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CASE REPORT

A novel PDCD10 gene mutation in cerebral cavernous malformations: a case report and review of the literature

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Abstract: Cerebral cavernous malformations (CCMs) are one of the most common types of vascular malformation, which are featured enlarged and irregular small blood vessels. The cavernous cavities are merely composed of a single layer of endothelial cells and lack other support tissues, such as elastic fibers and smooth muscle, which make them elastic. CCMs may develop in sporadic or familial forms with autosomal dominant inheritance. Mutations have been identified in three genes: *KRIT1, MGC4607,* and *PDCD10*. Here, we report a typical case of CCMs in a 44-year-old woman associated with a novel mutation in *PDCD10* gene. The patient, diagnosed with CCMs, has been suffering from headache for several months. Analyses of the Whole Exome Sequencing revealed a novel disease-associated mutation in the already known disease-associated *PDCD10* gene. This mutation consists a nucleotide deletion (c.212delG) within the exon 4, resulting in premature protein termination (p.S71Tfs*18). This novel mutation significantly enriches the spectrum of mutations responsible for CCMs, providing a new evidence for further clarifying the genotype–phenotype correlations in CCMs patients.

Keywords: cerebral cavernous malformations, hemorrhage, subcutaneous nodules, *PDCD10* gene, frameshift mutation

Introduction

Cerebral cavernous malformations (CCMs) are one of the most common types of vascular malformation. The prevalence rate in the general population has been estimated 0.4–0.6%[1] while it represents 5–15% of all vascular malformations of the central nervous system (CNS).[2] CCMs are featured enlarged and irregular small blood vessel. The cavernous cavities merely composed of a single layer of endothelial cells lack some support tissues, such as elastic fibers and smooth muscle, thus making vessel walls fragile and immature.[3,4] Because of the abnormally thin walls, the blood vessels are prone to recurrent hemorrhages that can cause various nervous system diseases, such as chronic headaches, epileptic seizures and stroke-like symptoms.[5] However, only 20–30% of individuals with CCMs experience some related health problems. Generally, CCMs predominantly affect the CNS, but that sometimes affect other vital tissues, including the retina, skin,[6] and even liver.[7]

Magnetic resonance imaging (MRI) has been historically regarded as the gold standard for CCMs diagnosis according to clinical guidelines.[8] Compared with computed tomography (CT), MRI possesses much more sensitivity and specificity

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toward the diagnosis of CCMs lesions, especially when gradient echo T2*-weighted (GRE T2*) and susceptibility-weighted imaging (SWI) sequences are utilized,[9] achieving an increased reported incidence of CCMs in the general population.[10] T2-weighted images reveal that lesions in patients with CCMs who had experienced repeated bleedings characterized by mulberry- or popcorn-like appearance and a dark rim due to hemosiderin deposition.

CCMs can develop in both sporadic and familial forms. The pattern of inheritance of the familial form is autosomal dominant. Usually the familial cases typically exhibit multiple lesions, whereas sporadic cases usually have a single lesion.[11] However, patients with no positive family history can also suffer from multiple lesions,[12] which may result from a de novo mutation or a mutation inherited from an asymptomatic parent.[13,14] Until now, mutations in three *CCM* genes, *CCM1/krev1* interaction trapped gene 1 *(KRIT1)*,[15,16] *CCM2/MGC4607*,[17] and *CCM3/*programmed cell death 10 (*PDCD10*),[18,19] have been identified. More than 90% of patients with autosomal dominant CCMs and over 60% for isolated cases with multiple malformations carry the loss of function mutations in one of the three genes.[20] About 200 distinct *CCM1-CCM3* mutations have been identified in familial CCMs to date.[21] *PDCD10/CCM3* has recently been found and its mutations cause 10–15% of familial CCMs.[22] According to the genotype–phenotype correlation analysis, patients with *PDCD10/CCM3* mutation may have a significant trend toward an earlier age at symptoms' onset (<15 years old), and a higher risk of cerebral hemorrhage during childhood.[23,24]

Here, we report a novel *PDCD10* frameshift mutation, detected in a heterozygous condition in a 44-year-old woman with CCMs. The study was conducted in accordance with the Ethics Committee of Peking University First Hospital and the case details were approved by the Ethics Committee of Peking University First Hospital to be published. Written informed consent was obtained from the patient to publish the case details and any accompanying images in medical journals.

Case presentation

A 44-year-old previously healthy woman presented to outpatient clinic with a 4-month history of progressively worsening headaches and dizziness, sometimes accompanied by vertigo, nausea, and vomiting. Headache was transiently controlled with trazodone, nigral, and paroxetine. Nonetheless, the headache was aggravated and ranged from the top of the head to the bilateral ankle and posterior occipital now, which seriously affected her sleeping. The patient did not have the history of hypertension, diabetes and psychosis, as well as the similar family history. Besides, the patient has a 7-year-history of multiple asymptomatic subcutaneous nodules located on right posterior occipital region (Figure 1A–B; red arrow) and the left posterior shoulder. Two years ago, the similar lesions subsequently appeared in the right nasal wing (Figure 1C; red arrow) and the right forehead. Skin examination found that the diameter of multiple nodular skin lesions was about 1 cm and their shape in T1WI sequence was oval with uniform low signal (Figure 1A; red arrow) while nearly round with popcorn-like appearance inside in T2WI sequence (Figure 1B; red arrow). The color of the overlying skin showed a very slightly bluish hue (Figure 1C) without any redness and swelling. The nodular mass was hard and no tenderness. By neurologic examination, it was shown that comprehension and computational abilities were slightly reduced. Near vision of the left eye is Jr5 and right eye Jr4. The right knee reflex was more active than the contralateral side. Both the bilateral palmar reflex and left side Rossolimo sign were positive. The head-MRI, including T2 Weight-sequence (Figure 2A), diffusion-weighted imaging-sequence (Figure 2B) and SWI-sequence in 2017 (Figure 2C) showed multiple abnormally low signals in the brain characterized by mulberry-appearance with a dark rim or oval shape, which were considered as micro-hemorrhagic lesions. The head-MRI SWI sequence from the re-examination at the local hospital in 2018 showed increased microhemorrhagic lesion number (Figure 2D, blue arrows) and extension of the lesion size (Figure 2D, red arrows) at the same position in the brain, involving the cerebral hemispheres, cerebellum, and brain stem. That is the reason why the patient had progressively worsening headaches. Additionally, SWI in 2018 showed the obvious filled veins than before (Figure 2D, red boxes). Head CT showed some high-density lesions in the left semi-oval center and the left basal ganglia, which were considered as bleeding. Unfortunately, none of these family members could be contacted and investigated for the presence of CCMs.

We extracted DNA from peripheral blood of the patient using DNA Isolation Kit (Bioteke, AU1802). DNA libraries were prepared with KAPA Library Preparation Kit (Kapa Biosystems, KR0453) following the manufacturer's instructions. And sample dilution, flowcell loading, and sequencing were performed according to the Illumina specifications. Whole exons were sequenced on the HiSeq2500 platform as paired-end 200-bp reads. The sequencing data was evaluated by Illumina Sequence

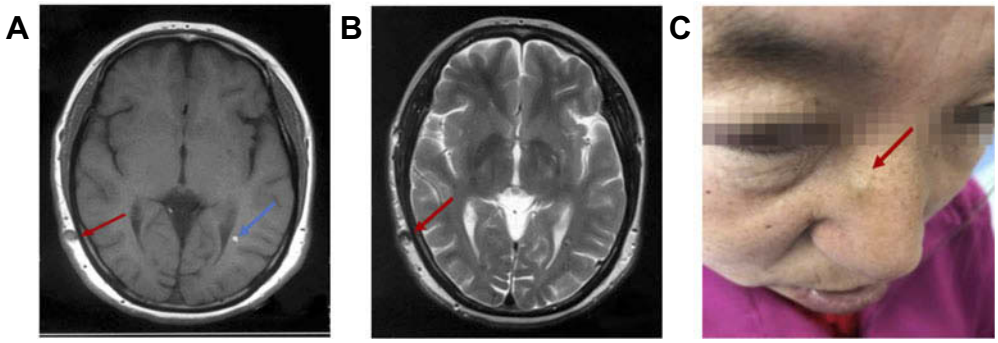

Figure 1 Representative subcutaneous nodular lesions of the patient. (**A**) The T1 Weight-sequence showed the subcutaneous nodules on right posterior occipital region with oval and uniform low signal (red arrow) and the new lesion in the brain (blue arrow). (**B**) The T2 Weight-sequence revealed the nodular skin lesions on right occipital with nearly round low signal and popcorn-like appearance inside (red arrow); (**C**) the nodular skin lesions on right nasal wing (red arrow).

Control Software to remove adapter sequences in the raw data and discard low-quality reads with low base quality. Finally, sequencing analysis of the patient's whole exomic DNA revealed a novel disease-associated mutation in exon 4 of the already known disease-associated PDCD10 gene. It is a frameshift mutation in the heterozygous state: the G nucleotide deletion at codon 212 position (Figure 3). According to HGMDpro database, the mutation site c.212delG has not been reported so far. Therefore, it is a novel mutation. The mutation leads to premature protein termination (p.S71Tfs*18). Effects of the novel mutation on PDCD10 tertiary structure were predicted by RaptorX

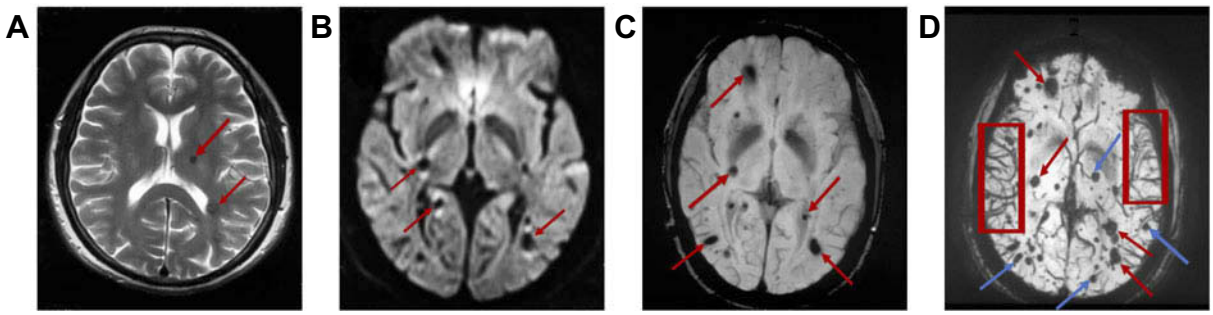

Figure 2 Representative cranial MRI of the patient. (**A**) The T2WI sequence showed the lesions were in round shape (long red arrow) or characterized by mulberry-appearance and a dark rim (short red arrow); (**B**) some low signal lesions in the diffusion-weighted imaging (DWI)-sequence were characterized by high signals around (red arrows); (**C**) the susceptibility-weighted imaging (SWI)-sequence (TR:19,TE:25) in 2017 revealed multiple oval or round lesions; (**D**) the SWI (TR:20,TE:26) in 2018 showed the old lesions in 2017 (Figure 2C; red arrows) had bigger diameters (red arrows) and the new low signal lesions (blue arrows) in the whole brain (cerebral hemispheres, cerebellum, and brain stem). The red box represented the filled veins.

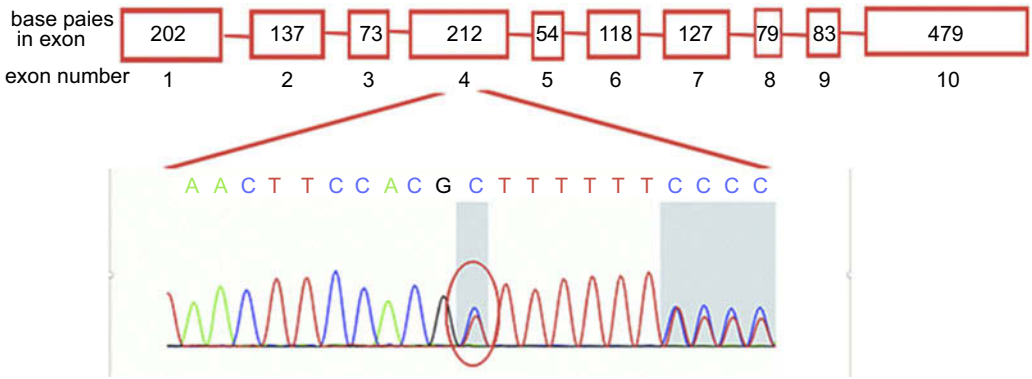

Figure 3 Graph showing *PDCD10* coding sequence and exon4 sequence analysis. Exons are represented graphically as boxes with the exon number below the boxes, the size of each exon is shown as the number of base pairs directly inside each box. Sequencing with forward primer shows a heterozygous frameshift mutation: the G nucleotide deletion at codon 212 position (red circle). The frameshift mutation (c.212delG) led to premature protein termination.

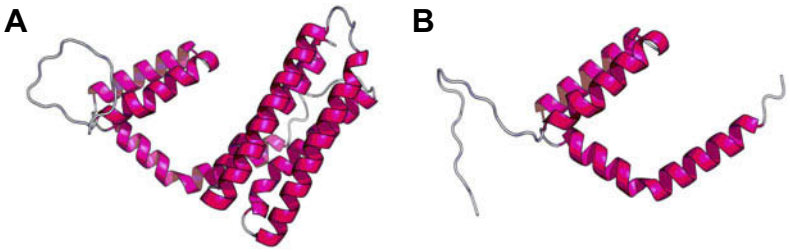

Figure 4 PDCD10 Tertiary structure alteration prediction by RaptorX tool. (**A**) Tertiary structure of wild-type protein. (**B**) Tertiary structure of p.S71Tfs*18 affected protein.

prediction tool.[25] The results reveal tertiary structure change in mutated protein (Figure 4B) compared to wild-type one (Figure 4A). And there are no mutations were identified when analysis of KRIT1 and MGC4607 genes was performed.

Discussion

CCMs are vascular lesions of the CNS characterized by abnormally enlarged capillary cavities.[26] CCMs can arise anywhere in the body, especially in the forebrain,[27] which can lead to focal neurological deficits, seizures, and hemorrhagic stroke. However, there is no effective pharmacologic therapy currently.[28] MRI has been historically regarded as the gold standard for CCMs diagnosis. CCMs can occur sporadically or be inherited in an autosomal dominant pattern. Mutations in one of three *CCM* genes, *KRIT1/CCM1, MGC4607/CCM2,* and *PDCD10/CCM3*, have been predicted to cause loss of function. Here, we present a case report on a 44-year-old woman whose clinical and radiological phenotype was remarkable of CCMs. The Analyses toward the Whole Exome Sequencing revealed a novel disease-associated mutation in the already known disease-associated *PDCD10* gene. This mutation consists of a nucleotide deletion (c.212delG) within the exon 4, resulting in premature protein termination (p.S71Tfs*18).

There are some significant features in the patient as following: (1) the patient's symptoms have been exacerbating during the progress of treatment. SWI sequence on 4/16/2018 showed more microhemorrhagic lesions and larger lesion size in the brain than SWI on 12/25/2017, which was the main reason for the progressively worsening headaches; 2) the patient with CCMs simultaneously suffered from multiple nodular skin lesions with a diameter of about 1 cm, which was rare in CCMs; 3) about 150 different mutations have been found in *CCM1* leading to CCMs.[29] 40 different mutations in *CCM2*[30] and 15 different mutations in *CCM3* have been identified in Western countries.[31] It appears that *CCM1* is the dominant genetic cause of CCMs in Chinese people. To date, no loss-of-function mutations have been identified in *CCM2* or *CCM3* in china. Thus, this novel mutation in *PDCD10* gene will be helpful to extensively understand the impact of *PDCD10* mutations on Chinese population and the difference between Chinese and Western.

Programmed cell death 10 (*PDCD10*) located on 3q26.1 is the third CCM locus and is also called *CCM3. CCM3* identified more recently is a highly conserved gene including seven coding and three non-coding exons, which code a 212-amino acid protein (*PDCD10*).[32] The protein, with an N-terminal dimerization domain and a C-terminal focal adhesion targeting (FAT)-homology domain,[33] is ubiquitously expressed. PDCD10 protein can interact with a variety of proteins including cell adhesion molecule Paxillin and Malcavernin (CCM2) through its C-terminal and also interact with Germinal Centre Kinase III (GCK III kinases) through its N-terminal,[34] promoting cell proliferation, modulating apoptotic pathways and increasing mitogen-activated protein kinase (MAPK) activity and STK26 activity.[35] In addition, a fully folded *CCM3* FAT-homology domain is vital for the stabilization of the expressed protein.[36] Loss-of-function mutations in *PDCD10* will lead to the onset of CCMs.[37,38]

So far, three known loci have accounted for 96% of CCM families. The frequency of identified mutations in the *PDCD10* gene is surprisingly low to 10% (3/29), especially given that this panel is heavily biased towards non-*CCM1*, non-*CCM2* probands.[39] However, *PDCD10* has a high proportion of de novo mutations. Furthermore, the clinical features of CCMs in *KRIT1, CCM2*, and *PDCD10* families have special differences. Patients with *CCM3* mutations are prone to have an earlier age at symptoms' onset and a higher risk of cerebral hemorrhage during childhood.[23] Although there are three genes responsible for CCMs, recent studies have demonstrated that the *CCM1* and *CCM2* proteins interact with each other and are part of a common signaling complex.[40] *CCM2* contains a phosphotyrosine-binding domain that can bind to *CCM1*, which can affect the p38 MAPK signaling cascade.[41] *PDCD10* also binds its C-terminal directly to *CCM2* as well

as several other signaling molecules.[42] A recent research showed that *CCM3* interacts with malcavernin, forming a part of the *Krit1*- malcavernin complex that participates in *CCM1*-dependent β1-integrin-mediated signal modulation and *CCM2*-mediated p38 MAPK signaling in response to cellular stress.[43] Thence, the interactions of *CCM1, CCM2*, and *CCM3* cooperatively contribute to the hypothesis that they function as a complex to affect a common signaling mechanism.[44,45] The hypothesis has already been proved by animal models. In the endothelium, both *KRIT1* and *CCM2* suppress the activity of the small GTPase RhoA. Deficient of *CCM1* or *CCM2* leads to RhoA activation and signal pathway through Rho kinase (ROCK), increasing actin stress fibers, impairing cell–cell interactions, increasing vascular permeability, and finally hindering the formation of the first branchial arch artery in mice.[46] A recent study showed a similar mechanism of Rho activation for *PDCD10*.[47] However, other signal pathways of *PDCD10* have also been studied. Advancements in genetic engineering and stem cell research will provide great potential for the interventional options for CCMs in the near future,[48] which can lead to further understanding about CCMs.

This novel mutation significantly enriches the spectrum of mutations responsible for CCMs, providing a new evidence for further clarifying the genotype–phenotype correlations in CCMs patients. However, there are also many questions remain to be solved. First, it is pity that none of her family members could be investigated for the presence of CCMs; second, we still have not figured out the mechanism of how this novel mutation causes CCMs. Therefore, further laboratory studies and clinical assessment are necessary to identify potential targets for pharmacologic therapy.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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