnB2 in endothelial cells. *Genesis*, 2011 49(10): p. 811–20. [PubMed: 21735541]
- [24]. Foo SS, et al., Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell*, 2006 124(1): p. 161–73. [PubMed: 16413489]
- [25]. Oike Y, et al., Regulation of vasculogenesis and angiogenesis by EphB/ephrin-B2 signaling between endothelial cells and surrounding mesenchymal cells. *Blood*, 2002 100(4): p. 1326–33. [PubMed: 12149214]
- [26]. Martin-Almedina S, et al., EPHB4 kinase-inactivating mutations cause autosomal dominant lymphatic-related hydrops fetalis. *J Clin Invest*, 2016 126(8): p. 3080–8. [PubMed: 27400125]
- [27]. Li D, et al., Pathogenic variant in EPHB4 results in central conducting lymphatic anomaly. *Hum Mol Genet*, 2018 27(18): p. 3233–3245. [PubMed: 29905864]
- [28]. Krebs LT, et al., Notch1 activation in mice causes arteriovenous malformations phenocopied by ephrinB2 and EphB4 mutants. *Genesis*, 2010 48(3): p. 146–50. [PubMed: 20101599]
- [29]. Preuss C, et al., Family Based Whole Exome Sequencing Reveals the Multifaceted Role of Notch Signaling in Congenital Heart Disease. *PLoS Genet*, 2016 12(10): p. e1006335.
- [30]. Revencu N, et al., RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation. *Hum Mutat*, 2013 34(12): p. 1632–41. [PubMed: 24038909]
- [31]. Amyere M, et al., Germline Loss-of-Function Mutations in EPHB4 Cause a Second Form of Capillary Malformation-Arteriovenous Malformation (CM-AVM2) Deregulating RAS- MAPK Signaling. *Circulation*, 2017 136(11): p. 1037–1048. [PubMed: 28687708]
- [32]. Yu J, et al., EPHB4 Mutation Implicated in Capillary Malformation-Arteriovenous Malformation Syndrome: A Case Report. *Pediatr Dermatol*, 2017 34(5): p. e227–e230. [PubMed: 28730721]
- [33]. Vivanti A, et al., Loss of function mutations in EPHB4 are responsible for vein of Galen aneurysmal malformation. *Brain*, 2018 141(4): p. 979–988. [PubMed: 29444212]
- [34]. Brambilla R, et al., Membrane-bound LERK2 ligand can signal through three different Eph-related receptor tyrosine kinases. *EMBO J*, 1995 14(13): p. 3116–26. [PubMed: 7621826]
- [35]. Sakano S, et al., Characterization of a ligand for receptor protein-tyrosine kinase HTK expressed in immature hematopoietic cells. *Oncogene*, 1996 13(4): p. 813–22. [PubMed: 8761303]
- [36]. Shin D, et al., Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. *Dev Biol*, 2001 230(2): p. 139–50. [PubMed: 11161568]
- [37]. Wang Y, et al., EPHB4 Protein Expression in Vascular Smooth Muscle Cells Regulates Their Contractility, and EPHB4 Deletion Leads to Hypotension in Mice. *J Biol Chem*, 2015 290(22): p. 14235–44. [PubMed: 25903126]

- [38]. Trahey M, et al., Molecular cloning of two types of GAP complementary DNA from human placenta. *Science*, 1988 242(4886): p. 1697–700. [PubMed: 3201259]
- [39]. Scheffzek K, et al., The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science*, 1997 277(5324): p. 333–8. [PubMed: 9219684]
- [40]. Eerola I, et al., Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet*, 2003 73(6): p. 1240–9. [PubMed: 14639529]
- [41]. Revencu N, et al., Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat*, 2008 29(7): p. 959–65. [PubMed: 18446851]
- [42]. Buhl T, et al., Multifocal capillary malformations due to RASA1 mutation misdiagnosed as cutaneous mastocytosis. *Arch Dermatol*, 2012 148(11): p. 1334–5. [PubMed: 23165854]
- [43]. de Wijn RS, et al., Phenotypic variability in a family with capillary malformations caused by a mutation in the RASA1 gene. *Eur J Med Genet*, 2012 55(3): p. 191–5. [PubMed: 22342634]
- [44]. Wooderchak-Donahue W, et al., RASA1 analysis: clinical and molecular findings in a series of consecutive cases. *Eur J Med Genet*, 2012 55(2): p. 91–5. [PubMed: 22200646]
- [45]. Durrington HJ, et al.; 1;, A novel RASA1 mutation causing capillary malformation-arteriovenous malformation (CM-AVM) presenting during pregnancy. *Am J Med Genet A*, 2013 161A(7): p. 1690–4. [PubMed: 23687085]
- [46]. Lapinski PE, et al.; 1;, Somatic second hit mutation of RASA1 in vascular endothelial cells in capillary malformation-arteriovenous malformation. *Eur J Med Genet*, 2018 61(1): p. 11–16. [PubMed: 29024832]
- [47]. Cai R, et al.; 1;, A novel RASA1 mutation causing capillary malformation-arteriovenous malformation (CM-AVM): the first genetic clinical report in East Asia. *Hereditas*, 2018 155: p. 24. [PubMed: 30026675]
- [48]. Eerola I, et al.; 1;, Locus for susceptibility for familial capillary malformation (‘port-wine stain’) maps to 5q. *Eur J Hum Genet*, 2002 10(6): p. 375–80. [PubMed: 12080389]
- [49]. Hershkovitz D, et al.; 1;, RASA1 mutations may cause hereditary capillary malformations without arteriovenous malformations. *Br J Dermatol*, 2008 158(5): p. 1035–40. [PubMed: 18363760]
- [50]. Hershkovitz D, Bergman R, and Sprecher E, A novel mutation in RASA1 causes capillary malformation and limb enlargement. *Arch Dermatol Res*, 2008 300(7): p. 385–8. [PubMed: 18327598]
- [51]. Kim C, et al.; 1;, Histopathologic and ultrasound characteristics of cutaneous capillary malformations in a patient with capillary malformation-arteriovenous malformation syndrome. *Pediatr Dermatol*, 2015 32(1): p. 128–31. [PubMed: 23829194]
- [52]. Thiex R, et al.; 1;, A novel association between RASA1 mutations and spinal arteriovenous anomalies. *AJNR Am J Neuroradiol*, 2010 31(4): p. 775–9. [PubMed: 20007727]
- [53]. Walcott BP, et al.; 1;, Pial arteriovenous fistulae in pediatric patients: associated syndromes and treatment outcome. *J Neurointerv Surg*, 2013 5(1): p. 10–4. [PubMed: 22213836]
- [54]. Grillner P, et al.; 1;, A spectrum of intracranial vascular high-flow arteriovenous shunts in RASA1 mutations. *Childs Nerv Syst*, 2016 32(4): p. 709–15. [PubMed: 26499346]
- [55]. Chida A, et al.; 1;, ACVRL1 gene variant in a patient with vein of Galen aneurysmal malformation. *J Pediatr Genet*, 2013 2(4): p. 181–9. [PubMed: 27625857]
- [56]. Heuchan A, et al.; 1;, RASA1 Mutations and Vein of Galen Arterial Malformations. *Arch Dis Child*, 2013 98 (Suppl 1): p. A16–A17.
- [57]. Henkemeyer M, et al., Vascular system defects and neuronal apoptosis in mice lacking ras GTPase-activating protein. *Nature*, 1995 377(6551): p. 695–701. [PubMed: 7477259]
- [58]. Boon LM, Mulliken JB, and Vikkula M, RASA1: variable phenotype with capillary and arteriovenous malformations. *Curr Opin Genet Dev*, 2005 15(3): p. 265–9. [PubMed: 15917201]
- [59]. Macmurdo CF, et al., RASA1 somatic mutation and variable expressivity in capillary malformation/arteriovenous malformation (CM/AVM) syndrome. *Am J Med Genet A*, 2016 170(6): p. 1450–4. [PubMed: 26969842]

- [60]. Lubeck BA, et al., Blood vascular abnormalities in *Rasa1*(R780Q) knockin mice: implications for the pathogenesis of capillary malformation-arteriovenous malformation. *Am J Pathol*, 2014 184(12): p. 3163–9. [PubMed: 25283357]
- [61]. Lapinski PE, et al., Generation of mice with a conditional allele of the p120 Ras GTPase-activating protein. *Genesis*, 2007 45(12): p. 762–7. [PubMed: 18064675]
- [62]. Lapinski PE, et al., *RASA1* maintains the lymphatic vasculature in a quiescent functional state in mice. *J Clin Invest*, 2012 122(2): p. 733–47. [PubMed: 22232212]
- [63]. Burrows PE, et al., Lymphatic abnormalities are associated with *RASA1* gene mutations in mouse and man. *Proc Natl Acad Sci U S A*, 2013 110(21): p. 8621–6. [PubMed: 23650393]
- [64]. Lapinski PE, et al., *RASA1* regulates the function of lymphatic vessel valves in mice. *J Clin Invest*, 2017 127(7): p. 2569–2585. [PubMed: 28530642]
- [65]. Brouillard P, et al., Mutations in a novel factor, glomulin, are responsible for glomuvenous malformations (“glomangiomas”). *Am J Hum Genet*, 2002 70(4): p. 866–74. [PubMed: 11845407]
- [66]. Pagenstecher A, et al., A two-hit mechanism causes cerebral cavernous malformations: complete inactivation of *CCM1*, *CCM2* or *CCM3* in affected endothelial cells. *Hum Mol Genet*, 2009 18(5): p. 911–8. [PubMed: 19088124]
- [67]. Timberlake AT, et al., Two locus inheritance of non-syndromic midline craniosynostosis via rare *SMAD6* and common *BMP2* alleles. *Elife*, 2016 5.
- [68]. Stahl S, et al., Novel *CCM1*, *CCM2*, and *CCM3* mutations in patients with cerebral cavernous malformations: in-frame deletion in *CCM2* prevents formation of a *CCM1/CCM2/CCM3* protein complex. *Hum Mutat*, 2008 29(5): p. 709–17. [PubMed: 18300272]
- [69]. Govani FS and Shovlin CL, Hereditary haemorrhagic telangiectasia: a clinical and scientific review. *Eur J Hum Genet*, 2009 17(7): p. 860–71. [PubMed: 19337313]
- [70]. McDonald MT, et al., A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33–34. *Nat Genet*, 1994 6(2): p. 197–204. [PubMed: 8162075]
- [71]. Shovlin CL, et al., A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nat Genet*, 1994 6(2): p. 205–9. [PubMed: 8162076]
- [72]. Johnson DW, et al., Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*, 1996 13(2): p. 189–95. [PubMed: 8640225]
- [73]. Gallione CJ, et al., A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in *MADH4* (*SMAD4*). *Lancet*, 2004 363(9412): p. 852–9. [PubMed: 15031030]
- [74]. Wooderchak-Donahue WL, et al., *BMP9* mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. *Am J Hum Genet*, 2013 93(3): p. 530–7. [PubMed: 23972370]
- [75]. Anaclerio S, et al., Conotruncal heart defects: impact of genetic syndromes on immediate operative mortality. *Ital Heart J*, 2004 5(8): p. 624–8. [PubMed: 15554034]
- [76]. Danarti R, Happle R, and Konig A, Paradoxical inheritance may explain familial occurrence of *Cutis marmorata telangiectatica congenita*. *Dermatology*, 2001 203(3): p. 208–11. [PubMed: 11701972]
- [77]. Kirkitadze MD and Barlow PN, Structure and flexibility of the multiple domain proteins that regulate complement activation. *Immunol Rev*, 2001 180: p. 146–61. [PubMed: 11414356]
- [78]. Carr CW, et al., 5q14.3 neurocutaneous syndrome: a novel contiguous gene syndrome caused by simultaneous deletion of *RASA1* and *MEF2C*. *Am J Med Genet A*, 2011 155A(7): p. 1640–5. [PubMed: 21626678]
- [79]. Flore LA, et al., *RASA1* analysis guides management in a family with capillary malformation-arteriovenous malformation. *J Pediatr Genet*, 2012 1(2): p. 125–9. [PubMed: 27625812]
- [80]. Hernandez F, et al., Mutations in *RASA1* and *GDF2* identified in patients with clinical features of hereditary hemorrhagic telangiectasia. *Hum Genome Var*, 2015 2: p. 15040. [PubMed: 27081547]
- [81]. Kwong Y, et al., Fetal MRI demonstrating vein of Galen malformations in two successive pregnancies--a previously unreported occurrence. *Childs Nerv Syst*, 2015 31(7): p. 1033–5. [PubMed: 25985841]

- [82]. Whitaker S, et al., Multifocal capillary malformations in an older, asymptomatic child with a novel RASA1 mutation. Clin Exp Dermatol, 2016 41(2): p. 156–8. [PubMed: 26132338]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HIGHLIGHTS

- Genetic and functional studies in model organisms have revealed the importance of ephrinB2-EphB4-RASA1 signaling in multiple aspects of cerebrovascular development.
- Recent whole exome sequencing studies in humans have identified mutations in *EFNB2*, *EPHB4*, and *RASA1* in multiple congenital cerebrovascular disorders.
- These diseases include capillary malformation-arteriovenous malformation (CM-AVM), Vein of Galen malformation (VOGM), and others.
- The localized nature and multifocality of lesions associated with ephrinB2-EphB4-RASA1 mutations implicates somatic mosaicism in disease pathogenesis.
- The ephrinB2-EphB4-RASA1 axis is a potentially targetable node for therapeutic intervention.

Box 1:**Cerebrovascular Malformations**

Cerebrovascular malformations are abnormal formations of blood vessels in the brain. They are classically subdivided into five common types: pial arteriovenous malformations (AVMs), dural arteriovenous fistulas (DAVs), cerebral cavernous malformations (CCMs), venous malformations, and capillary telangiectasias. AVMs and DAVs are considered to be fast-flow lesions. They are characterized by direct connections between arteries and veins within the brain and the dura mater respectively, which cause high-pressure arterial blood to flow quickly into the venous circulation without passing through an intervening capillary bed. CCMs are groups of thin-walled capillaries. Unlike AVMs and DAVs, they are slow-flow low-pressure malformations and are predominantly associated with mutations in the genes *CCM1* (*KRT1*), *CCM2*, and *CCM3* (*PDCD10*) [68]. Venous malformations (sometimes also called developmental venous anomalies or venous angiomas) are composed of one or more atypical enlarged veins. Finally, telangiectasias are enlarged capillaries that often occur in the brainstem. Cerebral arteriovenous malformations can occur sporadically, but they can also be observed in the context of inherited syndromes with known genetic causes, such as Capillary-Malformation Arteriovenous Malformation (CM-AVM) types 1 and 2 [31, 40] and Hereditary Hemorrhagic Telangiectasia (HHT) types 1 and 2 [69].

CM-AVM1 is classically caused by mutations in the *RASA1* gene and is transmitted in an autosomal dominant fashion [40, 48]. The disorder is characterized by capillary malformations located on the head, neck, trunk, and extremities along with AVMs in the skin, muscle, and brain [30, 40, 52, 58]. CM-AVM2 is associated with mutations in *EPHB4* [31]. CM-AVM2 presents with similar clinical features to CM-AVM1 including multi-focal capillary malformations but patients with CM-AVM2 display more frequent perioral and thoracic telangiectasia, a lower frequency of fast-flow lesions, and a lower frequency of central nervous system AVMs, though Vein of Galen Malformation, the most common and severe type of AVM in neonates, has been diagnosed in patients with both CM-AVM1 and CM-AVM2 [16, 30, 31, 41, 52].

HHT is an autosomal dominant disorder characterized by recurrent epistaxis, characteristic telangiectasias of the oropharynx, face, and hands, and multiple focal AVMs [69]. HHT is associated with a 10% prevalence of brain AVMs, but AVMs can also be found in the lungs, liver, gastrointestinal tract, and spinal cord. A number of subtypes of HHT have been described, each associated with a particular underlying genetic defect. Implicated genes include *ENG* (HHT1) [70-72], *ACVRL1/ALK1* (HHT2) [72], *SMAD4* (juvenile polyposis-HHT syndrome) [73], and *BMP9* (HHT5) [74].

CLINICIAN'S CORNER

- Damaging mutations in *EFNB2*, *EPHB4*, and *RASA1* have been identified in multiple congenital cerebrovascular disorders, including capillary malformation-arteriovenous malformation (CM-AVM) and Vein of Galen malformation (VOGM).
- The majority of the identified mutations in CM-AVM and VOGM are transmitted with incomplete penetrance and variable expressivity. Parents with mild cutaneous vascular lesions, which can be easily overlooked, are likely at higher risk for having children with a high-flow vascular lesion, including VOGM. Careful and thorough physical examination and prenatal genetic screen in pregnant women with family history of cutaneous vascular lesions and/or other vascular malformations could help the early diagnosis and treatment design of the disease.
- CM-AVM and VOGM have overlapping genetic underpinnings and phenotypes, with VOGM infrequently reported as an associated AVM in CM-AVM patients, and capillary malformations (CMs) often identified in patients with VOGM. Detailed genotype-phenotype studies, especially for VOGM, with emphasis on skin findings, associated heart defects, neuropsychiatric phenotypes, and radiological anatomy of cerebrovascular lesions are needed to gain more knowledge of the phenotypical overlap of these cerebrovascular disorders, which will in turn aid the genetic study in understanding their shared mechanism.
- Mutated ephrinB2-EphB4-RASA1 expression may cause excessive PI3K/mTORC1 pathway activity. Targeting PI3K-TORC1 signaling with clinically-available drugs like mTOR inhibitors might be a viable therapeutic approach for cerebrovascular diseases associated with ephrinB2-EphB4-RASA1 mutations, though much work is required to test this hypothesis.

OUTSTANDING QUESTIONS

What is the contribution of non-coding and regulatory regions of genes in the ephrinB2-EphB4-RASA1 signaling axis to human congenital neurovascular disorders?

What is the mechanism of the incomplete penetrance and variable expressivity of VOGM-associated ephrinB2-EphB4-RASA1 mutations?

What is the prevalence of CMs in VOGM? What are the phenotypic and genetic overlap between CM-AVM and VOGM?

Can other genes in the same pathway as *EFNB2*, *EPHB4*, and *RASA1* explain some of the remaining patients with neurovascular disorders that are negative of mutation in known genes?

Can drugs targeting the PI3K/mTORC1 pathway be used to treat patients with cerebrovascular disorders associated with ephrinB2-EphB4-RASA1 mutations?

Figure 1. An emerging paradigm of ephrinB2-EphB4-RASA1 mutations in human cerebrovascular disorders.

Schematic representation of the ephrinB2-EphB4-RASA1 signaling axis in vascular development. The membrane tethered ligand, ephrinB2, binds to its receptor, EphB4, which interacts with its downstream effector and binding partner, RASA1. Mutations disrupting the signaling axis potentially lead to excessive PI3K/mTORC1 pathway activity, causing dysregulation of downstream genes. EphrinB2-EphB4-RASA1 mutations identified in human patients with vascular disorders are mapped to their protein domains. Given the space limit, only mutation type distribution is provided for RASA1. Please refer to Table 2 for

detailed variant information. Mixed: a variant is reported in more than 1 inheritance models; TK = Tyrosine kinase domain.

Table 1.

Animal Models of EphrinB2-EphB4-RASA1 Signaling Axis

EFNB2 Animal Models			
Animal Model	Genotype	Phenotype	Description Ref
Mouse	ephrinB2 ^{flacZ}	Defects in angiogenesis by both arteries and veins in the capillary networks for the head and yolk sac as well as in myocardial trabeculation	Vasculogenesis occurred normally between E8.5 and E9.0; apparent pronounced disorganization at E10; defect in yolk sac angiogenesis by E9; absence of internal carotid arterial branches; defective angiogenesis of venous capillaries in the head; capillary ingrowth into the neural tube failed to occur [2]
Mouse	ephrinB2 ^{C/C} C ₁ (ephrinB2- C-HA fusion protein)	Able to restore guidance of migrating cranial neural crest cells, but have defects in vasculogenesis and angiogenesis and midgestation lethality	Lethal at midgestation (E10.5-E11) with growth retardation and inflation of the pericard; normal formation of branchial arches; defects in vascular morphogenesis [17]
Mouse	ephrinB2 ^{flacZ/loxp} ; Tie2-Cre ⁺ (endothelium and endocardium specific deletion of ephrinB2)	Angiogenic remodeling defect identical to ephrinB2 global knockout mice (Efnb2 ^{ml/ml})	Growth retardation; swollen but still beating heart with little blood flow; disorganized and less developed vasculature compared to WT; arrest in development of arteries and veins at the primary plexus stage in yolk sac; failure in anterior cardinal vein (ACV) assembly and remodeling; initial head plexus forms but remains in disorganized primitive stage [20]
Mouse	CAGp-ephrin-B2 T _g (global overexpression of ephrinB2)	Abnormal segmental arrangement of intersomitic vessels; die of acute aortic dissecting aneurysms at neonatal stages	Thin vascular wall in the ascending aorta and aortic arch; ECs have a flat morphology with a budlike structure; SMCs and elastic bands are missing, while microvessels are seen in these spaces; abnormal vasculature in ascending and arch portion of the aorta [25]
Mouse	Tie-2p-ephrin-B2 T _g (vascular ECs specific overexpression of ephrinB2)	Cranial hemorrhages with incomplete penetrance	Intracerebral bleeding [25]
Mouse	ephrinB2 ^{C-βgal/ C-βgal} (ephrinB2 C-terminal truncation and βgal fusion protein)	Severe hypospadias and incomplete midline fusion of the primitive cloaca with incomplete penetrance (heterozygotes) Failure in cloacal septation with complete penetrance (homozygotes)	Severe anorectal malformations with an absence of the terminal-most hindgut and formation of a fistula that aberrantly connects the intestines to the urethra at the base of the bladder. [21]
		Grossly normal with no early vascular defects, but die within the first day of birth due to cardiac defects	Normal chamber formation and vascular connection; thickened cardiac valves; disrupted pathfinding of anterior commissure axons; defective maturation of cardiac valve leaflets. [18]
Mouse	ephrinB2 ^{T/T} (ephrinB2 with C-terminal truncation)	Embryonic lethal with a failure in cardiovascular development	Visibly abnormal, appearing growth-retarded and necrotic, had enlarged pericardial, displayed abnormal heart and vascular structures [18]
Mouse	Efnb2 ^{PCvSMC} (mural cell specific deletion of ephrinB2)	Perinatal lethality; vascular defects in skin, lung, gastrointestinal tract, and kidney glomeruli; and abnormal migration of smooth muscle cells to lymphatic capillaries	Develop to term but die shortly after birth due to respiratory failure; edema and extensive hemorrhaging in the skin; pale intestines and blood filling mesenteric lymphatics; hemorrhaging in the lung; comma- and S-shaped bodies developed normally, but the more mature glomerular tufts were poorly organized [24]

EFNB2 Animal Models				
Animal Model	Genotype	Phenotype	Description	Ref
Mouse	<i>Efnb2</i> ^{ΔGFP/GFP} ; T _g (ECs specific expression of ephrinB2 in the background of <i>Efnb2</i> null)	Similar growth retardation, and heart morphogenesis defects as observed in <i>Efnb2</i> null mice, but the early lethality associated with <i>Efnb2</i> null mutation is partially rescued	Lethality earlier than E9.5 likely due to mixed genetic background of mice used in this study; partial rescue of the lethal phenotype of the <i>Efnb2</i> null mice by E10.5; bleeding in the pericardial cavity; intersomitic vessel network appeared fused dorsally and poorly branched	[23]
Mouse	<i>Efnb2</i> ^{CR/GFP} ; Tie2-Cre ^{Tg/+} (vascular ECs specific expression of ephrinB2 in the background of <i>Efnb2</i> null)	Perinatal lethality; normal angiogenesis; exhibit tracheoesophageal and urorectal fistulae as well as an incorrectly positioned thymus, while other bilateral structures including lungs, vena cava, and clavicles appear to be the same as controls.	Overtly normal at E9.5 without signs of angiogenic defects; normal remodeling of the primitive vascular plexus into hierarchically branched vessels at E9.5; survived to birth but died perinatally; no defects in branchial arches morphogenesis or cranial or trunk neural crest development	[22]
EPHB4 Animal Models				
Animal Model	Genotype	Phenotype	Description	Ref
Mouse	<i>EphB4</i> ^{4tailacZ/tailacZ}	Cardiovascular defects and embryonic lethality with very high penetrance	Growth retardation, arrested heart development, and lack of blood flow by E9.5-E10; degeneration and necrosis throughout the embryo by E10.5; incomplete heart looping at E8.75-E9.0; arrest of the remodeling of the primitive dilated vessels in head; disrupted development of the ACV and both arterial and venous intersomitic vessels; heart failed to increase in size, with incomplete cardiac looping and endocardium expansion; angiogenic remodeling arrest in the yolk sac with the phenotype being more severe on the venous than the arterial sides	[6]
Mouse	<i>Ephb4</i> ^{fl/fl} back crossed with <i>smMHC-Cre-IRES-eGFP</i> tg (SMC specific <i>EphB4</i> knockout)	Hypotension in male but not female knockout mice	significantly reduced systolic pressure and mean arterial pressure with normal heart rate compared to WT in male but not female knockout mice	[37]
RASAI Animal Models				
Animal Model	Genotype	Phenotype	Description	Ref
Mouse	Rasa1 ^{-/-} (RASAI null)	Embryonic lethal by day E10.5 with blood vessel abnormalities	16-somite stage; thinning of dorsal aorta, aberrant ventral branches resemble inter-segmental arteries; E9.5; yolk sacs visibly abnormal (rough wrinkled appearance), embryonic vasculature exhibits local ruptures	[57]
Mouse	Rasa1 ^{R780Q/R780Q} (knock-in expressing a mutant RASAI that lacks an arginine finger)	Mid-gestation embryonic lethal with blood vessel abnormalities	E9.5; embryos small and less developed, distended pericardial sac, wrinkled yolk sac, no organized blood vessel networks, irregularly shaped dorsal aorta, severely disrupted and irregularly shaped inter-segmental arteries	[60]
Mouse	RASA1 ^{fl/fl} /Ubp ^{er2cre} (conditional <i>rasa1</i> floxed allele and an inducible Cre-ERT2 transgene under the control of a ubiquitin promoter)	Lymphatic vessel abnormalities, death from chylothorax	8 weeks post-tamoxifen administration to adult mice; hyperplasia of initial lymphatics, dilation of initial and collecting lymphatics, contractility of conducting or collecting lymphatics, chylothorax, in some cases chylous ascites	[63]
		Complete loss of RasGAP expression	Complete loss of RasGAP expression, exon 17 spliced exclusively to exon 19 (not to other downstream exons)	[61]
		Death from chylothorax, lymphatic vessel abnormalities, increased density of lymphatic vessels within and inside the chest wall	Death by chylothorax by 8 months post-tamoxifen administration, chyle found in peritoneal cavity, dye injected into the footpads/tail leaked into peritoneal cavity instead of draining into thoracic duct, dye did not follow the typical route of lymph drainage to inguinal LN (drained into an expansive lymph vessel network instead of taking a direct route to the LN), increased density of lymphatic vessels	[62]

RASAI Animal Models				
Animal Model	Genotype	Phenotype	Description	Ref
		Impaired development of LV valves, chylothorax	within and inside chest wall facing the pleural cavity, dilated/increased number of lymphatic vessels in skin and lungs, no pulsatile activity in hyperplastic draining lymphatics, majority of their lymph vessels not lack smooth muscle	
			TM injection at 9 weeks: LVs ineffective as pumps, downstream valves re-opened inappropriately during the diastolic phase of vessel contraction and were unable to close properly in response to adverse intra-luminal pressure leading to back-leak, LV leaflets contained reduced amounts of ECM proteins; TM between E12.5-E14.5: complete absence of the LV system at E19.5 as a result of LEC apoptosis, TM at E15.5: mesenteric LVs that lack valves and appear dilated with increased # of cells	[64]
Mouse	RASAI ^{fl/fl} /Prox1 ^{er2cre} (conditional floxed rasal allele and lymph vessel specific inducible CreERT2 transgene)	Lymphatic vessel abnormalities, chylothorax	TM injection at 2 months: dilated increased number of lymphatic vessels in skin, no evidence of early lethality, chylothorax, or chyloous ascites; TM at E15: chylothorax, death, lymph vessel hyperplasia due to increased LEC proliferation, phenotype rescued by administration of anti-VEGF-3 antibody	[62]
		Lymphatic valve dysfunction and LV leaflet changes	The majority of valves dysfunctional including both mesenteric and popliteal LV valves, LV leaflets contained reduced amounts of ECM proteins	[64]
Mouse	RASAI ^{fl/R780Q} /Ubp ^{er2cre} (conditional floxed rasal knock in allele with an inducible CreERT2 transgene)	Chylothorax, LV hyperplasia	TM injection at E15.5: death by to chylothorax, mesenteric LVs lack valves, sometimes appear dilated with increased numbers of cells	[62]
Zebrafish	Knock down of RASAI homologs using antisense MOs	Cell death in the head region, improper formation of the caudal vascular plexus	Widespread cell death in head region and improper formation of caudal vascular plexus resulting in enlarged caudal vascular deformity (caused arterial blood flow to abruptly return to the posterior cardinal vein), phenotype rescued by administration of PI3K-TORC1 chemical inhibitors (but not MEK1/2 inhibitor)	[8]

Table 2. EphrinB2-EphB4-RASA1 Mutations Identified in Human Patients With Vascular Disorders

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>EFNB2</i>	Transmitted	c.830G>A	p.Arg277His	VOGM, hip dysplasia, neurodevelopmental delay, seizures, cutaneous capillary malformations	[16]
<i>EFNB2</i>	Transmitted	13q33.2-q33.3 deletion (entire EFNB2)		Proband 1: Pulmonary valve stenosis, developmental delay, pyramidal spasticity, myoclonic epilepsy heterogeneous retinal pigmentation, abnormal visually evoked response, bilateral sensorineural hearing loss, enophthalmia, hypertelorism; Proband 2: tetralogy of Fallot, developmental delay, enophthalmia, hypertelorism, Proband 3 (mother): learning disability, progressive bilateral hearing loss; Proband 4 (maternal grandfather): progressive bilateral sensorineural hearing loss present before age 18	[15]
<i>EFNB2</i>	<i>De novo</i>	c.239T>G	p.Met80Arg	Anal stenosis with fistula, hypoplastic center ventricle, hypotelorism, blepharimosis, mild psychomotor delay	
<i>EPHB4</i>	Transmitted	c.33delG	p.Leu12Trpfs*10	Cutaneous capillary malformation	[31]
<i>EPHB4</i>	Transmitted	c.123+3G>C; r.123_411del	p.Trp18Valfs*16	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.175G>A	p.Glu59Lys	Cutaneous capillary malformation, Parkes Weber Syndrome(left arm)	
<i>EPHB4</i>	Transmitted	c.221G>C	p.Arg74Pro	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.345_347delCTA	p.Tyr115del	Cutaneous capillary malformation, AVM (right face)	
<i>EPHB4</i>	<i>De novo</i>	c.389G>A	p.Trp130X	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.412-1G>T	Splicing	Cutaneous capillary malformation	
<i>EPHB4</i>	Parental DNA not sequenced	c.418_485delGTC AAinsTT	p.Val161_Lys162delinsLeu	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.447_448insGAA G	p.Arg150Glufs*74	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.560T>C	p.Leu187Pro	Cutaneous capillary malformation, Parkes Weber Syndrome(left leg)	
<i>EPHB4</i>	Transmitted	c.632_633delITG	p.Val211Alafs*11	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.730C>T	p.Gln244X	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.802T>C	p.Cys268Arg	Cutaneous capillary malformation, Parkes Weber Syndrome(right leg)	
<i>EPHB4</i>	Family 1: <i>De novo</i> , Family 2: Transmitted	c.1054C>T	p.Arg352X	Family 1: Cutaneous capillary malformation, Family 2: Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1077_1081delCC GCT	p.Leu59fs*19	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1123G>T	p.Gly375X	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.1177_1177delG	p.Val393Ffs*17	Cutaneous capillary malformation	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>EPHB4</i>	Transmitted	c.1291C>T	p.Arg431X	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1406T>G	p.Val469Gly	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1423-6G>A; r.1422_1423insAC AG	p.Gly475Thrfs*39	Cutaneous capillary malformation	
<i>EPHB4</i>	Family 1: transmitted, Family 2: unsure, Family 3: Transmitted	c.1546G>A	p.Gly516Arg	Family 1: cutaneous capillary malformation, AVM (left face and lip), Family 2: Cutaneous capillary malformation, AVM (left face), Family 3: Cutaneous capillary malformation	
<i>EPHB4</i>	Family 1: Unsure, Family 2: Unsure	c.1558C>T	p.Gln520X	Family 1: Cutaneous capillary malformation, Family 2: Cutaneous capillary malformation	
<i>EPHB4</i>	<i>De novo</i>	c.1588+5G>C	Splicing	Cutaneous capillary malformation, Parkes Weber Syndrome(right arm)	
<i>EPHB4</i>	Transmitted	c.1615delG	p.Ala539fs*2	Cutaneous capillary malformation, Parkes Weber Lesion (right leg)	
<i>EPHB4</i>	Transmitted	c.1733_1734insA	p.Gly579Argfs*10	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1788>G	p.Tyr596X	Cutaneous capillary malformation, Parkes Weber Syndrome(left arm)	
<i>EPHB4</i>	Transmitted	c.1870+2T>C	Splicing	Cutaneous capillary malformation, AVM (left face)	
<i>EPHB4</i>	Parental DNA not sequenced	c.1962-1G>A	Splicing	Cutaneous capillary malformation, AVM (left face)	
<i>EPHB4</i>	Unsure	c.1966C>T	p.Arg656Trp	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.1990G>A	p.Glu664Lys	VOGM, cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.2037_2038insT	p.Glu680X	Cutaneous capillary malformation, AVM (left arm), Parkes Weber Syndrome (right leg)	
<i>EPHB4</i>	Parental DNA not sequenced	c.2173G>A	p.Ala725Thr	Cutaneous capillary malformation	
<i>EPHB4</i>	Family 1: Transmitted, Family 2: unsure	c.2215C>T	p.Arg739X	Family 1: Cutaneous capillary malformation, family 2: cutaneous capillary malformation	
<i>EPHB4</i>	Family 1: <i>De novo</i> , Family 2: Transmitted	c.2233A>G	p.Asn745Asp	Family 1: cutaneous capillary malformation, Family 2: Cutaneous capillary malformation, Parkes Weber Syndrome (right arm)	
<i>EPHB4</i>	Transmitted	c.2365C>T	p.Pro789Ser	Cutaneous capillary malformation, AVM (left face)	
<i>EPHB4</i>	Transmitted	c.2366C>G	p.Pro789Arg	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.2379delC	p.Ala793fs*16	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.2418C>T	p.Tyr806X	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.2419G>A	p.Gly807Arg	Cutaneous capillary malformation, AVM (left ear)	
<i>EPHB4</i>	Transmitted	c.2458C>A	p.Pro820Thr	Cutaneous capillary malformation	
<i>EPHB4</i>	Transmitted	c.2459C>T	p.Pro820Leu	Cutaneous capillary malformation, AVM (lips)	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>EPHB4</i>	Unsure	c.2484+1G>A	Splicing	D8-L1 perimedullar AVF, cutaneous capillary malformation	[16]
<i>EPHB4</i>	Unsure	c.2512C>T	p.Arg838Trp	Cutaneous capillary malformation	
<i>EPHB4</i>	Unsure	c.2533T>C	p.Cys845Arg	Cutaneous capillary malformation, AVM (face and upper lip)	
<i>EPHB4</i>	<i>De novo</i>	c.2567G>A	p.Cys856Tyr	Cutaneous capillary malformation	
<i>EPHB4</i>	Family 1: Parental DNA not sequenced, Family 2: Transmitted	c.2590C>T	p.Arg846Trp	Family 1: Cutaneous capillary malformation, Family 2: Cutaneous capillary malformation	
<i>EPHB4</i>	Parental DNA not sequenced	c.2621T>C	p.Leu874Pro	Cutaneous capillary malformation, AVM (left face)	[16]
<i>EPHB4</i>	Transmitted	c.1295_1296del	p.Glu432fs1	VOGM, neurodevelopmental delay, seizures	
<i>EPHB4</i>	Transmitted	c.1526C>G	p.Ala509Gly	VOGM, Sternum excavatum	
<i>EPHB4</i>	Transmitted	c.1950G>T	p.Lys650Asn	VOGM, neurodevelopmental delay	
<i>EPHB4</i>	Transmitted	c.2599T>C	p.Phe867Leu	VOGM, cryptorchidism, strabismus, cerebral palsy	
<i>EPHB4</i>	Transmitted	c.319T>C	p.Cys107Arg	VOGM	[33]
<i>EPHB4</i>	<i>De novo</i>	c.570dupG	p.His191Alafs*32	Cutaneous capillary malformations, VOGM	
<i>EPHB4</i>	Transmitted	c.2484+1G>T	p.Met814_Val829del	Cutaneous capillary malformations, VOGM	
<i>EPHB4</i>	Transmitted	c.2484+2insT	Splicing	Neonatal cardiac failure, VOGM	
<i>EPHB4</i>	Transmitted	c.2609T>A	p.Val870Glu	Prenatal hydrocephaly and leukomalacia, VOGM, pregnancy terminated	
<i>EPHB4</i>	Familial	C.2334+1G>C	Splicing	Central conducting lymphatic anomaly	[27]
<i>EPHB4</i>	<i>De novo</i>	c.2405A>G	p.Asp802Gly	Cutaneous capillary malformations	[32]
<i>RASA1</i>	Transmitted	c.2603+5G>C	Splicing	Cutaneous capillary malformation	[42]
<i>RASA1</i>	Transmitted	c.616_620del	p.Ile206fs	Cutaneous capillary malformation, lymphedema	[63]
<i>RASA1</i>	Transmitted	c.3070A>G	p.Lys1024*	Proband: Asymmetric ears, erythema on head, neck, parotid area, ear, left forearm, and right knee, hypertrophy in epididymis, small tufted capillary malformations in superficial dermis, abnormal networks from ECA in right auricular region and neck cutaneous tissue; Father (carrier): erythema on neck, back, chest, waist, abdomen, right D5 dermatoma	[47]
<i>RASA1</i>	<i>De novo</i>	5q14.3 deletion (entire <i>RASA1</i>)		Cutaneous capillary malformation	[78]
<i>RASA1</i>	Familial	c.1678G>T	p.Glu560X	Mental and motor retardation, epilepsy	[55]
<i>RASA1</i>	Familial	c.734_737delinsAA A	p.Arg245fs*8	Cutaneous capillary malformation	[43]
<i>RASA1</i>	Transmitted	c.2927del	p.Asn976Metfs*20	Cutaneous capillary malformation, AVM (face)	[45]

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Familial	association with 5q14-21(entire RASA1) based on linkage analysis in 13 families		Cutaneous capillary malformation; 3 families have individuals with AVM	[48]
<i>RASA1</i>	Familial	association with RASA1 based on 6 families		Cutaneous capillary malformation; all 6 families have individuals with AVM	[40]
<i>RASA1</i>	Transmitted	c.1491_1492delAG	p.L498Lfs*2	Proband: micrognathia, bulbous nasal tip, multiple capillary malformations, patent foramen ovale, dilated right atrium, significant dilation of innominate, right carotid, and right subclavian arteries, lipoma above the left iliac crest, infratentorial AVM, complex posterior fossa AVF, multiple tortuous vascular structures in anterior thecal sac, mild delays in fine motor skills, mild stable left ventricular dilation; Mother (carrier): multipel cutaneous CMs, childhood febrile seizures, frequent bifrontal headaches	[79]
<i>RASA1</i>	Unsure	c.1279C>T	p.Arg427X	Cutaneous capillary malformation, AVM (intercranial)	[54]
<i>RASA1</i>	Transmitted	c.2125C>T	p.Arg709X	Cutaneous capillary malformation, VOGM, hydrocephalus	
<i>RASA1</i>	Transmitted	c.2707C>T	p.Arg903X	Cutaneous capillary malformation, AVM (intercranial)	
<i>RASA1</i>	Transmitted	c.1583A>G	p.Tyr528Cys	Multiple blanching telangiectasias on palms, fingers, tongue, and toes, reddened nasal mucosa with daily epistaxis, family history of epistaxis	[80]
<i>RASA1</i>	Transmitted	c.3043G>T	p.E1015X	Telangiectasias, epistaxis, family history of telangiectasias	
<i>RASA1</i>	Familial	c.829-9G>A	Splicing	Cutaneous capillary malformation, AVM (outer ear)	[49]
<i>RASA1</i>	Familial	c.853C>T	p.Arg285X	Cutaneous capillary malformation	
<i>RASA1</i>	Familial	c.2252_2255dupTC AT	p.Leu751Leufs*15	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1343_1346delins CATG	p.Gln448 Valfs*44 9	Cutaneous capillary malformation	[50]
<i>RASA1</i>	Parental DNA not sequenced	c.2119C>T	p.Arg707Cys	VOGM	[56]
<i>RASA1</i>	Parental DNA not sequenced	c.2912T>C	p.Leu971Ser	VOGM	
<i>RASA1</i>	Transmitted	c.2119C>T	p.Arg707Cys	Choroidal VOGM in two consecutive pregnancies of unaffected carrier mother	[81]
<i>RASA1</i>	Transmitted	c.1063A>T	p.Lys355X	Cutaneous capillary malformations, AVM (face)	[46]
<i>RASA1</i>	Transmitted	c.1269_1270insTA	p.Ile423X	Cutaneous capillary malformations, AVM (maxilla digit)	
<i>RASA1</i>	Unsure	c.1703G>A	p.Trp568X	Cutaneous capillary malformations, AVM (face), AVF (spine)	
<i>RASA1</i>	<i>De novo</i>	c.2125C>T	p.Arg709X	Cutaneous capillary malformations, AVM (neck), somatic c.1534C>T p.Arg512* mutation found in cutaneous capillary malformation epithelial cells in same patient	
<i>RASA1</i>	Unsure	c.2467dupG	p.Glu823Glyfs*6	20 CMs (macular and pink to brown in color with pale halo) mainly affecting face and neck	[82]

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Transmitted	c.1248T>G	p.Tyr416X	Proband: cardiomegaly, LVH, center extremity lymphedema and overgrowth of left thigh, inability to take food by mouth, multiple capillary malformations with hypertrophy of neck and lower face, recurrent left chylothoraces, intermittent pericardial effusions, congenital hypothyroidism, persistent compensatory severe metabolic alkalosis, GERD; Mother (carrier): multifocal capillary malformations with somatic c.2245C>T p.Arg749X in CM skin sample	[59]
<i>RASA1</i>	Transmitted	c.613_617delCTTA T	p.Leu205Lysfs*4	Cutaneous capillary malformation, optic glioma (before 12y), lipoma (before 10y)	[41]
<i>RASA1</i>	Unsure	c.656C>G	p.Ser219X	Cutaneous capillary malformation	
<i>RASA1</i>	<i>De novo</i>	c.806_810delTTTAC	p.Leu269Profs*11	Cutaneous capillary malformation, PKWS, cardiac overload	
<i>RASA1</i>	<i>De novo</i>	c.828+3A>T	Splicing	Cutaneous capillary malformation, AVM/AVF	
<i>RASA1</i>	Unsure	c.951dupG	p.Met318Aspfs*10	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.957G>A	p.Trp319X	Cutaneous capillary malformation, PKWS, cardiac overload	
<i>RASA1</i>	Transmitted	c.1017+1G>T	Splicing	Cutaneous capillary malformation, PKWS, cardiac overload	
<i>RASA1</i>	Transmitted	c.1192C>T	p.Arg398X	Cutaneous capillary malformation, AVM/AVF, epilepsy, hydrocephalus	
<i>RASA1</i>	<i>De novo</i>	c.1208dupC	p.Thr404Asnfs*14	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1277A>G	p.Tyr426Cys	Cutaneous capillary malformation	
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted	c.1279C>T	p.Arg427X	Family 1: Cutaneous capillary malformation, Family 2: Cutaneous capillary malformation, AVM/AVF	
<i>RASA1</i>	Transmitted	c.1332+5G>A	Splicing	Cutaneous capillary malformation	
<i>RASA1</i>	<i>De novo</i>	c.1336C>T	p.Gln446X	Cutaneous capillary malformation, PKWS	
<i>RASA1</i>	Transmitted	c.1350_1351insT	p.Asn451X	Cutaneous capillary malformation, PKWS	
<i>RASA1</i>	<i>De novo</i>	c.1362_1363insTC AGT	p.Asn455Serfs*30	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1480dupT	p.Tyr494Leufs*7	Cutaneous capillary malformation, PKWS	
<i>RASA1</i>	Unsure	c.1490T>G	p.Leu497X	Cutaneous capillary malformation, AVM/AVF	
<i>RASA1</i>	Transmitted	c.1572_1575dup	p.Ser526Metfs*8	Cutaneous capillary malformation, PKWS, cardiac overload, tetralogy of Fallot, superficial basal cell carcinoma (33y)	
<i>RASA1</i>	Unsure	c.1636C>T	p.Gln546X	Cutaneous capillary malformation, AVM/AVF	
<i>RASA1</i>	Unsure	c.1682_1683dup	p.Pro562Leufs*9	Cutaneous capillary malformation, PKWS, cardiac failure, epilepsy, hemorrhages, Atrial Septal Defect II, Patent Foramen Ovale	
<i>RASA1</i>	Transmitted	c.1698+3_1698+4i nsT	Splicing	Cutaneous capillary malformation, PKWS, pulmonary stenosis	
<i>RASA1</i>	Transmitted	c.1870C>T	p.Gln624X	Cutaneous capillary malformation, AVM/AVF	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Transmitted	c.2026C>T	p.Gln676X	Cutaneous capillary malformation	[30]
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted	c.2125C>T	p.Arg709X	Family 1: Cutaneous capillary malformation, AVM/AVF, PKWS, varicose veins, Family 2: Cutaneous capillary malformation, PKWS, cardiac failure	
<i>RASA1</i>	Transmitted	c.2184+1delG	Splicing	Cutaneous capillary malformation, PKWS, Patent Ductus Arteriosus, Atrial Septal Defect, pulmonary stenosis, tricuspid valve prolapse	
<i>RASA1</i>	Unsure	c.2185-1G>A	Splicing	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2288A>T	p.Glu763Val	Cutaneous capillary malformation, VOGM, cardiac failure, epilepsy, died rapidly after birth	
<i>RASA1</i>	Transmitted	c.2341G>T	p.Glu781X	Cutaneous capillary malformation, AVM/AVF, hydrocephalus	
<i>RASA1</i>	Transmitted	c.2365C>T	p.Arg789X	Cutaneous capillary malformation, AVM/AVF, angioliopoma(1y)	
<i>RASA1</i>	Transmitted	c.2422C>T	p.Gln808X	Cutaneous capillary malformation, PKWS	
<i>RASA1</i>	Transmitted	c.2450_2451delCT	p.Ser817Tyrfs*12	Cutaneous capillary malformation, AVM/AVF	
<i>RASA1</i>	Transmitted	c.2488-2A>G	Splicing	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2488-1delGTTA	Splicing	Cutaneous capillary malformation, AVM/AVF, non-small cell lung cancer (32y), hemoptysis	
<i>RASA1</i>	Unsure	c.2514_2515insA	p.Glu839Argfs*6	Cutaneous capillary malformation	
<i>RASA1</i>	<i>De novo</i>	c.2532_2536delTT AA	p.Leu845Thrfs*38	Cutaneous capillary malformation, spontaneous mutation in monozygotic twins, VOGM and cardiac failure in one	
<i>RASA1</i>	Unsure	c.2579_2582delTC AT	p.Phe860Trpfs*10	Cutaneous capillary malformation, AVM/AVF, ectopic thyroid and parathyroid	
<i>RASA1</i>	Transmitted	c.2603+1G>A	Splicing	Cutaneous capillary malformation, chylous ascites	
<i>RASA1</i>	Transmitted	c.2603+2_2603+3i nsT	Splicing	Cutaneous capillary malformation, ureteral reflux, epispadias	
<i>RASA1</i>	Transmitted	c.2603+4_2603+5i nsA	Splicing	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2603_5G>T	Splicing	Cutaneous capillary malformation, neurofibromas (53y)	
<i>RASA1</i>	Transmitted	c.3028C>T	p.Arg1010X	Cutaneous capillary malformation, vestibular schwannoma(26y)	
<i>RASA1</i>	Transmitted	c.3052delG	p.Ala1018Hisfs*6	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.365C>A	p.Ser122X	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.409dup	p.Leu137Profs*21	Cutaneous capillary malformation, AVM (right foot)	
<i>RASA1</i>	Transmitted	c.442_443delinsT	p.Ala148Trpfs*26	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.463G>T	p.Glu155X	Cutaneous capillary malformation	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted	c.475_476del	p.Leu159Glyfs*20	Family 1: Cutaneous capillary malformation, AVM (nose), renal dysplasia, left kidney ptosis, Family 2: Cutaneous capillary malformation, brain AVF (identified by chance at 12y)	
<i>RASA1</i>	Unsure	c.492C>G	p.Tyr164X	Cutaneous capillary malformation, pial AVM (deterioration in speech at 2, 5y), mental retardation	
<i>RASA1</i>	Transmitted	c.613_617del	p.Leu205Lysfs*4	Cutaneous capillary malformation, retinal vascular malformation in 2 individuals	
<i>RASA1</i>	Unsure	c.829_1G>A	Splicing	Cutaneous capillary malformation, AVM(left arm)	
<i>RASA1</i>	Family 1: <i>De novo</i> , Family 2: <i>De novo</i>	c.853C>T	p.Arg285X	Family 1: Cutaneous capillary malformation, AVM (upper lip), epilepsy, Family 2: Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.899+1G>T	Splicing	AVM (ear)	
<i>RASA1</i>	Family 1: Transmitted, Family 2: Unsure	c.1192C>T	p.Arg398X	Family 1: Cutaneous capillary malformation, pial AVM (gross motor delay at 2 y), AVM (left calf and foot), Family 2: Cutaneous capillary malformation, PKWS (left leg)	
<i>RASA1</i>	Transmitted	c.1220_1223del	p.Asn407Serfs*3	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.1290_1291del	p.Gln430Hisfs*3	Cutaneous capillary malformation, PKWS (center leg)	
<i>RASA1</i>	Unsure	c.1342C>T	p.Gln448X	Cutaneous capillary malformation, AVM (tongue)	
<i>RASA1</i>	Transmitted	c.1384_1388del	p.Thr462Profs*3	Cutaneous capillary malformation	
<i>RASA1</i>	<i>De novo</i>	c.1386_1387insCT	p.Ile463Leufs*21	Cutaneous capillary malformation, spinal AVM L1 (neurogenic bladder at 4y), atrial septal defect	
<i>RASA1</i>	Unsure	c.1440T>G	p.Tyr480X	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1453+1delG	Splicing	Cutaneous capillary malformation, spinal AVF	
<i>RASA1</i>	Unsure	c.1480dupT	p.Tyr494Leufs*7	Cutaneous capillary malformation, AVM (right foot)	
<i>RASA1</i>	Unsure	c.1494_1495dup	p.Gly499Argfs*22	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.1517_1520del	p.Tyr506Leufs*13	Cutaneous capillary malformation, PKWS (left leg)	
<i>RASA1</i>	Transmitted	c.1534C>T	p.Arg512X	Cutaneous capillary malformation, family history of VOGM with prenatal death at 32w	
<i>RASA1</i>	Transmitted	c.1583A>G	p.Tyr528Cys	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1589T>A	p.Val530Asp	Cutaneous capillary malformation, brainstem AVM (brain hemorrhage with facial palsy at 3w), left leg AVM, basal cell carcinoma in 2 individuals	
<i>RASA1</i>	Transmitted	c.1596_15797del	p.Asp532Glufs*17	Cutaneous capillary malformation, AVM (right hand)	
<i>RASA1</i>	Transmitted	c.1636C>T	p.Gln546X	Cutaneous capillary malformation	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Transmitted	c.1666_1698+15del	Splicing	Cutaneous capillary malformation, posterior fossa AVM, spinal AVF (flaccid paraplegia of lower extremities and neurogenic bladder at 6 months), family history of hydrops fetalis	
<i>RASA1</i>	Unsure	c.1698+1G>T	Splicing	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1717C>T	p.Gln573X	Cutaneous capillary malformation, spinal AVM, PKWS (right leg)	
<i>RASA1</i>	Transmitted	c.1726dup	p.Cys576Leufs*7	Cutaneous capillary malformation, AVM (left arm)	
<i>RASA1</i>	<i>De novo</i>	c.1870C>T	p.Gln624X	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.1877C>A	p.Ala626Glu	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.1989_1992del	p.Lys664Alafs*13	Cutaneous capillary malformation, AVM (center arm), macular degeneration in 1 individual	
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted, Family 3: Unsure,	c.2035C>T	p.Arg679X	Family 1: Cutaneous capillary malformation, AVM (right foot), Family 2: Cutaneous capillary malformation, AVM (right hand), Family 3: Cutaneous capillary malformation, Family 4: Cutaneous capillary malformation	
	Family 4: Transmitted				
<i>RASA1</i>	Transmitted	c.2081_2087del	p.Ser694Tyrfs*3	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2125C>T	p.Arg709X	Cutaneous capillary malformation, VOGM	
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted, Family 3: Transmitted, Family 4: <i>De novo</i>	c.2131C>T	p.Arg711X	Family 1: Cutaneous capillary malformation, AVM (lower lip), vertebral AVF (tetraplegia at 4y), Family 2: Cutaneous capillary malformation, posterior fossa AVM (engorged veins around face), family history of cerebral aneurysm, Family 3: Cutaneous capillary malformation, Family 4: Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2239C>T	p.Gln747X	Cutaneous capillary malformation, AVM (center cheek)	
<i>RASA1</i>	Unsure	c.2245C>T	p.Arg749X	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.2288A<T	p.Glu763Val	Cutaneous capillary malformation	
<i>RASA1</i>	<i>De novo</i>	c.2329G>T	p.Glu777X	Cutaneous capillary malformation, spinal artery pial fistula AVF (headaches and facial tics), right foot and calf flow lesion	
<i>RASA1</i>	Transmitted	c.2344+1G>C	Splicing	Cutaneous capillary malformation, AVM (face)	
<i>RASA1</i>	Family 1: Transmitted, Family 2: Transmitted	c.2365C>T	p.Arg789X	Family 1: Cutaneous capillary malformation, congenital heart defect, Family 2: Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.2422C>T	p.Gln808X	Cutaneous capillary malformation, AVM (right foot)	
<i>RASA1</i>	Unsure	c.2450_2451del	p.Ser817Tyrfs*12	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.2457_2460delins T	p.Leu819_Lys820de linsPhe	Cutaneous capillary malformation, AVM (face, right hand)	
<i>RASA1</i>	<i>De novo</i>	c.2500_2501del	p.Lys834Valfs*5	Cutaneous capillary malformation	
<i>RASA1</i>	Unsure	c.2532_2536del	p.Leu845Thrfs*38	Cutaneous capillary malformation, AVM (face)	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	Transmitted	c.2603+2dup	Splicing	Cutaneous capillary malformation, brain AVM, PKWS (right arm)	[30, 56]
<i>RASA1</i>	Transmitted	c.2632C>T	p.Gln878X	Cutaneous capillary malformation, PKWS (right leg, ulceration), hydrops fetalis	
<i>RASA1</i>	Transmitted	c.2707C>T	p.Arg903X	Cutaneous capillary malformation, parietal AVF (seizures at 5y)	
<i>RASA1</i>	Transmitted	c.2925+5G>C	Splicing	Cutaneous capillary malformation, spinal AVM D12-L1 (center leg paralysis at 2y)	
<i>RASA1</i>	Transmitted	c.2977del	p.Arg993Valfs*3	Cutaneous capillary malformation, VOGM (stroke), PKWS (left arm)	
<i>RASA1</i>	Transmitted	c.3024del	p.Glu1008Aspfs*16	Cutaneous capillary malformation, VOGM (stroke at 3y), developmental delay	
<i>RASA1</i>	Transmitted	c.3028C>T and c.3064G>T	p.Arg1010X and p.Gly22Cys	Cutaneous capillary malformation, pial AVM, family history of TOP at 7 months for VOGM	
<i>RASA1</i>	Unsure	c.3038_3054del	p.Ser1013Thrfs*27	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.3055C>T	p.Gln1019X	Cutaneous capillary malformation	
<i>RASA1</i>	Transmitted	c.3109_3112del	p.Gln1037Thrfs*63	Cutaneous capillary malformation, AVM (left leg), Naevus anemicus	
<i>RASA1</i>	Unsure	c.2125C>T	p.Arg709X	Heuchan: VOGM, CM-AVM rash, Revencu: cutaneous capillary malformation	[52]
<i>RASA1</i>	<i>De novo</i>	c.1386_1387insCT	p.Ile463Leufs*21	2 cutaneous capillary malformations at birth, more CMs appeared later, lower extremity weakness (non-ambulator), neurogenic bladder at 4y, AVM at conus medularis (L1)	
<i>RASA1</i>	Transmitted	c.1453+1delG	Splicing	Multifocal CMs at birth, AVF supplied by R vertebral artery and thyocervical trunk	
<i>RASA1</i>	Transmitted	c.1666_1698+15del	Splicing	Multifocal CMs at birth, flaccid paraplegia of lower extremities and neurogenic bladder at 16 months, AVM at conus medularis(L2)	
<i>RASA1</i>	Transmitted	c.1717C>T	p.Gln573X	Multifocal CMs at birth, acute sensorimotor deficits at 23y, AVM at L5-S1	
<i>RASA1</i>	<i>De novo</i>	c.2329G>T	p.Glu777X	CM on R plantar foot at birth, macular lesions appeared with time along with motor tics, AVF at level of C7-T1	
<i>RASA1</i>	Transmitted	c.1310T>G	p.Leu437Arg	Multifocal CMs on right palm, chest, and back, history of nosebleeds	
<i>RASA1</i>	Transmitted	c.1401_1402delAA	p.Thr467fs	Multifocal CMs, reddish-brown patch on head/face, extremities, AVF (high flow lesions underneath shins), bright red macule with telangiectasias on extremities	
<i>RASA1</i>	Transmitted	c.1491_1492delAG	p.Leu497fs	Large brain AVM, multifocal cutaneous vascular malformation on face, nape of neck, and occipital region	
<i>RASA1</i>	<i>De novo</i>	c.1617dupA	p.Asn539fs	AVM on right side of face and back, multifocal CMs	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	[44]
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	
<i>RASA1</i>	Transmitted	c.1771_2insC	p.Asn591fs	Brain AVM near carotid, multiple CMs near eye	

Gene	Type of Mutation	Coding Variant	Protein Variant	Phenotype	Ref
<i>RASA1</i>	<i>De novo</i>	c.1870C>T	p.Gln624X	Multifocal CMs on head, face, and chest, nose hypertrophy and telangiectasia	
<i>RASA1</i>	Transmitted	c.2026C>T	p.Gln676X	Multifocal CMs, macrocephaly	
<i>RASA1</i>	Unsure	c.2084A>T	p.His695Leu	Brain AVE, brain AVM, multifocal CMs on head/face	
<i>RASA1</i>	Transmitted	c.2225C>A	p.Ser724X	Brain AVM, multifocal CMs on head/face, trunk, and extremities, tumors (center nasal bridge and maxillary areas, center occipital lesion, multiple spots on face), macrocephaly, seizures	
<i>RASA1</i>	<i>De novo</i>	c.2239C>T	p.Gln747X	Facial AVM, multifocal CMs	