Biallelic Somatic and Germ Line CCM1 Truncating Mutations in a Cerebral Cavernous Malformation Lesion

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Background and Purpose—Cerebral cavernous malformations (CCMs) are focal dysmorphic blood vessel anomalies that predispose patients to hemorrhagic stroke and epilepsy. CCMs are sporadic or inherited and 3 genes (CCM1, CCM2, and CCM3) have been identified. However, the role of somatic mutation in CCM genesis has been disputed. The hypothesis that somatic mutations contribute to CCM lesion genesis is tested.

Methods—Mutations were identified by analysis of polymerase chain reaction (PCR) products spanning the 16 CCM1 coding exons with denaturing high-pressure liquid chromatography (DHPLC), cloning, and sequencing. Somatic mutation was verified 3 ways in lesion DNA and RNA samples. The somatic and germ line mutations were shown to be biallelic using allele specific reverse-transcribed PCR amplification and sequence analyses.

Results—A somatic 34-nucleotide deletion in CCM1 is identified in a CCM lesion along with a germ line CCM1 mutation (Q455X). The somatic mutation is not present in DNA or RNA isolated from the patient’s blood. These 2 genetic hits are biallelic.

Conclusions—Identification of biallelic CCM1 somatic and germ line truncating mutations strongly support the “two-hit” mechanism in this CCM lesion. (Stroke. 2005;36:872-874.)

Key Words: genetics ■ mutation ■ stroke, hemorrhagic ■ vascular malformations

A utosomal-dominant cerebral cavernous malformation (CCM) (OMIM #116860) is caused by germ line mutations in CCM1, CCM2, or the yet to be identified CCM3 gene. CCM lesions are found in 0.5% to 1% of the population.1 The “two-hit” mechanism of CCM formation is consistent with findings of new CCM lesions,2,3 as well as multiple lesions in familial CCM cases and single lesions in sporadic cases. The “two-hit” model of CCM lesion genesis has been difficult to prove. One report found 2 different somatic CCM1 mutations in DNA from a single CCM lesion that are predicted to change amino acids.4 However, it is not known whether these 2 somatic mutations lead to a truncated CCM1 protein as in all 90 different germ line mutations described to date,1,5,6 only 4 of which involve amino acid changes.7,8 In addition, it is not clear whether these somatic mutations are biallelic and in the same cells. In another study, somatic CCM1 mutations were not found in 72 CCM lesions (6 with multiple lesions), although the methods used may have lacked sufficient sensitivity.9

Materials and Methods

Samples
Informed consent was obtained and DNA isolated from blood and 3 pieces from a surgically excised CCM (QiAamp DNA Mini Kit; Qiagen Inc). Tissue (TRI Reagent; Molecular Research Center, Inc, Cincinnati, Ohio) and blood (mRNA Isolation Kit for Blood/Bone Marrow; Roche Molecular Biochemicals, Mannheim, Germany) RNAs were isolated.

Mutation Detection
Denaturing high-pressure liquid chromatography and automated sequence analyses of polymerase chain reaction (PCR) CCM1 exons 5 to 20 (accession number AC000120 and AF296765) products were used for mutation identification. Tissue, exon 15, had a subtle denaturing high-pressure liquid chromatography change and was fractioned and cloned (TOPO TA Cloning Kits with One Shot TOP10 Chemically Competent Escherichia coli; Invitrogen, Carlsbad, Calif).

Results

Tissue Sample
A 38-mm CCM lesion in the right dorsal midbrain was surgically excised because of significant hemorrhage (Figure 1A) and shown histologically to be a CCM (Figure 1B). Seven additional CCM lesions were visualized with magnetic resonance imaging. The patient has 4 other affected family members.

Mutations
DNA from blood and tissue from individual 354 showed a CCM1 C1363T germ line transition (Q455X) in exon 14 that has been identified in other Hispanic American individuals.1 A somatic 34-bp deletion in exon 15 was found in tissue DNA. This mutation was predicted to disrupt the open reading frame (Figure 2A to 2D).

Cells with Somatic Mutation
PCR products generated with a primer set outside the deletion resulted in 2 bands; the lower deleted allele was barely visible on agarose gel (not shown). Proportions of deleted and normal alleles were determined by denaturing high-pressure liquid chromatography-resolved peak heights (not shown). One sample from the end of the lesion evidenced no deletion, yet the deletion was found in 13.5% to 21% of the DNA (27% to 42% of cells) isolated from 2
other regions from the middle of the lesion, suggesting the somatic mutation is limited to a particular cell type and the ends of the lesion may include normal vessel.

**Verification**

An allele-specific primer spanning the deletion junctional fragment allowed preferential amplification of the deleted allele found only in tissue DNA (169 bp) and RNA samples (204 bp) (Figure 2B lane 1 and 2D lane 3, respectively). The wild-type allele is detected in the absence of the deleted allele in blood DNA (203 bp) and RNA (238 bp) from the patient (Figure 2B to 2E). The allele-specific primer (AGCTGAGGTGTT-TGCTTT/TG) includes 19 nucleotides common to both wild-type and deleted alleles and 2 nucleotides specific to the other end of the deleted junctional fragment. The RNA result is independent from the DNA results and not caused by PCR contamination.

**Biallelic Somatic and Germ Line Mutations**

The CCM1 mutations were biallelic in the tissue (Figure 3). Bidirectional sequence analysis of reverse-transcribed PCR products amplified specifically from the deleted allele revealed only wild-type sequence at exon 14 nucleotide 1363 (Figure 3A). Sequence near the exon 15 primer (−34 junction) confirmed that the generation of this lesion reverse-transcribed PCR product was only from transcripts with the deletion (not shown). Sequence analysis of the PCR product generated using an exon 15 primer within the 34-nucleotide deletion (GAGGATC- GATTAGTCAATTC) that anneals only to wild-type exon 15 alleles indicates lesion transcripts are heterozygous for the exon 14 C1363T mutation (Figure 3B). The nondeleted reverse-transcribed PCR products included transcripts from surrounding normal cells in the lesion.

**Discussion**

Although both mutations produced detectable transcripts in the lesion, they presumably truncate the 736-amino acid CCM1 protein to 454 and 525 amino acids. Consistent with a “2-hit” mechanism, these mutations are biallelic in the same

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**Figure 1.** A, Preoperative T1-weighted magnetic resonance imaging scan showing hemorrhaged CCM (arrows). B, CCM histopathology. Red indicates blood-filled vascular caverns (arrows), lined by endothelium (nuclei are black). Collagen is blue. Scale bar, 500 μm, 4× magnification.

**Figure 2.** A, Somatic mutation changes open reading frame. *Stop codon. Amino acids indicated by capital letters, nucleotide sequences indicated by lowercase letters.* B, Agarose gel of CCM1 exon 15 PCR DNA products. DNA Exon 15. Lane 1 shows lesion (169-bp deletion). Lane 2 shows blood (203-bp wild-type [wt]). Lane 3 shows clone with deletion (169-bp). Lane 4 shows 123-bp size standard. C, Expected intron 15 to exon 15 PCR products from genomic DNA using deleted allele-specific primer (top) versus less specific amplification of the wt allele (bottom). D, Agarose gel of reverse-transcribed PCR product RNA Exon 14 to 15. Reverse-transcribed PCR products. Lanes 1 and 4 show 100-bp size standard. Lane 2 shows blood (238 bp). Lane 3 shows lesion (204-bp). E, Schematic of the expected exon 14 to 15 reverse-transcribed PCR products from the deleted allele (top) and the wt allele (bottom).
cells and likely result in the complete loss of function of CCM1 protein in cells critical to maintaining vascular integrity. Identification of two CCM1 hits in a fraction of cells within a CCM lesion supports the hypothesis that CCM genesis is recessive at the cellular level, requiring homozygous inactivation as a primary event followed by clonal expansion to form dysmorphic vascular caverns. Further investigations are warranted to determine how common this mechanism is. Other mechanisms likely contribute to CCM genesis as well.

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