Cerebral cavernous malformations: clinical insights from genetic studies

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Familial disease is responsible for one third to one half of cerebral cavernous malformation (CCM) cases presenting to clinical attention. Much has been learned in the past decade about the genetics of these cases, which are all inherited in an autosomal dominant pattern, at three known chromosome loci. Unique features of inherited CCMs in Hispanic-Americans of Mexican descent have been described. The respective genes for each locus have been identified and preliminary observations on disease pathways and mechanisms are coming to light, including possible explanations for selectivity of neural milieu and relationships to endothelial layer abnormalities. Mechanisms of lesion genesis in cases of genetic predisposition are being investigated, with evidence to support a two-hit model emerging from somatic mutation screening of the lesions themselves and from lesion formation in transgenic murine models of the disease. Other information on potential inflammatory factors has emerged from differential gene expression studies. Unique phenotypic features of solitary versus familial cases have emerged: different associations with venous developmental anomaly and the exceptionally high penetrance rates that are found in inherited cases when high-sensitivity screening is performed with gradient echo magnetic resonance imaging. This information has changed the landscape of screening and counseling for patients and their families, and promises to lead to the development of new tools for predicting, explaining, and modifying disease behavior.

KEY WORDS • cavernous malformation • hemangioma • angioma • genetics • genomics

Cerebral cavernous malformations are discrete multilobed vascular malformations that consist of a cluster of thin-walled vascular sinusoids. Lined by a single layer of endothelium, they lack intervening neural parenchyma or identifiable mature vessel-wall elements. Histological analysis shows the lesions to lack an arterial wall smooth muscle layer and frequently reveals a peripheral hemosiderin deposition suggestive of chronic hemorrhage, a CCM hallmark. Electron microscopy analyses have implicated defective endothelial tight junctions as a potential explanation for the propensity for hemorrhage seen in these lesions.

Cavernous malformations are most commonly found in the cerebral cortex, although they may also occur in the brainstem, spinal cord, retina, cranial nerves, and cerebral ventricles. Although CCMs are often clinically silent, patients with these lesions may present with hemorrhage, focal neurological impairment, headache, and/or seizure. Cerebral cavernous malformations may also predispose patients to hemorrhagic stroke and epilepsy. The prevalence of these lesions has not been clearly elucidated. Data gathered from autopsy studies and retrospective cohort studies suggest that CCMs comprise from 5 to 13% of all cerebrovascular malformations and that from 0.3 to 0.6% of the general population harbors these lesions.

Molecular Genetics of CCMs

Researchers have made considerable progress in understanding the genetics of CCMs by focusing on familial forms of the disease, although both familial and sporadic cases are recognized. Using a positional cloning strategy founded on linkage analysis, investigators have identified three loci in inherited CCMs: CCM1 on human chromosome arm 7q; CCM2 on 7p; and, most recently, CCM3 on 3q.

In the general population, CCM1 is thought to be responsible for approximately 40 to 50% of inherited cases of CCM, and CCM2 and CCM3 are estimated to account for 10 to 20% and 40% of cases, respectively. The prevalence of CCMs is higher in Hispanic-Americans than in other ethnic groups. Gunel and colleagues compared the segregation of genetic markers and clinical cases of CCM in Hispanic-American kindreds with familial disease and concluded that virtually all cases of familial and sporadic CCM in this population were due to the inheritance of the same mutation from a common ancestor. The apparent paradox of sporadic cases being due to the same mutation as familial cases can be explained by incomplete penetrance and misdiagnosis of disease, especially in ear-

Abbreviations used in this paper: AVM = arteriovenous malformation; CCM = cerebral cavernous malformation; cDNA = complementary DNA; ICAP = integrin cytoplasmic domain-associated protein; Ig = immunoglobulin; KRIT = Krev interaction trapped; mRNA = messenger RNA; VEGF = vascular endothelial growth factor; STA = superficial temporal artery.
The use of gene, the first tumor suppressor gene. Several groups of mutation) or environmental effect predisposing individuals who are heterozygous for an inherited mutation exhibit a predilection for lesions. \( \text{CCMs} \) after craniospinal irradiation. Inherited \( \text{CCMs} \) are typically multiple lesions, whereas sporadic cases are mostly solitary lesions and often occur in association with developmental venous anomalies. Sporadic cases of multiple lesions may be due to unrecognized familiality (occult germ line mutations as seen in many cases involving Hispanic patients of Mexican descent), multiple \( \text{CCMs} \) in association with a single developmental venous anomaly (Fig. 2), or multiple \( \text{CCMs} \) after craniospinal irradiation. Several groups of researchers have supported this premise by demonstrating a distinct absence of \( !CCM1 \) or \( !CCM2 \) mutations in cohorts with sporadic single \( \text{CCM} \) lesions.

**Two-Hit Mechanism**

The pathogenesis of \( \text{CCMs} \) has been proposed to follow the two-hit model proposed by Knudson and colleagues in their description of retinoblastoma formation caused by mutations in the \( \text{RB} \) gene, the first tumor suppressor gene discovered. The two-hit mechanism has also been demonstrated in cyst formation in autosomal dominant polycystic kidney disease. This model, both alleles coding for a particular gene or factors affecting their function must be rendered inactive before lesion formation occurs.

Although germ line mutation of both copies of the \( \text{CCM} \) gene is embryonic lethal, individuals who are heterozygous for an inherited mutation exhibit a predilection for disease formation but require a second somatic mutation (second hit at the lesion site) for a \( \text{CCM} \) lesion to develop.

The mechanism of biallelic somatic mutation has recently been confirmed in humans. It is possible that the second hit might arise from mutation of any factor in a related disease pathway or in another disease gene (transheterozygous mutation), or from a genetic cause (for example, a \( p53 \) mutation) or environmental effect predisposing to mutation (for example, radiation exposure). Indeed, mice heterozygous for \( !CCM1 \) are more likely to develop \( \text{CCMs} \) if they also have a mutation of a \( p53 \) suppressor gene (Fig. 3).

The \( \text{CCM1} \) Locus at 7q

The \( \text{CCM1} \) gene was positionally cloned by linkage, haplotype, and mutation analyses mainly in patients with familial \( \text{CCM} \) and a Hispanic-American ancestral disease haplotype in the 7q11.2–21 region and a common mutation in \( \text{CCM1} \) that encodes the \( \text{CCM1} \) or \( \text{KRIT1} \) protein. The \( \text{CCM1} \) gene consists of 20 exons spanning 45,799 base pairs and maps to the 7q11.2–21 region. The start of translation seems to be in exon 5, and approximately 88 different germ line mutations distributed throughout the \( \text{CCM1} \) gene have been described in association with \( \text{CCM} \) in several different racial groups. The mutations in \( \text{CCM1} \) described to date are not likely to result in premature truncation of the \( \text{CCM1} \) protein with loss of function. Germ line \( \text{CCM1} \) mutations have been identified in apparently sporadic \( \text{CCM} \) cases that were caused by unrecognized familial or spontaneous germ line mutations.

**Fig. 1.** Axial gradient-echo MR image revealing multiple small lesions (small arrows) not seen on conventional T1- and T2-weighted MR sequences. The larger cerebellar lesion, which was symptomatic (large arrow), was seen on T1- and T2-weighted images and was thought to be solitary. Gradient echo sequences enhance detection for \( \text{CCM} \) lesions, leading to greater estimates of lesion multiplicity and penetrance in familial disease.
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![Fig. 2](image_url) Axial T2-weighted MR image showing a venous developmental anomaly associated with CCM lesions. Most cases of CCM associated with gross venous developmental anomaly are not associated with known germ line mutations or genetic disease.

result in impaired endothelial cell junctions during a critical phase of angiogenesis, and hence result in the dilated leaky capillaries of CCM lesions.

The CCM2 Locus at 7p

Recently, two independent groups identified a novel gene at the CCM2 locus by means of sequencing of positional candidate genes and loss-of-heterozygosity mapping. This gene, MGC4607, encodes a protein similar to the KRIT1 binding partner ICAP-1α; this protein, which contains a phosphotyrosine-binding domain, may be part of the complex pathway of integrin signaling that, when perturbed, causes abnormal vascular morphogenesis in the brain, leading to CCM formation.

It is likely that in addition to functioning as an ICAP1 binding partner, CCM1 influences CCM2 function. There is evidence to suggest that KRIT1 and the CCM2 protein (malcaverin) interact and share a common functional pathway. The CCM1/CCM2 association is dependent upon the phosphotyrosine-binding domain of CCM2. This interaction between these two proteins appears to be critical for p38 mitogen-activated protein kinase activation and/or for regulation of integrin-mediated adhesion by ICAP1. This premise is supported by the observation that the familial CCM2 missense mutation LI98R disrupts the CCM1–CCM2 interaction, suggesting that this interaction is pertinent to the pathogenesis of CCM.

The p38 signaling pathway plays an essential role in the pathogenesis of CCM, and mutations of the p38α gene have lead to embryonic death in mice due to placental defects ascribed to decreased vascularity, increased apoptosis, and aberrant angiogenesis.

Although the molecular functions of the CCM genes have begun to be elucidated, it remains unclear which cells within the brain ultimately result in the formation of CCMs. Uncertainty remains as to whether cavernous malformations are caused by a defect intrinsic to the endothelial cells or by defects in the brain parenchyma that surrounds the vessels. In an attempt to answer this question, Plummer and colleagues used the gene trap allele and in situ hybridization to characterize the expression pattern of CCM2 in the adult mouse brain. They found that CCM2 expression in the adult brain is primarily neuronal with additional expression in choroid epithelium and that CCM2 is not expressed at significant levels in vascular endothelium within the brain.

Therefore, cavernous malformations probably arise from abnormalities in surrounding neuronal and glial cells rather than any defect intrinsic to the endothelium.

The CCM3 Locus at 3q

Identification of PDCD10 as the CCM3 gene has been one of the most recent advances in unraveling the pathogenesis of CCMs. Using loss-of-heterozygosity mapping, Bergametti and colleagues found seven distinct mutations in eight unrelated families included on the basis of a negative KRIT1 (CCM1) and MGC4607 (CCM2) mutation screening. The nature of some of these mutations, particularly the deletion of the whole gene observed in one family, strongly suggests that one of the mechanisms that leads to cavernous angiomas might be PDCD10 haploinsufficiency.

On the basis of multiloci linkage data, investigators have...
suggested that in 40% of families with CCMs, the CCMs are linked to the CCM3 locus.9 Because the families screened in this study were included on the basis of negative results of KRT1 (CCM1) and MGC4607 (CCM2) mutation screening, a higher proportion of families with a PDCD10 mutation would have been expected. Several hypotheses have been proposed to explain this paradox, including the possibility that a fourth CCM locus may exist.4,30,59

Differential Gene Expression

Differential gene expression can be measured at the level of transcription (mRNA) or protein and can be used for both confirmation and discovery of gene involvement in disease. Gene transcription is quantitated on cDNA or oligonucleotide arrays allowing simultaneous assessment of the expression levels of thousands of genes. Complementary DNA arrays are generally more sensitive for measuring less abundant mRNA, and oligonucleotide arrays are generally more gene-specific due to less cross-hybridization. Gene-specific cDNA or oligonucleotide arrays are attached to quartz wafers, glass slides, or nylon membranes, and hybridized with cDNA that has been reverse transcribed from mRNA isolated from the tissues of interest. Hybridization can be separate, one chip per isolated mRNA or combined with one color for each cDNA set. Gene expression array results should provide confirmation that the known disease genes may actually be differentially expressed and may implicate previously unsuspected genes, including genes with unknown functions.

Results from gene expression array experiments must be verified independently using Northern Blot analysis and quantitative reverse transcription polymerase chain reaction to confirm gene expression differences and followed up with in situ hybridization using gene-specific probes; hybridizing to tissue sections is necessary to characterize tissue-specific gene expression. Confirmed differential gene expression does not necessarily mean that the protein is present at higher levels, and promising findings should be extended to include quantification of protein expression. Identifying differential protein expression is more labor intensive than measuring gene expression and depends on optimal separation of proteins. Proteomics allows the identification of hundreds of proteins, and mass spectrometric and other methods are now available for reliable and rapid quantification and identification of differentially expressed proteins in biologic tissues. When antibodies are available for specific proteins, immunoprecipitation, Western blot analysis, and immunohistochemical methods can verify protein quantification and determine tissue-specific expression. Proteomic studies in general would corroborate only a subset of the differential gene expression results (only proteins present in sufficient quantities that they can be resolved using two-dimensional gel electrophoresis), and gene transcription levels do not always correlate with protein levels.55

Gene and protein expression data are compiled and analyzed with computer programs, and the statistical analyses take thousands of comparisons into account. Normalization of expression levels per unit of tissue or mRNA, appropriate controls for differential expression, and the problem of tissue heterogeneity must each be considered in analyzing and interpreting the data. Advanced bioinformatics methods are being developed, including complex statistical approaches that can factor groups of functionally related genes and proteins (such as those involving related pathways), rather than assuming independent expression of each gene and protein.

The expectation that some vasculogenesis-, angiogenesis-, and disease-related genes are differentially expressed in cerebral vascular malformations has been partially confirmed.51,52 Preliminary gene-expression results identified 310 upregulated and 558 downregulated genes in both AVMs and CCMs compared with STAs (p = 0.012), including differences in genes involved in growth factor signaling (decreased ANGPT1; increased VEGF [a trend]; and increased ENG, endoglin, a TGFB receptor component), decreases in a cell adhesion gene (PECAM1, alias CD31), decreases in an endothelium-specific gap junction gene (GJA4), and decreases in extracellular matrix genes (LAMA3 and SMTN).51,52 Increased protein expression measured by immunohistochemical analysis of VEGF54,46, 54,55 and decreased expression of LAMA3 and SMTN in
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both AVM and CCM in comparison to STA is consistent with results of transcript quantitation. In addition, CCMs showed unique decreased expression of a VEGF receptor (KDR), cell adhesion molecules involved in integrin signaling (ITGB3, integrin β3; ROCK1) in comparison to AVMs and STAs. The AVMs showed specific differential gene expression of growth factor signaling molecules (increased FLT1, a VEGF receptor; decreased TIE and TEK, angiopoietin receptors), decreased integrin signaling molecules (ITGB5, integrin β5; ITGA6, integrin β6; CTNNAL, α-catenin) and decreased CCM1 gene expression.21 Notably, probe sets for 10 Ig genes and a distinct allele of the major histocompatibility complex (DOB1*06011) were upregulated in CCMs compared with AVMs and STAs, revealing possible evidence of a unique immune response within the CCM lesions. Expression for Ig genes that were upregulated in CCMs included the IgA heavy-chain gene of two accession numbers (AF067420 and S71043, with 26- and 17-fold increases, respectively); the IGJ gene (AI660656) for the IgJ chain with a 23-fold change; the IgGH3 gene (Y14737) for the heavy chain of IgG subclass 3 with a 20-fold change; three Ig light-chain genes, the IGL3 gene (M18645) with a 15-fold change; the IgL gene (X57869 and AF058075, with changes of 15- and threefold, respectively); the IGKC gene (M63438 and X72475, with changes of 14- and 12-fold, respectively); the IGHM gene (AI147237) for the heavy chain of IgM with a ninefold change; the Ig-related LOC91316 gene (A932613) with a 22-fold change; and the gene (U80114) for the hypothetical Ig MGC 27165 with a sixfold change. The increases in expression of the Ig genes were among the highest in the microarray analysis. In addition, the gene for the IgG Fc (CD64) receptor was upregulated in CCM with a 2.4-fold change. Among the upregulated genes in CCMs, the greatest degree of upregulation (43-fold) was for a probe set (M16276) for a 3′ untranslated region of the DOB1*06011 allele of the gene for HLA-DQB. Numerous observations have bolstered our confidence in the microarray results: 1) significant differential expression was shown in the microarrays for genes coding angiogenesis factors, receptors, and structural proteins that had previously been determined through immunohistochemical analysis to be expressed differentially in CCMs and AVMs; 2) downregulation of the expression of mature vessel wall components in CCMs, such as myosin, actin, calponin, desmin, transgelin, and filamin A endothelial actin binding protein; 3) differential expression of Igs was shown in CCMs but not in AVMs; and 4) constant expression of hemoglobin and leukocyte markers. The mean values ± the standard deviations for the expression in AVMs, CCMs, and STAs, respectively, were 109,144 ± 66,212, 62,611 ± 7,520, and 51,385 ± 18,489 for hemoglobin B and 264 ± 28, 246 ± 74, and 207 ± 33 for the B-cell marker CD19.

The CCM phenotype predisposes to vascular leakage and accumulation of blood products in adjacent brain tissue. This situation may create a special milieu for antigenic challenge and immune response, which may in turn contribute to lesion proliferation and clinical manifestations. The extent of inflammatory cell infiltration within lesions could motivate future studies on the imaging of these cells in vivo, with the aim of monitoring and modifying biological activity in the lesions.

Conclusions

The identification of the three CCM genes has provided significant insight into the molecular basis of CCM formation. Coupled with our knowledge about the mechanisms of vasculogenesis and angiogenesis, our understanding of the pathogenesis of these lesions is steadily expanding. The convergence of clinical and biological information holds promise of a new era in which clinicians will be better able to translate scientific advancement to clinical applications for the treatment of this lesion.

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References


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