

Capillary Malformation–Arteriovenous Malformation, a New Clinical and Genetic Disorder Caused by *RASA1* Mutations

Iiro Eerola,¹ Laurence M. Boon,^{1,2} John B. Mulliken,^{3,4} Patricia E. Burrows,^{3,5} Anne Domp Martin,⁶ Shoji Watanabe,⁷ Romain Vanwijck,² and Miikka Vikkula¹

¹Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology, and ²Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels; ³Vascular Anomalies Center, ⁴Division of Plastic Surgery, and ⁵Division of Interventional Radiology, Children's Hospital, Harvard Medical School, Boston; ⁶Division of Dermatology, CHU Caen, France; and ⁷Department of Plastic and Reconstructive Surgery, Showa University School of Medicine, Tokyo

Capillary malformation (CM), or “port-wine stain,” is a common cutaneous vascular anomaly that initially appears as a red macular stain that darkens over years. CM also occurs in several combined vascular anomalies that exhibit hypertrophy, such as Sturge-Weber syndrome, Klippel-Trenaunay syndrome, and Parkes Weber syndrome. Occasional familial segregation of CM suggests that there is genetic susceptibility, underscored by the identification of a large locus, *CMC1*, on chromosome 5q. We used genetic fine mapping with polymorphic markers to reduce the size of the *CMC1* locus. A positional candidate gene, *RASA1*, encoding p120-RasGAP, was screened for mutations in 17 families. Heterozygous inactivating *RASA1* mutations were detected in six families manifesting atypical CMs that were multiple, small, round to oval in shape, and pinkish red in color. In addition to CM, either arteriovenous malformation, arteriovenous fistula, or Parkes Weber syndrome was documented in all the families with a mutation. We named this newly identified association caused by *RASA1* mutations “CM-AVM,” for capillary malformation–arteriovenous malformation. The phenotypic variability can be explained by the involvement of p120-RasGAP in signaling for various growth factor receptors that control proliferation, migration, and survival of several cell types, including vascular endothelial cells.

Introduction

Dermal vascular development involves penetration of capillary vessels that is induced by vascular endothelial growth factor (VEGF) secreted by keratinocytes in the avascular epidermis (Brown et al. 1992; Ballaun et al. 1995). This invasion and the subsequent arterial differentiation is also guided by VEGF originating from sensory nerves in the dermis (Mukoyama et al. 2002). Defective cutaneous vascular development manifests as malformed vessels that vary in size, location, blood flow, and clinical severity (Mulliken and Glowacki 1982). Capillary malformation (CM), or “port-wine stain,” (MIM 163000) is the most common vascular malformation, occurring in 0.3% of newborns (Jacobs and Walton 1976). CM is a flat, cutaneous, slow-flow lesion that is composed of dermal capillary-venular-like channels that are dilated and/or increased in number (Jacobs

and Walton 1976; Barsky et al. 1980). Arteriovenous malformation (AVM) and arteriovenous fistula (AVF) are fast-flow vascular anomalies that can arise in skin, muscle, bone, internal organs, and the brain and can cause life-threatening complications such as bleeding, congestive heart failure, or neurologic consequences (Mulliken and Young 1988). Parkes Weber syndrome is characterized by a cutaneous blush with underlying multiple micro-AVFs, in association with soft-tissue and skeletal hypertrophy of the affected limb (Mulliken and Young 1988). Sturge-Weber syndrome can present with fast-flow leptomeningeal and choroid anomalies in association with a facial CM involving the V1 dermatomic area. Klippel-Trenaunay syndrome manifests as a slow-flow capillary lymphaticovenous malformation most commonly affecting lower extremities with soft-tissue hypertrophy.

In a previous study of 13 families with familial CM, we identified a susceptibility locus, *CMC1*, on chromosome 5q14-21, with a minimally linked region of 23 cM between markers D5S1962 and D5S652 (Eerola et al. 2002). In this study, a new family (family CM45) (figs. 1A and 2) made it possible to narrow the locus to 5 cM between markers D5S459 and GATA5F09. This interval contains eight characterized genes, three of which, *RASA1*, *EDIL3* (EGF-like repeats and discoidin I-like domains 3), and *MEF2C* (myocyte enhancer fac-

Received July 10, 2003; accepted for publication September 9, 2003; electronically published November 24, 2003.

Address for correspondence and reprints: Prof. Miikka Vikkula, Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, Avenue Hippocrate 74+5, bp 75.39, B-1200 Brussels, Belgium. E-mail: vikkula@bchm.ucl.ac.be

© 2003 by The American Society of Human Genetics. All rights reserved. 0002-9297/2003/7306-0004\$15.00

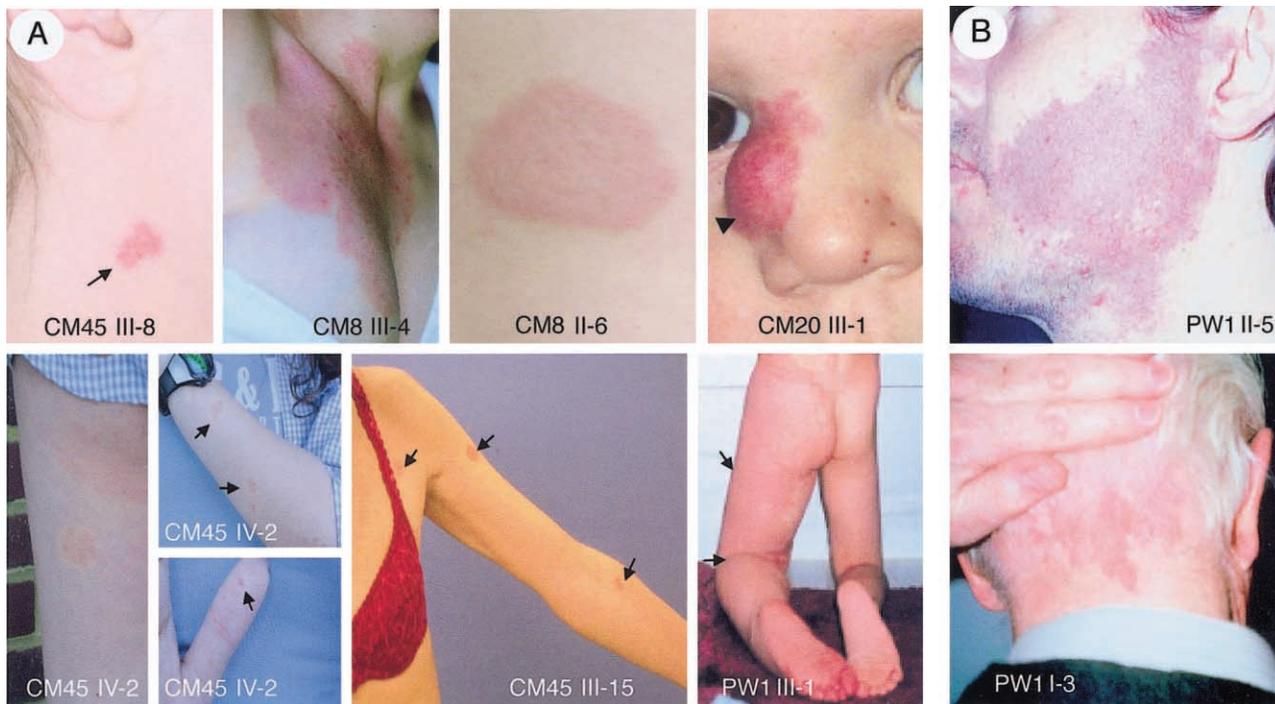


Figure 1 Photographs of vascular malformations linked to *RASA1* mutations. Individual numbers refer to pedigrees in figure 2. *A*, Atypical inherited pink-to-red, round-to-oval CMs (arrows) (patients CM45 III-8, CM45 III-15, and CM45 IV-2, CM8 III-4, and CM8 II-6); patient CM20 III-1, with a circumscribed nasal AVM and a cutaneous capillary blush (arrowhead); patient PW1 III-1, with Parkes Weber syndrome in the lower limb (arrows). *B*, Photographs of CMs not linked to *RASA1* mutations. Typical red-purple facial CM (patient PW1 II-5); typical red CM of the neck (patient PW1 I-3).

tor-2C), were considered to be candidates of functional interest.

The *RASA1* gene codes for p120-RasGTPase-activating protein (p120-RasGAP). This is a modular protein of 1,047 amino acids that contains two Src homology 2 (SH2) domains and one Src homology 3 (SH3) domain in the N-terminal region, a pleckstrin homology (PH) domain, and a protein kinase conserved region 2 in the central region, as well as a RasGTPase-activating domain in the C-terminal region (fig. 4A) (Trahey and McCormick 1987; Glanzer et al. 2002). p120-RasGAP's best-known function is as a negative regulator of the Ras/MAPK-signaling pathway, which mediates cellular growth, differentiation, and proliferation from various receptor tyrosine kinases (RTKs) on cell surfaces (fig. 5) (Ballester et al. 1990; Gibbs et al. 1990; Clark et al. 1993). p120-RasGAP and the other three known GAPs—NF1, GAPM1, and GAP1-like protein—switch the active GTP-bound Ras to the inactive GDP-bound form (Martin et al. 1990; Xu et al. 1990; Maekawa et al. 1994; Cullen et al. 1995). Effector functions downstream of RTKs have also been proposed for p120-RasGAP—for example, via the association with p190-RhoGAP that directs signaling to the cytoskeleton (Settleman et al. 1992). In addition, p120-

RasGAP binds to Rap1a (Frech et al. 1990; Hata et al. 1990), which has been shown to be involved in integrin-mediated cellular adhesion (Caron et al. 2000; Katagiri et al. 2000; Reedquist et al. 2000). Mutations that make Ras constitutively active and resistant to GAPs are often found in malignant tumors (Bos 1989). Inactivating mutations of the GAP proteins, in turn, can also lead to inordinately active Ras signaling and tumorigenesis, as in neurofibromatosis, caused by mutations in the *NF1* gene (Cawthon et al. 1990; Viskochil et al. 1990). Mutations in p120-RasGAP have also been implicated in basal cell carcinoma (Friedman et al. 1993). However, *RASA1*-deficient murine embryos exhibited early developmental arrest at E9.5 due to severe vascular defects but exhibited no signs of cellular proliferation (Henkemeyer et al. 1995).

Subjects and Methods

Subjects

Informed consent was obtained from all subjects participating in the study, as approved by the ethics committee of the medical faculty of Université Catholique de Louvain, Brussels, Belgium. Families CM8, CM11,

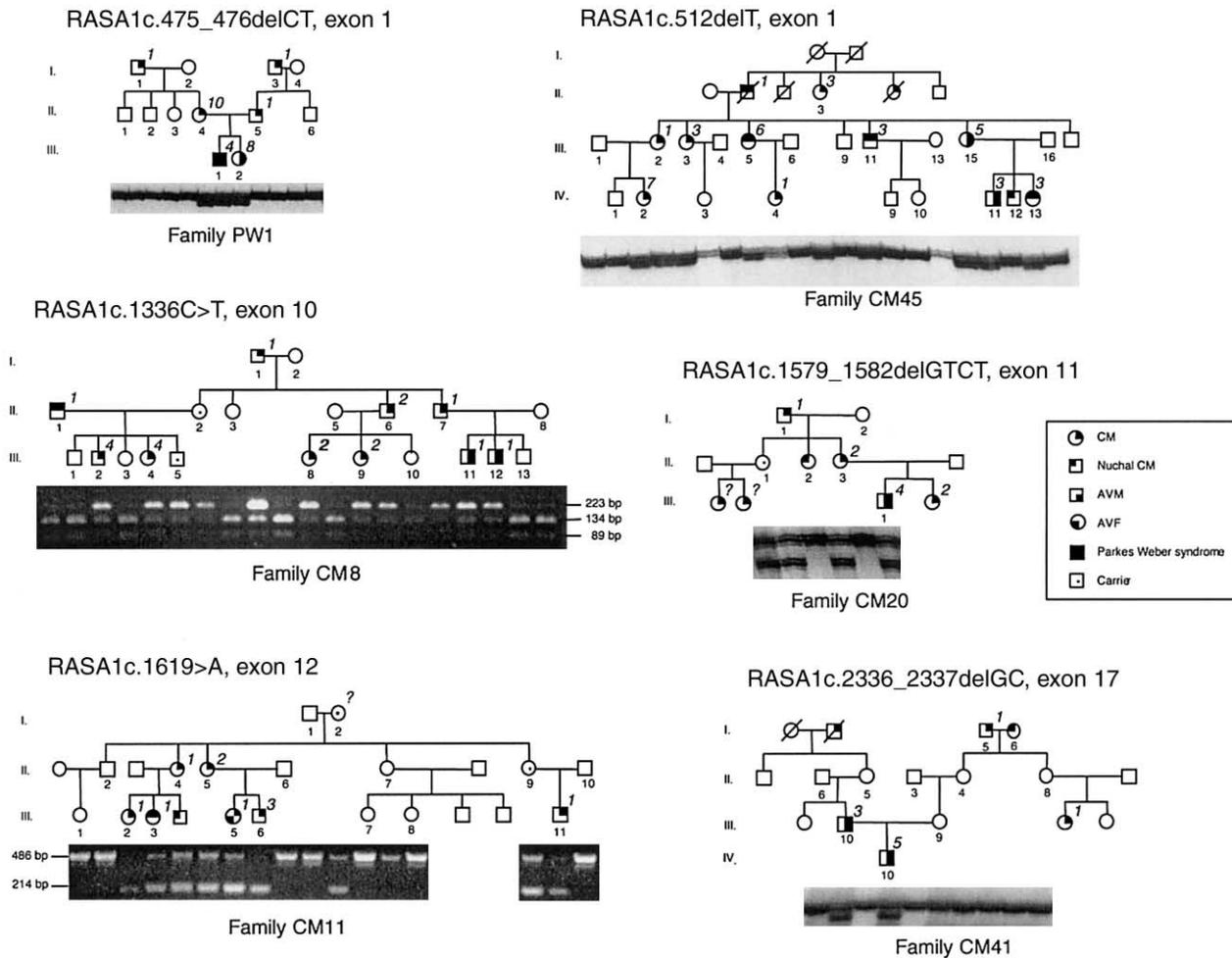


Figure 2 Pedigrees, vascular phenotypes, and cosegregation of the identified mutations. Pedigrees of families with detectable *RASA1* mutations are shown in order of occurrence of corresponding mutation in the *RASA1* gene. Numbered individuals were screened for mutations. The number of lesions is in italic superscript. In families PW1, CM45, CM20, and CM41, cosegregation of deletional mutations with the phenotype was performed using radioactive size-difference gel electrophoresis. In family CM8, the presence of *RASA1c.1336C→T* was detected by *Sau3A1* restriction digestion. The mutation destroys a *Sau3A1* restriction-enzyme cutting site that normally splits the 223-bp allele into 134-bp and 89-bp fragments. In family CM11, the allele containing *RASA1c.1619G→A* yielded a 214-bp fragment in mutation carriers only, in addition to the wild-type control fragment of 485 bp in allele-specific PCR. Question marks indicate that the number of CMs is unknown. *RASA1c.475_476delCT* and *RASA1c.2336_2337delGC* were de novo mutations. Altogether, four carriers were identified.

and CM20 have been reported elsewhere (as families C, D, and E, respectively) (Eerola et al. 2002). The cutaneous vascular lesions varied in appearance from a single, moderately large, pink macular stain in the nucha or central forehead to a single, purple-red lesion of variable shape and size, often located on the face (figs. 1 and 3), to multiple small, round-to-oval, pinkish red lesions, mainly present on an extremity (figs. 1 and 2). Some patients also had CM-associated vascular anomalies and soft- and skeletal-tissue hypertrophy, characterized as follows: In family PW1, patient III-1 had Parkes Weber syndrome (fig. 1A), and patient III-2 had an intracranial AVM, as well as multiple cutaneous CMs. In family CM45, patient III-15 had an intracranial AVM and five cutaneous CMs, whereas patient IV-11 had a

cutaneous AVM of the ankle and three cutaneous CMs. In family CM8, patient III-11 had a left maxillary AVM causing skeletal hypertrophy and an extensive hemifacial CM with soft-tissue hypertrophy, and patient III-12 had a lower labial CM with hypertrophy and an intramandibular AVM causing occlusal distortion. In family CM20, patient III-1 had a deep facial AVM with an overlying cutaneous capillary stain (fig. 1A). In family CM41, patient III-10 had an AVM of the forehead and three small cutaneous CMs, and patient IV-10 had a stage I cutaneous AVM of the right fifth finger. In family CM11, patient III-5 had a facial capillary stain and hypertrophy distal to an AVF, which was located between the left carotid artery and the jugular vein and caused cardiac overload, requiring medication since infancy.

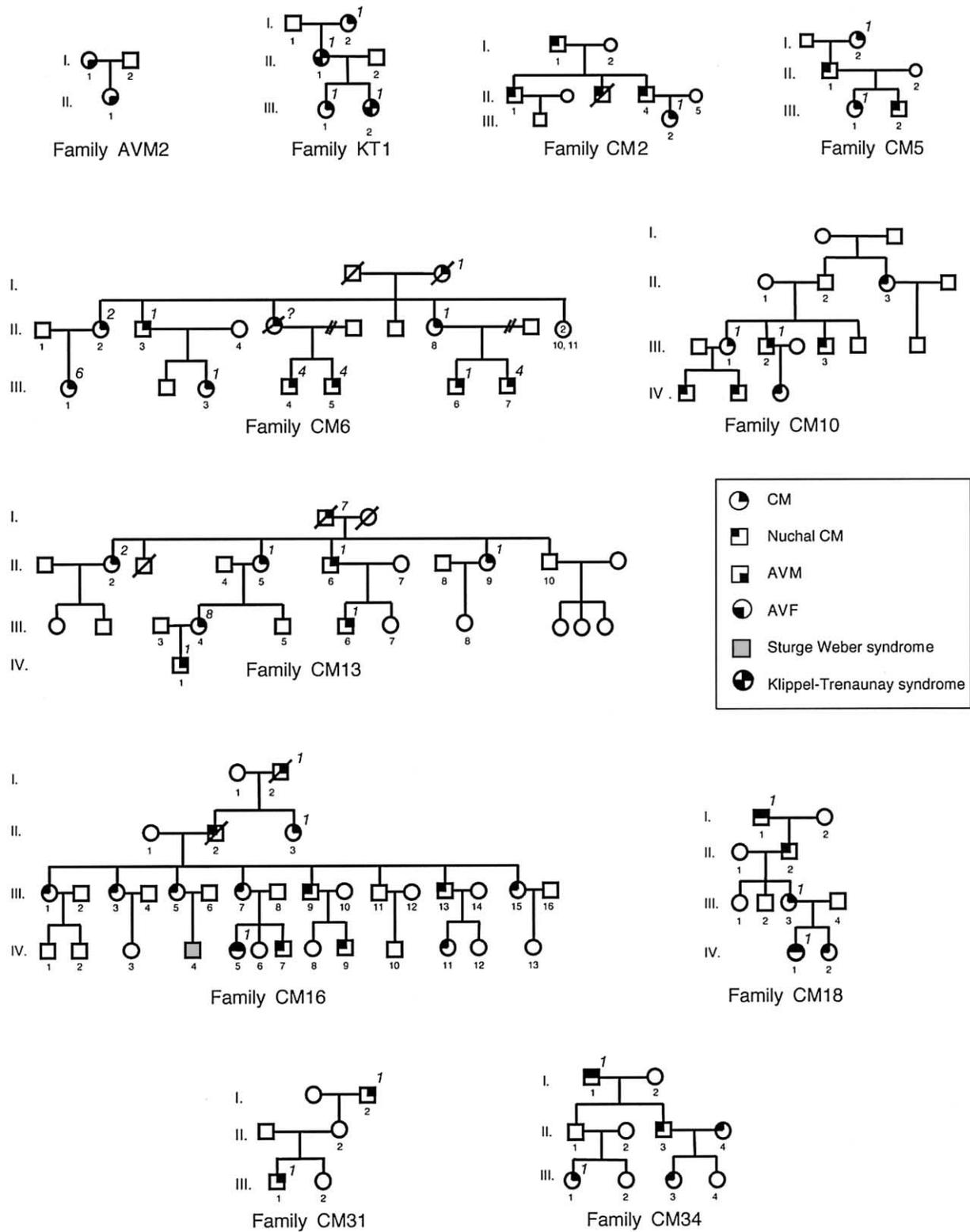


Figure 3 Pedigrees of families without detectable *RASA1* mutations. Numbered individuals were screened for mutations. The number of lesions is in italic superscript.

This patient also had a small CM on the left wrist. In family KT1, patients II-1 and III-2 had Klippel-Trenaunay syndrome in a lower extremity, and, in family CM16, patient IV-4 had Sturge-Weber syndrome. The two affected individuals in family AVM2 only had an AVM and no cutaneous CM.

Reduction of the CMC1 Locus

Linkage to the CMC1 locus was tested in family CM45 with 17 polymorphic markers: D5S1962, D5S646, D5S1501, D5S641, D5S2029, D5S2094, D5S428, D5S459, D5S617, D5S2103, D5S1725, D5S401, D5S2044, D5S2100, GATA5F09, AFM205, and D5S652, as described elsewhere (Eerola et al. 2002).

SSCP and Heteroduplex Analyses

The genomic sequences containing the RASA1 gene were identified with the RASA1 mRNA sequence (NM_002890.1) by performing a BLAST homology search on the human genome sequence at the National Center for Biotechnology Information (NCBI) Entrez BLAST server. The working draft sequence NT_037660.1 for homo sapiens chromosome 5, which contains the gene, was retrieved from the NCBI Entrez database. To amplify all the 25 exons, including exon-intron boundaries, 27 sets of primers (available on request) were designed. The isoform-2-specific exon 1 was also screened. PCR reactions, subsequent SSCP and heteroduplex analyses, and direct sequencing were performed, as described elsewhere (Brouillard et al. 2002). Numbering of nucleotides is based on the cDNA sequence NM_002890.1 with the adenosine of the ATG start codon marked as +1.

Cosegregation Analysis

The fragments with detected deletional mutations were amplified by radioactive PCR from genomic DNA from all family members and were analyzed by size-difference gel electrophoresis to detect cosegregation (families PW1, CM45, CM20, and CM41) (fig. 2). Cosegregation of the mutation RASA1c.1336C→T (p.Q446X), which destroys a *Sau3A1* restriction-enzyme cutting site, was screened by *Sau3A1* (Promega) digestion of exon 10 genomic amplicons for all individuals in family CM8 (fig. 2), according to the manufacturer's instructions. Mutation RASA1c.1619G→A (p.C540Y) in family CM11 did not change any restriction-enzyme cutting site. Thus, it was screened by allele-specific PCR (fig. 2) (primers available on request). A group of 105 unrelated, healthy controls was also screened for all mutations.

Results

Identification of RASA1 Mutations

SSCP and heteroduplex analyses were performed on DNA samples from 17 families manifesting cutaneous CM to screen all 26 exons and the exon-intron boundaries of the gene for RASA1 mutations. RASA1 mutations were found in six families. None of these mutations was identified in the 105 healthy controls. Of the 11 families without a detectable RASA1 mutation, 6 (KT1, CM2, CM13, CM16, CM18, and CM31) yielded a negative LOD score, and 2 small families (CM5 and CM10) were uninformative at the CMC1 locus in multipoint linkage analysis (90% penetrance; 0.3% phenocopy rate). In family CM6, one affected individual (III-6) excluded the locus. No linkage data was available for family CM34, and family AVM2 was uninformative for linkage analysis.

The identified alterations included four deletions in the coding region, RASA1c.475_476delCT, RASA1c.512delT, RASA1c.1579_1582delGTCT, and RASA1c.2336_2337delGC, and two substitutions, RASA1c.1336C→T, leading to a nonsense mutation, p.Q446X, and RASA1c.1619G→A, resulting in cysteine-to-tyrosine substitution at amino acid 540 (p.C540Y) (fig. 4A). The deletions caused reading-frame shifts and subsequent premature stop codons, predicted to result in a truncated protein (fig. 4A). The only amino acid substitution, p.C540Y, occurred in the PH domain (fig. 4), which has been shown to regulate interaction between p120-RasGap and RAS (Drugan et al. 2000). The adjacent C-terminal amino acid phenylalanine (541) is highly conserved in PH domains (fig. 4B). Cysteine is present at position 540 in 37 (15%) of 201 of the PH domains in the PROSITE database, whereas tyrosine at this position is only found in the PH domains of the guanine-nucleotide-releasing protein (RasGRF1/CDC25) of three species (fig. 4B). It is interesting that, unlike the PH domain of p120-RasGAP, the PH domain of RasGRF1/CDC25 did not inhibit Ras-induced transformation in an overexpression experiment on NIH3T3 cells (Drugan et al. 2000). Thus, the RASA1c.1619G→A mutation may also result in a functionless p120-RasGap; however, further functional studies are needed to look at the effect of this change on function. Two of the six mutations occurred de novo (family PW1 and CM41) (fig. 2).

RASA1 Mutations Cause a Distinct Phenotype: CM-AVM

Mutations cosegregated with vascular malformations in all six families (fig. 2). The affected individuals exhibited atypical CMs, usually characterized as pink-to-red, multiple, small (1–2 cm in diameter), round-to-oval lesions (fig. 1A). In each family, at least one individual had a high-flow lesion—either a soft-tissue ($n = 4$),

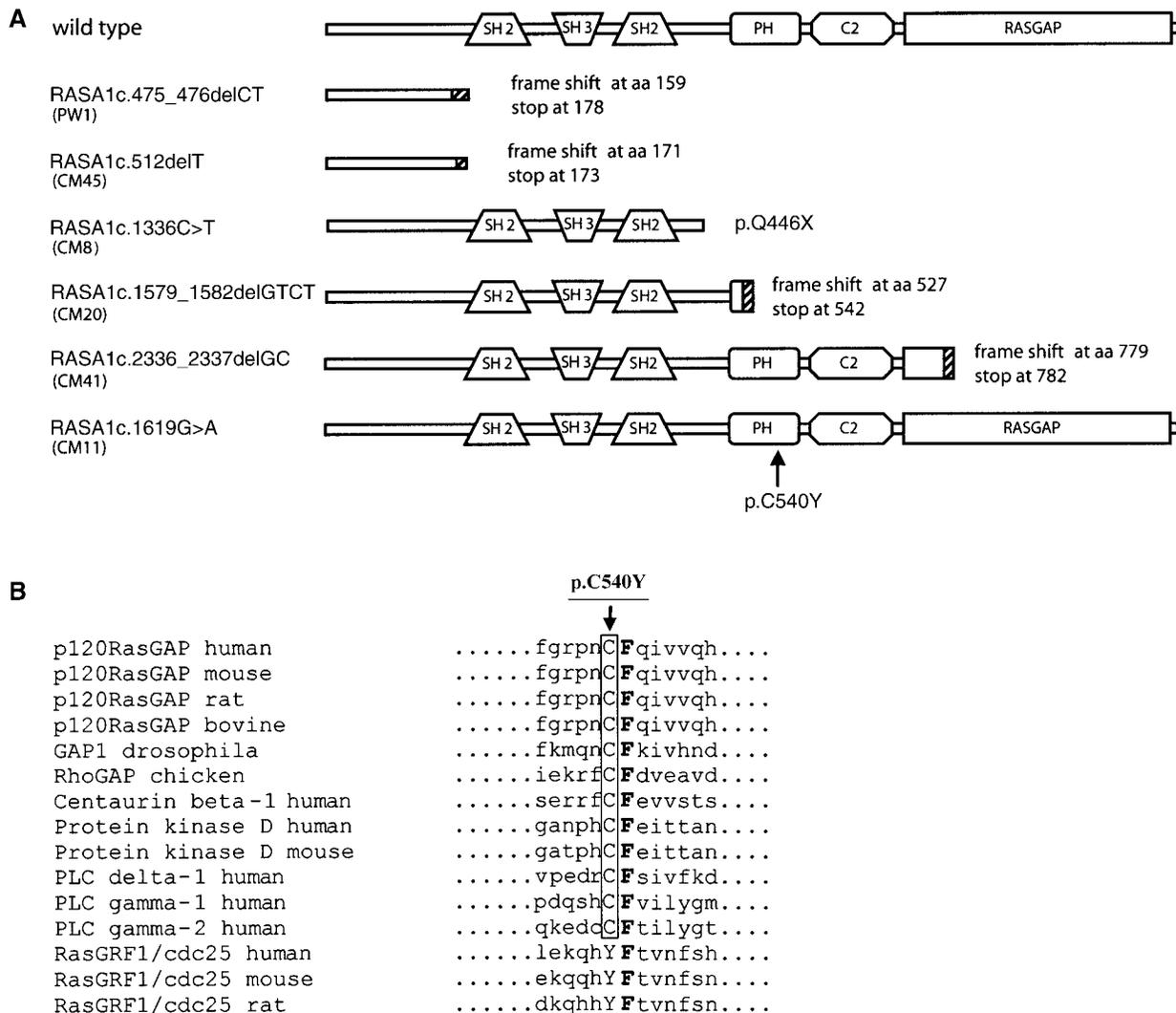


Figure 4 A, Schematic presentation of identified *RASA1* mutations. Four deletional mutations—*RASA1c.475_476delCT*, *RASA1c.512delT*, *RASA1c.1579_1582delGTCT*, and *RASA1c.2336_2337delGC*—cause frame shifts and subsequent premature stop codons. Resulting hypothetical proteins are illustrated. *RASA1c.1336C>T* is a nonsense mutation, whereas *RASA1c.1619G>A* results in a Cys-540-Tyr substitution in the PH domain at position 540. SH2 = Src homology 2 domain; SH3 = Src homology 3 domain; C2 = protein kinase conserved region 2; RASGAP = rasGTPase-activating domain. Altered amino acid sequences due to frame shifts are shown by diagonal lines. B, Multiple alignment of PH domains. The figure illustrates the alignment of 15 PH-domain amino acid sequences around the p.C540Y mutation. Highly conserved phenylalanine is represented by a boldface F. Only 12 of 37 PH domains containing the mutated cysteine p.C540Y at this position are shown (box). Also shown are the three orthologous PH-domain sequences, all from RASGRF1, that contain a tyrosine (Y) at this position.

brain ($n = 2$), or skeletal ($n = 2$) AVM or an AVF ($n = 1$) concurrently with an atypical CM. The AVF between the ipsilateral carotid artery and the jugular vein, as well as a skeletal AVM of the face, were associated with overgrowth. In addition, one patient (PW1 III-1) was diagnosed as having Parkes Weber syndrome, with multiple micro-AVFs and soft- and skeletal-tissue hypertrophy of the affected limb (fig. 1A). A mutation was found in four individuals who had no obvious vascular malformation, giving an overall penetrance of 89%. The eight phenocopies without a *RASA1* mutation

identified in these families had either a single pink CM of the nuchal region (4/8) (fig. 1B) or a single purple-red lesion reminiscent of common, sporadic CM (4/8). Four of the families with a *RASA1* mutation were from Belgium; one was from the United States; and one was from Canada.

In 9 of the 11 families without a detectable *RASA1* mutation, the cutaneous vascular lesions either were located in the nuchal region or they were moderately large or purple-red CMs of variable shape and location. In one of these families, two individuals had Klippel-Tren-

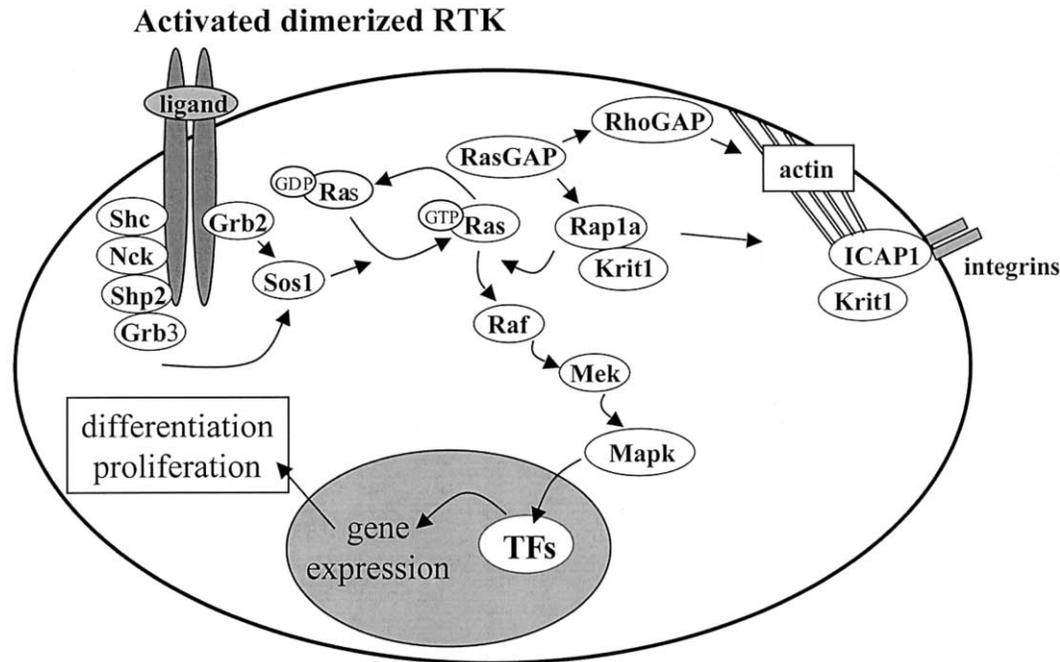


Figure 5 Signaling pathways implicating p120-RasGAP. Growth-factor binding to RTKs on the cell surface activates the Ras/MAPK-signaling pathway, which mediates signals to transcription factors in the nucleus. Changes in gene expression lead to cellular responses, such as differentiation and proliferation. p120-RasGAP (RasGAP) downregulates Ras signaling by catalyzing intrinsic GTPase activity of Ras. p120-RasGAP also is implicated in signaling to the cytoskeleton by binding Rap1a and/or p190-RhoGAP (RhoGAP).

aunay syndrome, and, in another, one individual had Sturge-Weber syndrome. In family CM6, some individuals had multiple small, pink-to-red CMs. No associated AVFs or AVMs were observed in these 10 families. In the 11th family, the two affected individuals had an AVM without cutaneous CM. The identification of atypical round-to-oval CMs and their association with high-flow arterial lesions and soft- and skeletal-tissue hypertrophy constitutes a newly recognized clinical and genetic entity that we named “CM-AVM,” for capillary malformation–arteriovenous malformation.

Discussion

Mosaic mice composed of wild-type and p120-RasGAP-deficient cells exhibit abnormal angiogenesis due to endothelial cell dysfunction in the reorganization of the capillary rete to a mature vascular network (Henkemeyer et al. 1995). Subsequent studies of *RASA1*-deficient cells have shown that p120-RasGAP is required for properly directed cellular movement (Kulkarni et al. 2000). The wide range of growth-factor receptors, the signaling of which can be affected by *RASA1* mutations, probably explains the murine phenotype, as well as the human phenotypic variability described in this article. The pathogenesis of the cutaneous lesions of CM-AVM could involve a defective migratory response of endo-

thelial cells to angiogenic factors, presumably a result of VEGF secreted by keratinocytes and sensory nerves during the development of dermal vasculature. The aberrant response to secreted VEGF could also explain an earlier observation of misalignment of capillaries and cutaneous nerves in CM (Smoller and Rosen 1986; Rydh et al. 1991). Furthermore, altered VEGF response could contribute to the abnormal arteriogenesis, resulting in AVMs, AVFs, and soft-tissue or skeletal hypertrophy. The formation of direct shunts in AVMs and AVFs could also be due to dysregulated signaling of EphB-receptor-mediated pathways in which p120-RasGAP has a role (Wang et al. 1998; Kim et al. 2002; Nagashima et al. 2002).

The localized nature of the vascular anomalies and incomplete penetrance of the mutations may suggest that a somatic second hit, resulting in a complete lack of activity of p120-RasGAP, could be necessary for a lesion to develop, as has been shown for *glomulin* in glomuvenous malformations (Brouillard et al. 2002). Thus, the variable phenotypes caused by the *RASA1* mutations might reflect the extent and identity of the affected cellular population. The soft-tissue and skeletal overgrowth could be caused by a complete loss of function of p120-RasGAP in mesenchymal cells, which would intensify the cellular effects of mitogens, such as FGF and EGF.

Another inherited vascular malformation, cerebral capillary malformation (CCM [MIM 116860]), has also been linked to misregulated Ras signaling (Laberge-le Couteulx et al. 1999; Sahoo et al. 1999). The mutated protein, KRIT1 (Krev interaction trapped-1), was originally identified as a binding partner of Rap1a (Serebriiskii et al. 1997), an antagonist of Ras transformation (fig. 5) (Kitayama et al. 1989). CCM lesions are located in the cerebral parenchyma and are composed of dilated capillary-like vessels and/or large cavernous channels lined by thin basal lamina. The number of lesions tends to increase over time (Labauge et al. 2000, 2001), which might be a result of the identified slight upregulation in the proliferative capacity of CCM-derived endothelial cells (Notelet et al. 1997). KRIT1 has also been shown to bind ICAP1 (integrin cytoplasmic domain-associated protein-1) (Zhang et al. 2001; Gunel et al. 2002; Zawistowski et al. 2002), a protein that links integrins and the actin cytoskeleton (Chang et al. 1997), which implies a process of integrin-signaling-mediated cellular adhesion in the pathogenesis of CCM (fig. 5). It is possible that CM-AVM and CCM are due to similar cellular processes, since p120-RasGAP can bind Rap1a, which is known to have an important role in integrin-mediated cellular adhesion (Caron et al. 2000; Katagiri et al. 2000; Reedquist et al. 2000; Nagashima et al. 2002). It is interesting that, in certain CCM families carrying KRIT1 mutations, some members also have cutaneous lesions characterized as hyperkeratotic capillary-venous malformations (Labauge et al. 1999; Eerola et al. 2000).

Our data show that an atypical cutaneous CM can be an indicator of a genetic susceptibility to more severe vascular high-flow anomalies—namely, AVM and AVF—which can occur alone or as part of a combined disorder, such as in Parkes Weber syndrome. Latent intracranial AVM and carotid AVF, which may cause life-threatening hemorrhaging, could be identified by screening at-risk individuals by MRI or echo-Doppler examination. Since four of the families were observed in the small Belgian population, many others probably exist. Extended studies are needed to further characterize the phenotypic variability in CM-AVM, to test the hypothesis of two-hit activation of the pathogenesis of the lesions, and to dissect the specific pathways altered by p120-RasGAP mutations. In theory, Ras activity modulators could have a therapeutic potential in normalizing the regulation of the Ras signaling pathway in these patients.

Acknowledgments

The authors are grateful to all the family members for their participation in this study. The work was supported by the Belgian Federal Service for Scientific, Technical, and Cultural Affairs, the F.N.R.S. (Fonds national de la recherche scienti-

fique), and the ARC (Actions de Recherche Concertées-Communauté française de Belgique) (all to M.V., a “chercheur qualifié du F.N.R.S.”). I.E. was supported by a Marie Curie Fellowship from the European Community Program Quality of Life, (contract number QLRI-CT-1999-51491). The authors thank Ana Gutierrez for technical assistance and Liliana Niculescu for secretarial help.

Electronic-Database Information

The URLs for data presented herein are as follows:

NCBI Entrez, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi> (for *RASA1* cDNA and genomic sequences)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for CM and CCM)

PROSITE, <http://us.expasy.org/prosite/> (for PH-domain searches)

References

- Ballaun C, Weninger W, Uthman A, Weich H, Tschachler E (1995) Human keratinocytes express the three major splice forms of vascular endothelial growth factor. *J Invest Dermatol* 104:7–10
- Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, Wigler M, Collins F (1990) The *NF1* locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. *Cell* 63:851–859
- Barsky SH, Rosen S, Geer DE, Noe JM (1980) The nature and evolution of port wine stains: a computer-assisted study. *J Invest Dermatol* 74:154–157
- Bos JL (1989) ras oncogenes in human cancer: a review. *Cancer Res* 49:4682–2689
- Brouillard P, Boon LM, Mulliken JB, Enjolras O, Ghassibe M, Warman ML, Tan OT, Olsen BR, Vikkula M (2002) Mutations in a novel factor, glomulin, are responsible for glomuvenous malformations (“glomangiomas”). *Am J Hum Genet* 70:866–874
- Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, van de Water L (1992) Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. *J Exp Med* 176:1375–1379
- Caron E, Self AJ, Hall A (2000) The GTPase Rap1 controls functional activation of macrophage integrin $\alpha M\beta 2$ by LPS and other inflammatory mediators. *Curr Biol* 10:974–978
- Cawthon RM, Weiss R, Xu GF, Viskochil D, Culver M, Stevens J, Robertson M, et al (1990) A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell* 62:193–201
- Chang DD, Wong C, Smith H, Liu J (1997) ICAP-1, a novel $\beta 1$ integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of $\beta 1$ integrin. *J Cell Biol* 138:1149–1157
- Clark GJ, Quilliam LA, Hisaka MM, Der CJ (1993) Differential antagonism of Ras biological activity by catalytic and Src homology domains of Ras GTPase activation protein. *Proc Natl Acad Sci USA* 90:4887–4891
- Cullen PJ, Hsuan JJ, Truong O, Letcher AJ, Jackson TR, Daw-

- son AP, Irvine RF (1995) Identification of a specific Ins(1,3,4,5)P₄-binding protein as a member of the GAP1 family. *Nature* 376:527–530
- Drugan JK, Rogers-Graham K, Gilmer T, Campbell S, Clark GJ (2000) The Ras/p120 GTPase-activating protein (GAP) interaction is regulated by the p120 GAP pleckstrin homology domain. *J Biol Chem* 275:35021–35027
- Eerola I, Boon LM, Watanabe S, Grynberg H, Mulliken JB, Vikkula M (2002) Locus for susceptibility for familial capillary malformation (“port-wine stain”) maps to 5q. *Eur J Hum Genet* 10:375–380
- Eerola I, Plate KH, Spiegel R, Boon LM, Mulliken JB, Vikkula M (2000) KRIT1 is mutated in hyperkeratotic cutaneous capillary-venous malformation associated with cerebral capillary malformation. *Hum Mol Genet* 9:1351–1355
- Frech M, John J, Pizon V, Chardin P, Tavitian A, Clark R, McCormick F, Wittinghofer A (1990) Inhibition of GTPase activating protein stimulation of Ras-p21 GTPase by the *Krev-1* gene product. *Science* 249:169–171
- Friedman E, Gejman PV, Martin GA, McCormick F (1993) Nonsense mutations in the C-terminal SH2 region of the GTPase activating protein (GAP) gene in human tumours. *Nat Genet* 5:242–247
- Gibbs JB, Marshall MS, Scolnick EM, Dixon RA, Vogel US (1990) Modulation of guanine nucleotides bound to Ras in NIH3T3 cells by oncogenes, growth factors, and the GTPase activating protein (GAP). *J Biol Chem* 265:20437–20442
- Glanzer JG, Liao L, Baker T, McMullen MH, Langan AS, Crandall LZ, Vorce RL (2002) Organization and regulation of the human rasGAP gene. *Gene* 285:149–156
- Gunel M, Laurans MS, Shin D, DiLuna ML, Voorhees J, Choate K, Nelson-Williams C, Lifton RP (2002) KRIT1, a gene mutated in cerebral cavernous malformation, encodes a microtubule-associated protein. *Proc Natl Acad Sci USA* 99:10677–10682
- Hata Y, Kikuchi A, Sasaki T, Schaber MD, Gibbs JB, Takai Y (1990) Inhibition of the ras p21 GTPase-activating protein-stimulated GTPase activity of c-Ha-ras p21 by smg p21 having the same putative effector domain as ras p21s. *J Biol Chem* 265:7104–7107
- Henkemeyer M, Rossi DJ, Holmyard DP, Puri MC, Mbamalu G, Harpal K, Shih TS, Jacks T, Pawson T (1995) Vascular system defects and neuronal apoptosis in mice lacking ras GTPase-activating protein. *Nature* 377:695–701
- Jacobs AH, Walton RG (1976) The incidence of birthmarks in the neonate. *Pediatrics* 58:218–222
- Katagiri K, Hattori M, Minato N, Irie S, Takatsu K, Kinashi T (2000) Rap1 is a potent activation signal for leukocyte function-associated antigen 1 distinct from protein kinase C and phosphatidylinositol-3-OH kinase. *Mol Cell Biol* 20:1956–1969
- Kim I, Ryu YS, Kwak HJ, Ahn SY, Oh JL, Yancopoulos GD, Gale NW, Koh GY (2002) EphB ligand, ephrinB2, suppresses the VEGF- and angiopoietin 1-induced Ras/mitogen-activated protein kinase pathway in venous endothelial cells. *Faseb J* 16:1126–1128
- Kitayama H, Sugimoto Y, Matsuzaki T, Ikawa Y, Noda M (1989) A *ras*-related gene with transformation suppressor activity. *Cell* 56:77–84
- Kulkarni SV, Gish G, van der Geer P, Henkemeyer M, Pawson T (2000) Role of p120 Ras-GAP in directed cell movement. *J Cell Biol* 149:457–470
- Labauge P, Brunereau L, Levy C, Laberge S, Houtteville JP (2000) The natural history of familial cerebral cavernomas: a retrospective MRI study of 40 patients. *Neuroradiology* 42:327–332
- Labauge P, Brunereau L, Laberge S, Houtteville JP (2001) Prospective follow-up of 33 asymptomatic patients with familial cerebral cavernous malformations. *Neurology* 57:1825–1828
- 3.0.CO;2-VLabauge P, Enjolras O, Bonerandi JJ, Laberge S, Dandurand M, Joujoux JM, Tournier-Lasserre E (1999) An association between autosomal dominant cerebral cavernomas and a distinctive hyperkeratotic cutaneous vascular malformation in 4 families. *Ann Neurol* 45:250–254
- Laberge-le Couteulx S, Jung HH, Labauge P, Houtteville JP, Lescoat C, Cecillon M, Marechal E, Joutel A, Bach JF, Tournier-Lasserre E (1999) Truncating mutations in *CCM1*, encoding KRIT1, cause hereditary cavernous angiomas. *Nat Genet* 23:189–193
- Maekawa M, Li S, Iwamatsu A, Morishita T, Yokota K, Imai Y, Kohsaka S, Nakamura S, Hattori S (1994) A novel mammalian Ras GTPase-activating protein which has phospholipid-binding and Btk homology regions. *Mol Cell Biol* 14:6879–6885
- Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haubruck H, Conroy L, et al (1990) The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. *Cell* 63:843–849
- Mukouyama YS, Shin D, Britsch S, Taniguchi M, Anderson DJ (2002) Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. *Cell* 109:693–705
- Mulliken JB, Glowacki J (1982) Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg* 69:412–422
- Mulliken JB, Young AE (1988) *Vascular birthmarks: hemangiomas and malformations*. WB Saunders, Philadelphia
- Nagashima K, Endo A, Ogita H, Kawana A, Yamagishi A, Kitabatake A, Matsuda M, Mochizuki N (2002) Adaptor protein Crk is required for Ephrin-B1-induced membrane ruffling and focal complex assembly of human aortic endothelial cells. *Mol Biol Cell* 13:4231–4242
- Notelet L, Houtteville JP, Houry S, Lechevalier B, Chapon F (1997) Proliferating cell nuclear antigen (PCNA) in cerebral cavernomas: an immunocytochemical study of 42 cases. *Surg Neurol* 47:364–370
- Reedquist KA, Ross E, Koop EA, Wolthuis RM, Zwartkruis FJ, van Kooyk Y, Salmon M, Buckley CD, Bos JL (2000) The small GTPase, Rap1, mediates CD31-induced integrin adhesion. *J Cell Biol* 148:1151–1158
- Rydh M, Malm M, Jernbeck J, Dalsgaard CJ (1991) Ectopic blood vessels in port-wine stains lack innervation: possible role in pathogenesis. *Plast Reconstr Surg* 87:419–422
- Sahoo T, Johnson EW, Thomas JW, Kuehl PM, Jones TL, Dokken CG, Touchman JW, Gallione CJ, Lee-Lin SQ, Kosofsky B, Kurth JH, Louis DN, Mettler G, Morrison L, Gil-Nagel A, Rich SS, Zabramski JM, Boguski MS, Green ED, Marchuk DA (1999) Mutations in the gene encoding KRIT1, a

- Krev-1/rap1a binding protein, cause cerebral cavernous malformations (CCM1). *Hum Mol Genet* 8:2325–2333
- Serebriiskii I, Estojak J, Sonoda G, Testa JR, Golemis EA (1997) Association of Krev-1/rap1a with Krit1, a novel ankyrin repeat-containing protein encoded by a gene mapping to 7q21-22. *Oncogene* 15:1043–1049
- Settleman J, Narasimhan V, Foster LC, Weinberg RA (1992) Molecular cloning of cDNAs encoding the GAP-associated protein p190: implications for a signaling pathway from ras to the nucleus. *Cell* 69:539–549
- Smoller BR, Rosen S (1986) Port-wine stains. A disease of altered neural modulation of blood vessels? *Arch Dermatol* 122:177–179
- Trahey M, McCormick F (1987) A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. *Science* 238:542–545
- Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, Culver M, et al (1990) Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell* 62:187–192
- Wang HU, Chen ZF, Anderson DJ (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 93:741–753
- Xu GF, Lin B, Tanaka K, Dunn D, Wood D, Gesteland R, White R, Weiss R, Tamanoi F (1990) The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements ira mutants of *S. cerevisiae*. *Cell* 63:835–841
- Zawistowski JS, Serebriiskii IG, Lee MF, Golemis EA, Marchuk DA (2002) KRIT1 association with the integrin-binding protein ICAP-1: a new direction in the elucidation of cerebral cavernous malformations (CCM1) pathogenesis. *Hum Mol Genet* 11:389–396
- Zhang J, Clatterbuck RE, Rigamonti D, Chang DD, Dietz HC (2001) Interaction between krit1 and icap1 α infers perturbation of integrin β 1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. *Hum Mol Genet* 10:2953–2960