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Genetic aspects of vascular malformations

Nicole Revencu[1], Julien Coulie[2,3], Laurence M. Boon[2,3], Miikka Vikkula[3,4]

[1] Center for Human Genetics, Cliniques universitaires Saint-Luc, University of Louvain, Brussels, Belgium; VASCERN VASCA European Reference Centre

[2] Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires Saint-Luc, University of Louvain, Brussels, Belgium; VASCERN VASCA European Reference Centre

[3] Human Molecular Genetics, de Duve Institute, University of Louvain, Brussels, Belgium

[4] WELBIO department, WEL Research Institute, Wavre, Belgium

Corresponding author : nicole.revencu@saintluc.uclouvain.be

Address :
Center for Human Genetics
Cliniques universitaires Saint-Luc
Avenue Hippocrate, 10
1200 Brussels, Belgium

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Abstract

Vascular anomalies are a heterogenous group of diseases that include vascular tumours and vascular malformations. Over the past two decades, significant progress has been made in elucidating the genetic basis of these anomalies, leading to improved classification, management and genetic counselling. Major

signalling pathways, such as RAS/MAPK/ERK, PI3K/AKT/mTOR, and G protein-coupled receptor pathways, have been identified as central to the pathogenesis of vascular anomalies. This article reviews the major types of vascular malformations, addresses the challenges associated with genetic testing and counselling, and explores the emerging potential for precision medicine in the treatment of these diseases.

Keywords : genetic testing, genetic counselling, hereditary, ISSVA, sporadic, vascular malformation

Introduction

Vascular anomalies encompass a broad spectrum of diseases affecting both blood and lymphatic vessels, with considerable variability in presentation, progression, and clinical outcomes. The International Society for the Study of Vascular Anomalies (ISSVA) provides a standardised, widely adopted classification system, originally proposed by John Mulliken and Julie Glowacki, which divides these anomalies into two main categories: vascular tumours, such as infantile haemangiomas, and vascular malformations [1] (https://www.issva.org/classification). Vascular malformations are further subdivided according to their complexity into simple forms, affecting a single vessel type (such as venous or lymphatic malformations), complex forms, involving multiple vessel types (such as capillary-venous or lymphatico-venous malformations), and vascular malformations associated with other anomalies (such as macrocephaly-capillary malformation or *PTEN*-hamartoma tumour syndrome).

Genetically, vascular malformations can be classified as hereditary or sporadic. Hereditary vascular malformations are caused by germline pathogenic variants that can be inherited or arise de novo, whereas sporadic forms are due to somatic variants that occur post-zygotically, resulting in mosaic patterns of affected tissues. Advances in genetic research, particularly over the last two decades, have shed light on the molecular basis of vascular malformations, and revealed the main pathways involved in their development: phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, RAS/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, and G protein-coupled receptor signalling pathway [2]. As these pathways control cell growth, proliferation, differentiation, movement, and survival, aberrations in these pathways can disrupt cell behaviour, vascular morphogenesis, and vessel stability, leading to the characteristic features of different vascular anomalies.

The pathways involved in vascular malformations are also involved in cancer. However, in vascular anomalies the pathogenic variants are limited to vascular cells, whereas in cancer, they occur in various cell types and often accumulate. Recognition of this similarity has led to new treatment concepts, by proposing targeted molecular therapies initially developed for cancer, for the treatment of patients with vascular malformations [3]. For example, the

discovery of activating variants in *PIK3CA* has led to the repurposing of targeted therapies that inhibit the PI3K-AKT pathway in patients with overgrowth syndromes and lymphatic and/or venous malformations. Some of these molecules are in clinical trial or off-label use [3]. Similarly, aberrant signalling through the RAS-MAPK pathway has been linked to arteriovenous malformations and complex lymphatic anomalies, providing further opportunities for pathway-specific interventions.

This article reviews the genetic aspects of vascular malformations, which together with clinical, radiological, and pathological data can support the clinician in the diagnostic process. It also addresses specific aspects such as phenotypic and genetic heterogeneity, and challenges of genetic testing and counselling. Additionally, the article provides an overview of the major signalling pathways and targeted therapies, which will be further detailed in the dedicated articles of this issue of *La Presse Médicale.*

1. Genetic and clinical aspects of vascular malformations

The major types of vascular malformations, their associated genes, mode of inheritance and main clinical features are summarised in Table 1. Further detailed clinical information is available in the dedicated articles of this issue of *La Presse Médicale.*

1.1 Capillary malformation

Capillary malformation (CM, also known as “port-wine stain”) is the most frequent vascular malformation, affecting 3 in 1,000 newborns. It is a slow-flow vascular malformation, characterised by a homogenous, pink, red or purple macule present at birth. It is typically located on the skin, but can also be found on mucosa. CM is most often located in the head and neck region, but can also be seen on the trunk or limbs (Figure 1A). The malformation grows proportionally with the child and persists throughout life. The lesion becomes darker and often thicker with age. The underlying tissues (skin, fat, muscle and bone) may become hypertrophic. CM is usually an isolated, sporadic and solitary malformation, but it can occur as part of more complex phenotypes or be associated with other anomalies, such as : 1) growth disturbance (usually overgrowth, but undergrowth may also be seen), 2) other

sporadic slow-flow vascular malformations, including venous and/or lymphatic, 3) other slow-flow vascular malformations and overgrowth in different entities grouped under the umbrella of *PIK3CA*-related overgrowth spectrum disorders (PROS), 4) neurological manifestations, as seen in Sturge-Weber syndrome, macrocephaly (megalencephaly) - capillary malformation or microcephaly-capillary malformation (Table 1). Although generally sporadic, the CM can be hereditary in certain diseases such as capillary malformation – arteriovenous malformation (CM-AVM) type 1 and 2, with autosomal dominant inheritance or in microcephaly-capillary malformation, with autosomal recessive inheritance. In these hereditary forms, the CMs are usually multiples (multifocal).

Somatic activating variants in *the GNAQ* gene account for the majority of cases of common CMs (port-wine stain) and Sturge-Weber syndrome [4]. Somatic variants in *GNA11* are observed in some patients with CM with or without overgrowth and in patients with atypical Sturge-Weber syndrome [5,6]. Both *GNAQ* and *GNA11* are involved in the G protein-coupled receptor signalling pathway with the likely consequence being the activation of the MAPK signalling pathway (Figure 2) [4]. A wide range of overlapping phenotypes including CM as a feature, are caused by somatic activating variants in the *PIK3CA* gene, such as Klippel-Trenaunay syndrome, macrocephaly capillary – malformation and CLOVES [7]. *PIK3CA* encodes phosphatidylinositol-3-kinase catalytic subunit alpha (p110α). The *PIK3CA* variants identified in vascular malformations are associated with activation of the PI3K-AKT- mTOR pathway (Figure 2). CM-AVM type 1 and 2 are caused by heterozygous loss-of-function variants in *RASA1* and *EPHB4*, respectively, which lead to activation of the RAS-MAPK pathway (Figure 2) [8,9].

1.2 Venous malformations

Venous malformations (VMs) are congenital, slow-flow, vascular lesions characterised by localised bluish to purple lumps of malformed veins. These lesions are soft and compressible and can affect any tissue or organ, including the skin, subcutaneous tissue, muscles, joints, bones, and internal organs, such as brain and gastro-intestinal tract. Chronic consumptive coagulopathy may occur [10]. VMs are mostly sporadic and characterised by solitary lesions (94%), which can vary in size. Multiple lesions can be observed in two rare sporadic entities : 1) Multifocal Sporadic Venous Malformation

(MSVM), which presents with small, bluish, usually cutaneous and subcutaneous lesions, and 2) Blue Rubber Bleb Naevus (BRBN) syndrome (also known as Bean syndrome), which is characterised by small, rubbery, blue to purple, disseminated cutaneous venous malformations, typically affecting the palms, soles, and gastrointestinal tract. In addition, multiple VMs on the skin and mucosa are seen in Mucocutaneous Venous Malformation (VMCM), a hereditary disorder with autosomal dominant inheritance (Table 1).

All these 4 entities are caused by activating variants in *TEK* : somatic variants in the solitary VM lesion, usually two somatic variants on the same allele in VM lesions from BRBN and two somatic or one somatic (affected tissue) and one mosaic (detected in blood at low fraction) in MSVM [11,12,13]. In contrast, patients with VMCM carry heterozygous *TEK* variants (present in all the cells). In addition, approximately 25% of the solitary VMs are caused by somatic activating variants in *PIK3CA* [14].

Glomuvenous malformation (GVM) accounts for about 5% of venous malformations. It is a congenital, distinct type of venous malformation characterised by multiple bluish-purple cutaneous and subcutaneous lesions, mainly located on the extremities. GVM can rarely affect the mucosa or deeper structures, such as muscles, joints or bones. Some patients may present with larger lesions, known as plaque-type GVM. Unlike VMs, GVM do not compress easily and tend to be painful on palpation. This disorder is inherited in an autosomal dominant manner, and it is caused by heterozygous, loss-of-function variants in *GLMN* [15].

1.3 Arteriovenous malformation

Arterio-venous malformations (AVMs) are congenital, fast-flow vascular malformations characterised by abnormal connections between arteries and veins. In an AVM, the normal capillary bed is replaced by a nidus, through which blood shunts from feeding arteries into draining veins. An arteriovenous fistula, in contrast, is a direct communication between an artery and a vein.

The AVMs are the most aggressive vascular malformations and the most difficult to treat. They can be localised in the skin, subcutaneous tissue, muscles, viscera or central nervous system. Like CM and VM, AVMs are mostly sporadic, but can also be part of hereditary diseases. Sporadic AVMs are typically caused by somatic activating variants in *MAP2K1*, *KRAS* and *BRAF* (Figure 2) [16,17]. Rare somatic variants have been reported in *HRAS* and *RIT1* [18,19]. Three hereditary diseases include AVM as part of the phenotype : CM-AVM, hereditary haemorrhagic telangiectasias (HHT) and PTEN hamartoma tumour syndrome (PHTS). All these three diseases follow an autosomal dominant inheritance with variable expressivity (Table 1).

The localisation of AVM, the imaging characteristics and other associated clinical features can help guide the diagnosis in the hereditary forms, though some overlap exists, especially between CM-AVM and HHT [9,20]. In CM-AVM, which is caused by *RASA1* or *EPHB4* loss-of-function, heterozygous variants, the hallmark feature is multifocal CMs often surrounded by a pale halo (Figure 1B). The AVMs in CM-AVM are usually cutaneous, subcutaneous and muscular and are located in the head/neck, extremities, or as Parkes Weber syndrome, or in the central nervous system [21]. In HHT, caused by heterozygous variants in *ENG*, *ACVRL1*, *SMAD4* or *GDF2*, mucocutaneous and gastrointestinal telangiectasias as well as recurrent epistaxis are common features [22]. AVMs in HHT are most commonly found in the lungs, liver or central nervous system. Telangiectasias and recurrent epistaxis may also occur in CM-AVM, particularly with *EPHB4* variants and may lead to confusion with HHT [9]. Finally, PHTS groups Bannayan–Riley–Ruvalcaba syndrome (BRRS) and Cowden syndrome. The phenotype of PHTS is highly variable and may include, macrocephaly, developmental delay, autism, skin lesions, increased risk for both benign and malignant tumours. Vascular malformations, especially with fast-flow, can be seen [23].

1.4 Lymphatic anomalies

Lymphatic anomalies include a wide range of diseases involving abnormal development and/or function of the lymphatic vessels. Like blood vessels malformations, lymphatic anomalies are mostly sporadic (such as lymphatic malformation and most of the complex lymphatic anomalies) or hereditary (mainly primary lymphedema) (Table 1). Lymphatic malformations (LMs) are focal lesions composed of dilated lymphatic channels that are disconnected from the lymphatic system, forming cystic lesions, that may vary in size and location. LMs are classified as macrocystic, microcystic or

mixed. Most LMs are caused by somatic activating *PIK3CA* variants [24]. Some macrocystic LMs are caused by somatic activating *BRAF* variants [25]. LMs can also occur in combination with other vascular malformations, such as CM, VM or a combination of CM and VM. LMs are frequently observed in diseases classified under the PROS umbrella [26].

Complex lymphatic anomalies (CLAs) are characterised by multifocal lymphatic anomalies or affect the central collecting lymphatic channels. They include generalised lymphatic anomaly (GLA), Gorham-Stout disease (GSD), Kaposiform lymphangiomatosis (KLA) and central conducting lymphatic anomalies (CCLA). CLAs are severe diseases, associated with high morbidity and mortality. The diagnosis and management are particularly challenging. Somatic *PIK3CA* variants have been identified in patients with GLA, whereas somatic variants in the RAS-MAPK pathway have been identified in GSD and KLA [27-31]. Both somatic and germline variants in the RAS-MAPK pathway have been identified in CCLA [31-33] (Table 1).

Primary lymphedema is characterised by the accumulation of lymph in the extracellular spaces, most commonly in the lower limbs, although the upper limbs and other parts of the body can also be affected. Over 30 genes have been identified in association with primary lymphedema, and pathogenic germline variants are identified in about one third of patients [34]. The diagnosis is based on the clinical presentation (including regions affected, and time of onset), associated features, radiological investigations, and genetic analysis (Table 1).

2. Genetic and clinical heterogeneity in vascular malformations : the challenges of clinicogenetic correlations

Vascular malformations are characterised by significant genetic and clinical heterogeneity, which complicates both diagnosis and clinical management. As genetic research has progressed, it has become increasingly clear that while some genes are associated with specific vascular malformations, such as *GLMN* with GVM, others are involved in several distinct or overlapping phenotypes. The most prominent example is *PIK3CA*. Variants in this gene can cause isolated vascular malformations, such as, venous malformations or lymphatic malformations, combined vascular malformations, such as lymphatico-venous malformations, but are also associated with complex syndromes that involve vascular, adipose and

musculoskeletal tissues, collectively classified under the umbrella of PROS [7]. In this group of diseases, the phenotypic severity and complexity seem to be explained by the timing and the location of the variant, and, to some extent, by the type of variant. Indeed, studies have shown that non-hotspot *PIK3CA* variants are more commonly associated with syndromic than with non-syndromic forms [35,36]. In addition, these non-hotspot variants tend to have a higher variant allele fraction (VAF; the percentage of alleles carrying the variant) in syndromic forms [36].

On the other hand, a single clinical phenotype can result from variants in different genes. For example, CM-AVM can be caused by variants in two genes, *RASA1* and *EPHB4*, and familial cerebral cavernous malformation can be caused by variants in three genes, *KRIT1*, *CCM2* and *PDCD10*. This overlap in clinical presentation, despite different genetic causes, highlights the importance of genetic testing to accurately identify the underlying aetiology, as this may influence prognosis. For example, the risk of intracranial AVM is higher in CM-AVM caused by *RASA1* variants than by *EPHB4* variants [9]. Similarly, in patients with HHT, the pulmonary and cerebral AVMs are more common in individuals with *ENG* variants, whereas hepatic AVMs are more common in individuals with *ACVRL1* variants [37].

3. Hereditary versus sporadic vascular malformations and genetic counselling considerations

As already mentioned, vascular malformations can be classified as either sporadic or hereditary, depending on the pattern of inheritance (Table 1). Most vascular malformations are sporadic (e.g. Sturge-Weber syndrome, lymphatic malformations, Klippel-Trenaunay syndrome), meaning that they occur without a family history and are not passed on to offspring. These malformations are caused by somatic (post-zygotic) genetic variants that are present only in some cells of the affected tissues, resulting in a mosaic pattern. These variants are usually not found in the blood. The VAF in the affected tissue is variable, but is often less than 10%.

Some vascular malformations, however, are hereditary, most commonly with autosomal dominant inheritance, such as CM-AVM, HHT, and GVM. The hereditary forms are characterised by incomplete penetrance, which means that some individuals with the genetic variant will not show any signs or symptoms throughout their lives. The penetrance varies among different diseases. For example, in HHT, the penetrance is high, around 95%, whereas in

Milroy-like disease (caused by *VEGFC* variants) the penetrance is notably lower, around 50% [38,39]. In addition, hereditary forms are also characterised by variable expressivity, which means that the clinical manifestations can vary in severity between individuals, even within the same family, with the same genetic variant. The clinical variability can be considerable, ranging from mild to severe clinical manifestations. For example, in CM-AVM, some individuals may have only multifocal, harmless CMs, while others may have life-threatening AVMs in addition [9,40]. Somatic second-hit is one explanation for this incomplete penetrance and variable expressivity. In this mechanism, a pathogenic variant in one allele of a gene is present in all cells, and a second variant is required in the other allele of the same gene for the malformation to develop. This phenomenon has been demonstrated in several hereditary vascular malformations, such as GVM, CM-AVM, VMCM, and CCM [12, 40–42].

Genetic counseling plays an important role in the management of vascular malformations, particularly when there is a suspected hereditary type. The primary role of genetic counseling is to provide individuals and families with general information about the condition, its genetic basis, inheritance patterns, and potential risks for future generations. It helps patients understand the implications of genetic testing, including the potential outcomes, limitations, and what the results could mean for both the individual and their family members.

Genetic counselling for vascular malformations can be challenging. The somatic variants present in sporadic vascular malformations are usually not present as germline variants. In fact, it is thought that if these variants were present in all cells, they would likely have severe life-threatening effects. For hereditary forms, the challenges are particularly due to the complexity introduced by incomplete penetrance and variable expressivity. These factors lead to considerable uncertainty in predicting the risk of developing clinical manifestations and their severity in individuals and their families. They also complicate family planning discussions, as couples have to make decisions based on the likelihood of developing symptoms and the potential severity of the disease if the variant is passed on. This uncertainty can have significant psychological and emotional impact and can be associated with confusion and anxiety. It is therefore important to provide as much clarity as possible, while acknowledging the limitations of current knowledge, so that families can make informed decisions despite the uncertainties.

In hereditary vascular malformations with autosomal dominant inheritance, there is not always a family history. In this case, the malformation appears to be sporadic (the only individual affected in the family). This can happen if the genetic variant has arisen de novo in the affected individual, meaning that it has not been inherited from either parent. The likelihood of de novo variants varies by disease : for example, about 25% of CM-AVM cases are due to de novo changes, whereas in HHT the figure is less than 5% [40,43]. If the variant is not present in either parent, there remains a small possibility of germline mosaicism, which confers a low risk of recurrence in subsequent pregnancies. Another possible explanation for the absence of a family history is incomplete penetrance, where the genetic variant is present in one parent but does not manifest clinically. It is therefore important to offer genetic testing to parents, even in the absence of clinical features. The results of these tests can provide accurate genetic counselling and guide better-informed reproductive decisions for future pregnancies. In addition, if the genetic variant is found in one parent, genetic testing can be offered in cascade to at-risk relatives. It is important to mention that genetic testing of unaffected at-risk minors requires careful ethical and medical consideration. Key questions to be addressed include : what are the potential benefits of early detection? are preventive measure or treatments available? [44].

4. Genetic testing

The primary role of genetic testing is to confirm or clarify the diagnosis, especially in cases where clinical features alone may not be sufficient to distinguish between different types of vascular malformations. It also helps guide management decisions, facilitates more precise therapeutic approaches, and supports the development of new treatments. However, it is important to note that genetic testing alone cannot always distinguish between diseases, as different forms can be caused by the same variants or variants in the same gene. For example, genetic testing does not distinguish capillary malformation from Sturge-Weber syndrome, both of which are caused by the R183Q variant in the *GNAQ* gene. In addition to these benefits, genetic testing is important for genetic counselling. Identifying the causal genetic variant in individuals with hereditary diseases allows personal and familial risk assessment for

having or developing the disease as well as the risk for offspring. Finally, genetic testing may offer the possibility of prenatal or preimplantation genetic diagnosis for couples at increased risk of having a child with potentially serious disease.

Genetic testing for both sporadic and hereditary vascular malformations can be performed using a single technique or separate approaches. However, it is important to note that testing for sporadic or germline variants involves technical differences and, although there is some overlap, the genes involved in sporadic vascular malformations are in most cases, different from those involved in hereditary forms. The choice of the optimal approach for a laboratory depends on factors such as local resources (e.g. sequencing platforms), the number of samples (to ensure optimal turnaround time), and cost considerations. A detailed clinical description and working diagnosis are important to guide the laboratory in selecting the appropriate genetic tests and interpreting the results.

Recommendations for genetic testing in sporadic vascular malformations have recently been published [45]. In these malformations, the genetic variants are present in a subset of cells, often with a VAF below 10%. Identifying variants with low VAF is challenging and requires highly sensitive techniques and careful selection of the tissues to be analysed. In most cases, blood analysis is unlikely to identify these variants. Several technical approaches can be used, each with its own advantages and limitations. An effective first-tier test for sporadic vascular malformations could be (droplet) digital PCR, a rapid technique capable of detecting variants with very low VAFs (as low as 0.1%). This method can be used to identify hotspot variants, which are common in sporadic vascular malformations. The laboratory selects the appropriate variant or set of variants for analysis based on the clinical information provided with the sample, which thus needs to be accurate and complete. For example, the R183Q variant would be first test in cases of common CM and Sturge-Weber syndrome, while the E542K, E545K, and H1047L/R variants would be relevant for PROS. Cell-free DNA (cfDNA) testing is a non-invasive, promising approach, particularly useful when the affected tissue is inaccessible or difficult to access, or in young patients. However, a major limitation is that the disease-causing variants often have a lower VAF than in the affected tissue, making them even more difficult to detect [31,46].

For broader genetic testing, deep exome sequencing or customised multi-gene panels offer more comprehensive solutions. Exome sequencing (sequencing of all protein-coding genes) not only allows the study of genes of interest, but also has the potential to uncover novel genetic contributors to vascular malformations. However, this method is often not performed at a depth sufficient to reliably detect mosaic variants with a VAF of 1% or less. In contrast, multi-gene panels offer deeper sequencing coverage at a lower cost and are currently the most widely used approach. These panels contain a defined set of genes or gene regions involved in vascular anomalies, and therefore require regular updates as new genes are identified. In addition, by definition, they do not allow for the identification of new genes. However, as large-scale sequencing technologies become more affordable and powerful, the cost-benefit balance may gradually shift from multi-gene panels to deep exome sequencing in the future.

In hereditary vascular malformations, variants are usually present in all cells. Except for the *TEK* gene, the variants identified in all the other genes involved in hereditary vascular malformations cause loss-of-function and can be located throughout the gene. Therefore, targeted approaches, such as (droplet) digital PCR are not useful. Exome sequencing or customised multi-gene panels are the techniques of choice. As with sporadic vascular malformations, the exome sequencing offers the possibility of more extensive analysis if needed, whereas multi-gene panels require regular updating to include newly identified genes. On the other hand, exome sequencing can present challenges, particularly in the detection of copy number variants and mosaic variants, which are rare, but can occur in patients with hereditary vascular malformations [47,48]. In addition, the coverage of the coding regions of relevant genes may be incomplete. These challenges are often more effectively addressed by multi-gene panels, which are specifically designed to target the relevant genes.

5. Pathways involved in vascular malformations and targeted treatments

The major signalling pathways involved in vascular malformations include : 1) the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, 2) the RAS/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase

(ERK) pathway, and 3) the G protein-coupled receptor (GPCR) pathway (Figure 2). Pathogenic variants in genes of the PI3K/AKT/mTOR pathway are primarily associated with slow-flow vascular malformations, such as lymphatic and venous malformations. In contrast, pathogenic variants in genes in the RAS/MAPK/ERK pathway are mainly associated with fast-flow vascular malformations, including intra- and extra-central nervous system sporadic AVMs and CM-AVM type 1 and 2. Pathogenic variants in this pathway are also associated with complex lymphatic anomalies. Pathogenic variants in genes of the GPCR pathway, are commonly found in CM and Sturge Weber syndrome and result in the activation of the downstream ERK pathway. The effect of the pathogenic variants in the vascular malformations is the activation of the PI3K/AKT/mTOR or RAS/MAPK/ERK or both (due to cross-talks between the 2 pathways). This has led to the idea that inhibitors of these pathways might be useful in the treatment of vascular malformations. In addition, many of the somatic genetic variants that cause sporadic vascular malformations are also common in different types of cancer, opening up therapeutic options for pathway-specific pharmacological inhibitors that are already used in cancer. These options are particularly important for patients for whom conventional treatments such as laser, surgery, embolisation, and sclerotherapy cannot be used or are insufficient to control the disease.

Sirolimus, an mTOR inhibitor, initially used to prevent renal allograft rejection, has been shown to be effective in treatment of patients with slow-flow vascular malformations. Following several successful phase II clinical trials, and several case reports/case series, preliminary results of a large, multicentric, prospective, phase III European study (VASE), show promising results [49]. Of the 250 patients with slow-flow vascular malformations refractory to standard treatments enrolled in VASE, 85% showed clinical improvement with decreased pain, bleeding and infections after one year of treatment. Alpelisib, a selective PI3Kα inhibitor, demonstrated efficacy in a study including 19 patients with CLOVES refractory to standard of care [50]. Clinical improvement was observed in all the patients. In 2022, Alpelisib received accelerated approval from the US Food and Drug Administration for the treatment of patients aged 2 years or older with severe manifestations of PROS, who require systemic therapy. Further confirmatory evidence is needed to sustain the approval. In AVMs, trametinib, a MEK inhibitor, has shown promising results in several case report studies. Preliminary results from the ongoing monocentric TRAmetinib in Arteriovenous Malformation trial (TRAMAV) study have shown efficacy in 10 patients with complex extracerebral

AVMs. Thalidomide was evaluated in a prospective study as a potential treatment for AVMs due to its anti-angiogenic and anti-inflammatory properties. Remarkable results were achieved in all 18 patients treated with rapid reduction of pain, cessation of bleeding, and healing of ulcers [51]. More information on pharmacological treatments can be found in the dedicated articles of this special issue of *La Presse Médicale*.

6. Conclusion and future perspectives

The identification of the genetic bases of vascular anomalies is fundamental for understanding their pathophysiology and improving our ability to classify, diagnose and treat these diseases more effectively. Significant progress has been made over the last 15 to 20 years, largely due to the widespread use of next-generation sequencing techniques in both research and diagnostic laboratories. It has become increasingly clear that these malformations exhibit both genetic and phenotypic heterogeneity, with variants in the same gene potentially leading to different phenotypes, and the same phenotype being caused by variants in different genes. This complexity highlights the fact that genetic testing alone is not automatically sufficient to guide the correct diagnosis. It also underlines the importance of regular gene-phenotype association evaluations to clarify which gene is associated with which phenotype and to assess the strength of these associations [45].

Targeted therapies have emerged as a promising option for patients with complex malformations that are refractory to conventional treatments. Important questions to be addressed in the near future include : should targeted therapies be combined in specific clinical contexts? what is the optimal dose? how can these therapies be integrated with standard treatment approaches? Further research is needed to develop even more precise therapeutic approaches and advance the field of precision medicine for these patients. At present, however, not all patients benefit from genetic testing. In some cases, the affected tissue may not be accessible or the genetic techniques, despite their high sensitivity, may fail to identify pathogenic variants, particularly those with very low VAF. There is a need for continued improvements in the sensitivity of genetic techniques, including testing on cell-free DNA.

Figure legends

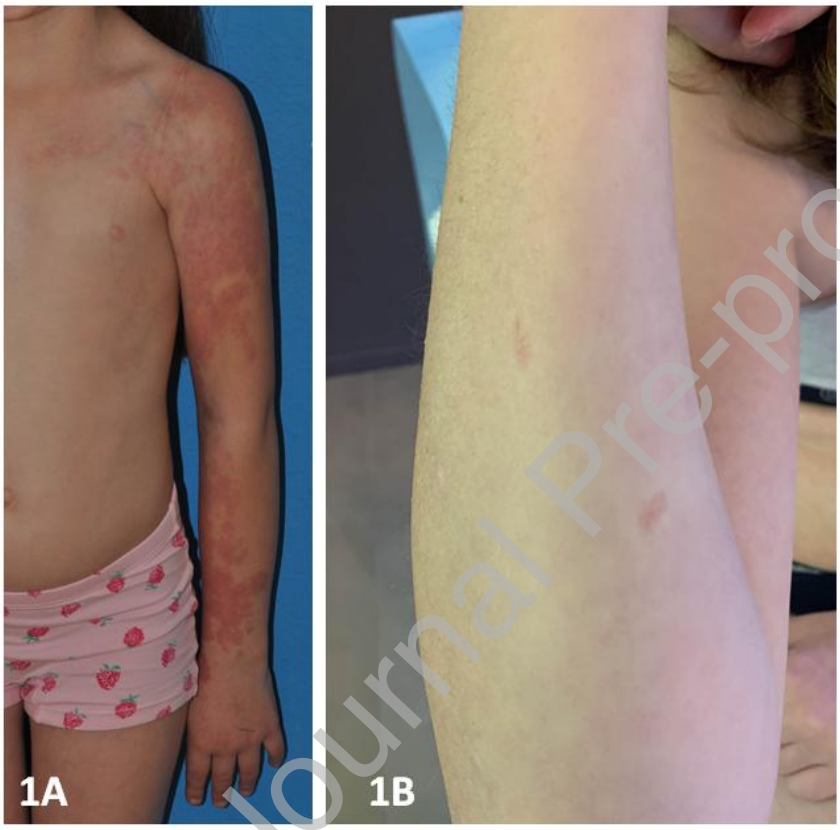

Figure 1 : Examples of vascular malformations

1A: common capillary malformation (port-wine stain); 1B : multiple capillary malformations with halo, typical of capillary malformation – arteriovenous malformation.

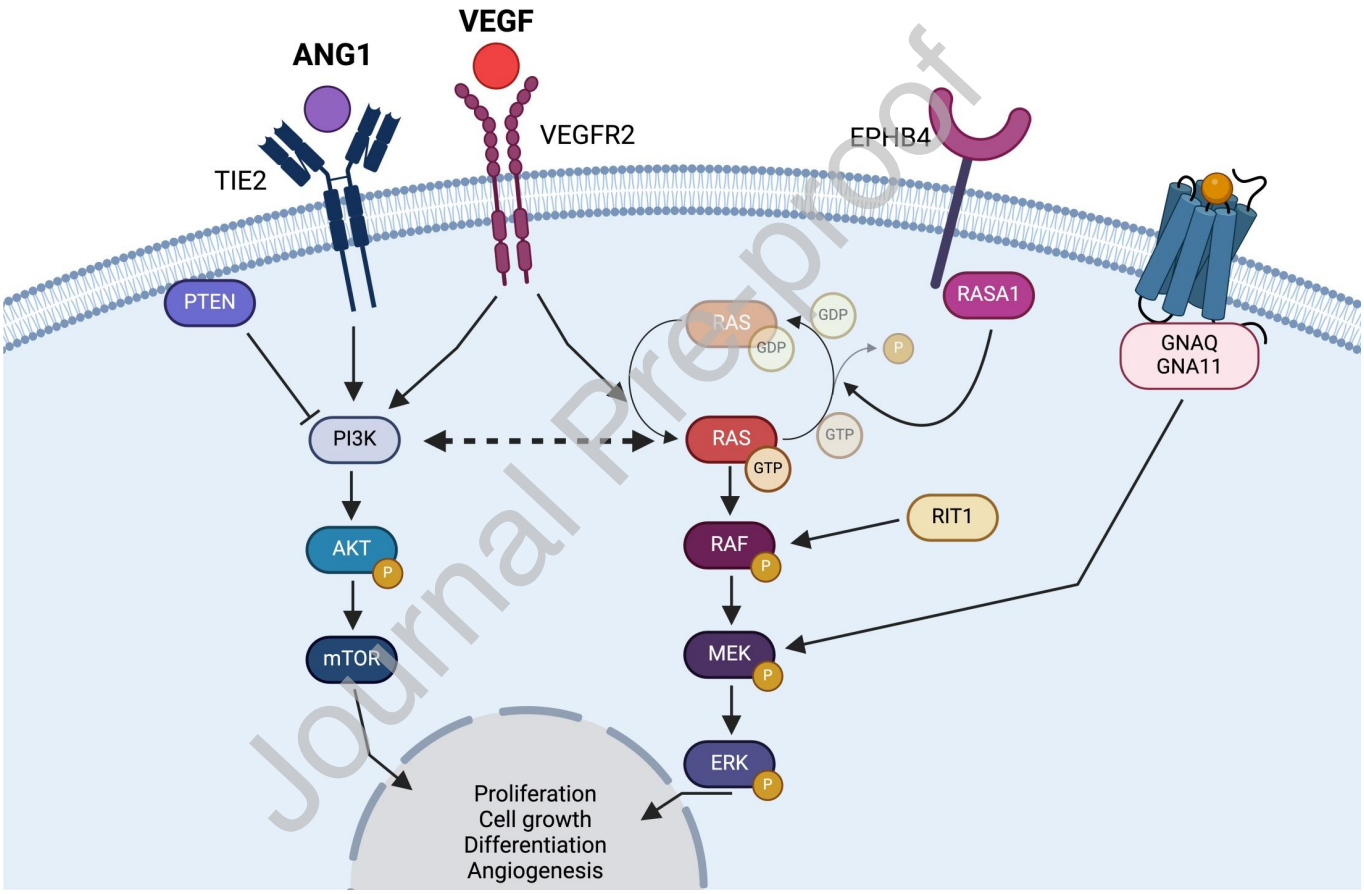

Figure 2 : Major pathways involved in vascular malformations

1) The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, primarily associated with slow-flow vascular malformations, such as lymphatic and venous malformations

2) the RAS/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, mainly associated with fast-flow vascular malformations, including intra- and extra-central nervous system sporadic AVMs and CM-AVM type 1 and 2. Pathogenic variants in this pathway are also associated with complex lymphatic anomalies

3) the G protein-coupled receptor (GPCR) pathway, primarily associated with CM and Sturge Weber syndrome

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Table 1. Vascular malformations, mode of inheritance, genes involved, and major clinical characteristics

Malformation	MOI	Gene	Major clinical characteristics
Slow-flow vascular malformations			
capillary malformation ("port-wine stain") (CM)	sporadic	*GNAQ / GNA11* somatic, activating	flat, red, homogenous, stain, usually present on skin sometimes on the mucosa, present at birth; becomes darker and thicker with time
capillary malformation with overgrowth (CMO)	sporadic	*GNAQ / GNA11* somatic, activating	purple or dark red CM of an extremity associated with soft tissue and bony hypertrophy, which develop over time;
diffuse capillary malformation with overgrowth (DCMO)	sporadic	*PIK3CA / GNA11* somatic, activating	diffuse, reticulated, pink-to-red CM on skin, spread over several anatomic regions, uni- or bi-lateral, present at birth, accompanied by tissue and/or bone proportionate overgrowth in affected areas, which develop over time
capillary malformation dilated veins (CMDV)	sporadic	*PIK3CA / PIK3R1* somatic, activating	purple, homogenous, regional CM with mild limb hypertrophy due to venous stasis and phlebectasia
cutis marmorata telangiectatica congenital (CMTC)	sporadic	-	bluish-red, reticulated, cutaneous lesions associated with skin atrophy; can ulcerate; spontaneous
cerebral cavernous malformation (CCM)	AD	*KRIT1 / CCM2 / PDCD10* loss-of-function	multiple, dilated capillary-like vessels in a dense collagenous matrix, mainly located in the central nervous system; possible retinal, cutaneous lesions
	sporadic	*MAP3K3* somatic, activating	solitary CCM lesion in the central nervous system

venous malformation (VM)	sporadic	*TEK / PIK3CA* somatic, activating	soft, compressible, light-to-dark blue lesions, mainly on skin or mucosa; can infiltrate the underlying tissues, muscles and joints.
multifocal sporadic venous malformation (MSVM)	sporadic	*TEK* somatic, mosaic, activating	multiple small, raised lesions of various hues of blue involving the skin and oral mucosa and, occasionally, the subcutaneous tissues and skeletal muscles
blue rubber bleb nevus syndrome (BRBN)	sporadic	*TEK* somatic, activating	many born with a dominant lesion, in addition multiple, small, cutaneous VMs, mainly on the palms and soles; visceral VMs, mainly gastrointestinal with high risk of bleeding

verrucous venous malformation (VVM)	sporadic	*MAP3K3* somatic, activating	raised purple hyperkeratotic papule or nodule on the skin; can extend in the subcutis
mucocutaneous venous malformation (VMCM)	AD	*TEK* activating	multifocal, small, bluish lesions, mainly involving skin and oral mucosa, can rarely affect the muscles and gastrointestinal tract
glomuvenous malformation (GVM)	AD	*GLMN* loss-of-function	multifocal, bluish-purple, raised, cobblestone-like, and hyperkeratotic nodules, painful on palpation, mainly on skin and subcutis
Fast-flow vascular malformations			
arteriovenous malformation (AVM)	sporadic	*KRAS / MAP2K1 / BRAF / HRAS / RIT1* somatic, activating	direct communication between feeding arteries and draining veins trough a bundle of abnormal vessels (nidus)
hereditary haemorrhagic telangiectasia (HHT)	AD	*ENG / ALK1 / GDF2* loss-of-function	cutaneomucosal and gastrointestinal telangiectasias; AVM/AVF mainly in lung, liver, brain and spine
hereditary haemorrhagic telangiectasia/juvenile polyposis	AD	*SMAD4* loss-of-function	HHT features and predisposition to hamartomatous polyps of the gastrointestinal tract; malignant transformation in up to 50% of patients
capillary malformation-arteriovenous malformation (CM-AVM)	AD	*RASA1 / EPHB4* loss-of-function	AVM/AVF (brain, spine, head and neck, extremities), Parkes Weber syndrome, associated with multifocal CMs
Vascular malformations associated with other anomalies			
Sturge-Weber syndrome	sporadic	*GNAQ / GNA11* somatic, activating	congenital capillary malformation on the forehead and the upper eyelid, ipsilateral leptomeningeal angiomatosis with high risk of seizures and ocular involvement, mainly glaucoma
cutis marmorata telangiectatica congenital (CMTC) - variant	sporadic	*AKT3* somatic, activating	reticulated or faint geographic CM, with deep reddish-purple hue, with or without brain abnormalities, such as megalencephaly, hemimegalencephaly or focal cortical dysplasia

macrocephaly (megalencephaly) - capillary malformation (M-CM; MCAP)	sporadic	somatic *PIK3CA* somatic, mosaic, activating	macrocephaly (megalencephaly) and capillary malformation, plus a variable combination of other features: developmental delay, brain and body asymmetry, syndactyly, polydactyly, and joint hyperlaxity
microcephaly-capillary malformation (MIC-CAP)	AR	*STAMBP* loss-of-function	multiple, pink-to-red, small, capillary malformations spread diffusely on the body, severe progressive microcephaly, early-onset refractory epilepsy and profound developmental delay
Klippel-Trenaunay syndrome (KTS)	sporadic	somatic *PIK3CA* somatic, activating	asymmetrical overgrowth in girth and length of an extremity with a vascular malformation consisting of combined capillary, lymphatic and venous malformation; there is often persistence of the embryonic vein of the lateral thigh (vein of Servelle) and anomalies of the deep venous system
congenital lipomatous overgrowth, vascular malformations, epidermal nevi, skeletal/scoliosis and spinal abnormalities (CLOVES) syndrome	sporadic	somatic *PIK3CA* somatic, activating	congenital, extensive, primarily truncal, VM, CM, LM or combined-type of vascular malformations, lipomatous overgrowth, epidermal nevi, enlarged, but not distorted, bony structures, scoliosis, asymmetric overgrowth of extremities with broad feet
lower lip capillary malformation, face and neck lymphatic malformation, asymmetry and partial/generalized overgrowth (CLAPO) syndrome	sporadic	somatic *PIK3CA* somatic, activating	capillary malformation of the lower lip, in the midline, symmetrical and well defined is the hallmark of CLAPO syndrome; lymphatic malformation, often associated with venous malformations, especillay in the face and neck region; partial or generalized overgrowth
fibro adipose vascular anomaly (FAVA)	sporadic	somatic *PIK3CA* somatic, activating	fibrofatty infiltration of skeletal muscle (mainly in the lower extremities), pain, functional restriction, venous and lymphatic malformations
Proteus syndrome	sporadic	somatic *AKT1* somatic, activating	asymmetric, progressive, disproportionate, severely deforming, overgrowth of body parts with bony hypertrophy, dysregulated adipose tissue, epidermal nevi, vascular malformations (CM, VM and/or LM) and increased tumour risk

PTEN Hamartoma Tumor Syndrome (Bannayan-Riley-Ruvalcaba syndrome/Cowden syndrome	AD	*PTEN* loss-of-function	highly variable phenotype, including macrocephaly, intellectual disability, penile lentigines, multiple hamartoma with a high risk of benign and malignant tumors of the thyroid, breast, and endometrium, kidney and colon; intramuscular vascular anomalies, predominately fast-flow, ectopic fat and severe
Maffucci syndrome	sporadic	*IDH1 / IDH2* somatic, activating	multiple enchondromas associated with superficial and deep slow-flow venous-like malformations (called spindle cell hemangiomas), containing areas with spindle cells; enchondromas are commonly found in the bones of the hands and feet, and can degenerate into chondrosarcomas
Parkes Weber syndrome	sporadic	*RASA1* somatic, loss-of-function *KRAS* somatic, activating	capillary malformation associated with diffuse micro-arteriovenous fistulas throughout an extremity with limb overgrowth due to bony and/or soft-tissue hypertrophy
	AD	*RASA1 / EPHB4* loss-of-function	same characteristics as the sporadic form plus associated multifocal CM distant from the Parkes Weber lesion

Lymphatic anomalies

lymphatic malformation	sporadic	*PIK3CA / BRAF* (rare) somatic, activating	dilated micro, macrocystic or mixte lymphatic channels with clear or serosanguineous fluid; most diagnosed during infancy
Nonne–Milroy lymphedema (Milroy disease)	AD, AR	*FLT4* loss-of-function	lower limb bilateral lymphedema, localised below the knees, and usually present at birth; hydrops (rare); absent or hypoplastic subcutaneous lymphatic vessels
Nonne–Milroy-like lymphedema	AD	*VEGFC* loss-of-function	neonatal or very early onset lymphedema of the lower legs, associated with dysplastic toenails, leg papillomatosis and hyperkeratosis; phenotype is similar to Milroy disease, but milder
lymphedema – distichiasis syndrome	AD	*FOXC2* loss-of-function	lymphedema in lower limbs at or after puberty; second row of eyelashes, varicose veins; hydrops
hypotrichosis-lymphedema-telangiectasia	AD	*SOX18* loss-of-function	lymphedema, sparse hair, telangiectasias; hydrops

Hennekam syndrome	AR	*CCBE1 / FAT4 / ADAMTS3* loss-of-function	lymphedema of the extremities, intestinal lymphangiectasia, variable intellectual disability, dysmorphic features
microcephaly, lymphedema, chorioretinopathy syndrome	AD	*KIF11* loss-of-function	microcephaly, mild to moderate developmental delay, congenital lymphedema, ocular abnormalities
Emberger syndrome	AD	*GATA2* loss-of-function	lymphedema of lower extremities and genitalia, immune dysfunction, cutaneous warts, deafness
lymphedema-choanal atresia syndrome	AR	*PTPN14* loss-of-function	lymphedema of lower limbs in children, lack of nasal airways
other primary lymphedema forms	AD	*GJC2* ?	lymphedema of the extremities, onset at <15 years of age
	AD, AR	*ANGPT2* loss-of-function	lymphedema occuring in the first year of life, mainly involving the lower extremities. The upper extremities can also be involved. Hydrocele is frequent in boys. Gradual resorption generally occurs, although some patients may experience progression complicated by
	AD	*CELSR1* loss-of-function	lower limb lymphedema, unilateral or bilateral, with almost complete penetrance with onset in childhood or adolescence in females, while in males, it shows incomplete penetrance and later onset
	AD	*HGF* loss-of-function	lower limb lymphedema with onset varying from early childhood to adulthood.
	AD	*EPHB4* kinase-inactivating variants	hydrops fetalis or late-onset lymphedema
	AR	*PIEZO1* ?	generalised lymphatic dysplasia with systemic involvement: pleural and pericardial effusion, ascites and intestinal and/or pulmonary lymphangiectasia. The onset is prenatally, with a high incidence of non-immune hydrops fetalis, or during childhood
	AD	*TIE1* *loss-of-function*	lower limb of four limb lymphedema occuring in puberty or adulthood

Gorham-Stout disease (GSD)	sporadic	*KRAS* somatic, activating	also known as, vanishing bone disease : progressive destruction and resorption of bones, which are replaced by lymphatic vessels and capillaries. It can also involve viscera and soft tissues
generalised lymphatic anomaly (GLA)	sporadic	*PIK3CA* somatic, activating	invasive lesions affecting multiples organs (bones, lungs, mediastinum, liver, spleen and soft tissues); in contrast with GSD, the bones lesions are not associated with cortical bone resorption
Kaposiform lymphangiomatosis (KLA)	sporadic	*NRAS* / *CBL* somatic, activating	subtype of GLA, histologically characterised by foci of spindle-shaped lymphatic endothelial cells; often associated with severe, life-threating coagulopathy (hypofibrinogenemia and thrombocytopenia)
central conducting lymphatic anomalies (CCLA)	sporadic	*BRAF* / *KRAS* / *MAP2K1* somatic, activating	dilation and dysfunction of large lymphatic vessels, mainly leading to ascites or pleural effusion or protein- losing enteropathy. It can be associated with peripheral lymphedema, and it can be part of GSD, GLA and KLA.
	AD	*EPHB4* kinase inactivating	

	AR	*MDFIC*	abnormalities in the development and function of major truncal lymphatic vessels, causing nonimmune hydrops fetalis, postnatal subcutaneous lymphedema and chylothorax, with pleural and pericardial effusions and ascites; stillbirth in some cases

Author Contributions

Nicole Revencu – Designed and wrote the manuscript
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