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Cutaneous Venous Malformations Related to *KRIT1* Mutation: Case Report and Literature Review

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Abstract Cavernous malformations (CMs) are vascular anomalies of the nervous system mostly located in the brain. Cerebral cavernous malformations can present sporadically or familial, as a consequence of an autosomal dominant condition, with incomplete penetrance and variable clinical expression. Occasionally, extraneural manifestations of CMs involving the skin have been described. We report the case of two siblings presenting in adulthood diffuse cutaneous vascular lesions associated with cerebral CMs that, after surgical excision and histopathologic analysis, resulted to cavernous haemangiomas. Genomic DNA was extracted from peripheral blood, and molecular evaluation of *KRIT1* gene was performed. Although no signs of neurological impairment were reported, cerebral MRI revealed multiple images in both patients, suggestive of cavernous haemangiomas. The genetic study demonstrated a nonsense mutation (c.535C>T) in the *KRIT1* (Krev-1/rap1 interaction trapped 1) gene. Few reports describe extraneural manifestations of Cavernous malformation syndrome (CMs) related to a *KRIT1* mutation; these involve the skin and are associated with hyperkeratotic cutaneous capillary–venous malformation. CMs should be suspected in patients developing multiple nodular cutaneous venous lesions in adulthood.

Keywords Vascular lesions · *KRIT1* gene mutation · Anti smooth muscle antibody · Skin nodules · Surgical excision

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Introduction

Cavernous malformations (CMs) are vascular disorders characterized by sinusoidal, dilated vascular cavities lined with a layer of endothelium and absence of intervening neural tissue. When the nervous system is involved, the brain is the most frequent localization, followed by the leptomeninges, spinal cord and retina. CMs occur in 0.4–0.8 % of the population (Ebrahimi et al. 2009). Clinical symptoms of cerebral CMs (CCMs) are headache, seizure, haemorrhage and focal neurological deficit (Labauge et al. 1998). CCMs may occur as sporadic or familial disorder (Labauge et al. 2007). Familial CCM (FCCM) is more frequent in the Hispanic-American population (50 %) than in the Caucasian (10–40 %) (Rigamonti et al. 1988; Labauge et al. 2007). FCCM is inherited in an autosomal dominant manner with incomplete clinical/neuroradiological penetrance and variable expressivity, both intrafamilial and interfamilial (Labauge et al. 1998; Ng et al. 2006). Mean age of disease onset is reported to be 30 years, but first symptoms can present in early infancy or in older age. Three CCM genes have been identified: *CCM1/KRIT1* on chromosome 7q21-22, *CCM2/MGC4607* on 7p13–15 and *CCM3/PDCD10* on 3q25.2–27. Almost 80 % of FCCM patients harbour a

heterozygous germline mutation in one of the three genes. More than 150 distinct *CCM1/CCM2/CCM3* mutations have been published to date (Sahoo et al. 1999; Liquori et al. 2006; Ortiz et al. 2007). Most mutations identified in the FCCM patients led to a premature termination codon through different mechanisms including nonsense, splice-site and frameshift mutational events, as well as large genomic rearrangements. These data suggest that loss of function is the pathophysiologic mechanism causing the disease (Lan et al. 2010). *KRIT1* is the gene most frequently involved in the FCCM and accounts for 40 % of the cases (Cau et al. 2009). *KRIT1* contains 16 exons and encodes for a 736-amino acid protein probably involved in signalling pathways for stabilization of cellular junction and normal angiogenesis (Lan et al. 2010).

Extraneural expressions of CMs, including skin involvement, have been described in familial cases of cerebral CMs. Cutaneous vascular malformation frequency in FCCM patients is unknown, although it has been reported in 9 % of patients, mostly mutated in the *KRIT1* gene (Sirvente et al. 2009).

Hyperkeratotic cutaneous capillary–venous malformations (HCCVMs), considered pathognomonic for FCCM, have been associated with *KRIT1* mutations (Labauge et al. 1999; Eerola et al. 2000) and described as bluish nodules, cherry angiomas, port-wine stains and angiokeratomas (Wood et al. 1957; Bartolomei et al. 1992; Wakabayashi et al. 2000).

In this paper, we report two siblings presenting with cutaneous and cerebral CMs without neurological symptoms and with a nonsense *KRIT1* mutation.

Case Report

We observed two siblings, respectively 63 and 64 years old, whose diagnosis of CMs was based on brain magnetic resonance imaging features, histopathologic analysis and genetic study.

A 63-year-old female (II:3) was admitted to the plastic surgery ward, complaining a long history of multiple, painful, slowly growing, irregularly shaped, varied in sizes, bluish purple easily compressible lumps progressively appearing in adulthood on the upper limbs (Fig. 1a), lower limbs and abdomen. Ultrasound evaluation showed several subcutaneous oval-shaped hyperechogenic lesions measuring 24 mm on the abdomen, 19.4 and 6 mm on the right arm and 14 mm on the left leg.

Patient medical history was significant for cerebral vascular lesions diagnosed serendipitously after a MRI exam performed, because of a cerebral contusion, 1 year before skin lesion referral. Cerebral MRI was suggestive of cavernous haemangiomas which are unknown until that moment, in the absence of neurological symptoms, mostly located in the left cerebellar vermis area (3 mm in diameter), with minor lesions in the right ponto-cerebellar area, left frontal and right parietal lobes. The biggest lesion of 1 cm in diameter was located at the left ponto-medullary (bulbo-pontine) junction.

The second patient (II:2), a male aged 64 years, presented one irregularly shaped, non-tender, easily compressible, bluish purple lump on the left elbow. Cerebral MRI was taken revealing multiple images suggestive of asymptomatic cavernous haemangiomas, as those presented by his sister. Vascular lesions were mostly located in the left cerebellar hemisphere with a diameter of 3.5 mm, in the left frontal and right parietal lobes with a diameter of 3 mm and in the left temporal–occipital lobes with a diameter of 5 mm.

Both patients received surgical treatment: in proband II:3, two lumps from the right arm and one from the left leg were removed; in patient II:2, one lesion was excised from the right arm. All specimens were sent to the pathologist for

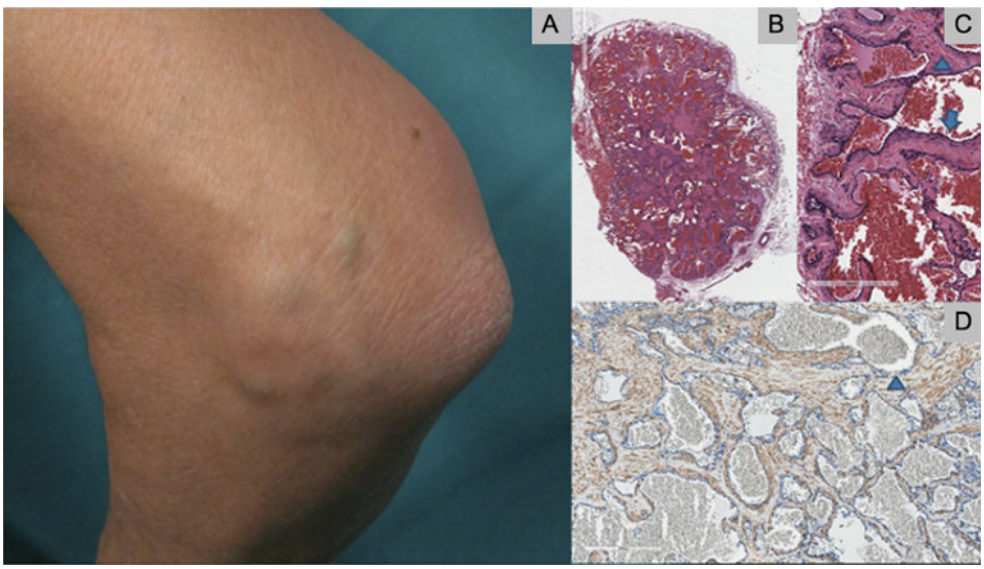

Fig. 1 **a** Left elbow of the 63-year-old female patient, showing irregularly shaped, painful, easily compressible lumps. **b** Well-circumscribed lesion (haematoxylin and eosin, magnification ×20). **c** Magnification of the previous figure (haematoxylin and eosin, magnification ×100), showing dilated vascular channel with a flat endothelial lining (*lower arrow*) and thick vascular walls (*arrowhead*). **d** Immunohistochemical stain identifies muscle fibres partially surrounding vascular structures (*arrowhead*) (anti-smooth muscle actin stain, magnification ×100)

examination and were formalin-fixed, paraffin-embedded and stained with haematoxylin and eosin.

All specimens showed the same histological pattern: a well circumscribed deep dermis lesion, with dilated, interconnected, thick-walled blood vessels lined by a single layer of non-atypical flat endothelial cells with very large vascular lumens (Fig. 1b, c).

Immunohistochemistry was performed on 4-μm-thick sections prepared from formalin-fixed, paraffin-embedded tissue using an automated immunostainer (Autostainer Link 48, Dako) and stained with an anti-smooth muscle actin antibody. The immunohistochemical stain identified muscle fibres around the vascular walls partially surrounding the vascular lumens (Fig. 1d). These findings were consistent with cavernous haemangiomas (venous malformation, nodular type).

Genetic Study

After written informed consent acquisition, genomic DNA was extracted from peripheral blood lymphocytes of patients II:2 and II:3. DNA samples from the other family members were not available, as they were not accessible for clinical and molecular evaluation. All the exons and splice junctions of the *KRIT1* gene were amplified using the primers designed by Cavé-Riant et al. (2002). Purified PCR products were sequenced in the forward and reverse directions using the BigDye Terminator chemistry and an ABI Prism 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA). The proband (II:3) and her brother II:2 were heterozygous for the nonsense mutation c.535C>T located in exon 8 of the *KRIT1* gene. Mutation c.535C>T leads to a premature stop codon (p.R179X), as the triplet for the arginine (R) is changed in a non-coding triplet.

Discussion

When family history is not available, some clinical features may help to identify familial cerebral CMs. Familial cases are characterized by a high prevalence of multiple lesions, while sporadic cases often have only one lesion (Rigamonti et al. 1988). Furthermore, extraneural involvement, as cutaneous localization, is more common in the familial form of cerebral CMs. Few reports of associations between CMs and cutaneous lesions exist, the last described as heterogeneous: bluish nodules (Bartolomei et al. 1992), cherry angiomas (Gass et al. 1971) or capillary vascular anomalies (Goldberg et al. 1979). Toll et al. (2009) suggested that mutations in the *KRIT1* gene may cause phenotypically heterogeneous cutaneous vascular lesions, other than those previously described as HCCVMs. Sirvente et al. (2009) reported in their series three distinct major cutaneous vascular phenotypes: HCC VMs (39 %), capillary malformations (34 %) and venous malformations (21 %), with *KRIT1* most frequently mutated when HCCVMs occur.

KRIT1 nonsense mutation c.535C>T, identified in heterozygosity in our patients, introduces a premature stop codon (p.R179X), giving rise to protein truncation. This alteration, known as pathogenic, has been previously described in two other unrelated patients (Cavé-Riant 2002); no data on their cutaneous manifestations were reported by the authors, suggesting a normal cutaneous phenotype. In patients II:2 and II:3, *KRIT1* nonsense c.535C>T mutation was associated with nodular venous malformations, thus confirming that mutations of *KRIT1* bring cerebral vascular anomalies. A large spectrum of different clinical manifestations even with the same mutation are described.

Neurological clinical manifestations are very often reported as associated to the CMs (Bacigaluppi et al. 2012). Nevertheless, our reported patients did not present any clinical neurological symptoms, although cerebral MRI showed neurological anomalies in both. Pain and discomfort were associated to the cutaneous lesions in the younger sibling only.

The cutaneous malformations observed in our patients suggest that CCM should be suspected in patients who develop multiple nodular cutaneous venous lesions in adulthood, even if there is no evidence of neurological symptoms. Skin or subcutaneous finding in those patients could hide a much more complex and severe clinical status, involving cerebral vascular anomalies. In these cases, an early diagnosis performed through genetic analysis is of great importance, because it provides an appropriate clinical surveillance to the CCM family.

Conflict of Interest All the authors disclose any sponsorship of funding arrangement related to this study and declare that there are no conflicts of interest.

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