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RESEARCH ARTICLE

Parkes Weber Syndrome, Vein of Galen Aneurysmal Malformation, and Other Fast-Flow Vascular Anomalies Are Caused by *RASA1* Mutations

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Capillary malformation-arteriovenous malformation (CM-AVM) is a newly recognized autosomal dominant disorder, caused by mutations in the *RASA1* gene in six families. Here we report 42 novel *RASA1* mutations and the associated phenotype in 44 families. The penetrance and de novo occurrence were high. All affected individuals presented multifocal capillary malformations (CMs), which represent the hallmark of the disorder. Importantly, one-third had fast-flow vascular lesions. Among them, we observed severe intracranial AVMs, including vein of Galen aneurysmal malformation, which were symptomatic at birth or during infancy, extracranial AVM of the face and extremities, and Parkes Weber syndrome (PKWS), previously considered sporadic and nongenetic. These fast-flow lesions can be differed from the other two genetic AVMs seen in hereditary hemorrhagic telangiectasia (HHT) and in phosphatase and tensin homolog (PTEN) hamartomatous tumor syndrome. Finally, some CM-AVM patients had neural tumors reminiscent of neurofibromatosis type 1

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or 2. This is the first extensive study on the phenotypes associated with *RASA1* mutations, and unravels their wide heterogeneity. Hum Mutat 29(7), 959–965, 2008.

KEY WORDS: arteriovenous fistula; arteriovenous; capillary; malformation; hereditary benign telangiectasia; hereditary hemorrhagic telangiectasia; *NF1*; *NF2*; Parkes Weber syndrome; p120RasGAP; *RASA1*; *RAS p21*

INTRODUCTION

Recognition of the association capillary malformation-arteriovenous malformation (CM-AVM; MIM# 608354) was the culmination of the search for a gene responsible for inherited capillary stains. Several affected families guided linkage studies and led to the identification of germline mutations in the *RASA1* gene (MIM# 139150) in six families (39 individuals) [Eerola et al., 2002, 2003]. These capillary malformations (CMs) were atypical in that they were multifocal, small, round-to-oval, pinkish-to-red, and randomly distributed. Interestingly, 9 out of 35 affected individuals had an arteriovenous malformation (AVM) or an arteriovenous fistula (AVF), two intracranial and seven peripheral (Table 1). The high frequency of fast-flow vascular malformations raised concern about the possibility of their presence in patients with harmless cutaneous capillary stains. Since the latter occur with a birth prevalence of 0.3%, it is important to recognize those patients with an elevated risk for fast-flow lesions.

One of the affected family members in the original study (Table 1) was diagnosed as having Parkes Weber syndrome (PKWS; MIM# 608355), characterized by a large cutaneous vascular stain with multiple underlying subcutaneous and intramuscular AVFs, and overgrowth of the affected extremity [Mulliken and Young, 1988]. This condition, described in 1918 by Parkes Weber [Parkes Weber, 1918], has been considered to be a sporadic overgrowth syndrome of unknown etiology. The phenotypic similarity among the overgrowth disorders often leads to misdiagnosis and improper management.

In this study we describe that fast-flow lesions associated with CM-AVM are frequent and different from those of hereditary hemorrhagic telangiectasia (HHT) and phosphatase and tensin homolog (*PTEN*) hamartomatous tumor syndrome. In addition, we demonstrate a genetic form of PKWS, and that patients with *RASA1* mutation may be at increased risk for specific neurologic tumors.

TABLE 1. **Clinical Characteristics of 140 Patients With CM-AVM**

	Number of individuals		
	Current study (44 families)	Previous study (six families)	Total (50 families) n (%)
Number of individuals with *RASA1* mutation	**101**	**39**	**140**
CM	**99**	**35**	**134 (95.7)**
Multifocal	**90**	**35**	**125 (89.3)**
Solitary atypical	**9**	**0**	**9 (6.4)**
AVM/AVF	**17**	**9**	**26 (18.5)**
Intracerebral	**8**	**2**	**10 (7.1)**
Vein of Galen aneurysmal malformation	**2**	**0**	
Other	**6**	**2**	
Extracerebral	**9**	**7**	**16 (11.4)**
Parkes Weber syndrome	**16**	**1**	**17 (12.1)**
Other			
Cardiac anomaly	**4**	**0**	**4 (2.8)**
Benign/malignant tumor	**7**	**0**	**7 (5)**
Chylous ascites	**1**	**0**	**1 (0.7)**
Ectopic thyroid and parathyroids	**1**	**0**	**1 (0.7)**

MATERIALS AND METHODS

Patient Recruitment

A total of 21 centers located in the European Union, North and South America, and Australia participated in this study. Informed consent was obtained from all subjects, and the research protocol was approved by the ethics committee of the medical faculty of Université catholique de Louvain, Brussels, Belgium. Inclusion criteria were multifocal CMs with/without a fast-flow lesion: AVM, AVF, PKWS. Individuals with PKWS without multifocal CMs were also included. A detailed clinical questionnaire was completed by each referring physician. A proband was the first affected individual detected in a family. We recruited 61 probands, and 120 family members. A total of 57 probands were Caucasian (Europe, Canada, United States, and Australia) and four were Hispanic.

Mutation Analysis of the *RASA1* Gene

Genomic DNA was extracted from blood leukocytes or buccal cells using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). A total of 26 sets of primers were designed to amplify all 25 exons of the *RASA1* gene, including exon-intron boundaries (isoform 1; University of California, Santa Cruz [UCSC] Genome Bioinformatics; http://genome.ucsc.edu). Genomic DNA was screened by denaturing high-performance liquid chromatography (DHPLC) on the WAVE 3500 HS system (Transgenomic, Omaha, NE). Each sample with an abnormal elution profile was sequenced on a CEQ2000 fluorescent capillary sequencer (Beckman Coulter, Analis, Belgium). DHPLC was used to assess cosegregation of the abnormal elution profile with the phenotype and to test control chromosomes. Numbering of nucleotides was based on cDNA sequence NM_002890.1 with the A of the ATG start codon marked as +1. Mutations were named according to the international recommendations (www.hgvs.org/mutnomen).

Whenever possible, false paternity was excluded in sporadic cases using 20 highly polymorphic Weber set 8 or laboratory-designed microsatellite markers. Genotyping was performed as described elsewhere [Eerola et al., 2002].

RESULTS

Genetic Studies

Multifocal CMs were noted in 56 out of 61 probands. Of the 56, 21 had multifocal CMs only, and 35 had an associated fast-flow lesion: 19 AVM/AVF and 16 PKWS. The five other probands had isolated PKWS (without multifocal CMs). A germline mutation was identified in 44 out of 56 probands with CMs: 15 out of 21 (71%) of those with CMs only, 16 out of 19 (84%) of those with AVM/AVF, and 13 out of 16 (81%) of those with PKWS. The genetic basis was ascertained in 14 out of 24 (58%) sporadic and 30 out of 32 (93.7%) familial cases (Table 2). A total of 41 of the probands with *RASA1* mutation were Caucasian and three were

TABLE 2. Phenotype of Individuals With CM-AVM with (I) or Without (II) RASA1 Mutation*

	RASA1 mutation			Phenotype of individuals with CM-AVM with (I) or without (II) RASA1 mutation			
Family	Nucleotide change	Putative effect at amino acid level	Mutation carriers[a]	CM[a]	AVM/AVF[a]	PKWS[a]	Symptoms and associated features[b]
1	c.613_617delCTTAT	p.Leu205LysfsX4	4	4	0	0	Optic glioma (before 12y); lipoma (before 10y)
2	c.656C>G	p.Ser219X	1	1	0	0	
3	c.806_810delTTTAC	p.Leu269ProfsX11	1(dn)	1	0	1	CO
4	c.828+3A>T	Splicing affected	1(dn)	1	1	0	
5	c.951dupG	p.Met318AspfsX10	1(dn?)	1	0	0	
6	c.957G>A	p.Trp319X	2	2	0	1	CO
7	c.1017+1G>T	Splicing affected	6	6	0	2	CO
8	c.1192C>T	p.Arg398X	2	2	1	0	Epilepsy; hydrocephaly
9	c.1208dupC	p.Thr404AsnfsX14	1(dn)	1	1	0	
10	c.1277A>G	p.Tyr426Cys	8	9	0	0	
11	c.1279C>T	p.Arg427X	2	2	0	0	
12	c.1279C>T	p.Arg427X	1	1	1	0	
13	c.1332+5G>A	Splicing affected	4	4	0	0	
14	c.1336C>T	p.Gln446X	1(dn)	1	0	1	
15	c.1350_1351insT	p.Asn451X	3	3	0	2	
16	c.1362_1363insTCAGT	p.Asp455SerfsX30	1(dn)	1	0	0	
17	c.1480dupT	p.Tyr494LeufsX7	2	2	0	1	
18	c.1490T>G	p.Leu497X	1(dn?)	1	1	0	
19	c.1572_1575dup	p.Ser526MetfsX8	4	4	0	1	CO;TOF, superficial basal cell carcinoma (33y)
20	c.1636C>T	p.Gln546X	1(dn?)	1	1	0	
21	c.1682_1683dup	Pro562LeufsX9	1(dn?)	1	1	0	CF; epilepsy; hemorrhages; ASDII/PFO
22	c.1698+3_1698+4insT	Splicing affected	2	2	0	1	P St
23	c.1870C>T	p.Gln624X	2	2	1	0	
24	c.2026C>T	p.Gln676X	3	3	0	0	
25	c.2125C>T	p.Arg709X	5	5	1	1	VV
26	c.2125C>T	p.Arg709X	6	5	0	2	CF
27	c.2184+1delG	Splicing affected	4	3	0	1	PDA, ASD, P St, prolapsed tricuspid valve
28	c.2185–1G>A	Splicing affected	1(dn?)	1	0	0	
29	c.2288A>T	p.Glu763Val	1(dn?)	1	1	0	VGAM; CF, epilepsy; died rapidly after birth
30	c.2341G>T	p.Glu781X	2	2	1	0	Hydrocephaly
31	C>T	p.Arg789X	3	3	2	0	Angiolipoma (1y)
32	C>T	p.Gln808X	2	2	0	1	
33	c.2450_2451delCT	p.Ser817TyrfsX12	2	2	1	0	
34	c.2488-2A>G	Splicing affected	2	2	0	0	
35	c.2488-1delGTTA	Splicing affected	1(dn?)	1	1	0	Non-small-cell lung cancer (32y); hemoptysis
36	c.2514_2515insA	p.Glu839ArgfsX6	1	1	0	0	
37	c.2532_2536delTTTAA	p.Leu845ThrfsX38	2(dn)	4	1	0	De novo mutation in monozygotic twins; VGAM and CF in one
38	c.2579_2582delTCAT	p.Phe860TrpfsX10	1(dn?)	1	1	0	Ectopic thyroid and parathyroid
39	c.2603+1G>A	Splicing affected	2	2	0	0	Chylous ascites
40	c.2603+2_2603+3insT	Splicing affected	2	2	0	0	Ureteral reflux, epispadias
41	c.2603+4_2603+5insA	Splicing affected	3	3	0	0	
42	c.2603+5G>T	Splicing affected	1	1	0	1	Neurofibromas (53y)
43	C>T	p.Arg1010X	3	3	0	0	Vestibular Schwannoma (26y)
44	c.3052delG	p.Ala1018HisfsX6	2	2	0	0	
45	No mutation identified			7	0	1	
46	No mutation identified			1	1	0	
47	No mutation identified			1	0	0	
48	No mutation identified			1	0	0	
49	No mutation identified			1	0	1	
50	No mutation identified			1	0	1	
51	No mutation identified			2	0	0	
52	No mutation identified			1	1	0	
53	No mutation identified			1	0	0	
54	No mutation identified			1	0	0	
55	No mutation identified			1	1	0	
56	No mutation identified			1	0	0	

*Numbering of nucleotides was based on cDNA sequence NM_002890.1 with the A of the ATG start codon marked as +1. Mutations were named according to the international recommendations (www.hgvs.org/mutnomen/).
[a]Number of individuals concerned.
[b]The age of onset for the tumors is in parentheses.
dn, de novo mutation; dn?, likely de novo mutation, but one or both parents' DNA was not available for testing; CO, cardiac overload; CF, cardiac failure; VV, varicose veins; TOF, tetralogy of Fallot; PDA, patent ductus arteriosus; ASD, atrial septal defect; PFO, patent foramen ovale; P St, pulmonary stenosis; VGAM, vein of Galen aneurysmal malformation.

Hispanic. No mutation was identified in the five patients with isolated PKWS; 3 out of 5 were sporadic and two had at least one first-degree relative with a solitary CM.

Genotyping the 44 probands' relatives identified 57 additional individuals with a *RASA1* mutation (in total 101; 58% females, 42% males). All mutations cosegregated with vascular anomalies and were absent in 200 control chromosomes. There were two unaffected carriers (Families F26 and F27) and three phenocopies with a typical solitary CM (Family 10; and two in Family 37) (Table 2).

We identified 42 distinct *RASA1* mutations: 39 were novel and private, two were novel and present in two apparently unlinked families, and one was previously published (Table 2; Fig. 1) [Eerola et al., 2003]. The genetic alterations were distributed in 15 out of

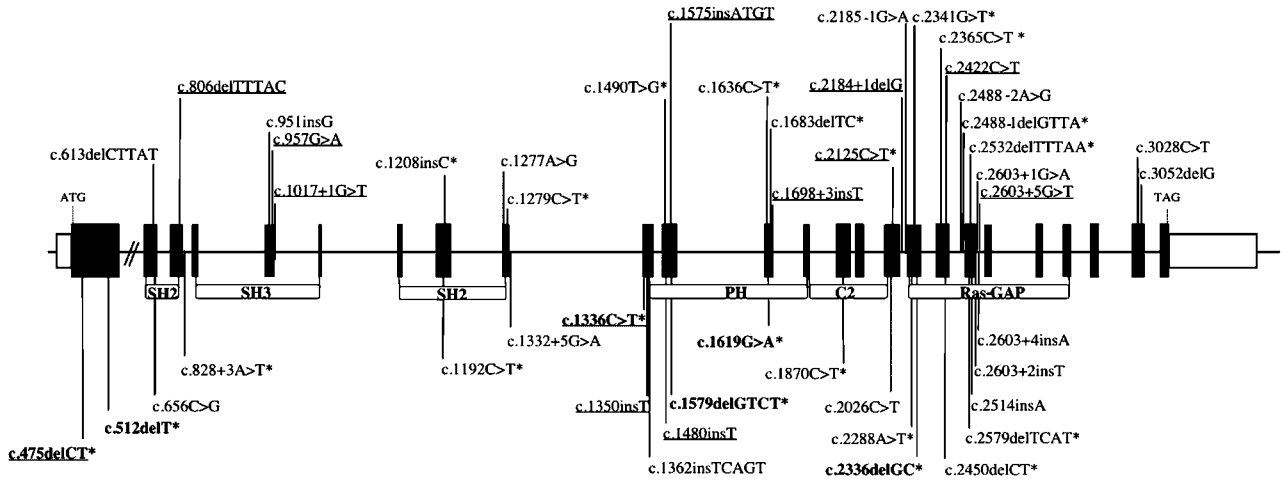

FIGURE 1. **Schematic representation of the *RASA1* gene and CM-AVM causing mutations. Exons (boxed) and introns (lines) are drawn to scale, except for intron 1 (longer than shown); untranslated regions, small open boxes. Known domains of p120-RasGAP protein marked below exons. Mutations in bold described previously [Eerola et al., 2003]. Mutations associated with PKWS are underlined and those associated with AVM/AVF are starred. Numbering of nucleotides based on the cDNA sequence NM_002890.1 with the adenosine of the ATG start codon marked as +1.**

the 25 exons and consisted of 19 insertions or deletions causing a frameshift, or a splice-site alteration, and 23 substitutions: 14 nonsense, seven splice-site, and two missense mutations involving conserved amino acids.

In 14 out of 44 families the *RASA1* mutation was likely de novo (32%). False paternity was excluded in six of them, using a large panel of polymorphic markers. In the remaining cases, DNA was not available for one or both of the parents, and the novo occurrence was based on the clinical examination and/or family history (Table 2). There was no difference among the distribution of de novo vs. inherited mutations.

Clinical Spectrum in Patients With a *RASA1* Mutation

The penetrance was very high, even when index cases were excluded; indeed, 55 out of 57 mutation carriers were affected (96.5%). There was a marked inter- and intrafamilial clinical heterogeneity (Table 2). In 16 of 44 families only multifocal CMs were seen. In the remaining families, some individuals had multifocal CMs only, while others had multifocal CMs and fast-flow lesions: AVM/AVF or PKWS.

Multifocal CMs

The most prominent feature among *RASA1* patients was small, usually multifocal, and randomly distributed cutaneous CMs (Fig. 2). A total of nine had a solitary lesion, the maximum 53 (Table 1). Some of the lesions were present at birth, others appeared in childhood. Many CMs were smaller than 1 cm in diameter, but some were large, up to 15 cm. Typically, the CMs were pink, sometimes red, brown, or gray; a narrow, white halo was present in about one-half of the lesions. Some were warm, but without a thrill, and continuous wave hand-held Doppler examination revealed an increased flow in some of the lesions.

Fast-Flow Anomalies

Fast-flow anomalies AVM/AVF or PKWS were present in 32.6% of the investigated patients (33/101).

PKWS. PKWS with multifocal CMs was the clinical diagnosis in 16 patients from 13 families (Tables 1 and 2). The Parkes Weber anomaly was localized in the upper extremity in six patients (three left, three right), and in the lower extremity in 10 patients (five left, five right). In some patients, the ipsilateral buttock or the thoracic region was also affected (Fig. 2). The patients exhibited unilateral capillary stain of various sizes. One had a single 5 × 5 cm lesion, some had patchy and others a homogenous diffuse lesion almost completely covering the affected extremity (Fig. 2). PKWS patients also had bony and soft-tissue hypertrophy, with the affected limb being longer and larger than the unaffected one. This was more pronounced when the lower extremity was involved. The cutaneous stains were pink-to-red, warm, and usually without a thrill. Often these lesions were geographic and well demarcated. No venous or lymphatic components were observed. The vascular lesions were present at birth and worsened with age; ischemia was not documented. Reduced hair density was seen in some adult patients in the area of the capillary stain. A total of five patients had signs of cardiac volume overload (31%) secondary to fast-flow, particularly if the lower extremity was involved. A total of four were well tolerated; however, one of them required medical treatment for cardiac failure.

AVM/AVF. A total of 18 AVMs and two AVFs associated with multifocal CMs were identified in 17 individuals from 16 families: 15 out of 17 had one fast-flow lesion, 1 out of 17 had two lesions, and 1 out of 17 three lesions. Of the 18 AVMs, 12 were extracranial and six, as well as the two AVFs, were intracranial. None of the extracranial AVMs were in viscera. The distribution was: nose (n = 2), right cheek (n = 4), right ear (n = 1), nose-lip-cheek (n = 1), thorax (n = 1), arm (n = 1), buttock (n = 1), and mediastinum (n = 1); they involved skin, subcutis, and sometimes muscles, and bones. Intracranial AVMs included: one asymptomatic pial lesion and five symptomatic lesions. Among them, two were vein of Galen aneurysmal malformations causing cardiac failure at birth in one, and cardiac failure, epilepsy, and death soon after birth in the other. The three other symptomatic intracranial AVMs comprised of two frontal lesions causing late onset seizures in one case and hydrocephalus in the second, and a posterior fossa lesion causing migraine, hemorrhage, epilepsy, and cardiac failure. A total of two individuals had an AVF: one, an asymptomatic pial lesion, and the other, a left temporal AVF causing epilepsy soon after birth, hydrocephaly, and delayed development. The initial symptoms were present at birth or appeared during the first year of

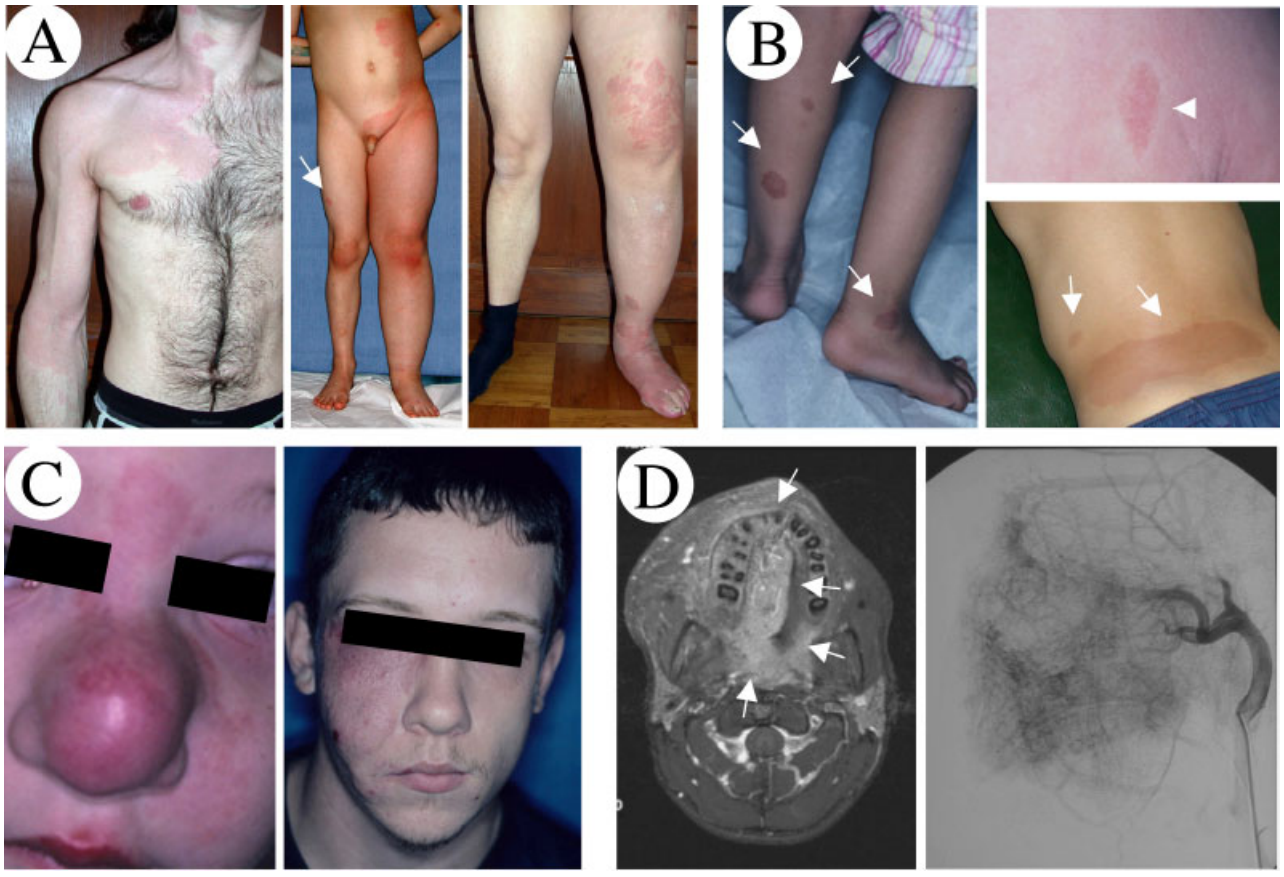

FIGURE 2. **Phenotypic variation in CM-AVM. A: PKWS in the upper or lower extremity; note hypertrophy in the lower limb. CM is homogenous and diffuse in first two patients and patchy in the third one. Hair density is reduced in area of capillary stain on the thoracic region. B: multifocal CMs; arrowhead, with halo. C: AVM on nose and right cheek. D: Imaging of an AVM on right cheek: axial T1-weighted (fat saturated) image after contrast infusion showed diffuse thickening (cheek, maxilla, and hard palate [arrows]) and enhancement. Selective arteriography of right facial artery (lateral view): enlargement of feeding artery, diffuse alteration of arteriolar and capillary bed.**

life in five intracranial AVMs/AVFs, and at age 7 years in the sixth case.

Radiological investigation of fast-flow lesions. Angiographic features of the extracranial AVMs were those considered typical of these lesions, including enlargement and tortuosity of the feeding arteries, AVM nidus or tissue "blush" with arteriolovenular microfistulas and arteriolovenous or AVFe and dilated draining veins (Fig. 2). There were no aneurysms and few varices. The intracranial AVMs had classical imaging features consistent with location, e.g., pial or Galenic. Patients with PKWS had diffuse microshunting, giving the angiographic appearance of a soft-tissue blush involving some of the muscles and areas of the subcutaneous fat with early opacification and dilation of the deep and superficial veins. Color Doppler ultrasonography with spectral analysis revealed enlarged subcutaneous arteries and veins with low-resistance arterial flow and pulsatile venous flow consistent with the diagnosis of fast-flow lesion. MRI of extracranial AVMs and PKWS documented vascular flow voids along with symmetrical overgrowth of affected muscle with mild hypersignal on T2-weighted sequences, and contrast enhancement. There was a variable degree of thickening of the subcutaneous fat. Bony hypertrophy was common (Fig. 2). No ectopic fat or disruption of the soft-tissue morphology was seen.

Other clinical features. Less common features included: cardiac structural defects (n = 4), chylous ascites (n = 1), and ectopic thyroid and parathyroids (n = 1) (Table 2). Interestingly, seven individuals had tumors; i.e., optic glioma, neurofibroma, vestibular schwannoma, non-small-cell lung cancer, superficial basal cell carcinoma, paraspinal angiolipoma, and paraspinal lipoma.

Clinical Phenotype of Patients Without a *RASA1* Mutation

No mutation in *RASA1* was found in 10 sporadic patients and two families (nine members) with a phenotype indistinguishable from those with a mutation (Table 2). All had multifocal CMs, and 6 out of 19 a fast-flow lesion: three had PKWS and three had an AVM. In the five patients with isolated PKWS, no *RASA1* mutation was found either. Clinically, these stains were less hypertrophic and darker, and sometimes staining was bilateral. No significant radiological differences were observed by MRI, angiography, or color Doppler ultrasonography, between the PKWS with or without a *RASA1* mutation.

DISCUSSION

Autosomal dominant multifocal CM is the cardinal feature of patients with *RASA1* mutation, and represents the clue to the diagnosis. These CMs are asymptomatic "red-flags," signaling the possible association of AVM/AVF. These stains are pink, red,

sometimes darker, multifocal, and randomly distributed. Half of them have a pale halo and some a slightly increased flow by hand-held Doppler examination. They could be considered pre-AVM, yet they remain stable throughout adulthood. These pathognomonic *RASA1* lesions have to be differentiated from punctuate, radiate, or arborizing telangiectatic lesions seen in hereditary benign telangiectasia (HBT; MIM# 187260) and hereditary hemorrhagic telangiectasia (HHT; MIM# 187300). One family initially reported to have HBT had, in fact, CM-AVM (Family 11 in this study) [Brancati et al., 2003].

Among a total of 140 individuals so far identified with a *RASA1* mutation, 45 (seven families) were from the French speaking part of Belgium with a population of 4 million people. Thus, one can estimate a minimum prevalence of 1 in 100,000 for CM-AVM in Caucasians. This translates roughly to 48,000 and 30,000 affected individuals in the European Union and the United States, respectively. This figure is probably an underestimate, as CM-AVM is a newly recognized phenotype that can be subtle, presenting as asymptomatic multifocal CMs. By comparison, the incidence of HHT, another autosomal dominant condition associated with AVMs, increased from 1 in 100, 000 to 1–2 in 10,000 once genetic diagnosis became available [Abdalla and Letarte, 2006].

One of the *RASA1* associated fast-flow lesions is PKWS. The high percentage of patients with PKWS in the study might be biased, since we recruited specifically PKWS patients with or without multifocal CMs. The etiology of the segmental overgrowth syndromes has been hypothesized to be nongenetic or caused by postzygotic (somatic) mutation [Happle, 1987]. We showed that PKWS with multifocal CMs is a genetic disease due to de novo or inherited *RASA1* mutations. This revises the current risk evaluation in genetic counseling. Importantly, these patients are at risk for cardiac overload/failure and ischemia [Robertson, 1957]. One third of our PKWS patients required cardiac follow-up and one patient active treatment.

PKWS seems to be clinically and etiologically heterogeneous, as we did not identify any *RASA1* mutation in patients with PKWS without multifocal CMs. This distinction suggests future study of the differences in evolution, prognosis, incidence, and management. Moreover, they can now be better distinguished from Klippel-Trenaunay syndrome (KTS; MIM# 149000). One family reported to have KTS with autosomal dominant inheritance had, in fact, CM-AVM (Family 27 in this study) [Ceballos-Quintal et al., 1996].

CM-AVM patients are also at risk for intra- and extracranial AVMs and AVFs. Interestingly, two patients had vein of Galen aneurysmal malformations; thus, at least some of them are due to a genetic anomaly. This malformation has not been reported in association with other familial AVMs (HHT or *PTEN* hamartoma tumor syndrome), and might be specific to *RASA1* alterations. Since both of our patients harbored de novo germline mutations, a similar mechanism could be involved in other seemingly sporadic vascular anomalies. Overall, the number of intracranial lesions among CM-AVM individuals is likely underestimated, as we did not do a routine MRI scan. Alternatively, it is possible that the percentage of AVMs is biased, as patients with symptomatic fast-flow lesions seek medical care, whereas those with only multifocal CMs usually do not. Most of the intracranial AVMs/AVFs in CM-AVM were macrofistulous, causing symptoms at birth or before the age of 1 year. It has been shown that due to an immature hydrovenous system during infancy, AVMs can lead to rapid brain damage, and, thus, they require more prompt treatment than AVMs in older children or adults [Suh et al., 2001]. In contrast, in HHT, intracranial AVMs are usually diagnosed in adults, although some pediatric cases have been reported [Morgan et al., 2002]. A standardized radiological protocol for CM-AVM patients is needed. Meanwhile, the use of cerebral MRI as a screening tool in patients, especially in children, with multiple CMs is a reasonable precaution.

Presently, we are aware of three groups of familial AVMs: CM-AVM, HHT, and PTEN-related fast-flow vascular anomalies. CM-AVM most resembles HHT, which also includes multiple cutaneous small-vessel malformations, AVF, and AVM. Both conditions can involve the brain, but while HHT involves the liver and lungs, CM-AVM affects the limbs and the face. PTEN-related fast-flow vascular anomalies are usually intramuscular and multifocal, and associate with ectopic fat and severe disruption of the tissue architecture. Mesenchymal (often lipomatous) overgrowth can be seen both at the site of the fast-flow vessels and elsewhere [Tan et al., 2007]. AVMs in CM-AVM do not differ radiologically from nonsyndromic AVMs.

The molecular mechanisms by which germline heterozygous mutations in *RASA1* lead to the localized vascular phenotype are unknown. The mutations were distributed throughout the gene and most of them are predicted to be truncating, which suggests loss-of-function. Neither the type nor the position of the mutation correlated with the phenotype. The localized nature, multifocality, and intrafamilial variability suggest that a somatic "second-hit" might be necessary. This mechanism has been shown in two patients with other inherited multifocal vascular malformations: one with glomuvenous malformation and the other with cerebral cavernous malformation [Brouillard et al., 2002; Gault et al., 2005]. In addition, a second-hit was identified in several tissues in a patient with PTEN mutation [Zhou et al., 2000]. In 12 patients with indistinguishable phenotype we could not identify any *RASA1* alterations with our exon-based screening approach. This could be explained by the nonsensitivity of the technique used (DHPLC), gross rearrangement of the gene, or alterations in promoter, deeper in introns or in the regulatory regions. Another gene might also be involved.

The protein encoded by *RASA1*, p120RasGAP, is an inhibitor of RAS p21, which controls cellular growth, proliferation, survival, and differentiation. P120RasGAP also functions independently from Ras [Kulkarni et al., 2000; Yue et al., 2004]. Its importance for vascular development is underscored by the phenotype observed in *Rasa1*$^{-/-}$ murine embryos [Henkemeyer et al., 1995]. Interestingly, these mice did not demonstrate an increased incidence of tumors, as expected based on Ras activation in tumors, [Bos, 1989] and somatic *RASA1* mutations in basal cell carcinoma [Friedman et al., 1993]. Among our patients, the number of tumors was low, but the cellular types are intriguing. One was a basal cell carcinoma, and three were tumors of the nervous system: a neurofibroma and an optic glioma, known to be associated with mutations in *NF1*, a *RASA1* homolog, and a vestibular schwannoma, which is either isolated (annual incidence of about 1 in 60,000) [Tos et al., 2004], or associated with mutations in *NF2*. Although the cellular expression of *RASA1* has not been completely elucidated, these observations suggest some overlapping functions between the proteins encoded by *RASA1*, *NF1*, and *NF2*. Interestingly, *Nf1* haploinsufficiency augments angiogenesis, [Kolanczyk et al., 2007; Wu et al., 2006] and AVMs have been observed in patients with neurofibromatosis type I [Parkinson, 1999; Rodriguez-Jadraque et al., 2000; Westacott et al., 1988]. Thus, *RASA1* could be involved in the pathogenesis of some of the complications associated with *NF1* and *2*, and, vice-versa, NF proteins, as well as other RasGAPs, could play a role in CM-AVM pathogenesis.

In conclusion, we showed that *RASA1* is an important angiogenic gene frequently mutated in patients with pathognomonic multifocal pinkish-red CMs that are often associated with fast-flow vascular anomalies. We also demonstrated that PKWS and vein of Galen aneurysmal malformation, in association with multifocal CMs, are genetic disorders, caused by germline *RASA1* mutations. Moreover, CM-AVM patients are at risk of specific neural tumors reminiscent of neurofibromatosis type 1 and 2. In addition, the frequent occurrence of de novo *RASA1* mutations underscores the need for genetic screening in patients with sporadic vascular anomalies. Finally, this study pinpoints RAS p21 as a possible target for controlling fast-flow lesions in CM-AVM.

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