Genetic syndromes with vascular malformations – update on molecular background and diagnostics

Adam Ustaszewski, Joanna Janowska-Głowacka, Katarzyna Wołyńska, Anna Pietrzak, Magdalena Badura-Stronka

1 Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland
2 Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland
3 Department of Neurology, Poznan University of Medical Sciences, Poznan, Poland

Submitted: 19 April 2018
Accepted: 9 September 2018

Arch Med Sci
DOI: https://doi.org/10.5114/aoms.2020.93260
Copyright © 2020 Termedia & Banach

Abstract

Vascular malformations are present in a great variety of congenital syndromes, either as the predominant or additional feature. They pose a major challenge to the clinician: due to significant phenotype overlap, a precise diagnosis is often difficult to obtain, some of the malformations carry a risk of life threatening complications and, for many entities, treatment is not well established. To facilitate their recognition and aid in differentiation, we present a selection of notable congenital disorders of vascular system development, distinguishing between the heritable germinal and sporadic somatic mutations as their causes. Clinical features, genetic background and comprehensible description of molecular mechanisms is provided for each entity.

Key words: arteriovenous malformation, vascular malformation, capillary malformation, venous malformation, arterial malformation, lymphatic malformation.

Introduction

Congenital vascular malformations (VMs) are disorders of vascular architecture development. They may involve any vessel type and are accordingly classified as capillary, venous, arterial, lymphatic and arteriovenous, any of which can occur alone or in combinations. Congenital VMs are not only a cosmetic issue, as they can lead to serious or even life-threatening complications including congestive heart failure, ischemia, brain and spinal cord stroke and glaucoma. Vascular malformations are a feature of numerous genetic syndromes. Due to the overlapping symptoms, the differential diagnosis of genetic syndromes with VMs may be troublesome [1, 2]. Moreover, the effect of a particular gene mutation is often pleiotropic, giving rise to multiple phenotypes. While most well-known VM syndromes are caused by a constitutional mutation, some are revealed to result from somatic mutations, present in only a fraction of cells in the body.
Capillary malformation-arteriovenous malformation syndrome

Capillary malformations (CMs, port-wine stains) are the most common VMs, occurring in 0.3% of newborns [1]. Capillary malformations present as lightly colored, reddish to pink, flat, slow-flow cutaneous lesions in the form of irregularly shaped macules. They may be confused with other cutaneous VMs such as naevus flammeus nevatorum, commonly known as angel’s kiss or salmon patch. Unlike hemangiomas, however, capillary malformations do not diminish with age but tend to darken and thicken. Capillary malformations usually are sporadic lesions, although recently, familial occurrence and genetic syndromes presenting with CMs have been reported [3].

The recently described capillary malformation-arteriovenous malformation (CM-AVM) syndrome is an underdiagnosed condition, often considered a sporadic collection of infantile hemangiomas or “port-wine stain”. The skin changes typical for CM-AVM syndrome are acquired macules or papules that are multifocal (present in more than one region or location of the body), small (ranging from less than 1 cm to 3 cm in diameter), round to oval in shape and pink to red in color [2]. They are often surrounded by a white halo suggesting vascular steal and show arterial flow on Doppler ultrasound. The CM-AVM is usually associated with high-flow arteriovenous malformations (AVMs) or arteriovenous fistulas (AVFs) in muscle, skin and other tissues (intracranial, intraspinal and interosseous AVMs have been described) [4]. Chee et al. reported on patients with skin CMs and nontraumatic cerebral hemorrhage due to intracranial AVM [5]. The authors estimate the frequency of the CM-AVM syndrome in patients with cerebral AVMs at between 15% and 50% [5]. Boon et al. suggested that patients with multiple atypical CMs should be assessed for high-flow lesions such as AVMs or AVFs [6]. In these patients, neuroimaging screening should be considered.

The differential diagnosis of CM-AVM syndrome should include Klippel-Trénaunay-Weber syndrome, Parkes Weber syndrome, hereditary hemorrhagic telangiectasia (HHT; Rendu-Osler-Weber syndrome) and hereditary benign telangiectasia (HBT), which is a mild variant of the HHT, and Sturge Weber syndrome. All these syndromes feature cutaneous VMs.

Mutations of the RASA1 gene are responsible for several forms of VMs: CMs, AVMs, AVFs, singularly and in complex combinations, as in the rare Parkes Weber syndrome (PWS) [7, 8]. In 2002, two loci for hereditary CMs were identified on chromosome 5 (5q14-21 and 5q13-22). A year later, RASA1 (RAS p21 protein activator 1; OMIM 139150) was identified as a candidate gene for atypical CMs with AVMs and AVFs; what is more, these alterations have been also observed sporadically in PWS [1, 9, 10]. This constellation of VMs related to RASA1 mutations was named capillary malformation – arteriovenous malformation syndrome (CM-AVM) [1]. Subsequently, a number of studies reported on atypical CMs caused by RASA1 mutations [2, 3, 5, 6, 11–23]. It was demonstrated that alterations of RASA1 might also act as an additional factor in other vascular development disorders, including vein of Galen malformations (VOGMs) and HHT [24–26]. RASA1 encodes RAS p21 protein activator 1 (p120-RasGAP) consisting of five domains. It is a negative regulator of the Ras/MAPK and MAPK/ERK pathway, converting the Ras protein into its inactive GDP-coupled form [6, 27]. The active GTP Ras form interacts with the Raf protein that is responsible for phosphorylation of proteins involved in cell growth, proliferation and differentiation (Figure 1) [28, 29]. The changes in p120-RasGAP concentration levels have an impact on angiogenesis [30]. The CM-AVM syndrome follows the pattern of autosomal dominant inheritance with incomplete penetrance and variable phenotype, in part due to the localized nature of VMs [31]. Interestingly, De Wijn reported 100% penetrance in patients with RASA1 deletion [14] while Carr et al. estimated the penetrance at 20% [32]. Eerola et al. suggested a predominant inheritance pattern (similar to the one in glomuvenous and cerebral cavernous malformations) that requires a somatic second hit mutation, resulting in complete loss of function of the p120 Ras-Gap protein [1].

Arguably, other non-synonymous alterations present in other members of Ras/MAPK pathway may lead to vascular development disorders. Indeed, published data indicate that inactivating mutation of the EPHB4 gene which recruits and activates RASA1 may result in CM-AVM syndrome in non-RASA1 patients [39–40]. The transmembrane kinase receptor EPHB4 is required for the proper functioning of several processes such as intervening capillary beds and morphogenesis, which are crucial for the development of the vascular system [39, 41]. However, mutations in RASA1 are still considered the main factor responsible for the CM-AVM syndrome. Table I contains a brief summary of molecular screening results in patients with CM-AVM syndrome.

Hereditary hemorrhagic telangiectasia

Owing to the similar clinical features, HHT may be mistaken for other CM-AVM syndromes. It has an autosomal dominant inheritance with high penetrance but considerable intrafamilial variability. The incidence of HHT is estimated at 1/8,000 in infants [46]. The hallmark of the syndrome is...
multiple AVMs without intervening capillaries, resulting in direct connections between arteries and veins, affecting mucosa, skin and internal organs with co-occurrence of telangiectases. The HHT presentation is age-dependent. While the first symptoms are usually reported in adolescence, infants may also be affected. Commonly, HHT initially manifests with epistaxis and cutaneous or mucosal CMs that rupture and bleed after a minor trauma [47]. Ninety-five percent of people with HHT suffer from recurrent epistaxis and 90% of affected individuals experience them before the age of 30 years [48]. The telangiectasias frequently affect the tongue, lips, buccal mucosa, face, chest, fingers, ears and conjunctivae. In adulthood, telangiectasias develop in gastrointestinal mucosa. Stomach and duodenal mucosa is most frequently affected. It is estimated that approximately 25% of patients with HHT suffer from gastrointestinal bleeding, which usually occurs after the age of 50 years [49, 50]. The AVMs localized in internal organs may have life-threatening manifestations. They are commonly found in lungs, liver and brain. In most cases, brain AVMs are already present at birth. They may manifest with intracerebral bleeding or epileptic seizures. It is estimated that 30–50% of patients with HHT syndrome bear pulmonary arteriovenous malformations [51]. Their rupture is the cause of hemoptysis. Pulmonary AVMs may also constitute a source of embolic material and cause vascular embolic incidents, including transient ischemic attacks (TIAs) and stroke, as well as brain and other organ abscesses; they may also lead to migraine-like headaches and polycythemia. Hepatic AVMs are usually asymptomatic. The common manifestations of hepatic vascular abnormalities are portal hypertension, high output heart failure and biliary disease. In the Lanora et al.

Figure 1. A simplified model of Ras activation as a part of the MAPK/ERK signaling pathway. Ras proteins play a key role in regulation of numerous subsequent signaling cascades and pathways. Mutations in the RASA1 gene may cause a change in Ras activity, resulting in development of CM-AVM syndrome and PWS. There is some evidence that KTWS might also be caused by abnormal Ras function. The complete genetic background of KTWS remains unknown [33–38]
study, 74% of patients were diagnosed with hepatic vascular malformations when systematic liver imaging (CT scan) was performed [52]. The other complications of HHT syndrome are anemia and pulmonary hypertension.

Hereditary hemorrhagic telangiectasia, also known as the Osler-Weber-Rendu disease (HHT; OMIM 187300), has an autosomal dominant inheritance pattern, like the other CM-AVM syndromes [53]. While, in fact, RASA1 mutations can cause HHT phenotype, making it part of the CM-AVM syndrome spectrum, current research indicates several other genes involved in HHT development and progress [47, 51]. So far, the reported genes include ENG, ACVRL1, GDF2 and SMAD4 [24, 47, 51, 53–58]. Interestingly, all these genes are members of the same signaling pathway (TGF-β) and apparently, various alterations in this pathway may lead to different variants of the disease (Figure 2) [54].

The most recognized causative gene for HHT, with a large number of known mutations, is ENG (OMIM 131195) [54, 60–62]. It encodes an auxiliary transmembrane glycoprotein that is responsible for signal transduction [63]. ENG is located in the 9q34 region. The product of expression (endoglin protein) is a receptor for TGF-β signaling proteins [64]. The main function of endoglin is signal modulation between ALK1 and ALK5 receptors that are responsible for the appropriate function of the Smad1/5/8 signaling pathways [63]. Those pathways are related to angiogenesis, the immune system response and cellular proliferation processes [65, 66]. Higher expression of ENG is noted in tumors [67]. In addition, increased levels of ENG expression accompany wound healing and tissue inflammation in general [68]. It has been established that mutations in ENG correlate with HHT type 1 [69].

Another gene widely described in the context of HHT is ACVRL1, also known as ALK1 (OMIM 601284). It encodes the anaplastic lymphoma receptor tyrosine kinase [60, 70]. It was presumed that alterations of this gene may lead to HHT type 2 [71]. The gene is located in region 2p23.2-p23.1. The ACVRL1 product is a component of the protein complex involved in the interaction with TGF-β family signaling proteins [72]. The ACVRL1
protein influences signaling pathways similarly as endoglin. Both *ENG* and *ACVRL1* are involved in modulation of the endothelial cell response that is responsible for angiogenesis and vascular development [73]. Several studies concerning the prevalence of mutations of these two genes in various patient cohorts have been published (Table II).

The genetic background of the other HHT types is not well understood. However, it was shown that anomalies of BMP9 protein, a product of the *GDF2* gene, may result in HHT type 5 [54, 74]. BMP9 was suggested to act as an antimetastatic factor [75]; on the other hand, it may be responsible for survival and intensified proliferation of hepatocellular carcinoma cells [76]. BMP9 interacts with both endoglin and ACVRL1, which explains the HHT phenotype in *GDF2* mutations [54, 74].

Another noteworthy gene is *SMAD4*, which also plays a role in angiogenesis and vascular remodeling as a member of the TGF-β signaling pathway [77]. Studies have demonstrated that mutations in *SMAD4* may result in HHT, but also thoracic aortopathy and autosomal dominant cancer predisposition syndrome (juvenile polyposis syndrome – JPS) [78]. These presentations can overlap, as some patients exhibit both typical HHT symptoms and JPS [78, 79]. Because of these ominous co-morbidities, cautious radiological surveillance was suggested in *SMAD4* and *GDF2* mutation carriers [79].

**Parkes Weber syndrome**

Parkes Weber syndrome (PWS) is characterized by a cutaneous, red or pink, large, flat patch with underlying quiescent AVMs and extremity overgrowth that affects both bones and soft tissues. It is due to the presence of multiple AVFs along the affected extremity. These AVFs are readily detected by Doppler ultrasound or magnetic resonance angiography [85]. As a rule, the lower extremities are
more often affected than the upper extremities. The AVFs usually develop in adolescence, spontaneously or after trauma, including any surgical procedures on the affected limb. The VMs are usually present from childhood. The angiogram reveals areas of hypervascularization with enlarged vessels.

Common life-threatening complications in PWS include bleeding and congestive heart failure [86]. RASA1 mutations are frequently described in patients with prior diagnosis of PWS. Several authors have reported on patients with atypical, multifocal CMs and findings suggestive of PWS, who had a detectable RASA1 mutation [1–3, 15]. However, a recent study has shown that symptoms typical for PWS combined with AVM may occur without alterations of RASA1 [87]. In fact, patients with PWS without CMs are unlikely to have a mutation in RASA1 [26]. Overall, RASA1 mutations account for about 12% of PWS causes [1, 15, 26].

The genetic background of PWS without CMs currently remains unknown. A specific autosomal dominant mutation of the AGGF1 gene, c.397G>A, p.Glu133Lys, has been suggested as a putative causal factor for PWS [88]. In addition, it is believed that alterations in genes that are members of PI3K may lead to some sporadic cases of PWS [89]. Little is known about the actual inheritance pattern and exact incidence of this disease.

Cerebral cavernous malformation

Cerebral cavernous malformations (CCMs; OMIM 116860) are VMs ominously localized within the central nervous system. It is estimated that CCMs

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Analyzed gene</th>
<th>Number of screened sporadic cases and individuals with positive family history</th>
<th>Individuals screened for ENG and ACVRL1 mutations</th>
<th>Number of mutations</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesca et al., 2004 [80]</td>
<td>ENG</td>
<td>Number of individuals with positive family history not shown</td>
<td>34/160</td>
<td>34</td>
<td>Each proband fulfilled at least three criteria characteristic for HHT (epistaxis, telangiectasias, visceral lesions, positive family history)</td>
</tr>
<tr>
<td>Schulte et al., 2005 [81]</td>
<td>ENG</td>
<td>28 sporadic cases and 65 individuals with positive family history</td>
<td>16/63</td>
<td>14</td>
<td>HHT</td>
</tr>
<tr>
<td>Bossler et al., 2006 [82]</td>
<td>ENG</td>
<td>121 of 200 tested individuals have positive family history</td>
<td>77/200</td>
<td>63</td>
<td>Each proband fulfilled at least one criterion characteristic for HHT (epistaxis, telangiectasias, AVMs, positive family history)</td>
</tr>
<tr>
<td>Olivieri et al., 2007 [83]</td>
<td>ENG</td>
<td>123 of 137 tested individuals have positive family history, other cases were characterized as sporadic</td>
<td>29/137</td>
<td>26</td>
<td>HHT</td>
</tr>
<tr>
<td>Plumitallo et al., 2018 [84]</td>
<td>ENG</td>
<td>All individuals belong to one family</td>
<td>3/3</td>
<td>1</td>
<td>HHT</td>
</tr>
</tbody>
</table>

*aNumber of symptomatic individuals subjected to molecular testing. *Individuals with mutation in specific gene. *Number of detected mutations (both novel and previously described). HHT – hereditary hemorrhagic telangiectasia, AVMs – arteriovenous malformations.
are present in 0.5% of the population worldwide. The thin-walled capillaries of CCMs are prone to traumatic rupture due to the lack of elastic fiber support. Cerebral cavernous malformations can remain clinically silent or be symptomatic. When symptomatic, CCMs present with headache, seizures and focal neurological deficits. Intracranial hemorrhage may occur. Unlike AVMs, CCMs are low flow malformations and are thus notoriously undetectable in angiography. Sometimes, computed tomography reveals calcifications within the vascular lesions, but the study of choice is magnetic resonance imaging [90, 91].

So far, research on molecular causes of CCMs has revealed three genes associated with this disorder: KRIT1, CCM2 (MGC4607) and PDCD10, which are located in 7q21.2, 7p13 and 3q26.1, respectively [92]. The KRIT1 (Krev interaction trapped 1) gene was identified in CMM type 1, CCM2 (malcavernin) mutations have been described in CCM type 2 and CCM3 (PDCD10) gene mutations in CCM type 3 (Figure 3) [93–96]. It has been proved by several authors that these three genes play a key role in mechanisms related to vascular development and angiogenesis. A summary of KRIT1, MGC4607 and PDCD10 mutation screening studies in CCM is presented in Table III.

The products of these genes are auxiliary membrane proteins that cooperate to secure the interaction between endothelial cells in order to prevent blood leakage [99, 100]. Stockton et al. demonstrated that KRIT1 and CCM2 are suppressors of the signaling protein RhoA [97, 101]. They inhibit the activity of Rho kinase (ROCK), a RhoA effector, in order to stabilize and strengthen the intercellular interactions of the endothelial cells. The RhoA/Rho kinase pathway is responsible for cell contraction and migration, and it also supports cellular adherence by controlling the cytoskeletal organization [99, 102, 103]. The KRIT1 migrates from the nucleus to the cytosol to recruit the elements necessary for cell-cell junctions [99, 104]. The CCM2 protein is responsible for transportation of KRIT1 from the nucleus to the cytosol. Depletion of CCM2 disrupts the translocation of KRIT1 [105]. CCM2 binds to E3 ubiquitin ligase (Smurf1), decreasing the expres-

Figure 3. A model of CCMs related proteins' interaction with VE-cadherin, HEG1 and integrin-β1. They are responsible for coupling of extracellular and intracellular signaling pathways. Alterations in KRIT1, PDCD10 and CCM2 may lead to CCMs type 1, 3 and 2, respectively [97, 98]
### Table III. Mutations in **KRIT1**, **MGC4607** and **PDCD10** genes in different cohorts of patients with cerebral cavernous malformations

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Analysed gene</th>
<th>Number of screened sporadic cases and individuals with positive family history</th>
<th>Individuals screened for <strong>KRIT1</strong>, <strong>MGC4607</strong> and <strong>PDCD10</strong> mutations</th>
<th>Number of mutations</th>
<th>Criteria for molecular testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patients with mutation1/ All symptomatic patients2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stahl et al., 2008 [116]</td>
<td>KRIT1</td>
<td>16 individuals with positive family history (mutation found in 15)</td>
<td>14/28</td>
<td>14</td>
<td>Multiple lesions (sporadic cases and cases with positive family history)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 sporadic cases (mutation found in 8)</td>
<td>8/28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MGC4607</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD100</td>
<td></td>
<td>1/28</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cigoli et al., 2014 [117]</td>
<td>PD100</td>
<td>Exact number of individuals with positive family history not shown</td>
<td>11/87</td>
<td>8</td>
<td>At least one affected relative and/or multiple CCMs</td>
</tr>
<tr>
<td>Mondéjar et al., 2014 [118]</td>
<td>KRIT1</td>
<td>231 individuals with positive family history (mutation found in 65)</td>
<td>53/254</td>
<td>20</td>
<td>At least one affected relative and/or multiple CCMs</td>
</tr>
<tr>
<td></td>
<td>MGC4607</td>
<td></td>
<td>26/254</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD100</td>
<td></td>
<td>5/254</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Spiegler et al., 2014 [112]</td>
<td>KRIT1</td>
<td>63 familial cases (mutation found in 55)</td>
<td>48/105</td>
<td>30</td>
<td>Multiple lesions in sporadic case or in patients with positive family history</td>
</tr>
<tr>
<td></td>
<td>MGC4607</td>
<td></td>
<td>14/105</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD100</td>
<td></td>
<td>17/105</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Cigoli et al., 2015 [119]</td>
<td>MGC4607</td>
<td>6 individuals from one family</td>
<td>5/5</td>
<td>1</td>
<td>Multiple lesions and positive family history</td>
</tr>
<tr>
<td>Hirota et al., 2016 [120]</td>
<td>KRIT1</td>
<td>12 individuals with positive family history (from three different families) have been tested</td>
<td>5/6</td>
<td>3</td>
<td>CCMs and spinal cavernous malformations diagnosed using MRI</td>
</tr>
<tr>
<td></td>
<td>MGC4607</td>
<td></td>
<td>0/6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD100</td>
<td></td>
<td>0/6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Scimone et al., 2017 [111]</td>
<td>KRIT1</td>
<td>4 individuals with positive family history, 12 sporadic cases where 3 manifest multiple lesions</td>
<td>6/7</td>
<td>2</td>
<td>Multiple lesions and positive family history</td>
</tr>
<tr>
<td></td>
<td>MGC4607</td>
<td></td>
<td>3/7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD100</td>
<td></td>
<td>0/7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Yang et al., 2017 [121]</td>
<td>KRIT1</td>
<td>21 individuals from 5 families</td>
<td>7/12</td>
<td>0</td>
<td>Multiple CCMs</td>
</tr>
</tbody>
</table>

*Number of symptomatic individuals subjected to molecular testing. 1Individuals with mutation in specific gene. 2Number of detected mutations (both novel and previously described). CCMs – cerebral cavernous malformations, MRI – magnetic resonance imaging.
sion of RhoA [101]. CCM3 (OMIM 609118) encodes a protein that interacts with CCM2 and serine/threonine kinase 25 (STK25), to form a three-protein complex which may be essential for vascular development. It was suggested that its inactivation may initiate CCM formation [106]. The main function of PDCD10 might be the promotion of cell apoptosis [107, 108]. The PDGFC gene expression product has a significant role in RhoA activation [109, 110]. Loss of the aforementioned protein has also been described in the context of neuronal development where it was linked to cytoskeleton defects [110]. The incidence of mutations in KRIT1, CCM2 and PDGFC in CCMs is 56%, 16% and 17% respectively [111, 112].

Regarding deletions in genes mentioned above, the distribution appears to differ among patients of different origins. When two cohorts of patients were tested using multiplex ligation-dependent probe amplification technique, it was found that proportions of deletions for KRIT1, CCM2 and PDGFC in an American group of patients were 5%, 95% and 0%, while the distribution in Italians was 50%, 40% and 10% respectively [113]. Genomic rearrangements of the region including CCM2 are a suspected cause of familial CCMs as well [114]. Another novel putative mechanism of CCM pathogenesis is related to polymorphisms of TLR4 and CD14 genes. It has been suggested that their activation due to Gram-negative bacteria may accelerate the development of CCMs [115].

Glomuvenous malformation

Glomuvenous malformations (GVMs, OMIM 13800), often described as glomangiomas or “venous malformations with glomus cells”, should be distinguished from mucocutaneous cavernous hemangiomas. The GVMs are commonly present at birth or appear during the first two decades of life. The hallmark features of the GVMs are their cobblestone appearance, hard consistency and tenderness. The histopathology examination shows pathognomonic rounded cells (glomus cells) around the blood-filled cavities. The glomus cells stain positively for smooth-muscle α-actin and vimentin, which suggests their muscular origin [122]. It is claimed that glomus cells may be incorrectly or incompletely differentiated vascular smooth muscle cells [122].

The occurrence of glomuvenous malformations (GVMs) was found to be associated with mutations in the GLMN gene (OMIM 601749), which is located in region 1p22 [123, 124]. The product of GLMN, glomulin, is a competitive inhibitor of a group of ubiquitin ligases (E3s), cullin-RING ligases (CRLs; Figure 4). These multisubunit enzymes contain one of two types of RING protein, RBX1 or RBX2, by which they interact with a ubiquitin-conjugating enzyme (E2) to complete ubiquitination. Glomulin binds to the RING domain of RBX1, acting as a regulator of ubiquitination by preventing the E2 accession to the SCF complex, and thus by inhibiting the E3 ligase activity. While the RING E3 ligase subunit of CRL may contain either RBX1 or RBX2, by which they interact with a ubiquitin-conjugating enzyme (E2) to complete ubiquitination. Glomulin binds to the RING domain of RBX1, acting as a regulator of ubiquitination by preventing the E2 accession to the SCF complex, and thus by inhibiting the E3 ligase activity. While the RING E3 ligase subunit of CRL may contain either RBX1 or RBX2, it appears that glomulin’s tri-HEAT-like platform binds exclusively to RBX1’s E2-interacting surface. The regulation of CRL by glomulin is responsible for ubiquitination of many proteins, including ones that are important for proper vascular development [125–129]. Interestingly, McIntyre et al. observed an increased level of expression of GLMN in vascular smooth muscle cells in mice, suggesting globulin’s ubiquitous involvement in numerous processes [130].

Figure 4. A – A simplified model of protein ubiquitination by RING-type E3 enzyme. The ubiquitination of target protein is completed by interaction with a ubiquitin-conjugating enzyme (E2). B – Due to glomulin binding to E3, E2 accession to the complex is prevented and the entire process is inhibited. Mutations of GLMN prevent inhibition of ubiquitination. This may lead to development of GVMs [125, 131–133].
In 1998 it was established that GVMs have an autosomal dominant inheritance pattern with 100% penetrance by the age of 30 years [134]. Further studies have indicated that penetrance is almost complete 10 years earlier, reaching 97.2% by 20 years [122]. Between 38 and 63.8% of cases of GVMs are inherited [135–137]. Published sources indicate that numerous GLMN variants are responsible for the disease [138]. Among 162 kindreds reported by Brouillard et al., the premature stop mutation which leads to truncation of the protein (c.157_161del, p.(Lys53*)) was the most frequent, making up almost half of the cases. Additionally, only 19 individuals in which the mutations had been found were considered sporadic [138, 139]. Several other studies dealing with molecular screening of GLMN in patients with GVMs were performed (Table IV). In addition to GLMN mutations sensu stricto, Ohata et al. also found a region with loss of heterozygosity (LOH) near the GLMN gene [139]. So far no other genes have been associated with this disease.

Venous malformations, multiple cutaneous and mucosal

The characteristic features of venous malformations, multiple cutaneous and mucosal (OMIM 600195, VMCMs) are multiple small venous malformations, including cavernous hemangiomas, located in any part of the body, mostly affecting the internal organs. The coincidence of additional abnormalities such as cardiac malformations (e.g. ventricular septal defects) has been reported. Venous malformations are found on the skin of the limbs and trunk, on mucosal surfaces (on lips, tongue and tonsils, in the larynx, stomach and large intestine) and in viscera, including the liver, pancreas and spleen. On the skin and mucosa, the typical lesions are small, multifocal, bluish vascular malformations. Bleeding may occur [142].

Histopathological examination shows ectatic and relatively thin vessels, lined with one layer of endothelial cells without surrounding smooth muscle cells or elastic tissue. The lack of mural cells may suggest a recruitment defect [143, 144]. VMCMs are associated with mutations in the TEK gene (protein receptor tyrosine kinase, epithelial specific; OMIM 600221), also known as TIE2, and are inherited in an autosomal dominant manner [20]. The TEK tyrosine kinase receptor interacts with ANGPT1, ANGPT2 and ANGPT4 (Figure 5) [145]. Interaction with ANGPT1 triggers many signaling pathways and cascades by AKT1 activation [146]. The activation of the AKT1 enables communication between endothelial cells and smooth muscle cells and stimulates appropriate angiogenesis [147]. It is widely accepted that TIE2/TEK is mandatory for the proper development of the vascular system and plays an important role in angiogenesis and vascular stability [148–150].

Wouters et al. found a mutation in the TEK gene (c.2545C>T, p.Arg849Trp) in 14 individuals out of 26 patients with VMCMs. The identified mutation affects an intracellular domain of the TEK protein [143]. It has been suggested that specific functional abnormalities depend on the type of mutation [152]. In gain-of-function mutations of TEK, there is an increase in autophosphorylation and subsequent activation of the STAT1 signaling pathway [148, 152, 153]. On the other hand, a loss of function mutation was also found in a tissue sample from an VMCM affected individual [148].

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Number of families and screened individuals with positive family history</th>
<th>Individuals screened for GLMN mutations</th>
<th>Number of mutations(^d)</th>
<th>Criteria for molecular testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brouillard et al., 2002 [122]</td>
<td>238 individuals from 20 families tested, mutations found in each family, and one sporadic case</td>
<td>238 + 1 sporadic</td>
<td>110 + 1 sporadic/110 + 1 sporadic (additionally 15 unaffected carriers tested)</td>
<td>14</td>
</tr>
<tr>
<td>Brouillard et al., 2005 [140]</td>
<td>52 individuals from 23 families tested, mutations found in each family</td>
<td>53</td>
<td>42/42 (additionally 5 unaffected carriers tested)</td>
<td>17</td>
</tr>
<tr>
<td>O’Hagan et al., 2006 [141]</td>
<td>36 individuals from 4 families tested</td>
<td>36</td>
<td>19/19</td>
<td>1</td>
</tr>
<tr>
<td>Brouillard et al., 2013 [138]</td>
<td>381 individuals from 162 families tested</td>
<td>465</td>
<td>344/344 (additionally 37 unaffected carriers tested)</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\)Number of all individuals subjected to molecular testing. \(^b\)Number of individuals subjected to molecular testing with diagnosed GVMs. \(^c\)Individuals with diagnosed GVMs and with mutation in GLMN gene. \(^d\)Number of detected mutations (both novel and previously described). GVMs – glomuvenous malformations, VMs – vascular malformations, BRBNS – blue rubber bleb nevus syndrome.
Sturge-Weber syndrome

Sturge-Weber syndrome (SWS) is one of the most widely known capillary malformation syndromes. The hallmark of SWS is the characteristic facial capillary malformation (port-wine stain) coupled with an intracranial malformation, spanning the meninges on the same side. The meningeal malformations are leptomeningeal angiomata that are mostly situated over occipital and posterior parietal lobes. Laminar cortical necrosis and calcification develop due to stasis and ischemia of the neighboring leptomeningeal angiomatosus [154]. The cutaneous manifestation of SWS is the port-wine stain [155]. This birthmark develops in the distribution of one of the branches of the trigeminal nerve, most commonly V1, the ocular nerve [155]. Bilateral or unilateral port-wine stains involving the branches V1, V2, V3 of the trigeminal nerve and the port-wine stains on the eyelids have a high probability of eye and CNS vascular malformation co-occurrence [156]. Ocular vascular malformations involving the choroidal vessels frequently occur in SWS and lead to glaucoma, buphthalmia and hemianopsia. The clinical presentation of SWS is that of intellectual disability, seizures and focal neurological signs in an individual with a distinct facial birthmark [157]. The clinical course is variable: the seizures may become intractable and recurrent stroke-like episodes may occur. The prevalence of SWS is estimated at 1/20,000–1/50,000.

Curiously, SWS appears to be congenital but not heritable. This, together with strikingly segmental distribution of anomalous tissue, raised a suspicion that SWS may be caused by an acquired, somatic mutation at an early developmental stage, resulting in a chimeric individual with affected cell populations determined to develop the malformations. This elegant theory found support in subsequent research. In 2002, Huq et al. described a 4q inversion and trisomy of the 10th chromosome, which were present in leptomeningeal angiomatosis tissue sample but not in cells cultured from blood and skin of affected individuals [158]. Recently, the genetic origin of SWS was

Figure 5. Interactions among ANGPT1, ANGPT2, ANGPT4 and Tie2. Several signaling pathways are triggered by this activation. The most important one in the context of vascular development is PI3K/AKT/mTOR with its numerous subsequent cascades. Alterations of TEK/TIE2 were demonstrated to result in VMCMs. This might suggest that VM has a genetic background similar to VMCMs [151]
further elucidated. In 2013 Shirley et al. identified a single nucleotide variant, i.e. a missense variant (c.548G>A, p.Arg183Gln) in the GNAQ gene within malformed tissue from SWS and nonsyndromic (isolated) port-wine stain, but neither in the patients' unaffected tissue nor healthy controls and unrelated vascular malformations. This particular variant was reported consistently by several other researchers [159–162], with high prevalence of approximately 90% in pathological samples from SWS and nonsyndromic port-wine stains alike (88% in 23 of 26 patients and 92% in 12 of 13 patients, respectively [159, 160]).

The GNAQ (G protein subunit α q; OMIM 600998) is located in the 9q21.2 region. The gene encodes an α subunit of guanine nucleotide binding protein, commonly known as Gq protein. This protein family is involved in important signaling pathways, such as RAS/MAPK, that are significant for cell proliferation, survival and apoptosis [163]. The α domain in inactive form binds to the 7-transmembrane domain receptor. After receiving an external signal, the Gq α domain turns into an active form by converting the GDP form to the GTP form. The active α domain activates other enzymes and effector proteins that trigger further signaling pathways [164]). Activating mutations of GNAQ may influence pathways such as RAS-MEK-ERK and phospholipase C (Figure 6) [149]. The GNAQ expression product activates phospholipase C that causes phospholipid cleaves typical for SWS [147]. The aforementioned mutation, c.548G>A, p.Arg183Gln, destabilizes an inactive GDP-bound conformation of the encoded protein, and thus causes overactivation of the protein and subsequently involved pathways.

The exact developmental stage at which the mutation occurs is not known. Uchiyama et al. demonstrated low prevalence somatic mutations in GNAQ in patients with SWS by using deep sequencing methods [166]. In this study the GNAQ mutation (again, c.548G>A, p.Arg183Gln) was identified in brain lesions, saliva and blood leukocytes. The authors suggested that GNAQ somatic mutation derives from a hemangioblast or an early endothelial cell. They claimed that cells harboring the mutation in blood leukocytes and saliva lymphocytes might actively seed the SWS lesions. Moreover, the latest research by Couto et al. revealed another two variants (both substitutions) of GNAQ (c.548G>T, p.Arg183Leu and c.547C>G, p.Arg183Gly), which were present at a low level.

Figure 6. A model for G protein activity and its role in triggering of RAS-MEK-ERK and phospholipase C pathways. The external signal causes activation of G protein by exchanging a GDP particle bound to the G protein for a GTP. The α subunit of G protein (a GNAQ expression product) is then released and activates enzymes and effector proteins involved in signaling pathways necessary for vascular development. Alterations of GNAQ are indicated in SWS pathogenesis [159, 164, 165].
Genetic syndromes with vascular malformations – update on molecular background and diagnostics

exclusively in tissue with capillary malformation [167, 168]. On the other hand, Sundaram et al. suggested that the variation p.Arg183Gln, which is the most common alteration in SWS, is not only linked with vascular malformations, but may also affect brain parenchyma, which could explain the brain pathology present in SWS [169, 170]. In addition to this, in several other studies an attempt to reveal the genetic background of SWS was made, including molecular screening (Table V). Published sources also indicate that mutation in GNA11 may also be responsible for capillary malformations due to the likely close association between this gene and GNAQ [167]. What is more, it has been observed that mutations in those two genes occur in tumors including melanomas [171–173]. Another activating mutation of GNAQ (c.626A>T; p.Gln-209Leu) has been reported in SWS, phakomatosis pigmentovascularis (a similarly sporadic condition combining port-wine stain and pigmentary lesion) but also in uncomplicated, congenital hemangiomas and in several melanocytic neoplasms and malignant intraocular tumors including uveal melanomas [160, 174, 175].

**PIK3CA related syndromes: congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies, fibroadipose hyperplasia, megalencephaly-capillary malformation**

Congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies (CLOVES syndrome) belongs to segmental or patchy overgrowth syndromes. The symptoms associated with CLOVES syndrome are progressive and complex. They involve VMs (capillary, arteriovenous, venous and lymphatic), abnormal adipose tissue distribution that may form lipomatous masses, varying degrees of scoliosis, overgrowth of bony tissue with bony distortion in areas that had undergone trauma or major surgical procedures [178].

Recently, the genetic cause of CLOVES syndrome has been identified. An activating somatic mutation in the PIK3CA gene (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α), a component of the PI3K-AKT pathway, is a plausible cause of CLOVES syndrome [179–181]. However, the studies suggest that several other diseases may be caused by PIK3CA mutations. These include PIK3CA-related overgrowth spectrum (PROS), fibroadipose hyperplasia or overgrowth (FAO), hemihyperplasia-multiple lipomatosis syndrome (HHML), macrodactyly and muscle hypertrophy, the related megalencephaly syndromes, megalencephaly-capillary malformation (MCAP), hemimegalencephaly as well as an array of neoplasms including colorectal, ovary, breast, lung and brain tumors [178, 180–190].

Fibroadipose hyperplasia (FAH) features overlap with CLOVES syndrome and are characterized by segmental overgrowth of skeletal, visceral, fibroadipose, subcutaneous and muscular tissues with muscle lipomatous infiltration, accompanied by adipose tissue dysregulation and regional lipohypoplasia. Typically disproportionate linear overgrowth is present. Additional findings in FH may include vascular malformations, epidermal nevi, polydactyly and testicular or epididymal cysts and

**Table V. Somatic mutations in GNAQ gene in different cohorts of patients with Sturge-Weber syndrome**

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Number of families, screened cases both sporadic and with family history</th>
<th>Individuals screened for GNAQ mutations</th>
<th>Criteria for molecular testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shirley et al., 2013 [159]</td>
<td>26 sporadic cases tested</td>
<td>23/26</td>
<td>1 SWS</td>
</tr>
<tr>
<td>Nakashima et al., 2014 [162]</td>
<td>15 sporadic cases tested</td>
<td>12/15</td>
<td>1 SWS</td>
</tr>
<tr>
<td>Uchiyama et al., 2016 [166]</td>
<td>15 sporadic cases tested</td>
<td>4/15</td>
<td>1 SWS</td>
</tr>
<tr>
<td>Huang et al., 2017 [176]</td>
<td>One family tested</td>
<td>3/3</td>
<td>1 SWS</td>
</tr>
<tr>
<td>Sundaram et al., 2017 [169]</td>
<td>9 sporadic cases tested</td>
<td>9/9</td>
<td>1 SWS</td>
</tr>
<tr>
<td>Hildebrand et al., 2018 [177]</td>
<td>4 sporadic cases tested</td>
<td>4/4</td>
<td>1 Forme fruste SWS type III</td>
</tr>
</tbody>
</table>

*Number of individuals subjected to molecular testing. †Individuals with SWS and somatic mutation in GNAQ gene. ‡Number of detected mutations (both novel and previously described). SWS – Sturge-Weber syndrome.
hydrocele [183]. Due to overlapping features, FAH and CLOVES may be considered different phenotypes of a single overgrowth syndrome.

The MCAP phenotype is diversified between affected individuals. The hallmark of the syndrome is an unusual growth dysregulation of the brain and multiple somatic tissues. The typical features in classic MCAP are congenital or early postnatal progressive megalencephaly (MEG) or hemimegalencephaly (HMEG), hypotonia, mild to severe intellectual disability and segmental to generalized somatic overgrowth with single or multifocal capillary malformations. Usually, midline facial capillary malformations – especially persistent naevoid flammus or cutis marmorata – are present. Megalencephaly may be accompanied by secondary overgrowth of the ventricles, corpus callosum and cerebellum followed by cerebellar tonsillar ectopia [188]. The additional findings in MCAP may include cortical malformations such as polymicrogyria, digital abnormalities in the form of polydactyly and syndactyly and connective tissue dysplasia.

The PIK3CA gene (OMIM 171834) is located on chromosome 3 and encodes the catalytic subunit of phosphoinositide-3-kinase (PI3K), which is necessary for enzyme activity control. PI3K is involved in phosphorylation of proteins, and takes part in the regeneration of phosphatidylinositol 3,4,5-trisphosphate (PIP3) by multiple phosphorylations of phosphatidylinositol [191, 192]. Phosphorylation of PDK1 by regenerated PIP3 and then finally phosphorylation of AKT by AKT leads to hyperphosphorylation of the TEK protein. These two genes are members of the PI3K/AKT-mTOR growth-signaling pathway, which is responsible for cell proliferation, growth and survival (Figure 7) [179, 181, 183].

Kurek et al. found three alterations (c.3140A>G, p.His1047Arg; c.1633G>A, p.Glu545Lys) in PIK3CA in all six tested patients with mutant allele frequencies between 3% and 30%. Michel et al. reported the aforementioned variants and a newly discovered one (c.3140A>T, p.His1047Leu) with the prevalence between 1.5% and 31% in affected tissues [180, 195]. The authors suggested that urine may be adequate material for detection of CLOVES causal mutations, which would be a good alternative for biopsy [195]. However, the results of the latest study have revealed that some patients with phenotype characteristic for CLOVES do not show the presence of any known mutations [196]. It may be caused by insufficient accuracy of the proposed experimental method. Alternatively, mutations of PIK3CA do not have to be only cause of the CLOVES phenotype. Regardless of the specific genetic alteration, most academics believe the mutations to be postzygotic and selectively present in the affected body regions [180, 183, 188, 197].

Venous malformations

Isolated venous malformation is a nonproliferating, slow-flow vascular abnormality that is present at birth. Venous malformations are blue, soft, compressible lesions without excessive warmness of skin covering the malformation [198]. The hallmark of VMs is enlargement of the lesion along with patient’s growth. Venous malformation grows from infancy to puberty. It can be located in any region of the body and may extend to neighboring areas. Facial VMs usually affect skin and subcutaneous tissue and often spreads to oral muscles and mucosa [199]. Temporal and cheek lesions may extend to the infratemporal fossa and parotid gland, whereas neck lesions tend to spread posteriorly to the trapezius muscle or anteriorly to the pharyngeal and laryngeal areas [198]. Arteriovenous malformations often present an aesthetic issue but may also lead to serious complications. The lesion of the head can obstruct the airway, be a source of recurrent bleeding and may interfere with speech. Venous malformations on extremities are segmental or localized, but extensive changes have been reported. Extensive VMs may have troublesome complications due to skeletal muscle and joint destruction that leads to either hypotrophy or hypertrophy of the affected extremity [200, 201].

Venous malformations were found to be caused by a somatic gain of function mutation in the TEK gene, which was already mentioned as the causative gene for venous malformations, multiple cutaneous and mucosal syndrome. Known mutations affect the intercellular domain of the TEK protein [143, 202], Limaye et al. discovered a somatic mutation in 28 of 57 patients diagnosed with sporadic VMs [148]. The characteristic mutation for VMs causes a substitution of leucine with phenylalanine (c.2740C>T, p.Leu914Phe). This sporadic variant has not been observed in patients with VMCMs. The p.Leu914Phe mutation leads to hyperphosphorylation of the TEK protein. It may have an impact on the PI3K/AKT pathway [20]. On the other hand, recent studies indicate that activating mutations of PIK3CA (causative for CLOVES) may be pivotal factors in the context of occurrence of VMs. PIK3CA mutations are mutually exclusive with alterations of the TEK-encoded receptor. These two genes are members of the same signaling pathway [203–205]. Some mosaic somatic mutations of PIK3CA (c.3140A>G, p.His1047Arg; c.1633G>A, p.Glu545Lys) are not related to overgrowth of tissues and abnormalities related to lymphatic vessels, but they may be an explicit factor in the pathogenesis of VMs [205]. In summary, the vast majority of VM cases carry mutations in TEK gene but the alterations of PIK3CA may also be responsible for sporadic cases of VMs [204, 205].
Klippel-Trénaunay-Weber syndrome

The characteristic feature of Klippel-Trénaunay-Weber syndrome (KTWS) is overgrowth of a limb and concurrent large, superficial, cutaneous hemangioma. The limb overgrowth is associated with both soft tissue and bone hypertrophy. The affected limb bears varicose veins with or without concurrent deep vein abnormalities. Lower limbs are usually affected. The coincidence of upper and lower extremity involvement may occur in 10% to 15% of cases and is usually ipsilateral [206]. The VMs affect the entire limb and commonly are present from infancy. The main complications of KTWS are deep venous thrombosis (possibly complicated by life-threatening pulmonary embolism), cellulitis, Kasabach-Merritt syndrome, lymphedema and internal bleeding from abnormal blood vessels. Polydactyly, oligodactyly, syndactyly, macrodactyly and, quite unexpectedly, seizures and intellectual disability have also been reported. The differential diagnosis of the KTWS involves not only CM-AVM syndrome and PWS, but also Servelle-Martorell syndrome associated with capillary macules and varicose veins with relative hypotrophy of the affected extremity [207].

Klippel-Trénaunay-Weber syndrome is estimated to affect 1 in 10,000 newborns. The inheritance pattern of KTWS is unknown, but paradominant inheritance is under consideration. Some authors propose the hypothesis that KTWS may be caused by a mosaic mutation that otherwise would be lethal under non-mosaic conditions [208].

Currently, the exact inheritance of KTWS is unknown. It has been suggested that the disease is sporadic [209–211], but familial cases and a putative autosomal dominant model of inheritance were also considered [212–214]. The researchers have proposed AGGF1, a gene encoding a potent angiogetic factor, as a candidate gene for KTWS [215]. AGGF1 (angiogenic factor with G patch and FHA domains 1, OMIM 608464), also known as VG5Q, is located on chromosome 5. The expression product of AGGF1 binds to the surface of endothelial cells and promotes their proliferation [216]; however, recent studies have also shown increased expression of AGGF1 in plump endothelial cells and mast cells [217]. The protein is mainly present on endothelial cells, where it functions as an anti-inflammatory factor, but has also been detected in kidney, heart fibroblasts and ovarian cancer cells [218, 219].
Zhang et al. stated that the gene mentioned above is indispensable for angiogenesis as well as tumor growth [220] integrity. Finally, it has been proposed that genes AGGF1 and PIK3CA may be related due to mutual involvement in the AGGF1-PI3K-AKT pathway crucial for vascular system development and also a putative pathogenic factor for KTWS. On the other hand, Barker et al. tested individuals with KTWS for mutations in the AGGF1 gene [221]. Only one patient from a group of 24 tested individuals KTWS carried a heterozygous variant in the AGGF1 gene (c.397G>A, p.Glu133Lys). The authors then demonstrated the presence of this variant in 9 individuals out of 275 in the control group [221]. They concluded that the p.Glu133Lys mutation in AGGF1 is probably a nonpathogenic polymorphism of AGGF1 [222].

The second gene suggested to be associated with KTWS was PIK3CA (mentioned above as a cause of CLOVES syndrome and in sporadic cases of VMs) [180]. Firstly, the aspect of overlapping symptoms among patients with KTWS and those with mutations in PIK3CA may suggest a significant connection between this gene and KTWS pathogenesis [223]. Valerie L. Luks et al. used new generation sequencing (NGS) and digital droplet PCR methods for molecular testing of 21 patients with KTWS and lymphatic malformations. Mutations in PIK3CA (previously described in CLOVES – c.3140A>G, p.His1047Arg; c.3140A>T, p.His1047Leu; c.1624G>A, p.Glu542Lys; c.1258T>C, p.Cys420Arg) have been found in 13 patients with KTWS (90%) [196].

Eerola et al. and Wooderchak-Donahue et al. have been investigating patients with prior suspicion of KTWS for the presence of mutations in the RASA1 gene, but all have tested negative [1,15]. De Wijn et al. reported on three patients with multiple capillary malformations and limb overgrowth, albeit without varicose veins, which most likely represented PWS and four patients with atypical CMs and early-onset varicose veins with suspicion of KTWS [14]. In this study a novel heterozygous RASA1 mutation in exon 3 was identified [14]. In 2013 Revencu et al. tested patients with KTWS and limb overgrowth with and without varicose veins that could mimic PWS on RASA1 mutations. The results did not reveal any pathogenic changes. Thus, Revencu et al. suggested that RASA1 testing could be a diagnostic method for differentiation between PWS, CM-AVM syndrome and KTWS [208]. Whelan et al. reported on a patient with recognized KTWS with balanced translocation t(5;11)(q.3;p15.1) that encompasses the locus for RASA1. This could explain the rare positive results in probands with the KTWS diagnosis [224]. Other genetic abnormalities have been reported as a suspected cause of KTWS, namely translocation t(8;14)(q22.3;q13), de novo supernumerary ring chromosome 18 and terminal deletion 2q37 [225,226]. However, none of the above-mentioned changes has been confirmed to be associated with KTWS in further studies.

Summary and results

In this work, we gathered and summarized available data concerning a selection of ten notable disorders of vascular system development. We presented the clinical picture and molecular background of these relatively rare conditions. The notoriously overlapping symptoms have been listed and assigned to particular syndromes, in the most unambiguous way. Both the familial and sporadic character of selected syndromes as well as their inheritance patterns have been explained. Known mutations and their impact on cellular signaling have been described. Moreover, simplified schemes of pathways involved in vascular system development have been presented and explained. We performed a meta-analysis to determine the frequency of mutations in different cohorts of patients. The results are shown in Tables I–V. Finally the most important data have been placed in the table below (Table VI) for the convenience of both clinicians and geneticists.

In conclusion, the diagnosis of diseases and syndromes related to vascular system development and angiogenesis remains a great concern for both clinicians and genetics. These varied conditions have distinct etiologies, pathomechanisms and prognosis, and thus often require different treatment strategies. Imprecise diagnosis may therefore lead to inadequate therapy. Unfortunately, as demonstrated in this article, those diseases often have overlapping features, making the differential diagnosis difficult or even impossible on clinical grounds alone. So far, both the diagnosis and therapy are based on interventional radiology and surgery. Nevertheless, the whole procedure may encompass genetic testing in addition to thorough clinical and radiological assessment. This multidisciplinary approach, with cooperation between genetics and clinicians, will likely lead to a decrease of mortality and morbidity of affected individuals. That is exemplified by the case of RASA1, which has become a new tool helping in differentiation of patients with PWS, KTWS and CM-AVM syndrome. Further genetic testing should be developed in order to enhance recognition of specific vascular malformation syndromes and to guide therapeutic decisions.

Conflict of interest

The authors declare no conflict of interest.
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene mutations</th>
<th>Inheritance</th>
<th>Penetration</th>
<th>Prevalence</th>
<th>Age of presentation</th>
<th>Description of typical findings</th>
<th>Locations of changes</th>
<th>Mucosa involvement</th>
<th>AVMs and AVFs</th>
<th>Distribution of AVMs and AVFs</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM-AVM syndrome</td>
<td>RASA1, EPHB4</td>
<td>Autosomal dominant</td>
<td>Incomplete</td>
<td>1/100,000</td>
<td>Childhood, infancy</td>
<td>Acquired, small, round to oval, pink to red with approximately &lt; 1 cm to 3 cm diameter macules or patches, often manifest with concurrent white halo suggesting the vascular steal or with the arterial flow on Doppler ultrasound</td>
<td>Multifocal (present in more than one region or location of the body); mostly face and limbs</td>
<td>Yes</td>
<td>Yes</td>
<td>Muscles, skin, and other tissues (intracranial, intraspinal, interosseous); often in head and neck</td>
<td>KTWS, Parkes-Weber syndrome, HHT (Rendu-Osler-Weber syndrome) or HBT, Sturge-Weber syndrome</td>
</tr>
<tr>
<td>HHT (Rendu-Osler-Weber syndrome)</td>
<td>ENG, ACVRL1, GDF2, SMAD4</td>
<td>Autosomal dominant</td>
<td>Intrafamilial variability, high penetrance</td>
<td>1/8,000</td>
<td>Adolescence, infancy</td>
<td>Multiple AVMs without intervening capillaries with direct connections between arteries and veins, co-occurrence telangiectasias, age-dependent features, commonly manifests with epistaxis and cutaneous or mucosal CMs that rupture and bleed after a slight trauma</td>
<td>Tongue, lips, buccal mucosa, face, chest, fingers, ears and conjunctivae; gastrointestinal mucosa telangiectasias in GI mucosa – most frequently stomach and duodenal mucosa</td>
<td>Yes</td>
<td>Yes</td>
<td>Lungs, liver, brain</td>
<td>Ataxia, telangiectasia, CRST syndrome, CM-AVM syndrome, HHT (Rendu-Osler-Weber syndrome), chronic liver disease, pregnancy</td>
</tr>
<tr>
<td>Parkes Weber syndrome</td>
<td>RASA1, mutation p.E133K of AGGF1</td>
<td>Autosomal dominant</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Adolescence, after trauma, VMS in childhood</td>
<td>Cutaneous, red or pink, large, flat patch with underlying quiescent AVMs, extremity over-growth</td>
<td>Soft tissue, bone tissue, lower extremities affected more often than upper extremities</td>
<td>Yes</td>
<td>Yes</td>
<td>Mostly lower limbs</td>
<td>KTWS, CM-AVM syndrome, HHT (Rendu-Osler-Weber syndrome) or HBT, Sturge-Weber syndrome</td>
</tr>
</tbody>
</table>
### Table VI. Cont.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene mutations</th>
<th>Inheritance</th>
<th>Penetrance</th>
<th>Prevalence</th>
<th>Age of presentation</th>
<th>Description of typical findings</th>
<th>Locations of changes</th>
<th>Mucosa involvement</th>
<th>AVMs and AVFs</th>
<th>Distribution of AVMs and AVMs</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCMs</td>
<td>KRIT1, MGC4607, PDCD10</td>
<td>Autosomal dominant</td>
<td>88%, 100%, 63%</td>
<td>0.5%</td>
<td>Infancy and childhood, more often between 2nd and 5th decades</td>
<td>Enlarged, clustered capillaries (caverns) with single layer of endothelium and without mature vessel wall, without normal intervening brain parenchyma, diameter of few millimetres to few centimetres; hyperkeratotic capillary – venous malformation in skin in CCM2</td>
<td>Brain, spinal cord, retina</td>
<td>No</td>
<td>Yes</td>
<td>Skin</td>
<td>Sturge-Weber syndrome, Von Hippel-Lindau syndrome, hypertensive angiopathy trauma, multiple hemorrhagic metastases, myeloid angioptathy (with lacunar stroke), pneumocephalus, cysticercosis</td>
</tr>
<tr>
<td>GVMs</td>
<td>GLMN</td>
<td>Autosomal dominant</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Infancy, first two decades of life</td>
<td>Benign cutaneous neoplasms with cobble – stone appearance, hard consistency, partially compressible, painful; histopathology: rounded cells (glomerus cells) around the blood-filled cavities; cells stain positively with smooth – muscle α-actin and vimentin</td>
<td>Skin, multifocal</td>
<td>No</td>
<td>Yes</td>
<td>Skin</td>
<td>Mucocutaneous cavernous hemangiomas, VMCMs</td>
</tr>
<tr>
<td>VMCMs</td>
<td>TEK</td>
<td>Autosomal dominant</td>
<td>90%</td>
<td>Unknown</td>
<td>Infancy</td>
<td>Small, multifocal, bluish vascular malformations with slow blood flow on Doppler ultrasound, soft, compressible lesions, reported cases with coincidence of cardiac malformations (ventricular septal defect), cavernous hemangioma as included</td>
<td>Tongue, lips, limbs, trunk</td>
<td>Yes</td>
<td>Yes</td>
<td>Tongue, lips, larynx, tonsils, stomach, liver, pancreas, spleen, large intestine</td>
<td>GVMs, VMs</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene mutations</td>
<td>Inheritance</td>
<td>Penetration</td>
<td>Prevalence</td>
<td>Age of presentation</td>
<td>Description of typical findings</td>
<td>Locations of changes</td>
<td>Mucosa involvement</td>
<td>AVMs and AVFs</td>
<td>Distribution of AVMs and AVFs</td>
<td>Differential diagnosis</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------</td>
<td>--------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>--------------</td>
<td>-------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sturge-Weber</td>
<td>GNAQ, GNA11</td>
<td>Unknown</td>
<td>Unknown</td>
<td>1/20,000–1/50,000</td>
<td>Infancy, early adulthood</td>
<td>Intracranial vascular malformations, leptomeningeal angiomata, facial vascular malformations (port-wine stains), glaucoma, buphthalmos, neurological impairment, seizures and intellectual disability, migraine headaches</td>
<td>Occipital and posterior parietal lobes; port-wine stains in distribution of the three branches of the trigeminal nerve, most commonly in the V1 branch distribution</td>
<td>No</td>
<td>Yes</td>
<td>Occipital and posterior parietal lobes, eyes</td>
<td>Isolated facial port-wine birthmark, megalencephaly-capillary malformation-poly-microgyria, KTWS, Parkes Weber syndrome</td>
</tr>
<tr>
<td>CLOVES syndrome</td>
<td>PIK3CA</td>
<td>Mosaic somatic mutation</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Infancy, prenatal</td>
<td>Capillary, arteriovenous, venous and lymphatic malformations, epidermal nevi, abnormal adipose tissue: lipomatous masses, varying degrees of scoliosis, overgrowth of bony tissue with bony distortion in areas that had undergone major surgical procedures, macrodactyly, and plantar or palmar overgrowth, renal anomalies</td>
<td>Trunk</td>
<td>No</td>
<td>Yes</td>
<td>Within and around the lipomatous mass and in the paraspinal regions</td>
<td>Hemimegalencephaly, MPPH syndrome, KTWS, BRRS, Proteus syndrome, HHML, SOLAMEN syndrome, FH, MCAP</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene mutations</td>
<td>Inheritance</td>
<td>Penetration</td>
<td>Prevalence</td>
<td>Age of presentation</td>
<td>Description of typical findings</td>
<td>Locations of changes</td>
<td>Mucosa involvement</td>
<td>AVMs and AVFs</td>
<td>Distribution of AVMs and AVFs</td>
<td>Differential diagnosis</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
<td>------------</td>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-------------------</td>
<td>--------------</td>
<td>-------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Fibroadipose hyperplasia</td>
<td>PIK3CA</td>
<td>Mosaic somatic mutation</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Infancy, progressed to adulthood</td>
<td>Segmental overgrowth of skeletal, visceral, fibroadipose, subcutaneous and muscular tissues with muscle lipomatous infiltration, accompanied by adipose tissue dysregulation and regional lipohypoplasia, vascular malformations, epidermal nevi, polydactyly, testicular or epididymal cysts and hydrocele, epidermal nevi, cutaneous capillary vascular malformations</td>
<td>Muscle, visceral, subcutaneous fibroadipose tissue</td>
<td>Yes</td>
<td>Yes</td>
<td>Muscle, visceral, subcutaneous fibroadipose tissue</td>
<td>Hemimegalencephaly, MPH syndrome, KTWS, BRRS, Proteus syndrome, HHML, SOLAMEN syndrome, FH, MCAP</td>
</tr>
</tbody>
</table>

References


play between endoglin and ALK-1, two components of the endothelial transforming growth factor-beta receptor complex. J Cell Physiol 2005; 204: 574-84.


106. Voss K, Stahl S, Schleider E, et al. CCM3 interacts with CCM2 indicating common pathogenesis for cerebral


