Familial cerebral cavernous malformation presenting with epilepsy caused by mutation in the CCM2 gene

A case report

Kazuhiro Ishii, MD, PhD
Naoki Tozaka, MD
Satoshi Tsutsumi, MD
Ai Muroi, MD
Akira Tamaoka, MD, PhD

Abstract

Rationale: Cerebral cavernous malformation (CCM) of the familial type is caused by abnormalities in the CCM1, CCM2, and CCM3 genes. These 3 proteins forming a complex associate with the maintenance of vascular endothelial cell-cell junctions. Dysfunction of these proteins results in the development of hemangiomas and abnormal intercellular junctions.

Patient concerns: We report a 68-year-old man with familial cerebral cavernous malformation with initial presentation as convulsions at an advanced age. Brain magnetic resonance imaging revealed multiple cavernous hemangiomas in the right occipital lobe. The convulsions were considered to be induced by hemorrhage from cavernous hemangioma in the right occipital lobe.

Diagnoses: Genetic screening of the CCM1, CCM2, and CCM3 genes revealed a novel mutation in the CCM2 gene (exon4 c: 359 T>A, p: V120D). No abnormalities were found in CCM1 or CCM3. Therefore, we diagnosed the patient with familial CCM caused by a CCM2 mutation.

Interventions: This patient was treated with the administration of levetiracetam at a dosage of 1000 mg/day.

Outcomes: No seizures have been observed since the antiepileptic drug was administered. We performed brain magnetic resonance imaging (MRI) regularly to follow-up on appearance of new cerebral hemorrhages and cavernous hemangiomas.

Lessons: This report reviews cases of familial cerebral cavernous malformations caused by abnormalities in the CCM2 gene. This mutation site mediates interactions with CCM1 and CCM3. The mutation occurs in the phosphotyrosine binding (PTB) site, which is considered functionally important to CCM2.

Abbreviations: CCM = cerebral cavernous malformation, del = deletion, fs = frame shift, LD-like motif = leucine-aspartic acid like motifs, MRI = magnetic resonance imaging, PCR = polymerase chain reaction, PTB = phosphotyrosine binding, PTC = premature termination codon, TGF-β1 = transforming growth factor-β1, uk = unknown, VEGF-A = vascular endothelial growth factor-A.

Keywords: CCM1, CCM2, CCM3, cerebral cavernous malformation, phosphotyrosine binding domain

1. Introduction

Cerebral cavernous malformations (CCMs) are a type of low-flow vascular malformation; imaging studies have estimated their prevalence to be 0.4% to 0.8%.[1] Diagnosis of CCM is determined by imaging in 47% of cases, convulsions in 25%, intracerebral hemorrhage in 12%, and the identification of focal neurological defects in 15%.[2] CCMs are either sporadic or familial and recur in approximately 20% of patients. Mutations in the genes encoding CCM1, CCM2, and CCM3 have been associated with familial CCMs.[3] These 3 proteins, present in vascular endothelial cells, form a complex that regulates signal transduction proteins associated with the maintenance of adjacent vascular endothelial cell-cell junctions.[4] Dysfunction of these proteins results in the development of hemangiomas and abnormal intercellular junctions. The former increase in number and size over time, which increases the risk of intracerebral hemorrhage.[5,6] The development of interventions that target proteins associated with CCMs has therefore garnered increasing attention.[3]

Here, we report a case of familial CCM, first indicated by convulsions, in which we identified a novel mutation in the CCM2 gene through genetic testing. We further summarize previously reported CCM2 mutations, infer functionally impor-
tant sites of CCM2, and discuss potential interventions for progressive CCMs. The patient has provided informed consent for publication of the case.

2. Case presentation

A 68-year-old man was transported to the nearest hospital by an ambulance and was admitted due to impaired consciousness and a tonic seizure that had spread from the left leg while the patient was driving 3 months prior. Brain magnetic resonance imaging (MRI) revealed hemorrhages in the right occipital lobe and multiple CCMs. He was diagnosed with secondary convulsions caused by intracerebral hemorrhage. The patient received orally administered levetiracetam at a dosage of 1000mg/day, and no convulsive seizure occurred thereafter. He was referred to our hospital and admitted for advanced examination. Family history revealed that his second son and grandson had both exhibited CCMs.

A physical examination on admission showed no abnormalities, such as hemangioma or nevus, on the body surface. No retinal hemangioma was observed in the fundus. A neurological examination showed alertness and consciousness, and the Hasegawa Dementia Scale-Revised and Mini-Mental State Examination scores were 26 and 27, respectively. There were no abnormalities in the central nervous system, cerebellar symptoms, pyramidal signs, signs of Parkinson’s disease, autonomic symptoms, or sensory abnormalities. Posture/walking was normal, and no meningism was observed.

A urine test revealed no abnormalities. A blood test showed no liver dysfunction, kidney dysfunction, or glucose tolerance abnormalities. Autoantibodies were negative. A thoracoabdominal contrast-enhanced computed tomography scan showed no hemangioma or venous malformation. T1-weighted MRI of the brain showed a hemangioma of 20mm in diameter in the right occipital lobe and a relatively new hemorrhage at the same site. T2-star imaging revealed new and old hemorrhages (Fig. 1). An MRI of the cervical, thoracic, and lumbar spines did not demonstrate any vascular anomalies, such as hemorrhage. An electroencephalogram showed neither abnormal basic waves nor any epileptiform wave patterns.

Using the PAXgene Blood DNA kit, peripheral venous blood was collected to extract genomic DNA; this was amplified by polymerase chain reaction (PCR) to determine the DNA sequence according to a previously reported method, revealing a novel mutation in CCM2 (c, 359; T>A; p, V120D). No abnormalities were found in either CCM1 or CCM3. Based on the results, we diagnosed the patient with familial CCM caused by a CCM2 mutation.

Figure 1. Brain magnetic resonance imaging (MRI). T2-star MRI revealed multiple micro-bleedings as low-signal spotty lesions (upper). T1-weighted MRI was used to visualize relatively new bleeding in the right occipital lobe as a high-signal lesion (lower).
No seizures occurred after the continuous administration of levetiracetam 1000 mg/day. In addition, regular brain MRI scans have been performed to monitor the cerebral cavernous hemangioma and check for the appearance of new hemorrhages.

3. Discussion

CCM2 is composed of 10 exons that encode a 444-amino acid protein (CCM2/malcavernin).\(^6\) CCM2 features an N-terminal phosphotyrosine binding (PTB) domain and a C-terminal helical domain,\(^9\) which bind to CCM1 (KRIT1). CCM2 is a scaffold protein that contributes to several signal transduction cascades, including the p38 mitogen-activated protein kinase (MAPK) and Rho-kinase signaling pathways, which maintain the integrity of blood vessels.\(^12,13\) In addition, CCM2 binds to CCM3 with a leucine-aspartic acid like motifs (LD-like motif), which consists of amino acids 223 to 238, to form a complex of 3 proteins: CCM1, CCM2, and CCM3.\(^14\)

Our search of the National Center for Biotechnology Information ((NCBI) URL: https://www.ncbi.nlm.nih.gov/gene/83605) database and The Human Gene Mutation Database (URL: http://www.hgmd.cf.ac.uk/docs/login.html) revealed that 57 abnormal sites on \(\text{CCM2}\) have been reported, 40 of which are pathogenic mutations.\(^7\) As shown in Figure 2, these abnormalities are concentrated in the CCM1 binding site, the PTB domain, and the N-terminus, all of which are functionally important regions of CCM2.

When the function of CCM1, CCM2, or CCM3 is impaired by a genetic mutation, vascular endothelial cells in the central nervous system assume the features of mesenchymal and stem cells; this causes the loss of VE-cadherin organization and the polarity of vascular endothelial cells, impairment of endothelial intercellular adhesion, and abnormal vascular lumen, further inducing hemangioma and bleeding.

Effective treatment of familial CCMs associated with CCM2 has not been established. Hemangiomas progressively increase in number and grow larger over time, which elevates the risk of intracerebral hemorrhage.\(^5\)\(^,\)\(^6\) Interventions preventing their increase and growth would further forestall the occurrence of intracerebral hemorrhage and improve functional prognosis. Based on pathological mechanisms underlying the formation of hemangioma, agents that can eliminate inflammation and oxidative stress, such as Vitamin D3 and Tempol, have been developed. This same approach has yielded agents that suppress the transforming growth factor-\(\beta\)1 (TGF-\(\beta\)1) signaling such as DMH1, LY364947, SB431542, and Sulindac; RhoA inhibitors, such as statins and Fasudil; and anti-angiogenic agents, such as rapamycin, sorafenib, and vascular endothelial growth factor-A (VEGF-A).\(^3\)\(^,\)\(^12\) Statins such as simvastatin and atorvastatin are well known lipid-lowering medications, and are particularly

---

**Figure 2.** Reported mutations in CCM2 and consequent abnormalities in CCM2. The phosphotyrosine binding (PTB) domain (58–220 amino acids) in the CCM2 protein and exons are shown: protein abnormalities, upper; genetic mutations, lower. Pathogenic mutations are shown in red. Pathogenic mutations are concentrated in the region from the N-terminus to the vicinity of the PTB domain. The genetic variants were expressed in accordance with the guidelines of the Human Genome Variation Society (HGVS) in 2000. del: deletion; fs: frame shift; PTC: premature termination codon; uk: unknown.
practical from the perspective of drug repositioning; clinical trials using these agents are currently underway.[1]

4. Conclusion
We present a case of familial CCM with a novel mutation in CCM2 (c. 359; T>A; p. V120D). Forty abnormal pathogenic sites have been reported in CCM2. These mutation sites are predominantly found in the N-terminus, PTB site, and the LD-like motif of CCM2, which mediate interactions with CCM1 and CCM3. Because these regions are considered functionally important to CCM2, their dysfunction may account for the development of CCMs.

Acknowledgments
We appreciate Mrs. Ikuko Ogino for her assistance in the genetic study. We thank Jyunuko Itoh, PhD, and Mrs. Mioko Iseki for searching the genetic banks. We would like to thank Editage (www.editage.jp) for English language editing.

Author contributions
Data curation: Naoki Tohsaka, Satoshi Tsutsumi, Ai Muroi.
Investigation: Kazuhiro Ishii, Satoshi Tsutsumi.
Project administration: Kazuhiro Ishii.
Supervision: Akira Tamaoka.
Writing – original draft: Kazuhiro Ishii.
Writing – review & editing: Kazuhiro Ishii.

References
[1] Al-Holou WN, O’Lynnger TM, Pandey AS, et al. Natural history and imaging prevalence of cavernous malformations in children and young adults. J Neurosurg Pediatr 2012;9:198–205.
[2] Al-Shahi Salman R, Hall JM, Horne MA, et al. Untreated clinical course of cerebral cavernous malformations: a prospective, population-based cohort study. Lancet Neurol 2012;11:217–24.
[3] Chohan MO, Marchio S, Morrison LA, et al. Emerging pharmacologic targets in cerebral cavernous malformation and potential strategies to alter the natural history of a difficult disease: a review. JAMA Neurol 2019;76:492–500.
[4] Fischer A, Zalvide J, Faurobert E, et al. Cerebral cavernous malformations: from CCM genes to endothelial cell homeostasis. Trends Mol Med 2013;19:302–8.
[5] Gross BA, Lin N, Du R, et al. The natural history of intracranial cavernous malformations. Neurosurg Focus 2011;30:E24.
[6] Shenkar R, Shi C, Rebeiz T, et al. Exceptional aggressiveness of cerebral cavernous malformation disease associated with PDCD10 mutations. Genet Med 2015;17:188–96.
[7] Tsutsumi S, Ogo S, Miyajima M, et al. Genomic causes of multiple cerebral cavernous malformations in a Japanese population. J Clin Neurosci 2013;20:667–9.
[8] Denier C, Goutagny S, Labauge P, et al. Mutations within the MGC4607 gene cause cerebral cavernous malformations. Am J Hum Genet 2004;74:326–37.
[9] Liquori CL, Berg MJ, Siegel AM, et al. Mutations in a gene encoding a novel protein containing a phosphotyrosine-binding domain cause type 2 cerebral cavernous malformations. Am J Hum Genet 2003;73:1459–64.
[10] Fisher OS, Zhang R, Li X, et al. Structural studies of cerebral cavernous malformations 2 (CCM2) reveal a folded helical domain at its C-terminus. FEBS Lett 2013;587:272–7.
[11] Baranoski JF, Kalani MY, Przbylowski CJ, et al. Cerebral cavernous malformations: review of the genetic and protein-protein interactions resulting in disease pathogenesis. Front Surg 2016;4:60.
[12] Morrison L, Aker A, Adams MP, Ardinghe HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A. Cerebral cavernous malformation, familial. GeneReviews® [Internet] University of Washington, Seattle, WA: 1993–2019.
[13] Haasdjik RA, Cheng C, Maat-Kievit AJ, et al. Cerebral cavernous malformations: from molecular pathogenesis to genetic counselling and clinical management. Eur J Hum Genet 2012;20:134–40.
[14] Draheim KM, Li X, Zhang R, et al. CCM2–CCM3 interaction stabilizes their protein expression and permits endothelial network formation. J Cell Biol 2015;208:987–1001.
[15] Riant F, Cecillon M, Saugier-Veber P, et al. CCM molecular screening in a diagnosis context: novel unclassified variants leading to abnormal splicing and importance of large deletions. Neurogenetics 2013;14:133–41.
[16] Stahl S, Gaetzner S, Voss K, et al. Novel CCM1, CCM2, and CCM3 mutations in patients with cerebral cavernous malformations: in-frame deletion in CCM2 prevents formation of a CCM1/CCM2/CCM3 protein complex. Hum Mutat 2008;29:709–17.
[17] D’Angelo R, Alafaci C, Scimone C, et al. Sporadic cerebral cavernous malformations: report of further mutations of CCM genes in 40 Italian patients. Biomed Res Int 2013;2013:459253.
[18] D’Angelo R, Marini V, Rinaldi C, et al. Mutation analysis of CCM1, CCM2 and CCM3 genes in a cohort of Italian patients with cerebral cavernous malformation. Brain Pathol 2011;21:215–24.
[19] Mondéjar R, Solano F, Rubo R, et al. Mutation prevalence of cerebral cavernous malformation genes in Spanish patients. PLoS One 2014;9:e86286.
[20] Verlaan DJ, Laurent SB, Rochefort DL, et al. CCM2 mutations account for 13% of cases in a large collection of kindreds with hereditary cavernous malformations. Ann Neurol 2004;55:737–8.
[21] Pileggi S, Buscone S, Ricci C, et al. Genetic variations within KRIT1/CCM1, MGC4607/CCM2 and PDCD10/CCM3 in a large Italian family harbouring a Krit1/CCM1 mutation. J Mol Neurosci 2010;42:235–42.
[22] Du Q, Shi Z, Chen H, et al. Two novel CCM2 heterozygous mutations associated with cerebral cavernous malformation in a Chinese family. J Mol Neurosci 2019;67:467–71.