Cutaneous Venous Malformations in Familial Cerebral Cavernomatosis Caused by *KRIT1* Gene Mutations

Agustí Toll^a Elisabet Parera^a Ana M. Giménez-Arnau^a Alejandro Pou^b
Josep Lloreta^c Nisha Limaye^d Miikka Vikkula^d Ramon M. Pujol^a

Departments of ^aDermatology, ^bNeurology, and ^cPathology, Hospital del Mar, IMAS, Barcelona, Spain;

^dLaboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium

Key Words

Cutaneous venous malformation • Cerebral cavernomatosis • *KRIT1* gene mutation

Abstract

Background: Cerebral cavernous malformations (CCMs) are vascular lesions characterized by abnormally enlarged capillary cavities without intervening brain parenchyma. Although often asymptomatic, seizures, cerebral haemorrhages and focal neurological deficits are well-documented complications. Mutations in the *CCM1* (7q21–22), *CCM2* (7p13–15) and *CCM3* (3q25.2–27) genes have been identified in familial CCM. In rare instances, the association of congenital hyperkeratotic cutaneous capillary-venous malformations (HCCVMs) with *CCM1* has been reported. **Observations:** We studied 6 members of a family with CCMs. Four members of the family developed late-onset multiple, tiny, bluish, soft, cutaneous papules, mainly located on the face, arm and abdominal area, corresponding histologically to venous malformations. A splice donor site mutation in intron 4 (c. 1146 + 1 G→A) in the *CCM1* gene was identified. **Conclusions:** Our findings suggest that mutations in the *KRIT1* gene may cause phenotypically heterogeneous cutaneous vascular lesions other than those previously described as HCCVMs.

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Introduction

Cavernomas or cerebral cavernous malformations (CCMs) are fairly common (0.5%) vascular lesions involving the central nervous system (CNS) characterized by abnormally dilated capillary beds lined by a single layer of endothelium without intervening neural structures. The lesions may be solitary or multiple. Although often asymptomatic, 20–30% of individuals may experience symptoms including seizures, headaches, stroke or haemorrhage, making CCMs potentially fatal lesions.

Patients with CCM may develop vascular malformations in a wide range of anatomical areas other than the CNS, such as vertebrae [1], retina [2] and skin [1, 3, 4]. Both cerebral and extracerebral lesions show similar histological findings, consisting of malformed capillary and venous-like channels.

CCMs may occur either as a sporadic process or as a familial disorder with an autosomal dominant pattern of inheritance (10–20% of cases). Genetic linkage to 3 chromosomal loci, 3q25.2–27, 7p13–15 and 7q21–22, has been detected, and mutations in the *CCM1* gene (located on 7q21–22), which encodes a KRIT1 protein (acronym for the Krev Interaction Trapped 1 gene), as well as the *CCM2* (encoding MGC4607) and *CCM3* (encoding PDCD10)

Table 1. Clinical, pathological and radiological findings

Case/age/gender	Cutaneous manifestations	Histopathological samples	Neurological manifestations	Neuroradiological studies (MRI)	Outcome	Genetic studies
1/85/F	Five violaceous papules on malar areas	NA	None	Hypo-intense images distributed in both cerebral hemispheres, brain stem and cerebellum (aged 75)	Alive	Yes
2/80/F	Four violaceous papules on malar areas	NA	None	NA	Death (IC haemorrhage)	NA
3/54/M	Five violaceous papules on malar areas, 1 violaceous papule on the epigastria and 3 subcutaneous nodules on the left leg	Yes (cutaneous)	Locked-in syndrome (aged 32); right sixth-nerve palsy and contralateral body hyperaesthesia	Hypo-intense images with central hyperintensity in the left parietal region and right protuberance (aged 42)	Alive	Yes
4/49/M	Ten violaceous papules in malar areas and 1 on the arm	Yes (cutaneous)	No symptoms	Hypo-intense images with central hyperintensity located in the subcortical right parieto-occipital, left thalamic and right protuberance areas (aged 49)	Death (IC haemorrhage)	NA
5/37/M	NA	Yes (cerebral)	NA	NA	Death (IC haemorrhage)	NA
6/53/F	None	NA	None	Normal cerebral CT	Alive	Yes

IC = Intracerebral; NA = not available.

genes have been identified, including frameshift, nonsense and missense mutations, as well as splice junction mutations [5–7]. These proteins seem to be involved in both angiogenesis and vascular remodelling [8].

The development of hyperkeratotic cutaneous capillary-venous malformations (HCCVMs; MIM 116860) has been reported in families harbouring *KRIT1* mutations [3, 4].

We describe a family whose members showed coexisting CCM and cutaneous venous malformations. We identified a mutation in the *CCM1* gene in 3 members of this family. These findings suggest that mutations in the *KRIT1* gene may cause phenotypically heterogeneous cutaneous vascular lesions, differing from those previously described as HCCVMs.

Material and Methods

Patients

A kindred with CCMs and cutaneous vascular malformations segregating over 3 generations in an autosomal dominant manner was evaluated (fig. 1). Clinical, neuroradiological (MRI and gradient echo sequences) and/or pathological (biopsies from cutaneous and cerebral tissue) features were available for 6 members. A thorough cutaneous and mucosal examination for vascular

Fig. 1. Pedigree of the present family. Filled symbols = Cutaneous and cerebral lesions; open symbols = not known to be affected; asterisk = patients showing the mutation in *KRIT1*; open circle = no genetic studies available; slash = deceased.

lesions was performed in 5 members of the family (cases 1–4 and 6).

A signed informed consent was obtained from those wanting to participate in the genetic study, as approved by the ethics committee of the Medical Faculty of the Université catholique de Louvain, Brussels, Belgium. Blood samples were collected for individuals 1, 3 and 6. Genomic DNA was extracted from buffy coats using the Qiagen DNA purification kit (Westburg, The Netherlands).

Fig. 2. Patients 3 and 4 showing multiple, tiny, bluish papules in the left malar areas.

Mutation Analysis of the KRIT1 Gene

All 20 transcribed exons of the *KRIT1* gene, including exon-intron boundaries, were amplified by PCR using genomic DNA as template. Primer sequences and cycling conditions were performed as previously reported [3, 9]. Amplified products were screened by denaturing high-performance liquid chromatography using the Wave 3500 HT system (Transgenomic Inc., Omaha, Nebr., USA). The fragments showing abnormal migration patterns were further characterized by direct cycle sequencing, using a Beckam CEQ2000 capillary sequencer (Analisis, Belgium).

Results

The results are summarized in table 1. Five individuals presented cutaneous and/or neurological manifestations. Cases 1–4 showed cutaneous vascular lesions. Two individuals with cutaneous involvement also manifested concurrent cerebral complications (cases 3 and 4). One patient (case 5) had a fatal outcome due to intracerebral haemorrhage in 1985, and no dermatological exploration could be performed.

Cutaneous Clinical Manifestations

Cutaneous physical examination revealed multiple, tiny, bluish, easily depressible papules in the malar area in 4 patients (cases 1, 2, 3 and 4). Four to 10 bluish bilateral papules measuring 2–3 mm were noted in these areas (fig. 2). Patients 3 and 4 showed similar individual lesions in the upper abdominal region and on the arm, respectively. Patient 4 also had a bluish papule measur-

Fig. 3. MRI examination from patient 1 showing cerebral hypointense images corresponding to cavernomas.

ing 5 mm in diameter on the lower lip suggestive of venous-lake angioma (fig. 2b). The diagnosis of blue rubber bleb naevus syndrome (BRBNS) was clinically suspected. These lesions were strikingly subtle, and the patients had not sought medical advice for them. Patient 3 also complained of 3 asymptomatic subcutaneous nodules measuring 1.5 cm on the left leg. Patient 6 had not developed cutaneous manifestations at the age of 60.

Fig. 4. **a** Histology of a facial lesion from patient 4 showing a single large dilated vascular channel with a flat endothelial lining in the upper dermis. Haematoxylin-eosin stain. $\times 40$. **b** Histology of a vascular lesion on the arm from patient 4. Haematoxylin-eosin stain. $\times 40$. **c** Histology of a post-mortem brain sample (patient 5) showing a juxtaposition of dilated vascular structures lined by a single layer of endothelial cells and collagen. Haematoxylin-eosin stain. $\times 100$.

Neurological Clinical Manifestations

Patient 3 had suffered a subacute bleeding in the protuberance that resulted in a long-term locked-in syndrome at the age of 32 that progressively improved in the following years. At present, this patient suffers from a right sixth-nerve palsy and a contralateral hypo-aesthesia. Patient 4 died at the age of 49 due to progressive bleeding from a brain cavernoma. Patient 5 died in 1985 at the age of 37 due to postsurgical complications of a growing cavernoma of the left cerebral hemisphere.

Neuroradiological Studies

Three patients (patients 1, 3 and 4) underwent axial T₂-weighted gradient echo sequence MRI examinations. These studies revealed multiple hypo-intense images,

suggestive of cavernous angiomas in all patients (fig. 3). The lesions were located in both cerebral hemispheres, the cerebellum or the thalamic and/or protuberance region (table 1).

Histopathological Findings

Skin biopsies of 2 papules on the face (patients 3 and 4) and on the arm (patient 4) were performed. Two different histological patterns were observed (fig. 4). The facial lesions showed a single large dilated vascular channel with a flat endothelial lining in the upper dermis consistent with a venous lake (fig. 4a). The lesion on the arm also had a deeper component, with dilated vascular structures affecting the subcutaneous fat (fig. 4b), and closely resembled the changes observed in a cerebral tis-

sue sample (fig. 4c). The deep lesion on the leg from patient 3 was not biopsied. Immunohistochemical studies showed positive staining for CD34 and smooth-muscle actin, but negativity for elastic fibres, desmin and CD23 stainings. These findings were consistent with venous malformations.

A post-mortem brain sample from a cerebral lesion (patient 5) showed a juxtaposition of dilated vascular structures lined by a single layer of endothelial cells and collagen without intervening brain parenchyma (fig. 4c).

Genetic Findings

Mutational screening of DNA of individuals 1, 3 and 6 resulted in the identification of a heterozygous nucleotide change in the intron four 5' donor splice site sequence (c. 1146 + 1 G→A) that disrupts the invariant splice donor site from *gt* to *at* in all of them. Allele-specific PCR screening confirmed the sequencing results for all patients. Consensus splice site substitution is commonly related to exonic splicing defects, but as no lymphoblasts were available, this could not be tested.

Discussion

Isolated reports of 'cherry angiomas' [10], 'cutaneous bluish nodules' [11], capillary vascular anomalies [12], cavernous 'angiomas' [13] and angiokeratoma-like lesions [14] have been reported in patients with multiple CCM or belonging to families with multiple cavernomas. However, histological data were only rarely available [14], and genetic studies have rarely been performed in patients presenting this association.

Isolated observations of *CCM1* gene mutations in patients with CCMs and cutaneous lesions have been reported [1, 3]. These cutaneous lesions share similar clinical and histological features and have been termed HCCVMs [15]. HCCVM lesions are described as congenital crimson-coloured or red-to-purple, irregularly shaped macules, plaques or patches, which can extend to several centimetres. Crimson-coloured tiny papules (3–10 mm) with a hyperkeratotic epidermis have also been reported [15, 16]. They may appear on the limbs, buttocks and forearms [1, 3, 15].

Labauge et al. [15] studied 4 families (10 individuals) with CCMs who showed congenital, single lesions localized on the lower limbs in 9 patients and on the arm in 1. These lesions were crimson-coloured tiny papules (3–10 mm) or larger macules (20–65 mm) with a hyper-

keratotic epidermis. The histology, available in 4 patients, revealed orthokeratosis and hyperkeratosis, abundant dilated capillaries, and blood-filled spaces in papillary and reticular dermis extending to the hypodermis.

Eerola et al. [3] demonstrated mutations in the *KRIT1* gene in 2 patients with HCCVM. They found a deletion of G in the first exon of the *KRIT1* gene (*KRIT1*Δ^{G103}). No mutations in exon 1 had been previously described in patients with CCMs so that they hypothesized that an earlier truncation of the *KRIT1* gene could have triggered the cutaneous manifestations.

In 2002, Chen et al. [17] reported a family of Chinese origin with CCM and a mutation in exon 19 in the *CCM1* gene. A male member of this family developed cutaneous lesions on both forearms and right cheek at the age of 67. These vascular lesions were purple-black, round, raised, non-tender, 2–10 mm in diameter, resembling our patients' lesions. However, the patient also showed a flat reddish discolouration surrounding the angioma-like lesions. A skin biopsy was not performed. Four other members of the family developed cerebral manifestations without skin lesions.

Clatterbuck et al. [1] reported a 53-year-old woman with CCMs and a red-to-purple large patch and nodules ranging in size from 2 to 4 mm prior to treatment with laser. Microscopic examination showed multiple, endothelium-lined vascular channels in the dermis and hypodermis. Clatterbuck et al. described a tandem duplication 1487–1497 in exon 4 of *KRIT1* in this patient.

In contrast to HCCVMs, our patients presented acquired, multiple, tiny, non-confluent bluish papules, mainly located on the face, and clinically suggesting the diagnosis of venous lakes or BRBNS. No signs or symptoms of gastro-intestinal involvement were present. As far as we are concerned, venous malformations clinically suggesting BRBNS or venous lakes secondary to *KRIT1* mutations have not been reported previously.

Taking into account the similarities between the lesions observed in our patients and those observed in BRBNS, a possible relationship between both disorders may be suggested. Interestingly, sporadic BRBNS patients developing symptomatic cerebral and/or spinal vascular lesions have occasionally been described [18–20]. Although some authors have suggested that BRBNS and the familial venous malformation syndrome, caused by mutation in the *TIE* gene (9p21; MIM 600221), are the same condition, the underlying genetic mechanism responsible for BRBNS is not established (MIM 600195) [21]. A possible role of *KRIT1* gene mutations could be hypoth-

esized. In a recent report, Chen et al. [17] ruled out *CCM1* mutations in an 80-year-old man with multiple bilateral CNS cavernous angiomas and multiple, small, bluish, soft, non-tender skin lumps consistent with the BRBNS [20].

Loss of KRIT1 protein function due to gene mutations could be explained by haplo-insufficiency or a two-hit model. The clinical phenotype of multiple lesions in familial cases and single lesions in sporadic cases, the preponderance of nonsense mutations and the structure of the protein suggest that CCMs may be caused by somatic mutations of the remaining functional gene (two-hit model) in familial cases in a tumour-suppressor-like mechanism. Some patients with CCMs harbour segmental manifestations, which would also support a two-hit model [1], as already observed for glomuvenous malformation, another cutaneous vascular anomaly [22]. In our opinion, the observation of patients who progressively develop skin lesions during adulthood would also be consistent with loss of function of *KRIT1* and a two-hit model of the disease.

The single base pair transition at the exon-intron border in intron 4 (previous nomenclature: IVS4 + 1G→A; now: c. 1146 + 1 G→A) was previously reported by Sahoo et al. [23] in a study that allowed the identification of *KRIT1* as the *CCM1* gene. This mutation was found in a non-Hispanic Caucasian family showing CCM lesions. Coincident with patient 6 from our study, no cutaneous

lesions were reported in any of the families included in the study by Sahoo et al. These findings underline the heterogeneous clinical manifestations associated with *KRIT1* mutations and would also support the hypothesis of a second-hit model.

In conclusion, *KRIT1* gene mutations may cause heterogeneous vascular skin lesions including non-hyperkeratotic acquired lesions. CCM should be suspected in patients with a familial history of neurological anomalies who also develop multiple, tiny, cutaneous venous lesions. An early diagnosis of familial cases with this condition is of great importance as it may cause severe CNS manifestations that may be amenable to surgical correction.

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