RASA1: variable phenotype with capillary and arteriovenous malformations
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Capillary malformation-arteriovenous malformation (CM-AVM) is a newly discovered hereditary disorder. Its defining features are atypical cutaneous multifocal capillary malformations often in association with high-flow lesions: cutaneous, subcutaneous, intramuscular, intraosseous and cerebral arteriovenous malformations and arteriovenous fistulas. Some patients have Parkes Weber syndrome — a large congenital cutaneous vascular stain in an extremity, with bony and soft tissue hypertrophy and microscopic arteriovenous shunting. In the past, arteriovenous malformations and arteriovenous fistulas had been considered non-hereditary. A classical genetic approach was used to identify the locus. Candidate gene screening pinpointed mutations in RASA1 (p120-RASGAP) — a Rasa1GTPase. RASA1 reverts active GTP-bound Ras into inactive GDP-bound form. Murine Rasa1 knockout and tetraploid-aggregated embryos with RNA interference inhibited cell motility, possibly through inhibition of cell motility, possibly through p190-RhoGAP. Thus, RASA1 defects probably cause abnormal angiogenic remodeling of the primary capillary plexus [2]. These lesions are divided into four major categories on the basis of clinical, rheological and histological features: capillary, lymphatic, venous and arteriovenous malformations [3].

Vascular malformations have several features of potential heuristic value. Most importantly, they are nearly always localized (i.e. the majority of vessels are normal and the malformed vessels arise in a limited area). Although the majority of vascular malformations do not seem to be inherited in a Mendelian way, there is wide clinical variability and high penetrance (around 90%). Expressivity varies from small, harmless lesions to large, symptomatic ones [4*]. Moreover, it is the location of the lesion, rather than its size, that determines morbidity.

Genes for several vascular anomalies have been identified: TIE2 receptor tyrosine kinase for mucocutaneous venous malformation (VMCM; Online Mendelian Inheritance in Man (OMIM) 600195) [5]; glomulin for glomuvenous malformation (GVM; OMIM 138000) [6]; KRIT1 (Krev1 interaction trapped 1), malcavernin and prolymphatic, venous and arteriovenous malformations [7,8]. Additionally, in the families with hereditary predisposition, there is a somatic second-hit in the glomulin gene responsible for multiple GVMs, which have led to the discovery of a somatic mutation in the glomulin gene [9**].

CM-AVM is a similar, newly identified vascular disorder [16**]. Here, we discuss its phenotypic variations, differ-
ential diagnosis and the molecular effects of the causative mutations on the function of RASA1.

Capillary malformation-arteriovenous malformation phenotype

The hallmark of CM-AVM is a small, round-to-oval, pink-red CM, typically multiple and in a haphazard distribution (Figure 1) \[16^{*},17^{*}\]. In the six identified families, 39 individuals carried a RASA1 mutation: four of them were unaffected, 25 had atypical CMs and 10 had a high-flow lesion in addition to the atypical CMs: two localized cutaneous and subcutaneous facial AVMs (one nasal and one frontal); one subcutaneous and intramuscular AVM of the foot; two extensive hemifacial AVMs with intraosseous involvement (Figure 1); two cerebral AVMs causing epilepsy in one patient, and hemiparesis (see Glossary) and congestive heart failure in another; Parkes Weber syndrome (OMIM 608355); and a carotid-jugular arteriovenous fistula (AVF) causing heart failure and hemifacial hypertrophy. Although four of the six families were identified in Belgium, we suggest that CM-AVM is likely to be a relatively frequent genetic disorder, because several clinicians have contacted us about suspected CM-AVM patients. It is not yet known whether all these patients have the same entity — a Ras p21 protein activator 1 (RASA1) mutation; there could be locus heterogeneity.

This newly described entity raises several interesting questions. What is the prevalence of CM-AVM? Is there genetic predisposition to AVM, a lesion once thought to be sporadic? Is there a predilection for localization of AVM or AVF in CM-AVM? Do patients with AVMs and AVFs with CM-AVM differ from patients with AVMs and AVFs without CM-AVM?
AVFs without CM-AVM? Is Parkes Weber syndrome an expression of CM-AVM? Given that physicians have, generally, paid little attention to possible small cutaneous stains in family members (for example, in a patient with a cerebral AVM) it might well be that CM-AVM is relatively common. However, two of the six reported mutations occurred de novo, which suggests that RASA1 mutations might also cause sporadic vascular anomalies. Clearly, these questions stress the importance of detailed family history in all patients with a vascular anomaly.

There are curious features of CMs co-existing with an underlying AVM. Some CMs have a small white halo, suggesting vascular steal (see Glossary) (Figure 1), and others have fast-flow by Doppler (see Glossary), perhaps an early step towards development of an AVM (pre-AVM). To understand better, we would need histology as well as more clinical and radiological follow-up on such lesions.

**Differential diagnosis**

There are families reported to have hereditary benign telangiectasia (HBT) with CM-like lesions [18]. HBT consists of cutaneous telangiectasias similar to HHT, but without evolution of high-flow lesions in the lungs, brain or gastrointestinal tract. These telangiectasias are different from the more homogeneous capillary macules in CM-AVM. In addition, the telangiectasias in HBT are more often on the face, upper trunk and upper extremities and, in contrast to HHT, do not affect the mucosa, except the labial vermis. Given that HBT has not been reported to be caused by mutations of the three HHT genes or the RASA1 gene, it is probably a genetic entity separate from HHT and CM-AVM. A family reported to have HBT with linkage to the RASA1 locus most likely has CM-AVM [18].

Because of the serious sequela (see Glossary) of an internal AVM, the identification of CM-AVM raised new questions about management of these patients. In the first six genetically confirmed RASA1-mutated families, the frequency of patients with a high-flow lesion was around 26% (10 AVM for 39 CM-AVM patients). Thus, patients with atypical CMs similar to CM-AVM should be carefully assessed for possible high-flow lesions. Cerebral magnetic resonance imaging (MRI) is probably not indicative in these patients unless they exhibit neurological signs or symptoms.

**RASA1**

The germ line mutations reported in RASA1, on chromosome 5q13.1–14.3 (p120-RASGAP, OMIM 139150), cause premature termination codons (Figure 2) [16**]. The earliest STOP is in the amino-terminal side of the known protein–protein interaction domains: the two SH2s, an SH3, a plekstrin homology domain and a protein kinase conserved region 2 [19,20]. We have identified additional families with similar mutations (Revencu et al., unpublished). Thus, CM-AVM mutations are most likely to cause loss of function. The only missense substitution identified alters cysteine to tyrosine (C540Y) in the plekstrin homology domain. Given that this residue is highly conserved among RasGAP proteins, even between species, it is probably important for RASA1 function.

Loss of RASA1 should inhibit conversion of active GTP-bound Ras to the inactive GDP-bound form. Increased activated Ras, however, has been linked to various tumors [21], and RASA1 missense mutations have been identified in basal cell carcinoma [22]. These findings suggest that activating Ras mutations escape inhibitory GAP activity, whereas (heterozygous) loss of RASA1, because of residual RASA1 activity or compensation by other GAPS, keeps Ras under control and so tumors do not evolve. Neither homozygous Rasa1−/− embryonic stem cells nor Rasa1−/− murine embryos show increased cellular proliferation. In fact, localized apoptosis was observed in the embryos [23]. RNA interference-induced knockdown of RASA1 caused similar vascular defects to those seen in the RASA1-null embryos [24**]. The RASA1 missense mutations in basal cell carcinoma should alter RasGAP

**Figure 2**

Schematic representation of the RASA1 gene and CM-AVM-causing mutations. Exons, boxed; introns, simple line; untranslated region, small box. Known domains are marked below exons. Amino acid numbering marked at borders of certain exons. Four of the six known mutations cause a frameshift with a premature termination codon, one is a nonsense mutation (asterisk) and one a missense mutation leading to cysteine being replaced by a tyrosine (C540Y) in the plekstrin homology domain.
function differently. Furthermore, CM-AVM might be caused by a mechanism that is independent of Ras.

It is not yet known whether complete localized loss of function, similar to GVM, is needed for CM-AVMs to form [6]. In mouse, loss of one germinal Rasa1 allele does not cause any observable phenotype, whereas homozygous loss causes embryonic death at E10.5, with defects in vascular development [23]. Remarkably, murine embryonic mosaic for Rasa1−/− cells developed localized vascular defects [23], which suggest that CM-AVM in human could result from paradigmatic inheritance.

RASA1 belongs to a family of four homologous proteins: RASA1, RASA2 (GAPM1), NF1 (neurofibromin) and RASAL (GAP1-like protein) [25–28]. They all contain a GTPase domain, which is central for their function as Ras inactivators. RASA1 has also been associated with p190-RhoGAP, which is involved in signaling in the formation of cytoskeleton [29], and with Rap1a [30,31], linking it to integrin-mediated cell adhesion [32–34] similar to CCM genes [35]. RASA1 is modulated at least by GAP SH3 domain-binding protein (G3BP; OMIM 608431), which is recruited under Ras activation [36], and by elevated levels of focal adhesion kinase (FAK), which may diminish Ras inactivation by recruiting RASA1 [37]. All these are good candidate genes for related, non-RASA1, vascular phenotypes.

Intriguingly, the close homolog of RASA1, neurofibromin, mutated in neurofibromatosis (NF1), is thought to act as a classical tumor suppressor gene. Homozygous loss of NF1 increases proliferation and development of fibromatosus cutaneous tumors. Interestingly, neurofibromin, which is largely expressed in the nervous system, is also found in vascular endothelial and smooth muscle cells, and NF1−/− murine embryos show cardiac dysmorphogenesis. Patients with neurofibromatosis sometimes have vascular defects, such as aortic coarctation, arterial occlusion and vascular neurofibromatosis (increased vessels in a plexiform tumor). Could RASA1 or the other RasGAPs be involved as modifiers? When Rasa1−/− Nf1−/− compound heterozygous mice were crossed, the double homozygotes Rasa1−/− Nf1−/− had a more severe phenotype (e.g. in the heart and pharyngeal arches) than did the simple homozygotes. Moreover, there was overproliferation of hindbrain neuroepithelium, which was not present in Rasa1−/− embryos. These differences suggest interaction between RASA1 and NF1 function [23]. Given that RASA1 expression has not been characterized, it remains to be seen how extensive this interaction might be.

Because RASA1 germ line mutations cause vascular defects by loss of function, NF1 and the other two members cannot replace for lost RASA1 function during angiogenesis. This might be because of differences in patterns of expression or non-redundant function. The bony and soft tissue hypertrophy seen in the Parkes Weber patient could also be a direct effect of altered RASA1 function (e.g. through fibroblast growth factor receptor (FGFR) signaling in osteoblasts or fibroblasts) rather than a secondary effect caused by the increased blood flow. This would imply more widespread function for RASA1, in addition to lack of redundancy between RasGAPs.

Conclusions

The identification of RASA1 mutations in a newly recognized disorder (CM-AVM) is an exciting discovery. The next steps include phenotypic delineation of the variable vascular phenotypes, including possible soft tissue and bony changes, and tumors; genotype–phenotype correlating studies; clinical follow up of genetically defined patients for prevalence of bleeding, tumors and so on; testing the paradigmatic inheritance model; and identification of possible signaling pathways that could be targeted in therapeutic trials. The future holds the promise of unravelling the genetic events that lead to vascular anomalies, thus enabling precise genetic counseling and understanding of the role of RASA1 and other RasGAPs in angiogenesis.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study provides the first systematic comparison of the phenotype of individuals affected by a venous anomaly with or without a known genetics affect.


17. RASA1. This is the first report describing the CM phenotype with capillary and arteriovenous malformations. Am J Hum Genet 2004, 73:1459-1464. See annotation [9*].


