Clinical, Magnetic Resonance Imaging, and Genetic Study of 5 Italian Families With Cerebral Cavernous Malformation

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Background: Cerebral cavernous malformations (CCMs) are congenital vascular anomalies of the central nervous system that can result in seizures, hemorrhage, recurrent headaches, and focal neurologic deficits. These CCMs can occur as sporadic or autosomal dominant conditions, although with incomplete penetrance and variable clinical expression. Three CCM loci have been identified, on chromosomes 7q21-22 (CCM1; Online Mendelian Inheritance in Man [OMIM] 116860), 7p13-15 (CCM2; OMIM 603284), and 3q25.2-27 (CCM3; OMIM 603285), and 3 genes have been cloned, KRIT1 on CCM1, MGC4607 on CCM2, and PDCD10 on CCM3. Mutations in KRIT1 account for more than 40% of CCMs.

Objective: To describe the results of a comprehensive evaluation of 5 Italian families affected with CCM.

Design: Clinical, magnetic resonance imaging, and KRIT1 gene analysis.

Setting: University academic teaching hospitals.

Results: Three novel and 2 described mutations were found in KRIT1. The families included 33 KRIT1 mutation carriers, 57.6% of whom had no symptoms. Magnetic resonance imaging revealed CCM lesions in 82.3% of symptom-free mutation carriers.

Conclusions: The data confirm both incomplete clinical and neuroimaging penetrance in families with the KRIT1 mutation. This consideration is important in genetic counseling. Moreover, the data emphasize both the importance of magnetic resonance imaging in the diagnosis of CCM and the potential for DNA-based diagnosis to identify subjects at risk.

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with a single lesion. All KRIT1 mutations identified to date are highly stereotyped, leading to premature stop codons, suggesting that KRIT1 loss of function may be the underlying mechanism in patients with CCM. KRIT1 is a protein of unknown function binding to integrin cytoplasmic domain-associated protein-1α, a protein containing a phosphotyrosine binding domain that also binds to the β3-integrin cytoplasmic domain. A second disease gene, MGC4607, encoding the protein malcavernin with a phosphotyrosine binding domain, was identified in families with CCM2. Mutations in this gene account for about 20% of familial cases, whereas no mutations were found in a cohort of sporadic cases with single and multiple lesions. Recently it was shown that KRIT1 and malcavernin interact in a common signaling complex and that loss of this interaction could contribute to CCM pathogenesis. Families with CCM3 have recently been shown to have mutations in PDCD10 (programmed cell death 10), a gene up-regulated in the TF-1 premeloid cell line after apoptosis induction. The frequency of PDCD10 mutations identified in families with CCM3 was lower than expected on the basis of linkage, suggesting the possibility of a fourth CCM gene.

Although the disease gene products for all 3 loci have been identified, the pathologic mechanism remains unknown. At least 2 possible mechanisms have been postulated for malformation development: a Knudson second-hit hypothesis and a haploinsufficiency model. We performed clinical, neuroradiologic, and genetic analyses in 5 Italian families with familial CCM to further characterize the clinical and neuroimaging features of familial CCM segregating a KRIT1 mutation.

Figure 1. Pedigrees of families with KRIT1 mutations. A-E, Families 1 through 5, respectively. Filled circles and squares indicate affected, symptomatic individuals; half-filled circles and squares, affected, asymptomatic individuals; open circles and squares, unaffected individuals; question mark inside circles and squares, individuals not known to be affected (no magnetic resonance imaging performed); asterisk, mutation; small open circle, no mutation; arrow, index patient; diagonal line, individual deceased; diamond, sex unknown; and small diamond, in utero death.
Five unrelated, clinically affected CCM probands (index patients) were consecutively enrolled on the basis of 1 of the 2 following criteria: each proband had at least 1 affected relative and/or had multiple cerebral cavernous angiomas. Diagnosis was based on brain magnetic resonance (MR) imaging features and, when possible, postsurgery histopathologic analysis findings. Detailed clinical and brain MR imaging data were collected for all patients with symptomatic CCM through direct interview and review of medical records. Clinical assessment focused on the occurrence of seizures, cerebral hemorrhage, focal neurologic symptoms, and headache. Subjects who gave written informed consent underwent review of their medical records, brain MR imaging, and blood sampling for genetic analysis, and their medical records were reviewed. Subjects with cavernomas seen on MR images were considered affected and those with no abnormalities seen on MR images were considered unaffected; those who did not undergo MR imaging were classified as “unknown.” The clinical and MR imaging details for 1 kindred (family 1) included in this study were published, in part, before KRIT1 gene identification. The current study was approved by the local ethics committee.

The MR imaging was performed on a high-field brain magnet (1.5T) and included standard spin echo and fast turbo spin echo T1- and T2-weighted axial, coronal, and/or sagittal images in all participants. Gradient-echo T2-weighted axial MR images were obtained in patients with symptoms and in most symptom-free patients.

Genomic DNA was extracted from peripheral blood using standard procedures. In the probands, all 16 coding exons of KRIT1 were amplified with the polymerase chain reaction with a specific subset of 17 primer pairs.12 Direct DNA sequencing was then performed with an automated sequencer (model ABI310, Applied Biosystems, Foster City, Calif). Numbering of nucleotides was according to the full-length KRIT1 complementary DNA (accession number AF296765).

### RESULTS

#### PATIENT CHARACTERISTICS

A cohort of 60 Italian individuals, 15 with symptomatic CCMs and 45 at-risk, symptom-free relatives, was investigated. Pedigrees of the families are shown in Figure 1. Patients with symptomatic CCMs included 11 women and 4 men. The mean±SD patient age at clinical onset was 15.9±7.5 years (age range, 4-36 years). Among the first clinical manifestations, seizure was reported in 10 patients (67%), recurrent headache in 3 (20%), and cerebral hemorrhage in 2 (13%). At clinical follow-up, 11 patients were totally independent with no permanent neurologic disorders, 1 had symptomatic cerebral hemorrhages, 1 had left-sided hemiparesis, and 2 had intractable seizures.

#### MR IMAGING FINDINGS

Brain MR imaging was performed on 49 individuals, 15 patients with symptomatic CCMs and 34 at-risk, symptom-free relatives (Table 1). Eleven at-risk, symptom-free relatives declined brain MR imaging. Thirty-one subjects, 22 females and 9 males, exhibited cavernous angiomas at MR imaging and were classified as affected; 15 of these had symptomatic CCMs, and 16 had normal findings on neurologic examinations. All but 3 asymptomatic individuals were older than 20 years (mean±SD age, 40.4±22.3 years; age range, 6-79 years). In 18 symptom-free relatives, no abnormalities were seen on MR images, and they were considered unaffected. Multiple lesions were found in 28 (90.3%) of 31 affected patients, and a single lesion was found in 3 (9.6%) of 31 affected patients aged, respectively, 5, 19, and 26 years, although a correlation between age and number of lesions was not observed in our patients.

#### MUTATION ANALYSIS

Fifty-three individuals, 14 with and 39 without symptoms, were screened for KRIT1 gene mutations. Seven individuals, 1 with and 6 without symptoms, refused genetic testing (Table 1). In family 1, a previously described deletion, 1204delAACAA,11,20 was found. A novel 5-base pair (bp) deletion, 1306delTTGGA, was found in family 2, and a novel 2-bp insertion, 658insTT, was identified in family 3. Two nucleotide substitutions were also detected: a previously described splicing mutation,21 Q201E, in family 4, and a new nonsense mutation, Q482X, in family 5. All mutations were heterozygous and introduced a premature stop codon (Figure 2).

Thirty-three KRIT1 mutation carriers, 14 with and 19 without symptoms, and 20 noncarriers without symptoms, were identified. Among the 53 individuals molecularly screened, MR images were available for 42 (Table 1). The mutations identified in the 5 families, and the clinical and MR imaging features in the 31 mutation carriers are given in Table 2.

#### COMMENT

We describe 5 unrelated Italian families affected with CCM in which 3 novel and 2 previously reported mutations in the KRIT1 gene were identified. All mutations were...
in the coding sequence, 3 located within the second half of the gene (exons 12, 13, and 14), as in most cases reported in the literature, and 2 in a gene region (exon 8) in which mutations are relatively rare. All mutations introduce a premature termination codon, confirming the stereotypical nature of KRIT1 mutations.

Six different mutations in the KRIT1 gene have been previously described in Italian families affected with CCM. One of these families has been clinically described in detail, with a syndrome of cerebral angiomomas, hepatic hemangiomas, and retinal cavernous angiomas. Segregation of the KRIT1 mutation has been found in members of the family with cerebral or retinal lesions but not hepatic lesions.

A few KRIT1 mutations have been associated with lesions outside of the brain concurrently with cerebrovas-

**Figure 2.** Sequence chromatograms of KRIT1 mutations. Mutations are indicated by arrows. A, Family 1: deletion of 5 base pairs (bp) at nucleotide 1204. B, Family 2: deletion of 5 bp at nucleotide 1306. C, Family 3: insertion of 2 bp at nucleotide 658. D, Family 4: C→G substitution at nucleotide 601 (Q201E). E, Family 5: C→T substitution at nucleotide 1444 (Q482X). All changes lead to a premature stop codon through frame shifts (families 1, 2, and 3), aberrant splicing (family 4), or nonsense mutations (family 5).
circular lesions, suggesting that KRIT1 mutations could cause vascular abnormalities in tissues outside the nervous system. In family 1, a large kindred, the proband (III-20) and an affected family member (IV-6) with the mutation on exon 12 (1204delAACAA) had, in addition to cerebral cavernous angiomas, a renal angioma and a retinal angioma, respectively. This mutation has been previously described; however, no detailed clinical information is available. In the family we observed, only 2 affected members had noncerebral lesions; thus, segregation of the mutation with lesions outside the brain could not be demonstrated.

In our families positive for KRIT1, we observed a distribution of affected individuals consistent with an autosomal dominant mode of transmission, although with a variable phenotypic expression that is both intrafamilial and interfamilial. In family 3, for example, the 6-year-old proband (IV-3), with multiple cerebral cavernomas on MR imaging, had seizures at age 4 years, whereas his mother (III-6, aged 41 years) and a maternal aunt (III-4, aged 38 years) had no symptoms despite multiple cerebral lesions. In family 4, the proband (III-4) had seizures at age 20 years, whereas her affected mother (II-2, aged 79 years) was symptom free.

Among the 33 identified mutation carriers, 19 (57.6%) were, at the time of ascertainment, asymptomatic with normal findings at neurologic examination. This confirms incomplete penetrance of neurologic symptoms associated with CCM, as previously reported in families with the KRIT1 mutation. Among the 31 mutation carriers for whom MR images were available, lesions were detected in 28 (90.3%). Magnetic resonance images showed lesions in 82.3% of asymptomatic mutation carriers (Table 1 and Table 2). Three asymptomatic mutation carriers had normal MR images and were considered unaffected. Two of them did not exhibit any lesions; however, gradient echo MR imaging sequences were not obtained. The first symptom-free mutation carrier was a man (II-1, family 3) who died at age 67 years from a cerebral tumor; no autopsy was performed. The second symptom-free mutation carrier was an 8-year-old girl (IV-10, family 1). Her mother (III-10, aged 44 years) and sister (IV-11, aged 5 years) exhibited lesions on MR imaging. The sister had a cerebral hemorrhage at age 4 years, and both the mother and sister carried the mutation. The third symptom-free mutation carrier was a woman (IV-5, family 1, aged 29 years) who at age 22, before KRIT1 gene identification, underwent MR imaging including a gradient echo sequence that showed no abnormalities. She refused to undergo follow-up brain MR imaging to confirm her unaffected status. Thus, based on these observations, it cannot be ruled out that in these 3 symptom-free mutation carriers, considered unaffected, small CCM lesions could be present but undetected or might develop later. On the other hand, another symptom-free mutation carrier (III-10, family 1) had a normal gradient echo MR imaging sequence at age 37 years; a gradient echo MR imaging sequence at age 44 years revealed CCM lesions.

In conclusion, the 5 families with CCMs investigated had a KRIT1 mutation, suggesting that, although the number of families analyzed is small, KRIT1 may account for more than 40% of the genetic form of CCM, as reported in the literature. In our series of mutation carriers, clinical penetrance, defined as the percentage of individuals with neurologic symptoms among mutation carriers, was incomplete. In addition, 90.3% of KRIT1 mutation carriers had lesions on MR imaging, suggesting that neuroradiologic penetrance was also incomplete and age dependent. Clinical and neuroradiologic studies and follow-up of larger cohorts of patients carrying the KRIT1 mutation should enable better delineation of the disease penetrance and natural history.

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Table 2. KRIT1 Mutations Identified in Our Families, and Clinical and MR Imaging Features of Mutation Carriers

<table>
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<tr>
<th>Family</th>
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<th>Subjects Screened</th>
<th>Mutation Carriers</th>
<th>Subjects Who Underwent MR Imaging (n = 31)</th>
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Abbreviation: MR, magnetic resonance.

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References


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