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The frameshift Leu220Phefs*2 variant in *KRIT1* accounts for early acute bleeding in patients affected by cerebral cavernous malformation

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ABSTRACT

Background and Objectives: Cerebral cavernous malformation (CCM) is a neurovascular disease characterized by abnormally expanded and tortuous microvessels with increased predisposition to thrombosis and focal hemorrhage. Its incidence is estimated to range between 0.4% and 0.8%. Sporadic and familial forms of CCM are described. The first one is characterized by single lesion, while the familial form is defined by multiple malformations. In this scenario, more than 300 mutations affecting the CCM genes have been described to date, but the exact pathogenic mechanism is yet unknown. Most of the causative variants of KRIT1 gene are frameshift but there are many missense and nonsense variants and they have been found some splicing mutations. The diagnosis is based on magnetic resonance images (MRI) and genetic testing.

Case report: A 15-year-old male presented with a two weeks duration worsening headache accompanied by vomiting and three months behavioral changes. Computer tomography revealed a large right temporal lesion with other smaller in left parietal and left cerebellar region. At the time of diagnosis, the two siblings of the proband were asymptomatic. Nevertheless, four months later, the 7-years-old brother was admitted to the emergency room for balance deficit, diplopia, right-hitting nystagmus and stiff neck with deviation of the head. A cerebral CT revealed polylobate hyperdense mass of the middle cerebral pedicle associated to acute bleeding. A genetic testing for hereditary cavernous brain malformation was carried out.

Results: The molecular analysis identified a 2-bp duplication (NM_194456.1:c.658_659dupTT) as heterozygous within the exon 8 of *CCM1/KRIT1* gene (Fig. 1C). This duplication leads to a frameshift variant, resulting in a premature stop codon (p.Leu220Phefs*2).

Discussion: The clinical data collected confirm the variable phenotypic expression of CCM and suggest a greater severity of symptoms in the youngest patients.

1. Introduction

Cerebral cavernous malformation (CCM, # 116860) is a neurovascular disease characterized by abnormally expanded and tortuous microvessels covered by a single layer of endothelial cells with increased permeability, and predisposition to thrombosis and focal hemorrhage. Its incidence is estimated to range between 0.4% and 0.8% in the general population [1]. There are sporadic and familial forms of CCM. The first one usually is characterized by only one lesion, even if sporadic cases with multiple malformations were described. Instead, the familial form is defined by multiple malformations, or one CCM associated with at least one other affected family member and/or a pathogenic variant in three genes, i.e. *CCM1/KRIT1*, *CCM2/Malcavernin*, and *CCM3/PDCD10* [2]. Heterozygous germline loss-of-function mutations in one of these genes cause alterations in the signaling pathway that regulates cell proliferation, network formation, and growth in the endothelial layer,

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with the formation of abnormally dilated capillary cavities [3]. More than 300 mutations affecting the CCM genes have been described to date. Recent transcriptome analysis of CCM-derived endothelial cells (ECs) allowed to focus on impaired physiological processes related to angiogenesis, extracellular matrix (ECM) signal-ling, neuro-inflammation and reactive oxygen species (ROS) metabolism. Taken together, these results suggest novel mechanisms involved in CCM development, interpreted as EC response to damaging stimuli [4].

The diagnosis is clinical and neuroradiological. Magnetic resonance images (MRI) define the typical "popcorn appearance" of the lesion with the T2*/GRE and SWI/VenBold [5].

Genetic testing and counseling play a key role in the management of CCM. Patients with CCM may have multiple cerebral cavernomas and similar vascular changes in skin, lung, and cardiovascular system. Cerebral cavernomas may increase in number and size with age, be asymptomatic or associated with intracerebral hemorrhage, headache, epilepsy, and/or behavioral changes. The number and the site of lesions seem to be correlated to the risk of acute bleeding [6].

The decision of surgical treatment and the timing depends on the neurological status of the patient, the multiplicity and the location of the lesions, and the surgical experience of the treating team. Surgery is usually reserved for symptomatic, easily accessible CCM patients with a high risk of bleeding and low surgical-related mortality.

In this article, we describe a case of CCM *KRIT1*-related characterized by precocious acute bleeding.

2. Case report

A 15-year-old male from non-consanguineous parents (Fig. 1A) presented with a two weeks duration worsening headache accompanied by vomiting and three months behavioral changes. Computer tomography (CT) revealed a large right temporal lesion with other smaller in left parietal and left cerebellar region.

General and neurological examinations were unremarkable except for an ataxic gait and a bilateral superior temporal quadrantopsia at manual visual field testing. Subsequent MRI was obtained with and without contrast, integrated with tractography. All lesions exhibited significant signal dropout on gradient-echo sequences. Heterogeneous enhancement with signals of recent bleeding was noted in the right temporal mass (Fig. 1B).

The patient underwent a temporal craniotomy and the right temporal lesion was resected. The lesion demonstrated hemosiderin deposition. Few hours after surgery, the patient reported an improvement of the bilateral quadrantopsia. Histopathological examination confirmed the diagnosis of cavernous malformation. The postoperative course was uneventful, and he was discharged on postoperative day three with the resolution of ataxic gait and vomiting.

Genetic counseling revealed a paternal family history of vascular malformations (Fig. 1A). Indeed, the proband's father was operated at the age of 49 years for multiple malformations, and the following year, he presented with brain bleeding. Furthermore, a paternal uncle and a paternal aunt presented CMM-related symptoms (headache and cerebral hemorrhage respectively) and cavernomas seen on MRI. Therefore, a clinical diagnosis of a familial form of CMM was hypothesized.

At the time of diagnosis, the two siblings of the proband were asymptomatic. Nevertheless, four months later, the 7-years-old brother was admitted to the emergency room for balance deficit, diplopia, right-hitting nystagmus, and stiff neck with deviation of the head. A cerebral CT revealed polylobate hyperdense mass of the middle cerebral pedicle associated with acute bleeding. MRI confirmed the presence of multiple cavernomas. He underwent surgical treatment of the cavernoma malformation with interstitial laser. He was discharged 8 days after the procedure with the disappearance of the stiff neck, improvement of the balance deficit, and persistence of the diplopia.

In agreement with the clinical features and the imaging findings, the proband and his father were suspected to suffer from CCM. Accordingly, genetic testing for hereditary cavernous brain malformation was carried out.

DNA samples were obtained after signed informed consent of the parents and according to the Declaration of Helsinki. The local University Ethical Committees approved the DNA sampling and the collection of patient samples ('Federico II' University of Naples). Genomic DNA preparation and validation of the variant after targeted next-generation sequencing (t-NGS) were performed as previously described [7]. Genetic testing has been performed by a t-NGS analysis of a multigene panel composed of greater than 5000 genes associated with Mendelian diseases [SureSelect custom Constitutional Panel 17 Mb]. Sample preparation was performed using target enrichment

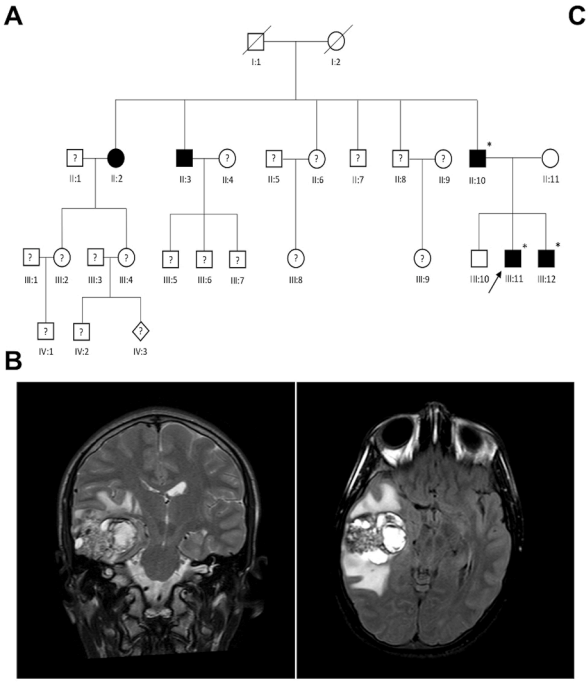

Fig. 1. A. Genetic pedigree of the family. Squares, males; circle, female; solid symbols, affected person; arrow, index case; empty circles and squares indicate unaffected female and male (absence of neurological signs and symptoms and cavernomas seen on MRI); rhombus indicate unknown sex; question mark inside circles and squares indicate individuals who have not performed MR and are asymptomatic; diagonal line indicates deceased individuals; asterisk indicates mutation in the *KRIT1* gene. B. Coronal and axial MRI T2*weighted sequence from proband. C. Upper panel. Visual inspection of the alignment track of next-generation sequencing analysis of the proband showing presence of the c.658_659dupTT variant as heterozygous, as obtained by Alissa Align and Call software. Bottom panel. The electropherogram, as obtained by direct sequencing analysis of the exon region encompassing the duplication, confirms the duplication identified by NGS.

(SureSelectQXT) for the Illumina platform (SureSelect Custom Tier1 1–499 kb; Agilent Technologies), according to the manufacturer's instructions. High-throughput sequencing was performed using an Illumina NextSeq 500 platform. The alignment of sequencing reads to the genomic locations, quality control metrics, and identification of variants were achieved using the Alissa Align and Call software (v1.1.2–2; Agilent Technologies). Variant annotation and analysis were performed using the Alissa Interpret software (v5.2.6; Agilent Technologies). As previously described and according to the guidelines of the American College of Medical Genetics and Genomics (ACMG), the pathogenicity of each variant was evaluated by gathering evidence from various sources: population data, computational and predictive data, functional data, and segregation data [7].

The molecular analysis identified a 2-bp duplication (NM_194456.1: c.658_659dupTT) as heterozygous within the exon 8 of the *CCM1/KRIT1* gene (Fig. 1C). This duplication leads to a frameshift variant, resulting in a premature stop codon (p.Leu220Phefs*2). It has been already described as a disease-causative variant of CCM (HGMD CI073755), and it is predicted as pathogenic according to the ACMG guidelines. The data obtained by t-NGS were confirmed by Sanger sequencing (Fig. 1C).

According to the dominant inheritance pattern, we also confirmed the t-NGS data by Sanger sequencing analysis on DNA from both the father (II.10) and the 7-years-old brother (III.12) of the proband (Fig. 1A).

3. Discussion

The *KRIT1* gene mutations (locus CCM1) are responsible for about 60% of familial forms of CMMs [8]. *KRIT1* is located on 7q21.2 and encodes a protein of 736 amino acids. The encoded protein is expressed in vascular endothelium, pyramidal cells, and astrocytes. It is involved in mechanisms of endothelial cell–cell and cell-ECM interactions since it binds to the ICAP1α protein but the same binding site on ICAP1α is competed by KRIT1 and β1 integrin, suggesting a possible regulatory mechanism. In addition, this interaction is dependent on the phosphotyrosine-binding domain of CCM2. The products of the *KRIT1* and *CCM2* genes can activate kinase MEKK3 and downstream p38 kinase pathway, playing an essential role in endothelial cell survival and angiogenesis [9]. Mutations of *KRIT1* result in an impairment of important signaling pathways and contribute to the formation of cavernomas although the precise pathogenic mechanism is not yet known.

The detection of mutations in *KRIT1/CCM2/PDCD10* is sufficient to make a diagnosis of FCCM. Alternatively, or in addition, the diagnosis of FCCM is given by the presence of multiple CCMs or one CCM in a patient and one or more lesions in at least one kindred [10].

Most of the causative variants of the *KRIT1* gene are frameshift but there are many missense and nonsense variants and they have been found some splicing mutations.

In our case, we investigated a frameshift variant that introduced a premature stop codon. The variant found in the proband's DNA has been previously described in an Italian family with CCM. In this family, DNA from six individuals was analyzed and the *KRIT1* c.658_659dupTT was detected. Among these six subjects, one had neither neurological signs and symptoms nor cavernomas seen on MRI, four were asymptomatic but presented cavernomas seen on MRI brain images and one presented both neurologic symptoms and cavernomas seen on MRI [11]. About penetrance (percentage of individuals with neurologic signs and symptoms and cavernomas seen on MRI in mutation carriers), this study indicates an incomplete penetrance of clinical features associated with CCM (16%) and an almost full neuroradiological penetrance of cavernomas seen in MRI (83%). Within the family herein described, five individuals exhibited vascular lesions at MR imaging and clinical signs while other family members didn't perform brain MR. Three of them performed genetic analysis and resulted in carriers of the mutation. Therefore, our case suggests a full clinical and neuroradiologic penetrance of the disease, although the variable expression of this condition. Indeed, about clinical data, one patient (paternal uncle) suffered from headache, three (index patient's father, younger brother, and paternal aunt) had a cerebral hemorrhage and two of them need surgical treatment; the index patient and his father suffered from recurrent headache and short temper, the father also presented dizziness associated with nausea and vomiting. The cerebral hemorrhage occurs in two young individuals (7 e 15 years old respectively) and one person of adult age (49 years old). This finding suggests an inverse correlation between onset age and severity of symptoms and confirms the results of previous studies that suggest an increased risk of bleeding in younger patients than adults although in our case the brain bleeding also occurred in an adult person. [12–14]

In addition, these works showed that the main clinical sign at the onset was the brain bleeding in *CCM3* mutation carriers and the seizure in *CCM1/CCM2* mutation carriers [11]. This finding confirmed the result of a previous work that had shown that cerebral hemorrhage is the second most common initial clinical feature in the *KRIT1/CCM2* carrier mutations after the seizure. In our case, the *KRIT1* mutation carriers didn't show seizure but they confirmed the variable expression of the CCM (intrafamilial as well as interfamilial) and the important role of the brain bleeding as a clinical feature at the onset of the disease.

In any case, a careful follow-up will allow us to evaluate and compare the prognosis of younger and older patients. Moreover, all members of the family should be examined to assess the clinical and neuroradiologic penetrance of the disease and to know the patients at risk to ensure an adequate follow-up and estimate of the risk of recurrence (50% for the offspring of the mutation carriers, independently from the sex of the unborn child). When the mutation is known, it is possible to perform neurosurgical treatment and MRI follow-up. Moreover, it is important to perform prenatal genetic counseling to evaluate appropriate treatment for pregnant women suffering from CCM and to research familial mutation through invasive prenatal diagnosis.

4. Conclusion

In this report, we have described the case of a family in which five individuals exhibited vascular lesions at MRI, and three of them performed molecular testing. The clinical data collected confirm the variable phenotypic expression of CCM and suggest a greater severity of symptoms in the youngest patients. The exact pathogenic mechanism is under investigation but the neuroradiological diagnosis of this pathology is fundamental for an adequate treatment. Molecular testing is important to detect the patients at risk and to recommend the appropriate follow-up. More than 300 mutations are known in *KRIT1/CCM2/PDCD10* but they don't seem to be the only genes involved in familial forms. Further studies allow to improve early diagnosis and provide an adequate risk of recurrence to pregnant couples in which one of the two partners is affected.

CRediT authorship contribution statement

Autilia Tommasina Buonagura: Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Teresa Somma:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Francesca Vitulli:** Conceptualization, Methodology, Investigation, Data curation. **Giuseppina Vitiello:** Methodology, Data curation, Supervision. **Immacolata Andolfo:** Methodology, Data curation, Writing - review & editing. **Felice Esposito:** Supervision. **Roberta Russo:** Methodology, Data curation, Writing - review & editing. **Achille Iolascon:** Supervision. **Paolo Cappabianca:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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