

# The role of GPRASP1 mutations in the pathogenesis of arteriovenous malformations

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## Abstract

Arteriovenous Malformation (AVM) is a sporadic vascular disease caused by the aberrant direct connection between arterioles and veins, resulting in disorganized blood shunting and oxygen level disparities. Previous research has elucidated some of the genetic underpinnings of AVM and related vascular anomalies. Despite its significant prevalence, current therapeutic approaches remain largely conservative, underscoring the need for a deeper understanding of AVM pathogenesis. This study focuses on the GPRASP1 gene, which encodes the G-protein coupled receptor-associated sorting protein 1 (GASP-1), a molecule implicated in vascular development. Utilizing western blotting and cellular assays, we explored the role of GPRASP1 in angiogenesis, neoplastic formation, and other mechanical aspects of AVM. Our findings suggested that mutations in the GPRASP1 gene may contribute to elevated levels of angiogenic factors and altered cellular functions related to angiogenesis, such as enhanced proliferative and migratory capabilities. This research lays the groundwork for identifying novel therapeutic targets in the management of AVM and offers insights into precision medicine approaches.

**Keywords:**GPRASP1 gene, arteriovenous malformation, G protein-coupled receptor, Germline mutations

## 1. Introduction

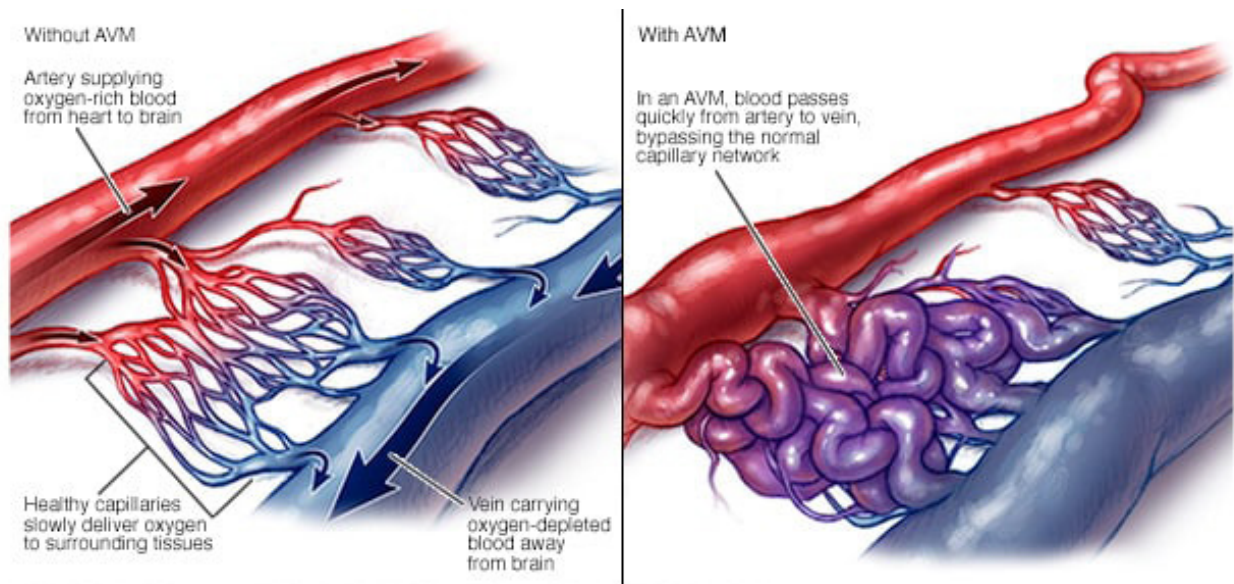
Arteriovenous malformation (AVM) is a congenital vascular disease, mostly sporadic onset. This disease comprises a mass of arteries, veins, and arterialized venous blood vessels that lack a capillary network, resulting in a blood flow disorder. AVM is caused by the absence of the capillary network structure between the arteries and veins, resulting in a direct blood connection, allowing a disorderly diversion of blood with different oxygen levels, causing serious damage in the body. Recent therapies include surgical resection and radiation therapy etc.; however, there are no appropriate therapies that can eradicate this disease in present clinical treatment. Therefore, researchers must start with the pathogenesis to provide a reference and basis for the treatment study. Recently, some studies conducted at the genetic level on the pathogenesis of different kinds of AVM are relatively mature. Still, more mechanisms need to be elucidated by further research.

GPRASP1 encodes G-protein coupled receptor-associated sorting protein, which regulates G-protein coupled receptors and can cause downregulation of gene expression. Abnormal GPRASP1 gene expression has been found in arteriovenous malformations of studied family lines. On this basis, we discussed the mechanism of GPRASP1 involved in developing brain arteriovenous malformations. Thus, this literature review will introduce the basic information about AVM first and then discuss

the current research on the genetic pathogenesis of AVM; finally, it will focus on the information and previous research about the GPRASP1 gene that will be studied in this work.

## 2. Pathology, Symptoms, Types and of AVM

The four types of vascular malformations include venous malformations (VM), cavernous malformations (CM), telangiectasis, and AVM(Lawton et al., 2015). AVM is one of the most common types and is considered to be a congenital disease. AVM belongs to the type of high-throughput vascular malformation caused by the partial or total deletion of the capillary network to form a malformed vascular mass, which directly connects the blood supply artery and the drainage vein. The blood is misdirected from the artery and quickly shunted into the vein (Laakso & Hernesniemi, 2012). Arteriovenous malformations can occur in almost any organ and are characterized by superficial, deep, or combined distribution(Uller et al., 2014). Therefore, the location, size, and severity of different arteriovenous malformations are very different(Lawton et al., 2015), mainly in the head and neck, followed by limbs, trunks, and organs(Greene & Orbach, 2011).



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**Fig 1 Pathological demonstration of AVM (Brain AVM (Arteriovenous Malformation) - Symptoms and Causes, n.d).**

The left picture is of normal blood vessels, and the right picture is of arteriovenous malformation blood vessels. Capillary network loss: artery and vein directly connected. AVM is usually a congenital disease born in infants but usually becomes obvious in childhood (Greene & Orbach,

2011). The lesions begin to appear as pink skin spots, and then the AVM lesions expand caused by angiogenesis and angiogenesis. Most people will have symptoms such as tissue overgrowth, congestion, pain, bleeding, ischemia, pain, ulcers, etc., in adore later (Greene & Orbach, 2011).

**Table 1. Schrodinger staging of AVM (Greene & Orbach, 2011).**

Stage	Clinical Findings
I (Quiescence)	Warm, pink-blue, shunting on Doppler
II (Expansion)	Enlargement, pulsation, thrill, bruit, tortuous veins
III (Destruction)	Dystrophic skin changes, ulceration, bleeding, pain
IV (Decompensation)	Cardiac failure

## 2.1 Arteriovenous malformation-related diseases

Arteriovenous malformations are divided into many types, mainly including hereditary hemorrhagic telangiectasia (HHT), capillary malformation-arteriovenous malformation complex (CM-AVM), and brain arteriovenous malformations (BAVM)

### 2.1.1 Hereditary Hemorrhagic Telangiectasia (HHT)

HHT is an autosomal dominant hereditary vascular disease known as Rendu-Osler-Weber syndrome. The incidence is about 1 / 5000 (Mamai et al., 2022, p. 14). The main symptoms are frequent rhinorrhagia, mucosal skin telangiectasia, lung, liver, and brain AVMs, and gastrointestinal bleeding caused by telangiectasia (Lesca et al., 2007). Telangiectasia is usually caused by abnormal vascular remodeling, leading to capillary rupture and direct shunt from the small artery to the small vein (Guttmacher et al., 1995) "properties": {"formattedCitation": "(Guttmacher et al., 1995. In general, the symptoms of HHT in the superficial epidermis are lighter and less harmful to patients. However, HHT occurring in organs has a high risk of rupture because AVM lesions in their corresponding organs are usually accompanied by HHT (McDonald et al., 2015) ENG and ACVRL1, were discovered almost two decades ago, and mutations in these genes have been reported to cause up to 85% of HHT. In our experience, approximately 96% of individuals with HHT have a mutation in these two genes, when published (Curação. For example, approximately 60 % of patients with pulmonary arteriovenous malformations have hereditary hemorrhagic telangiectasia (McDonald et al., 2015) ENG and ACVRL1, were discovered almost two decades ago, and mutations in these genes have been reported to cause up to 85% of HHT. In our experience, approximately 96% of individuals with HHT have a mutation in these two genes, when published (Curação. As a genetic disease, the main pathogenic gene mutations of HHT occur on ENG, ACVRL1, SMAD4, and GDF2. (Shovlin et al., 2020)

### 2.1.2 Capillary Malformation-Arteriovenous Malformation (CM-AVM)

CM-AVM is a rare vascular malformation with autosomal dominant inheritance. The prevalence of CM-AVM syndrome is about one in 100,000 (Alluhaibi et al., 2021). It was first discovered and named in 2003 by Iiro Eerola et al. Its lesion structure has both the phenotypic characteristics of capillary malformation and the characteristics of rapid flow high-flux arteriovenous malformation, which can occur in most parts of the body (Eerola et al., 2003). RASA1 mosaic is caused

by loss-of-function mutations in RASA1 or EPHB4 genes (Amyere et al., 2017) they also can be multifocal in autosomal-dominant disorders, such as hereditary hemorrhagic telangiectasia and capillary malformation (CM).

### 2.1.3 Brain Arteriovenous Malformation (BAVM)

BAVM are high-flow vascular malformations that occur in the brain. Most of them are sporadic, but a few family cases are reported. Sporadic BAVM can be caused by KRAS / BRAF and MAPK / ERK mutations (Hong et al., 2019) (Bameri et al., 2021). BAVM is the most common type of arteriovenous malformation, occurring in approximately 15 out of 100,000 people with an estimated incidence of 1.3 per 100,000 patients per year (Lawton et al., 2015). BAVM can easily lead to intracranial hemorrhage (50 %), which is the most common cause of intracranial hemorrhage in 20-40 young people (Lawton et al., 2015). In addition, patients without hemorrhage will have epilepsy, headache, stroke, and focal neurological deficits (Laakso & Hernesniemi, 2012). Because the surgical treatment of BAVM has the risk of increasing vascular rupture, non-interventional follow-up or invasive treatment is mainly used (Lawton et al., 2015). The current treatment methods mainly include microsurgery, embolization, and radiosurgery.

## 3. The Genetic Pathogenesis of AVM

### 3.1 Somatic Mutation

Somatic mutations causing AVM mainly include KRAS / BRAF and MAPK / ERK. Firstly, KRAS / BRAF is a common tumor mutation that leads to abnormal cell growth and proliferation. This mutation was first reported in the study of Nikolaev *et al.*, who analyzed tissue and blood samples from patients with BAVM and eventually detected the KRAS mutation in most sporadic patients (Nikolaev et al., 2018). Subsequently, Hong *et al.* performed ultra-deep next-generation sequencing tissue analysis of 422 common tumor genes in 31 patient samples and found that KRAS / BRAF somatic mutations in the brain and spinal arteriovenous malformations, and the mutation rates reached 81 % and 100 %, respectively (Hong et al., 2019). Priemer *et al.* found that KRAS p. G12 V is one of the most common mutations (Priemer et al., 2019). Subsequently, Fish *et al.* used postpartum and adult mice and embryonic zebrafish to demonstrate that KRAS mutations ( G12D or G12V ) were sufficient to induce brain but required active MAPK-MEK (mitogen-activated protein kinase kinase1) signal transduction activity (Fish et al., 2020).

Second, MAPK-ERK is located in one of the downstream



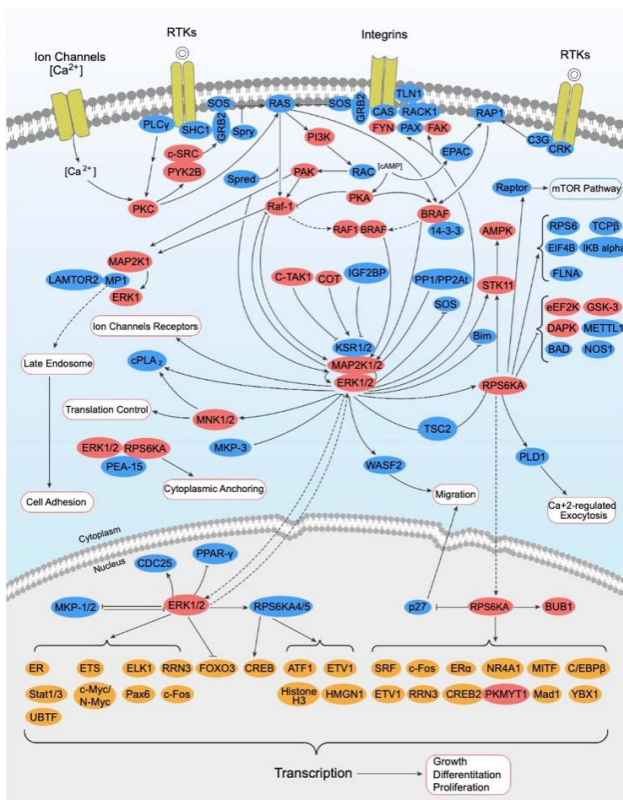
gene pathways of KRAS / BRAF. Increased MEK1 activity causes endothelial dysfunction, and its mutation is a common cause of extracranial AVMs(Couto et al., 2017, p. 1). Therefore, Nikolaev *et al.* detected KRAS somatic mutations in tissue samples from 45 of 72 patients and verified that BAVM was caused by activation of the MAPK-ERK signaling pathway in brain endothelial cells induced by KRAS(Nikolaev et al., 2018). Also, MAP2K1 may have somatic mutations by itself. In the study of Couto *et al.*, they detected and confirmed the presence of this mutation by using exome sequencing (WES), whole genome sequencing (WGS), and ddPCR(Couto et al., 2017, p. 1) and found that Endothelial MAP2K1 further determined the mechanism of the effect of mutant MAP2K1 on EC signal and vascular network formation. MAP2K1 inhibitor drug therapy could prevent the formation and development of AVM(Smits et al., 2020, p. 1). In summary, both KRAS / BRAF and MAPK / ERK somatic mutations play a role in the pathogenesis of arteriovenous malformations.

### 3.2 Germline Mutation

#### 3.2.1 TCR-β Signaling Pathway

The germline mutation ENG, ACVRL1, and SMAD4 in the TGF-β / BMP signaling pathway cause HHT, which regulates cell proliferation, differentiation, and apoptosis. Vascular endothelial cell transforming growth factor TGF-β family ligands (including BMP, TGF-β, etc.) first activate the TGF-β type II receptor (R-II ) in the pathway(Ruiz-Llorente et al., 2017)mucocutaneous telangiectases, arteriovenous malformations (AVMs. Subsequently, by phosphorylation, R-II activates the TGF-β type I receptor (R-1), also known as ALK1 (activin-like kinase). Endothelial glycoprotein (ENG) is a membrane-binding receptor that interacts with the ligand to regulate RI and R-II binding and signal through RI and R-II(Bernabeu et al., 2010). Phosphorylated R-1 activates Smads (R-Smad: Smad1,2,3,5,8) to transmit signals by binding to Smad4 and moving to the nucleus, thereby regulating the transcriptional activity of the target gene. The gene pathway is regulated by the negative feedback of inhibitory Smads (Smad6 and Smad7). Therefore, mutations in the TGF-β / BMP signaling pathway will lead to continuous activation of the pathway and cause abnormal cell function. BMP9, ENG, ALK1, and Smad4 proteins are encoded by GDF2, ENG, ACVRL1, and MADH4 genes, and their pathogenic mutations lead to HHT5, HHT1, HHT2, and JP / HT (juvenile polyposis / HHT syndrome ) (Ruiz-Llorente et al., 2017)mucocutaneous telangiectases, arteriovenous malformations (AVMs).

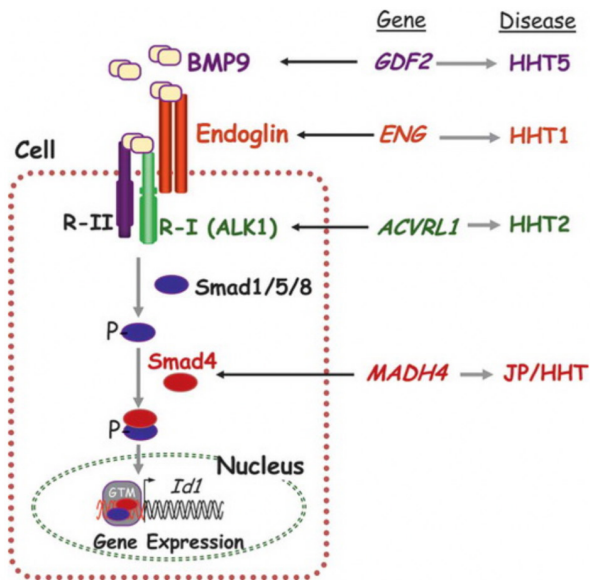
There has been sufficient evidence that mutations in this pathway lead to HHT. Heimdal *et al.* performed in 113 Norwegian families with suspected or confirmed HHT and found that ENG ( HHT1 ) or ACVRL1 ( HHT2 ) gene mutations could cause HHT in about 85 % of families(Heimdal et al., 2016). As early as 1994, McAllister *et al.*, identified the endothelial glycoprotein gene on chromosome 9q3 as an HHT 1 pathogenic gene(McAllister et al., 1994). Subsequently, in 1996, Johnson *et al.* reported a link between the activin receptor-like kinase 1 gene (ACVRLK1 or ALK1 ) and HHT 2(Johnson et al., 1996)or Osler-Rendu-Weber (ORW. Gallione *et al.* collected blood samples from seven unrelated families for testing and found MADH4 mutations in patients without ENG or ACVRL1 mutations, and it was speculated that MADH4 mutations could lead to JP / HT(Gallione et al., 2004)an inherited gastrointestinal malignancy predisposition, is caused by mutations in MADH4 (encoding SMAD4). In summary, TGF-β / BMP signaling pathway mutations play a key role in the pathogenesis of HHT, which can increase the



**Fig.2 Intracellular signal transduction pathway diagram(MAPK-ERK signaling pathway - Elabscience, n.d.)**

**Signaling pathways such as KRAS / BRAF and MAPK / ERK are shown in the figure, with the center portion circled by a blue line.**

incidence of HHT.



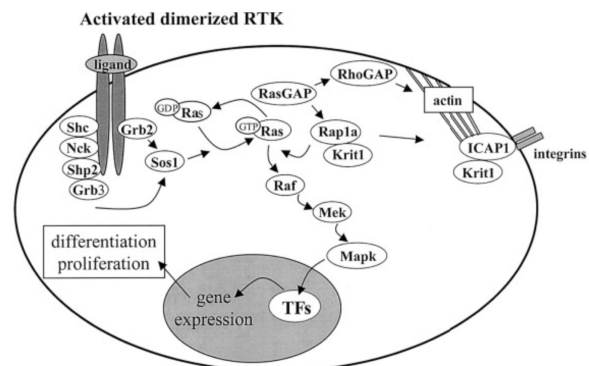
**Fig.3 TGF- $\beta$  / BMP pathway diagram (Ruiz-Llorente et al., 2017) mucocutaneous telangiectases, arteriovenous malformations (AVMs)**

**3.2.2 G Protein-Coupled Receptor Signaling Pathway**

RASA1, the main gene mutation causing CM-AVM, is a germline mutation. The RASA1 gene encodes a p120-RasGAP protein, whose main function is to hydrolyze active GTP into inactive GDP, which acts as a negative regulator to control the RAS / MAPK signaling pathway(Eerola et al., 2003). This pathway controls cell growth, proliferation, maturation, differentiation, and movement by transmitting signals from the extracellular to the nucleus. The pathway should be closed without external signal stimulation(Eerola et al., 2003). But if the RAS gene mutations lead to GAP protein dysfunction that cannot play a hydrolyzing role, it will lead to G protein continuing to transmit signals so that its downstream signaling pathways will be constantly activated, which will play a role in promoting cell growth, differentiation, and division, Eventually, it will lead to arteriovenous malformation

In 2003, Eerola *et al.* used RASA1, which encodes p120-RasGAP, as a candidate gene to screen mutations in 17 families, among which RASA1 mutations were detected in six families with atypical CM disease. Also, the researchers observed that the symptoms of these family members were similar to those of AVM, so the new association caused by RASA1 mutations was named CM-AVM(Eerola et al., 2003). Subsequently, Revencu *et al.* were screened 261 AVM patients, and finally, 58 different RASA1 mutations were found in 68 CM-AVM patients.

Some researchers have also suggested that RASA1 germline mutations required a second strike to lead to treatment(Revencu et al., 2013). In addition, Amyere *et al.* conducted a genome-wide linkage study in another 50 % of non-RASA1 patients and found EPHB4 germline mutations and p120-RasGAP encoded by RASA1 is a direct effect factor of EPHB4, therefore; they pointed out that the EPHB4-RAS-ERK signaling pathway was the main cause of AVM(Amyere et al., 2017)they also can be multifocal in autosomal-dominant disorders, such as hereditary hemorrhagic telangiectasia and capillary malformation (CM. RASA1 functions used zebrafish experiments and found that RASA1 mutations play a role in the EPHB4 / RASA1 / mTORC1 signaling axis downstream of endothelial cells, causing AVM(Kawasaki et al., 2014). In conclusion, although other genetic mutations have been identified(Walcott et al., 2018)and we investigated potential mutations in a 14-year-old girl who developed a recurrent brain AVM. Whole-exome sequencing (WES, the major genetic mutation that causes CM-AVM is the EPHB4-RAS-ERK G protein-coupled receptor signaling pathway.



**Fig.4 RAS / MAPK pathway diagram(Eerola et al., 2003)**

At the same time, G protein-coupled receptor 124 (GPR124) mediates angiogenesis in the central nervous system of embryos. In a genome-control analysis of 195 Caucasian BAVM patients and 243 Caucasian subjects, Weinsheimer *et al.* found that GPR124 is associated with the formation of BAVM and that GPR124 may serve as a potential genetic background contributing to the pathogenesis of sporadic AVM(Weinsheimer et al., 2012)we investigated the association of single nucleotide polymorphisms (SNPs). Subsequent studies have pointed out that GPR124 overexpression can cause excessive proliferation of blood vessels in the central nervous system and increase the risk of AVM diseases such as BAVM. In addition, there is a GNAQ mutation that can lead to

Sturge-Weber syndrome. This gene encodes a member of the G protein  $\alpha$  subunit, mediates the signal between the G protein-coupled receptor and the downstream gene pathway, and then promotes GTP hydrolysis(Shirley et al., 2013). Shirley *et al.* showed that the substitution of cysteine at the position of this gene leads to a decrease in intrinsic GTPase activity, increasing downstream signal activity(Shirley et al., 2013). In summary, G protein-related cytokines and pathway mutations are key in forming AVM.

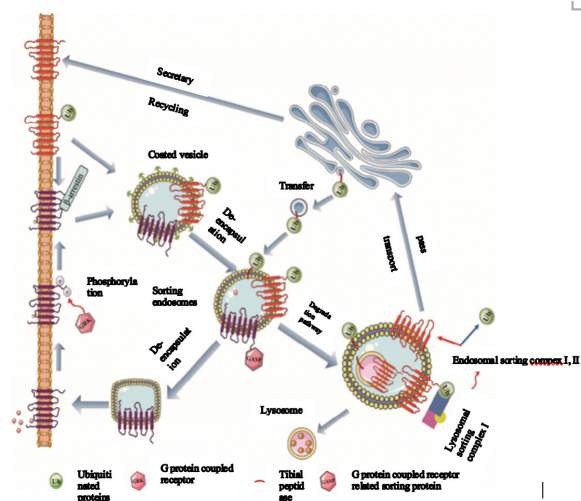
#### 4. The Recent Study of GPRASP1

The GPRASP1 gene encodes G-protein coupled receptor-associated sorting protein 1 (GASP-1) in the human body. This protein is responsible for lysosomal sorting and functional down-regulation of various G-protein coupled receptors. It is the first discovered and named in the family(*GPRASP1 G Protein-Coupled Receptor Associated Sorting Protein 1 [Homo Sapiens (Human)] - Gene - NCBI, n.d.*). Whistler et al., used the carboxyl tail of  $\delta$ -opioid receptor (DOR) cytoplasm as bait to screen GASP-1 for the first time and identified it as a novel sorting protein by using yeast two-hybrid screening(Whistler et al., 2002). Currently, 10 family members have been found, namely GASP-1—GASP-10, which are involved in regulating GPCR activity, the GPCR transport process, signal transduction, and related gene transcription(M. Zhang et al., 2020). Still, the mechanism of action is mostly unclear.

GASP-1 can regulate the sorting process of GPCRs after endocytosis. GPCRs, the most extensive signal body, are responsible for the extracellular signal transduction into the cell. After receiving external stimulation, GPCRs can activate the intracellular signal transduction pathway through the G protein pathway. Then, the phosphorylated GPCRs are uncoupled with the G protein under the action of  $\beta$ -arrestins and transmitted to the sorted endosomes to complete the endocytosis(M. Zhang et al., 2020). After that, GPCRs can be recycled to the cell membrane to re-function through the recycling pathway or after the phosphorylation or ubiquitination of GPCRs by GASPs and enter the degradation pathway. For example, GASP-1 can interact with some types of GPCRs through its carboxyl terminus and mediate the degradation of receptors into lysosomes(Simonin et al., 2004). In addition, GASPs both include the ability to control external signals to activate intracellular signaling pathways by regulating the number of GPCRs that can function(Hirata et al., 2019).

Therefore, GASP-1 can regulate the lysosomal sorting process and down-regulate the function of various

GPCRs. The functional defect of GASP-1 will affect its normal regulation of the endocytosis and sorting of GPCRs, resulting in a large number of GPCRs in the activated state in the cells to continuously activate the downstream signaling pathways, resulting in serious consequences and causing many diseases in the human body. At the same time, current studies have also shown that GASP1 can enhance some GPCRs-related signal transduction by promoting endosome formation, such as the US28 receptor. In addition, individual studies have shown that GASP1 can also directly regulate gene transcriptional expression, thereby affecting cell survival and proliferation.



**Fig.5 Schematic diagram of the GASPs working principle(M. Zhang et al., 2020)**

Researchers have shown that GASP1 dysfunction is associated with the pathogenesis of some diseases. For example, GPRASP1 plays an important role in tumorigenesis. Using 2-D HPLC technology, Xiaoyi Zheng *et al.* first detected GASP1 in tumor extracts of 7 stage 2 and stage 3 breast cancer cases but not in adjacent normal tissues. Subsequently, this team detected GASP-1 overexpression in brain cancer, pancreatic cancer, and breast cancer patients, which was about 4-7 times higher than that in normal healthy individuals and observed by immunohistochemical (IHC) staining that many GPRASP1 particles were attached to the cell membrane and nuclear membrane of cancer cells, making GASP1 a potential tumor marker(Zheng et al., 2012). Tao Zhang *et al.* showed that GPRASP1 is associated with the occurrence, progression, and prognosis of head and neck cancer (HNC) by analyzing many transcriptomic and genomic databases(Zhang et al., n.d.)GPRASP1 specific role has not been clarified in head and neck cancer (HNC). In conclusion, although no study exists to elucidate the pathogenesis of GASP1 in cancer, the high expression of



GASP1 tumors in various cancers demonstrates its impact on cancer. It can be a potential candidate biomarker and therapeutic target for cancer. In addition, GASPs are also associated with neurological diseases; for example, acetylcholine muscarinic receptors (mediating cholinergic neurotransmission to regulate participation in learning and memory) belong to GPCRs and can be within the scope of GASP1 regulation (Jakubik & El-Fakahany, 2010).

GASP1 is the most completely studied one in the GASPs family. Its main function is to sort GPCRs after endocytosis and control their activation of downstream pathways. Therefore, once GPRASP1 has a mutation that leads to loss of function or inactivation of its encoded GASP1, etc., it will lead to sustained activation and expression of downstream signaling pathways, causing serious effects, such as uncontrolled cell proliferation. At present, there are several studies have shown that it has a certain impact on the pathogenesis of diseases such as cancer, neurological diseases, and deafness, such as the level of overexpression in cancer, but few studies have been able to fully explain the specific causes of GASP1 dysfunction in the pathogenesis of these diseases.

## 5. Conclusion

In conclusion, current research has adequately studied sporadically arteriovenous malformations, one of the most serious vascular malformations, identifying and validating multiple gene pathways for treatment, including somatic and germline. However, there is still a problem with current research: family AVM cases have been reported besides Sporadic cerebral arteriovenous malformation cases. However, whether it is because of some genetic factor or just a coincidence is still being determined. Also, in some research, some sporadic patients cannot find common mutations such as KRAS in their samples. Some researchers hypothesized that it might be because of a germline mutation. They found germline mutations of G protein-coupled receptor sorting protein (GPRASP1) in three families with arteriovenous malformations but missed the relevant provided on the molecular and cellular levels (Yan, 2019).

Overall, some studies have found that GPRASP1 mutation occurs regularly in family genetics by bioinformatics methods, but it has not been proven. To further verify the pathogenesis of GPRASP1 in AVM at the molecular and cellular levels, this study investigated whether GPRASP1 is involved in angiogenesis and its mechanism of action at the molecular and cellular levels and whether mutations in the GPRASP1 gene affect altered vascular function.

## Reference

- Alluhaibi, R., Alkhatay, L. N., & Aqeeli, W. (2021). Capillary Malformation-Arteriovenous Malformation Syndrome. *Cureus, 13*(1), e12562. <https://doi.org/10.7759/cureus.12562>
- Amyere, M., Revencu, N., Helaers, R., Pairet, E., Baselga, E., Cordisco, M., Chung, W., Dubois, J., Lacour, J.-P., Martorell, L., Mazereeuw-Hautier, J., Pyeritz, R. E., Amor, D. J., Bisdorff, A., Blei, F., Bombei, H., Domp Martin, A., Brooks, D., Dupont, J., ... Vikkula, M. (2017). Germline Loss-of-Function Mutations in EPHB4 Cause a Second Form of Capillary Malformation-Arteriovenous Malformation (CM-AVM2) Deregulating RAS-MAPK Signaling. *Circulation, 136*(11), 1037–1048. <https://doi.org/10.1161/CIRCULATIONAHA.116.026886>
- Bameri, O., Salarzaei, M., & Parooie, F. (2021). KRAS/BRAF mutations in brain arteriovenous malformations: A systematic review and meta-analysis. *Interventional Neuroradiology, 27*(4), 539–546. <https://doi.org/10.1177/1591019920982810>
- Bernabeu, C., Blanco, F., Carmen, L., Garrido Martin, E., & Botella, L. (2010). Involvement of the TGF- $\beta$  superfamily signalling pathway in hereditary haemorrhagic telangiectasia. *Journal of Applied Biomedicine, 8*. <https://doi.org/10.2478/v10136-009-0020-x>
- Brain AVM (arteriovenous malformation)—Symptoms and causes.* (n.d.). Mayo Clinic. Retrieved December 10, 2022, from <https://www.mayoclinic.org/diseases-conditions/brain-avm/symptoms-causes/syc-20350260>
- Couto, J. A., Huang, A. Y., Konczyk, D. J., Goss, J. A., Fishman, S. J., Mulliken, J. B., Warman, M. L., & Greene, A. K. (2017). Somatic MAP2K1 Mutations Are Associated with Extracranial Arteriovenous Malformation. *The American Journal of Human Genetics, 100*(3), 546–554. <https://doi.org/10.1016/j.ajhg.2017.01.018>
- Eerola, I., Boon, L. M., Mulliken, J. B., Burrows, P. E., Domp Martin, A., Watanabe, S., Vanwijck, R., & Vikkula, M. (2003). Capillary Malformation-Arteriovenous Malformation, a New Clinical and Genetic Disorder Caused by RASA1 Mutations. *The American Journal of Human Genetics, 73*(6), 1240–1249. <https://doi.org/10.1086/379793>
- Fish, J. E., Flores Suarez, C. P., Boudreau, E., Herman, A. M., Gutierrez, M. C., Gustafson, D., DiStefano, P. V., Cui, M., Chen, Z., De Ruiz, K. B., Schexnayder, T. S., Ward, C. S., Radovanovic, I., & Wythe, J. D. (2020). Somatic Gain of KRAS Function in the Endothelium Is Sufficient to Cause Vascular Malformations That Require MEK but Not PI3K Signaling. *Circulation Research, 127*(6), 727–743. <https://doi.org/10.1161/CIRCRESAHA.119.316500>
- Gallione, C. J., Repetto, G. M., Legius, E., Rustgi, A. K., Schelley, S. L., Tejpar, S., Mitchell, G., Drouin, E., Westermann, C. J. J., & Marchuk, D. A. (2004). A combined syndrome of juvenile polyposis and hereditary hemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4).

- Lancet (London, England)*, 363(9412), 852–859. [https://doi.org/10.1016/S0140-6736\(04\)15732-2](https://doi.org/10.1016/S0140-6736(04)15732-2)
- GPRASP1 G protein-coupled receptor associated sorting protein 1 [Homo sapiens (human)]—Gene—NCBI*. (n.d.). Retrieved December 10, 2022, from <https://www.ncbi.nlm.nih.gov/gene/9737>
- Greene, A. K., & Orbach, D. B. (2011). Management of Arteriovenous Malformations. *Clinics in Plastic Surgery*, 38(1), 95–106. <https://doi.org/10.1016/j.cps.2010.08.005>
- Guttmacher, A. E., Marchuk, D. A., & White, R. I. (1995). Hereditary Hemorrhagic Telangiectasia. *New England Journal of Medicine*, 333(14), 918–924. <https://doi.org/10.1056/NEJM199510053331407>
- Heimdal, K., Dalhus, B., Rødningen, O. K., Kroken, M., Eiklid, K., Dheyaudeen, S., Røysland, T., Andersen, R., & Kulseth, M. A. (2016). Mutation analysis in Norwegian families with hereditary hemorrhagic telangiectasia: Founder mutations in ACVRL1. *Clinical Genetics*, 89(2), 182–186. <https://doi.org/10.1111/cge.12612>
- Hirata, Y., Enoki, R., Kuribayashi-Shigetomi, K., Oda, Y., Honma, S., & Honma, K. (2019). Circadian rhythms in Per1, PER2 and Ca<sup>2+</sup> of a solitary SCN neuron cultured on a microisland. *Scientific Reports*, 9, 18271. <https://doi.org/10.1038/s41598-019-54654-5>
- Hong, T., Yan, Y., Li, J., Radovanovic, I., Ma, X., Shao, Y. W., Yu, J., Ma, Y., Zhang, P., Ling, F., Huang, S., Zhang, H., & Wang, Y. (2019). High prevalence of KRAS/BRAF somatic mutations in brain and spinal cord arteriovenous malformations. *Brain: A Journal of Neurology*, 142(1), 23–34. <https://doi.org/10.1093/brain/awy307>
- Jakubík, J., & El-Fakahany, E. E. (2010). Allosteric Modulation of Muscarinic Acetylcholine Receptors. *Pharmaceuticals*, 3(9), 2838–2860. <https://doi.org/10.3390/ph3092838>
- Johnson, D. W., Berg, J. N., Baldwin, M. A., Gallione, C. J., Marondel, I., Yoon, S. J., Stenzel, T. T., Speer, M., Pericak-Vance, M. A., Diamond, A., Guttmacher, A. E., Jackson, C. E., Attisano, L., Kucherlapati, R., Porteous, M. E., & Marchuk, D. A. (1996). Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nature Genetics*, 13(2), 189–195. <https://doi.org/10.1038/ng0696-189>
- Kawasaki, J., Aegerter, S., Fevurly, R. D., Mammoto, A., Mammoto, T., Sahin, M., Mably, J. D., Fishman, S. J., & Chan, J. (2014). RASA1 functions in EPHB4 signaling pathway to suppress endothelial mTORC1 activity. *The Journal of Clinical Investigation*, 124(6), 2774–2784. <https://doi.org/10.1172/JCI67084>
- Laakso, A., & Hernesniemi, J. (2012). Arteriovenous Malformations: Epidemiology and Clinical Presentation. *Neurosurgery Clinics*, 23(1), 1–6. <https://doi.org/10.1016/j.nec.2011.09.012>
- Lawton, M. T., Rutledge, W. C., Kim, H., Stapf, C., Whitehead, K. J., Li, D. Y., Krings, T., terBrugge, K., Kondziolka, D., Morgan, M. K., Moon, K., & Spetzler, R. F. (2015). Brain arteriovenous malformations. *Nature Reviews Disease Primers*, 1(1), Article 1. <https://doi.org/10.1038/nrdp.2015.8>
- Lesca, G., Olivieri, C., Burnichon, N., Pagella, F., Carette, M.-F., Gilbert-Dussardier, B., Goizet, C., Roume, J., Rabilloud, M., Saurin, J.-C., Cottin, V., Honnorat, J., Coulet, F., Giraud, S., Calender, A., Danesino, C., Buscarini, E., & Plauchu, H. (2007). Genotype-phenotype correlations in hereditary hemorrhagic telangiectasia: Data from the French-Italian HHT network. *Genetics in Medicine*, 9(1), Article 1. <https://doi.org/10.1097/GIM.0b013e31802d8373>
- Mamai, O., Belefrod, D. T., Taylor, M., Basu, S., Cen, X., Trilok, S., Zhang, J., Balmain, A., & Akhurst, R. J. (2022). *PTPN14, a modifier of HHT, protects SMAD4 from ubiquitination and turnover to potentiate BMP9 signaling in endothelial cells* (p. 2021.09.29.462397). bioRxiv. <https://doi.org/10.1101/2021.09.29.462397>
- MAPK-ERK 信号通路—Elabscience. (n.d.). Retrieved December 10, 2022, from [https://www.elabscience.cn/signaling\\_pathways-mapkerk\\_signaling\\_pathway-239.html](https://www.elabscience.cn/signaling_pathways-mapkerk_signaling_pathway-239.html)
- McAllister, K. A., Grogg, K. M., Johnson, D. W., Gallione, C. J., Baldwin, M. A., Jackson, C. E., Helmbold, E. A., Markel, D. S., McKinnon, W. C., Murrel, J., McCormick, M. K., Pericak-Vance, M. A., Heutink, P., Oostra, B. A., Haitjema, T., Westerman, C. J. J., Porteous, M. E., Guttmacher, A. E., Letarte, M., & Marchuk, D. A. (1994). Endoglin, a TGF- $\beta$  binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nature Genetics*, 8(4), Article 4. <https://doi.org/10.1038/ng1294-345>
- McDonald, J., Wooderchak-Donahue, W., VanSant Webb, C., Whitehead, K., Stevenson, D. A., & Bayrak-Toydemir, P. (2015). Hereditary hemorrhagic telangiectasia: Genetics and molecular diagnostics in a new era. *Frontiers in Genetics*, 6, 1. <https://doi.org/10.3389/fgene.2015.00001>
- Nikolaev, S. I., Vetiska, S., Bonilla, X., Boudreau, E., Jauhiainen, S., Rezai Jahromi, B., Khyzha, N., DiStefano, P. V., Suutarinen, S., Kiehl, T.-R., Mendes Pereira, V., Herman, A. M., Krings, T., Andrade-Barazarte, H., Tung, T., Valiante, T., Zadeh, G., Tymianski, M., Rauramaa, T., ... Radovanovic, I. (2018). Somatic Activating KRAS Mutations in Arteriovenous Malformations of the Brain. *New England Journal of Medicine*, 378(3), 250–261. <https://doi.org/10.1056/NEJMoa1709449>
- Priemer, D. S., Vortmeyer, A. O., Zhang, S., Chang, H. Y., Curless, K. L., & Cheng, L. (2019). Activating KRAS mutations in arteriovenous malformations of the brain: Frequency and clinicopathologic correlation. *Human Pathology*, 89, 33–39. <https://doi.org/10.1016/j.humpath.2019.04.004>
- Revenu, N., Boon, L. M., Mendola, A., Cordisco, M. R., Dubois, J., Clapuyt, P., Hammer, F., Amor, D. J., Irvine, A. D., Baselga, E., Dompmartin, A., Syed, S., Martin-Santiago, A., Ades, L., Collins, F., Smith, J., Sandaradura, S., Barrio, V. R., Burrows, P. E., ... Vikkula, M. (2013). RASA1 mutations



- and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation. *Human Mutation*, 34(12), 1632–1641. <https://doi.org/10.1002/humu.22431>
- Ruiz-Llorente, L., Gallardo-Vara, E., Rossi, E., Smadja, D. M., Botella, L. M., & Bernabeu, C. (2017). Endoglin and alk1 as therapeutic targets for hereditary hemorrhagic telangiectasia. *Expert Opinion on Therapeutic Targets*, 21(10), 933–947. <https://doi.org/10.1080/14728222.2017.1365839>
- Shirley, M. D., Tang, H., Gallione, C. J., Baugher, J. D., Frelin, L. P., Cohen, B., North, P. E., Marchuk, D. A., Comi, A. M., & Pevsner, J. (2013). Sturge–Weber Syndrome and Port-Wine Stains Caused by Somatic Mutation in GNAQ. *New England Journal of Medicine*, 368(21), 1971–1979. <https://doi.org/10.1056/NEJMoa1213507>
- Simonin, F., Karcher, P., Boeuf, J. J.-M., Matifas, A., & Kieffer, B. L. (2004). Identification of a novel family of G protein-coupled receptor associated sorting proteins. *Journal of Neurochemistry*, 89(3), 766–775. <https://doi.org/10.1111/j.1471-4159.2004.02411.x>
- Smits, P. J., Konczyk, D. J., Sudduth, C. L., Goss, J. A., & Greene, A. K. (2020). Endothelial MAP2K1 Mutations in Arteriovenous Malformation Activate the RAS/MAPK Pathway. *Biochemical and Biophysical Research Communications*, 529(2), 450–454. <https://doi.org/10.1016/j.bbrc.2020.06.022>
- Uller, W., Alomari, A. I., & Richter, G. T. (2014). Arteriovenous malformations. *Seminars in Pediatric Surgery*, 23(4), 203–207. <https://doi.org/10.1053/j.sempedsurg.2014.07.005>
- Walcott, B. P., Winkler, E. A., Zhou, S., Birk, H., Guo, D., Koch, M. J., Stapleton, C. J., Spiegelman, D., Dionne-Laporte, A., Dion, P. A., Kahle, K. T., Rouleau, G. A., & Lawton, M. T. (2018). Identification of a rare BMP pathway mutation in a non-syndromic human brain arteriovenous malformation via exome sequencing. *Human Genome Variation*, 5, 18001. <https://doi.org/10.1038/hgv.2018.1>
- Weinsheimer, S., Brettman, A. D., Pawlikowska, L., Wu, D. C., Mancuso, M. R., Kuhnert, F., Lawton, M. T., Sidney, S., Zaroff, J. G., McCulloch, C. E., Young, W. L., Kuo, C., & Kim, H. (2012). G Protein-Coupled Receptor 124 (GPR124) Gene Polymorphisms and Risk of Brain Arteriovenous Malformation. *Translational Stroke Research*, 3(4), 418–427. <https://doi.org/10.1007/s12975-012-0202-9>
- Whistler, J. L., Enquist, J., Marley, A., Fong, J., Gladher, F., Tsuruda, P., Murray, S. R., & von Zastrow, M. (2002). Modulation of Postendocytic Sorting of G Protein-Coupled Receptors. *Science*, 297(5581), 615–620. <https://doi.org/10.1126/science.1073308>
- Zhang, M., Liang, L. H., Lu, Y. J., & Cao, X. (2020). G protein-coupled receptor-associated sorting proteins: Function and relevant disorders. *Yi Chuan = Hereditas*, 42(8), 713–724. <https://doi.org/10.16288/j.ycz.20-020>
- Zhang, T., Liu, G., Zhang, J., Chen, S., Deng, Z., & Xie, M. (n.d.). GPRASP1 is a candidate anti-oncogene and correlates with immune microenvironment and immunotherapeutic efficiency in head and neck cancer. *Journal of Oral Pathology & Medicine*, n/a(n/a). <https://doi.org/10.1111/jop.13376>
- Zheng, X., Chang, F., Zhang, X., Rothman, V. L., & Tuszyński, G. P. (2012). G-protein coupled receptor-associated sorting protein 1 (GASP-1), a ubiquitous tumor marker. *Experimental and Molecular Pathology*, 93(1), 111–115. <https://doi.org/10.1016/j.yexmp.2012.03.013>