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

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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, seizures, cerebral haemorrhages and focal neurological deficits are well-documented complications. Mutations in the \textit{CCM1} (7q21–22), \textit{CCM2} (7p13–15) and \textit{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 \textit{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\rightarrow A) in the \textit{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.

Key Words
Cutaneous venous malformation \cdot Cerebral cavernomatosis \cdot KRIT1 gene mutation

Abstract

\textbf{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 \textit{CCM1} (7q21–22), \textit{CCM2} (7p13–15) and \textit{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 \textit{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\rightarrow A) in the \textit{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.
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 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).

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

IC = Intracerebral; NA = not available.

**Table 1. Clinical, pathological and radiological findings**

**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.
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 (Analis, 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 measuring 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.
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 T<sub>2</sub>-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. 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 (KRIT1ΔG103). 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 discoulouration 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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**References**


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