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# Cerebral cavernous malformations with diffuse manifestation: A benign entity?

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ABSTRACT

*Purpose:* Cerebral cavernous malformations (CCMs) are a distinct cerebrovascular disease. A fraction of CCMs present as diffuse manifestations distributed over the cerebral hemispheres, cerebellum, and brainstem. The purpose of the present study was to explore the clinical picture of such CCMs.
*Methods:* This study assessed the appearance of CCMs on magnetic resonance (MR) images, the presence of genetic mutations using the polymerase chain reaction method, and disease course over long-term follow-up in a total of 10 patients with diffuse CCMs.
*Results:* The 10 patients were Japanese and comprised 5 males and 5 females with a mean age of 48.7 years. Three of them presented with seizures, two with headache and intracerebral hemorrhage, two with numbness, and one with dizziness, while the remaining two were asymptomatic. Genetic analysis revealed *CCM1* mutations in four patients, *CCM2* mutations in three, and a *CCM3* mutation in one. In a family with 2 *CCM2* patients, the appearance of sustained diffuse CCMs on MR images significantly differed between the 2 patients despite the mutation being identical. During the mean follow-up period of 13.7 years, none of the 10 patients showed evidence of neurological deterioration or symptomatic hemorrhage. The appearance of their CCMs on MRI did not show significant changes. Eight patients maintained normal neurological function.
*Conclusions:* CCMs with diffuse manifestation is a hereditary disease with satisfactory prognosis. Unrecognized genomic mutations may be involved in the genesis of these CCMs.


## 1. Introduction

Cerebral cavernous malformations (CCMs) are a distinct entity of cerebrovascular diseases that affect approximately 0.5% of the general population [1]. The annual hemorrhage rate of CCMs has been reported to be 0.7–2.4%, which were based on inconsistent approaches for evaluating the clinical picture and neuroimaging classifications of the hemorrhages [2–4]. Prior hemorrhage, brainstem location, and associated developmental venous malformations are thought to be the significant risk factors for symptomatic hemorrhage from CCMs [5,6]. CCMs can occur in sporadic or familial form with autosomally dominant trait. Mutations in the *CCM1*, *CCM2*, and *CCM3* genes, located in the 7q, 7p, and 3q, respectively, are thought to be responsible for the genesis of familial CCMs [7–12]. Annual hemorrhage rate in patients with familial CCMs is estimated to be 2.5%, reflecting the higher proportion of patients developing clinical symptoms in the familial compared to sporadic type [13,14]. Neuroimaging penetrance of familial CCMs is reported to be much higher than the clinical penetrance [14,15]. If a patient without obvious family history of CCM has been found to have multiple CCMs on imaging examination, in 75% the case is actually a familial type [14]. T2-weighted gradient-echo magnetic resonance (MR) images and susceptibility-weighted sequence are thought to be highly sensitive for detecting CCMs and accompanying hemosiderin deposition [15–17].

A fraction of familial CCMs show unusually diffuse and extensive manifestations involving the cerebral hemispheres, cerebellum, and brainstem, which can perplex clinicians when encountered these patients. To our knowledge, there have been few reports documenting these CCMs [18]. The present study aimed to explore the clinical picture of these diffusely manifesting CCMs based on their appearance on magnetic resonance imaging, the presence of genetic mutations, and long-term follow-up.

## 2. Materials and methods

### 2.1. Patients

This prospective study initially evaluated 30 familial CCM patients who presented to our institution between November 2006 and March 2016. Their symptoms at the onset were seizure, headache, numbness, and dizziness. The diagnosis of familial CCMs was based on a detailed

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interview of family history, medical records provided by referring physicians, and MR imaging examinations of the brain and spinal cord using a 3.0T MR scanner (Achieva R2.6; Philips Medical Systems, Best, The Netherlands), including T2*-weighted images and/or susceptibility-weighted sequences. Of these 30 patients, 10 with an unusual presentation of multiple CCMs involving the cerebral hemispheres, cerebellum, and brainstem were diagnosed as patients with diffuse manifestation of CCM. Patients with multiple CCMs who did not present involving all the above-mentioned brain areas were excluded. In 9 of these 10 patients, peripheral blood was collected from the antecubital vein for genetic analysis, carried out as described below. The present study was performed in accordance with our institution's guidelines for human research. Written informed consent for this study was obtained from all 10 patients.

### 2.2. Genetic analysis

1. DNA extraction, polymerase chain reaction (PCR), and sequencing

Whole venous blood was collected in a PAXgene DNA Tube (PreAnalytiX, Hombrechtikon, Switzerland) and the genomic DNA was extracted using the PAXgene Blood DNA Kit (PreAnalytiX). Mutations in the *CCM1*, *CCM2*, and *CCM3* genes were identified using a previously documented PCR method [7,9,11]. Amplifications were carried out in 25 μl 2× AmpliTaq Gold 360 Master Mix (Applied Biosystems), with 20 pmol of each primer and 100 ng of DNA. Cycling conditions consisted of an initial 12.5-min denaturation step at 95 °C and subsequent extension for 7.5 min at 72 °C. PCR products were visualized by ethidium bromide staining on 2% agarose gel. Amplicons were purified using Microcon (Millipore, Bedford, MA, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were loaded on ABI3100 capillaries (Applied Biosystems) and analyzed with Seqscape v2.6 (Applied Biosystems).

2. Reverse transcription PCR

Whole venous blood was collected in a PAXgene RNA Tube (PreAnalytiX) and the RNA extracted with RNA purification kit (PAXgene Blood RNA Kit; PreAnalytiX). PCR products were visualized by ethidium bromide staining on 1.5% agarose gels. Amplicons were purified using Microcon and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit. Sequencing reactions were loaded on ABI3100 capillaries and analyzed using Seqscape v2.6.

## 3. Results

### 3.1. Demographics and clinical symptoms at onset

The study population comprised 5 males and 5 females aged between 20 to 68 years (mean: 48.7 years). Their age at the initial onset or presentation was between 10 to 58 years (mean: 39.4 years). All the subjects were Japanese and belonged to 6 different families in total. Three of these 10 patients presented with seizures, 2 with headache and intracerebral hemorrhage, 2 with numbness in the face or upper extremity, and 1 with dizziness. The other two were asymptomatic and found coincidentally on MR images performed for a brain check-up and minor head trauma, respectively.

### 3.2. Neuroimaging findings

On T2-weighted gradient-echo images and susceptibility-weighted sequences, all patients showed a diffuse CCMs represented by many or innumerable hypointense spots and nodular masses of varying sizes. Most of the brainstem lesions were tiny with a diameter of less than 3 mm. Notably, CCMs were not compressive to the surrounding brain, regardless of the size and location. Concurrent vascular malformations were not found. Spinal cord involvement was identified in 4 patients; the cervical cord was affected in 1, thoracic cord in 2, and cervicothoracic cord in 1. In 4 patients, spinal cord involvement was not identified. The remaining 2 patients did not undergo spinal MR imaging (Fig. 1).

### 3.3. Genetic features: presence of CCM gene family mutations

Genetic analysis revealed a *CCM1* mutation in 4 patients, *CCM2* mutation in 3 patients, and *CCM3* mutation in 1 patient. In another patient belonging to a CCM1 family, genetic analysis was not conducted and the type of *CCM* mutation was defined as “unknown” (Patient No. 3 in Table 1). In one of the families, 3 patients (Nos. 1, 2, and 4) had the same *CCM1* mutation and their CCM lesions exhibited a characteristic appearance on MR images that was represented by innumerable nodular lesions of varying sizes over the whole brain (Fig. 2). By contrast, two patients in another family (Nos. 5 and 6) had an identical *CCM2* mutation, but the appearance of CCMs on neuroimaging was significantly different between the two cases (Fig. 3).

### 3.4. Follow-up results and disease course

Five (Nos. 2, 3, 4, 5, and 6) of the 10 patients were initially treated by outside physicians. These individuals were diagnosed with familial CCMs based on an interview of the family history and MR imaging examination. Until referral to our institute, all had followed an uneventful course without neurological deterioration or symptomatic hemorrhage. The period from the diagnosis of familial CCMs to the latest interview ranged from 1.4–29 years (mean: 13.7 years). The follow-up period at our institution ranged from 1.4–9.4 years (mean: 7.1 years). During the period, no patient showed neurological deterioration or a symptomatic hemorrhage. Appearance of CCMs on MR images did not show significant growth of CCMs or compressive hemorrhage in any location of the brain, which was consistently observed for all CCM types (Figs. 4-6). These results and details of *CCM* mutations are summarized in Table 1. Two patients who presented with seizures as the initial symptom were well controlled during the follow-up period with constant medication. Modified Rankin Scale (mRS) scores at the latest interview were 0 in 8 patients and 1 in 2; 1 patient exhibited a sustained fine movement disturbance in the right hand and another presented intermittent trigeminal pain.

## 4. Discussion

The present investigation suggests that patients with diffuse CCMs may follow a stable clinical course without neurological deterioration. In spite of the impressive appearance on MR images, patients with diffuse CCMs may maintain favorable neurological function for a long time even after initial symptomatic onset. In our study, these patients did not sustain neurological deterioration or symptomatic hemorrhages during a long-term follow-up. MR appearance of these CCMs did not show significant changes in any location of the brain. Furthermore, all the patients who manifested diffuse CCMs and underwent genomic analysis possessed genomic mutations in *CCM1*, *CCM2*, and *CCM3*. Thus, we surmise that diffuse CCMs are typically a hereditary disease with a reasonably favorable prognosis.

Relative to other locations in the central nervous system, CCMs located in the brainstem are thought to be more likely to cause a symptomatic hemorrhage [5,6]. The10 patients in our study did not show significant growth or symptomatic hemorrhages even when there were multiple, pre-existing lesions in the brainstem. Most of the lesions were identified as tiny spots on initial MR images and did not enlarge thereafter, which may contribute to the good functional prognosis of patients with diffuse CCMs. This is in contrast to a 36-year-old female who was in the group of 30 patients evaluated at the beginning of the study but not enrolled because brainstem lesions were absent. This patient