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Cerebral cavernous malformations: from genes to proteins to disease

Clinical article

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Over the past half century molecular biology has led to great advances in our understanding of angio- and vasculogenesis and in the treatment of malformations resulting from these processes gone awry. Given their sporadic and familial distribution, their developmental and pathological link to capillary telangiectasias, and their observed chromosomal abnormalities, cerebral cavernous malformations (CCMs) are regarded as akin to cancerous growths. Although the exact pathological mechanisms involved in the formation of CCMs are still not well understood, the identification of 3 genetic loci has begun to shed light on key developmental pathways involved in CCM pathogenesis. Cavernous malformations can occur sporadically or in an autosomal dominant fashion. Familial forms of CCMs have been attributed to mutations at 3 different loci implicated in regulating important processes such as proliferation and differentiation of angiogenic precursors and members of the apoptotic machinery. These processes are important for the generation, maintenance, and pruning of every vessel in the body. In this review the authors highlight the latest discoveries pertaining to the molecular genetics of CCMs, highlighting potential new therapeutic targets for the treatment of these lesions. *(DOI: 10.3171/2011.8.JNS101241)*

Key Words • **cerebral cavernous malformation** • **stroke** • **gene** • **protein** • **cerebrovascular disease** • ***CCM1*** • ***KRIT1*** • ***CCM2*** • ***MGC4607*** • ***CCM3*** • ***PDCD10*** • **vascular disorders**

The estimated prevalence of stroke in the US in 2006 was 6,400,000 cases. Although ischemic stroke is by far the more frequent presentation, 10% of all strokes are hemorrhagic in nature.[62] One of the causes of hemorrhagic stroke is the presence of vascular malformations with friable vessels that are prone to rupture due to changes in systemic blood pressure or stressors. Cerebral cavernous malformations account for 5%–15% of all vascular malformations in the CNS.[19,31,63,73,84] In this review we focus on the entity of the CCM in terms of the causal interactions of genes and proteins that lead to disease. For the reader it is important to note terminology: mouse and other animal genes are notated differently from their human counterparts. For example, *CCM2* names the human gene, while *Ccm2* is the *Mus musculus* (mouse) gene and *ccm2* is the *Danio rerio* (zebrafish) gene. The protein is signified by CCM2.

Cavernous malformations are slow-flow anomalies characterized by densely packed vascular sinusoids embedded in a collagen matrix without intervening neural tissue.[71,86] Cavernous malformation clusters are well defined and consist of enlarged capillary channels, lined by a thin endothelium and rare subendothelial cells, without smooth muscle and elastic tissue (Figs. 1 and 2). The capillary channels of cavernous malformations may be filled with blood at various stages of thrombosis and organization, producing a mulberry-like appearance.[11] Generally, CCMs lack tight junctions between endothelial cells and astrocyte foot processes, have diminished laminin and collagen IV within their endothelial cells, and are associated with a hypertrophic surrounding basal lamina.[12,42,109]

Cavernous malformations are usually occult lesions that are discovered by an episode of symptomatic hemorrhage causing intraluminal thrombosis and usually subsequent recanalization.[97,110] Usually, patients clinically present with CCMs between the 2nd and 5th decades of life, but symptoms can start in early infancy or in old age.[16,73,84,110] Following their first episode of hemorrhage, patients with

Abbreviations used in this paper: BAC = bacterial artificial chromosome; CCM = cerebral cavernous malformation; ERK = extracellular signal-regulated kinase; ICAP-1 = integrin cytoplasmic domain–associated protein 1; lod score = logarithm of odds score; MAPK = mitogen-activated protein kinase; MEKK3 = MAPK-ERK kinase kinase 3; MLPA = multiplex ligation-dependent probe amplification; PAC = plasmid artificial chromosome; PTB = phosphotyrosine binding; YAC = yeast artificial chromosome.

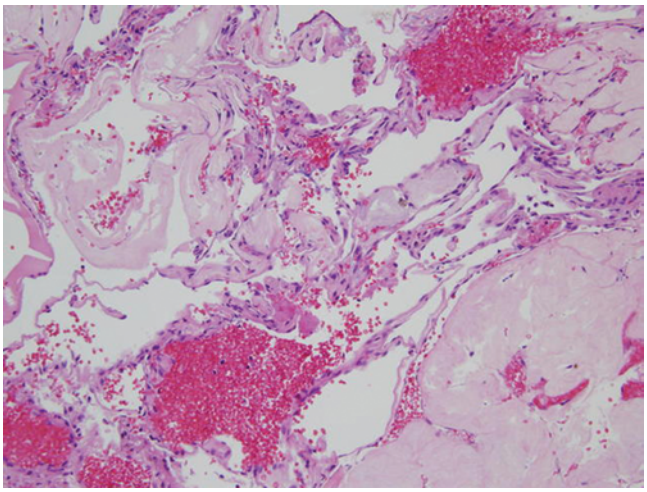

Fig. 1. Photomicrograph of CCM section highlighting the enlarged capillary channels lined by a thin endothelium, a characteristic finding in this disease. H & E. Original magnification × 40.

CCM may be relatively asymptomatic or they may be neurologically devastated. The onset of symptoms is usually abrupt, although patients may present with gradual and nonspecific symptoms. The most common presenting signs include headaches, seizures, and focal neurological deficits caused by cerebral hemorrhages. Seizures are the most common symptom in various series, representing 40%–60% of the symptoms at presentation.[20,30,93,100,110]

The prevalence of CCM in the general population has been estimated to be about 0.3%–0.5%, accounting for approximately 24 million people worldwide. In the US, more than 1 million people (mostly of Hispanic origin) are known to harbor CCMs and are subject to a 1%–5% per year cumulative risk (estimated 50%–70% lifetime risk) of hemorrhage, epilepsy, and other neurological sequelae.[16,71,76,84] In youth, CCM hemorrhage accounts for more than 10% of intracerebral hemorrhages.[1,25,74,76]

Cerebral cavernous malformations can be both sporadic and familial (further discussion to follow).[5,11,49,53,72] Approximately half of CCM cases are familial in nature and are inherited in an autosomal dominant fashion with variable penetrance.[5,43,83,110] Labauge et al.[55] demonstrated that 75% of sporadic cases of CCMs are actually familial cases. Similarly, others have identified familial mutations in nearly 60% of sporadic cases with multiple lesions, further demonstrating the variable penetrance of this autosomal dominant trait.[18]

Familial CCMs are characterized by the presence of multiple lesions identified on cerebral MR imaging.[83,110] A multiplicity of lesions is characteristic in up to 84% of familial cases,[20,37,43,57,110] whereas it is reported in 10%–33% of supposed sporadic cases.[16,83,84] The annual symptomatic hemorrhage rate in familial cases can reach 6.4%,[110] a significantly higher rate than reported in series of sporadic cases (range 1.6%–3.1%).[51,73,81]

Hispanic populations of Mexican descent appear to be more susceptible to dominantly inherited CCMs.[4,43,69,110] Linkage mapping in family-based studies in the mid-1990s aided in localizing the first gene responsible for CCMs to the long arm of chromosome 7.[54,68] Gunel et al.[38]

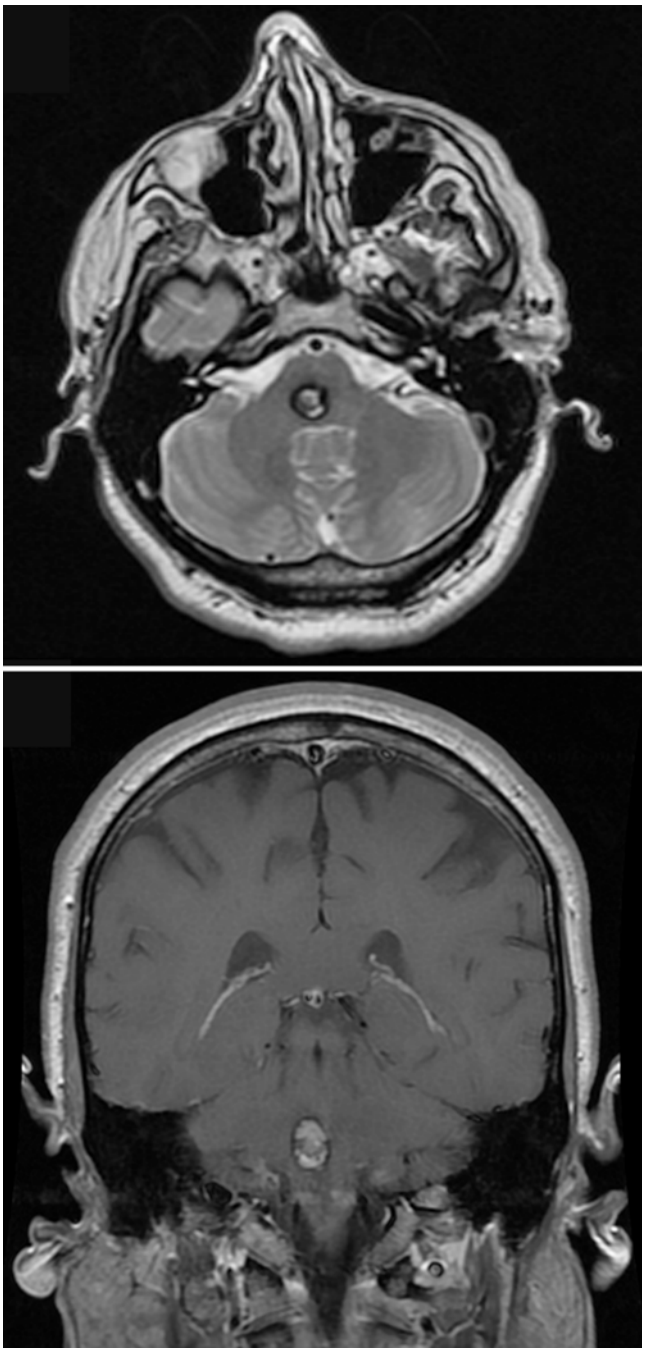

Fig. 2. Axial **(upper)** and coronal **(lower)** T1-weighted MR images of a cavernous malformation in the brainstem. The lesion demonstrates the heterogeneous signal characteristics of hemorrhage in various stages of resolution.

identified a strong founder effect when studying several unrelated Hispanic kindreds affected by familial CCM. This study and others identified identical haplotypes over a region of at least 22 cM over the short segment of chromosome 7q.[29,46] A large study assessing French families also revealed linkage to the *CCM1* locus, but without a founder effect.[56]

Although the *CCM1* locus initially explained the cause of CCMs in certain cohorts, it became apparent

that these malformations were genetically heterogeneous and caused by mutations at other loci. The identification of 2 additional loci on 7p and 3q partially reconciled clinical findings in other patient populations.[13] To date, 3 distinct loci have been mapped in different families, and 3 CCM genes, namely *CCM1*/*KRIT1*, *CCM2*/*MGC4607,* and *CCM3*/*PDCD10,* have been identified. Germline mutations in these 3 genes have been shown to lead to the development of CCM. Although the precise functions of these genes are still not fully understood, they seem to orchestrate angiogenesis throughout embryonic development[12,39,57,106] and vascular pruning in the postnatal stages of development.

The 3 identified loci account for 70%–80% of all cases of familial CCMs.[18,23,26] Multilocus linkage analysis showed that *CCM1* accounts for nearly 40% of inherited cases, *CCM2* for 20%, and *CCM3* for 40%.[3,8,13,17] However, genetic screening data have revealed a higher frequency of *CCM2* mutations; a lower frequency of mutations in *CCM3* has also been reported as well, suggesting the existence of additional genes involved in the pathogenesis of CCM.[18,59,60] Clinical penetrance has been shown to vary according to the involved locus, with an estimated 100% penetrance in *CCM2* families and 63% penetrance in *CCM3* members.[13] Clinical penetrance in *CCM1* families varies between 60% and 88%.[15] Generally, kindreds affected with *CCM1* mutations exhibit little clinical variance,[64] but this is not the rule.[37,38]

The products of CCM genes, CCM1/Krit1, CCM2/malcavernin, and CCM3/PDCD10, have been shown to be specifically expressed in the endothelium, neurons, and astrocytes and their foot processes.[91,96] Negative immunostaining of defective CCM proteins suggests that the endothelial cells are the cell of origin for causing CCM.[32,77]

Identification of a Genetic Locus Responsible for CCM Formation: *CCM1*

In 1982, an initial attempt at linkage mapping of the CCM genes by using 12 biochemical and serological markers in 36 Hispanic individuals was unsuccessful.[21] In 1994, Kurth et al.[54] mapped a gene for CCM to the q11-q12 region of chromosome 7 by using linkage analysis and short tandem repeat polymorphism analysis in a large Hispanic family. Broad shared haplotypes among affected individuals in this group allowed for further restriction of the gene to an interval of approximately 33 cM on the map from D7S502 to D7S479. Concurrently, Marchuk et al.[68] identified linkage between CCM and genetic markers on the proximal long arm of chromosome 7 in studying a Hispanic and an Italian-American family. They identified a locus between D7S502 and D7S515, with an interval of 41 cM as harboring the gene of interest. This interval was further refined to 15 cM by Gil-Nagel et al.[29] who bracketed the interval of interest between D7S660 and D7S558 while studying a large 4-generation non-Hispanic family harboring the familial form of the disease. Performing multipoint linkage analysis, Günel et al.[37] found an lod score of 6.88 for linkage of CCM and locus D7S699 at a recombination fraction of 0. Moreover, their data placed the CCM gene in a 7-cM region within an interval centromeric to D7S802 and telomeric to *ELN* (elastin) in 7q11.2-q21.

Further refinement of the techniques for the large-scale study of DNA allowed the region likely to contain the *CCM1* gene to be reduced to a 4-cM segment of the human 7q21-q22. It was later shown that D7S2410 and D7S689 bounded the *CCM1* critical region.[34,35,46] Using a YAC-based sequence-tagged site content mapping strategy, all markers within the refined chromosomal segment were located on a single YAC contig estimated to be 2 Mb in size.

The *CCM1* gene was finally identified with a positional cloning strategy based on genomic sequencing of the involved candidate interval within the human chromosome, using then-new microsatellite markers to provide fine haplotype analysis of families linked to the *CCM1* locus.[88] A detailed physical map of chromosome 7 was crucial to identify the disease gene, *KRIT1* or *CCM1*.[7] Aligning the genomic DNA sequence with the *KRIT1* cDNA sequence again revealed its organization in 12 exons distributed over approximately 37.7 kb of DNA. Fluorescent in situ hybridization allowed *KRIT1* cDNA to be mapped to 7q21-q22.[92] Interestingly, the 7q21-q22 locus is often deleted or amplified in various malignant tumors.[48,75]

In 1999, identification of the *CCM1* gene was confirmed in French families with hereditary CCM.[57] Using a published YAC contig and genomic sequence information on chromosome 7,[7] BAC and PAC contigs spanning the *CCM1* interval were constructed. Twenty families with a high probability of linkage to *CCM1* were assessed for high-resolution mapping of this locus, with polymorphic markers identified in BAC/PAC sequences. Using this technique, investigators were successful in reducing the previously determined interval from MS2456 and D7S689 because of a recombination event observed in an affected individual. In addition, they mapped 574 expressed sequence tags, clustering them in 53 potential transcriptional units comprising 6 characterized human genes, including *CCM1*. They also showed the 12 exons aligning the *KRIT1* cDNA sequence to the BAC HSAC000120 sequence.[92] Later, it was demonstrated that the gene has 20 exons, the first 3 of which are noncoding sequences.[8,22,87,113]

CCM1 encodes Krit1, a 736-amino acid microtubule-associated protein containing 4 ankyrin domains, a C-terminal FERM domain (that is, band 4.1, ezrin, radixin, moesin), and multiple N-terminal NPXY (Asn-Pro-X-Tyr) motifs.[2,39,87,114]

Seven different germline mutations within *CCM1* have been identified, consisting of 2 single base transversions and 1 transition leading to nonsense (stop) codons, 2 single base transitions (splice site mutations), and 2 single base deletions leading to frameshift mutations and premature termination; all of these changes are loss-of-function mutations. Laberge-le Couteulx et al.[57] also searched for mutations in *CCM1* in persons belonging to the aforementioned French families by using single-strand conformation polymorphism analysis, direct genomic DNA sequencing, and screening of *CCM1* cDNA

for deletions. Accordingly, they found point mutations leading to premature stop codons and deletions resulting in frameshift and premature chain termination. All of these lesions resulted in loss of function via an early termination codon, generation of unstable mRNA, or truncated Krit1 proteins completely or partially devoid of the putative Rap1A-interacting region. Screening several unrelated families from different countries, Davenport et al.[15] reported 10 new mutations related to *CCM1*, all of them leading to the loss of Krit1 protein function. Interestingly, a de novo germline mutation was reported in a patient harboring 2 CCMs, corresponding to a deletion of 2 base pairs in exon 6.[65]

Uncovering the total genomic structure of *CCM1* allowed for mapping of mutations to the critical domains in the protein product. Mutational analysis revealed that 75% of mutations in 52 probands linked to *CCM1* occurred in the C-terminus of the gene, mainly within exons 13, 15, and 17.[8] Nonsense or frameshift mutations leading to a premature stop codon are responsible for 96.2% of all mutations, with the other 2 cases causing small in-frame deletion in the C-terminus. Other studies[99] have revealed that point mutations result in the production of truncated proteins without biological function. Finally, the introduction of MLPA screening refined the identification of large deletions and duplications that might not be seen by the direct sequencing of genomic DNA.[26,89] Thus, large deletions spanning the entire coding sequence of the *CCM1* gene and producing loss of function have been reported in German families following MLPA.[26] Concurrently, Liquori and colleagues[59,61] also identified large deletions using MLPA in American and Italian CCM families that tested negative for mutations in *CCM1*, *CCM2,* and *CCM3*; only 5% of such deletions in the American cohort occurred within *CCM1,* whereas 50% of them in the Italian cohort were found within the *CCM1.* A rare multiexon duplication from exon 7 to 17 was identified in a Swiss patient.[23]

The elevated number of loss-of-function mutations leading to an mRNA decay of the mutated allele may imply that *CCM1* acts as a tumor suppressor where somatic mutations resulting in a loss of the balancing wild-type allele predispose to the loss of growth control and tumor formation.[15,57,88] For this reason and because of the dissimilarity between the characteristic multiplicity of lesions in the inherited form and the presence of a solitary lesion in a majority of sporadic cases, a 2-hit mechanism similar to the Knudson model for tumor suppressor gene (Fig. 3) has been proposed to explain CCM.[47,50,77] Indeed, further studies have revealed biallelic mutations in *CCM1* in the same cells, characterized by a germline transition within exon 14 and a somatic 34-nucleotide deletion within exon 15, truncating Krit1.[27] The consistent finding of the complete and specific absence of CCM proteins, within cavernous malformation endothelial cells in patients carrying germline mutations, supports the 2-hit model.[77]

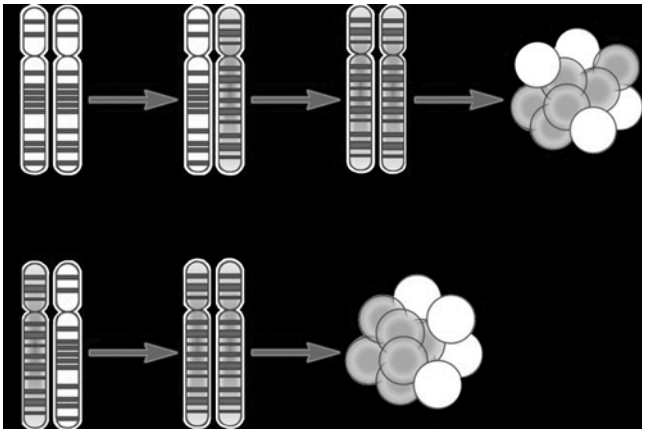

Fig. 3. Schematic representation of the Knudson 2-hit hypothesis for the generation of cancer. The Knudson hypothesis proposes that multiple "hits" are needed for the transformation of a cell into an uncontrolled state of growth. The initial genomic changes can be inherited or acquired during development. It is the accumulation of multiple genomic hits rendering critical growth and remodeling pathways that results in the loss of cell cycle control and unregulated expansion of cell populations in cancer.

Identification of Two New Loci: *CCM2* and *CCM3*

The assessment of some non-Hispanic families harboring familial CCM without evidence of involvement of *CCM1* pointed to the possibility of the involvement of other loci.[13,17]

Therefore, a genome-wide linkage search across all 22 autosomes was undertaken in 7 non-Hispanic families, which did not reveal linkage to *CCM1*.[13] After genotyping 312 loci, CCM inheritance in that group could not be linked to a single locus. When 3 large kindreds were evaluated separately, evidence began to emerge for linkage to a segment on 7p in 1 family and to a small interval on 3q in 2 others. Being aware of the 3 genetic intervals involved with CCM, a multipoint analysis of linkage was performed in 17 more kindreds, which provided an interesting distribution, with 40% of families being linked to 7q, 20% to 7p, and 40% to 3q (maximum multilocus lod score of 14.11). Such robust evidence denoted locus heterogeneity for CCM. The use of a 4-locus model initially failed to produce evidence of a fourth locus.[13]

The CCM2 *Gene*

The *CCM2* gene has been mapped to 7p15-p13, spanning a region of 11 cM defined by markers D7S2846 and D7S1818.[13] From 55 known or putative genes then identified within this region, Liquori et al.[58] selected 8 that were probably involved in the genesis of CCM (*CAMK2B, CDC10, CDC2L5, HIP-55, MGC4607, MYLC2A, RALA,* and *STK17A*). Among them, *MGC4607* contains a PTB domain and was predicted to interact with Krit1.

In 2004, Denier et al.[17] confirmed the identification of *CCM2* or *MGC4607*, a gene containing 10 coding exons on 7p13. First, these authors reduced the previously reported *CCM2* interval from 22 to 7.5 cM via genetic linkage analysis by using 32 microsatellite markers on families not linked to both *CCM1* and *CCM3*.[13] They identified 2 large deletions involving at least exon 1 as well as 8 different point mutations, all resulting in loss of function.[17] More recently, the use of MLPA allowed a significant increment in the identification of large dele-

tions within *CCM2*;[59,61] combining these data with previous DNA sequencing results produced a mutation rate of approximately 40%, indicating that the prevalence of *CCM2* is much higher than the value provided by a previous large multilocus linkage study.[13] Therefore, large mutations within *CCM2* may represent a major mechanism in the pathogenesis of CCM. Identification of a deletion specifically encompassing exons 2 to 10, primarily found in American families, was not reproduced in French, German, and Swiss series, pointing to a founder effect.[3,17,23,26,57] Altogether, the mutations encountered so far invariably lead to loss of function.

The CCM2 protein, or malcavernin, is required for normal cytoskeletal structure, cell-cell interactions, and lumen formation in endothelial cells. The 444-amino acid product of *CCM2*, containing a PTB domain, is a scaffold protein in a signaling cascade that controls the activation of p38 MAPK (by binding to MAPK kinase 3).[58,98,111,112] It also interacts with Krit1, actin, and Rac1, a small GTPase involved in signal transduction pathways that controls proliferation, adhesion, actin cytoskeleton reorganization, and migration of cells.[70,112]

The CCM3 *Gene*

Genetic analysis of patients with familial CCM helped map a third autosomal dominant locus. The third CCM locus, *CCM3*, is located on 3q25.2-27. This locus was identified within a 22-cM interval flanked by D3S1763 and D3S1262.[13] In 2005, Bergametti et al.[3] screened 20 unrelated families harboring the familial form of CCM but lacking mutations within the *CCM1* and *CCM2* loci. A high-density microsatellite genotyping of that interval allowed searching for putative null alleles, as identified in *CCM2*.[17] Once they found a de novo deletion in an interval bracketed by markers D3S3668 and D3S1614 and overlapping D3S1763, the authors could outline the interval containing the candidate gene within a 970-kb region, now bracketed by D3S1763 and D3S1614. Of 5 genes already mapped to this interval (*FLJ33620*, *GOLPH4*, *LOC389174*, *PDCD10*, and *SERPINI1*), the programmed cell death 10 gene, *PDCD10,* appeared as a likely candidate because of its putative role in apoptosis.[3]

The *PDCD10* gene was initially identified in relation to its upregulation in the TF-1 premyeloid cell line granulocyte during deprivation of the granulocyte-macrophage colony-stimulating factor.[103] The highly conserved gene has 7 coding exons and 3 noncoding ones on 3q26.1, producing a 212-amino acid protein. Examples of point mutations identified on genomic DNA sequence analysis include direct nonsense mutations and splice-site mutations leading to a frameshift.[3,60] Interestingly, the frequency of mutations in *CCM3* has been reported to be low in large studies assessing non*CCM1*, non*CCM2* kindreds,[3,18,59,60] in contrast with a 40% expected frequency in inherited cases reported in the classic aforementioned linkage analysis by Craig et al.[13] Additionally, the frequency of affected members per family is significantly lower in *CCM3* families than in families harboring *CCM1* and *CCM2*.[18]

Apparently, the CCM3 or PDCD10 protein takes part in the aforementioned complex involving Krit1 and CCM2.[91,94,101] This can be explained by the fact that apoptosis within smooth muscle cells is mediated by the β1 integrin signaling pathway,[104] which interacts with the other CCM proteins. Furthermore, Chen et al.[10] demonstrated that such protein is both necessary and sufficient to promote apoptosis, and thus CCM may originate from aberrant apoptosis, impairing the equilibrium between neural cells and endothelium within the CNS.

Are Other Genes Involved?

The existence of a fourth CCM gene was postulated after unexpected low frequencies of *CCM3* mutations had been obtained in families without a *CCM1* and *CCM2* gene mutation.[3,18,59,60] The existence of an additional gene within a short distance of the 3 other loci or missed in the original report on multilocus linkage analysis for familial CCM could explain such a fact.[3] The examination of haplotypes in kindreds linked to *CCM3* locus given that linkage mapping revealed a particular recombination event excluding *CCM3* in a family with strong linkage; *CCM4* would be located in or close to 3q26.3-3q27.2.[3,13]

A balanced translocation involving the long arms of chromosomes 3 and X, t(X;3)(q22.3;q12.3) was encountered in a patient with CCM, without any mutation within the known CCM genes.[28] After narrowing the breakpoint position to a 200-kb interval on 3q12.3, a potential interval of interest, the zona pellucida–like domain containing 1 gene *(ZPLD1)* was found to span this region. This highly conserved gene comprises 19 exons, spanning approximately 500 kb of genomic DNA. The 2 isoforms, ZPLD1a and ZPLD1b, contain 431 and 415 amino acids, respectively. Expression levels of ZPLD1 mRNA were reduced in that patient as compared with controls, with an allelic loss of gene expression. However, mutation screening of this gene in CCM families negative for mutations in known CCM genes was unsuccessful. Finally, the translocation did not promote dysregulation of the expression of any CCM genes. Future studies are necessary to assess the structure and role of *ZPLD1* products and their possible interaction with the CCM proteins and a link to a fourth locus.

Other targets of interest are the protein products of the ephrin genes. Ephrin-B2 *(EFNB2)* is associated with vascular development, mainly in arterial differentiation.[102] *Efnb2* is downregulated within arterial endothelium in mice lacking *Ccm1*, indicating a compromised arterial identity.[106] Members of the transforming growth factor β signaling pathway, notably Notch genes, have been shown to interact with *EFNB2* and to regulate critical steps during embryonic vascular development and remodeling.[52] *Dll4* and *Notch4* expressions are also downregulated within arteries in *Ccm1*$^{-/-}$ embryos, even before the onset of blood flow.[106] Importantly, a corresponding reduced expression of *Notch 4* was seen in surgical specimens obtained from patients carrying mutations in *CCM1,* suggesting that mutations in the transforming growth factor β signaling pathway may cause the formation of CCMs.

Lessons Learned From Various Animal Models of CCM

Given the rare nature of CCMs, animal models have

been the key source for studying CCM pathology. Animal models have helped to establish the role of CCM genes in vascular development and the pathobiology of CCM lesion development and to explain their focal nature. Zhang et al.[114] mapped the mouse *Krit1* gene to a region of chromosome 5 that shares homology of synteny with human 7q21-q22. Further, knockout alleles of the murine orthologs of *CCM1* and *CCM2* have been generated.[106]

Heterozygous mutant animals, which are the appropriate genotype for the autosomal dominant disorder, do not recapitulate the disease phenotype with appreciable penetrance.[106] A *Ccm1* mutant allele was generated by disrupting the gene at the first ankyrin repeat. Mice lacking *Ccm1* suffer from generalized developmental arrest after E9.5, retaining a primitive yolk sac vascular network, and thus dying by E11 with prominent vascular defects associated with inappropriate angiogenic remodeling but apparently normal neuronal development.[106] Pathological study of these animals revealed that they possessed numerous vascular dilations related to altered arterial fate and increased endothelial mitosis. The animals failed to recruit arterial smooth muscle cells and generate a normal vascular channel. Importantly, the $Ccm1^{-/-}$ model contributed to defining the role of *CCM1* in arterial morphogenesis; primary defects in such a model were vascular, without preceding impairment in neural or cardiac development.

An alternative mouse model was developed by gene trap insertion in the *Ccm2* gene. The $Ccm2^{-/-}$ mice are embryonic lethal. On the other hand, $Ccm2^{+/-}$ revealed the expected phenotype, albeit with a low penetrance.[80] Expression of *CCM2* is mostly neuronal but is also present in the choroid plexus and vascular endothelium.[40]

Based on the Knudson 2-hit mutation mechanism, Plummer et al.[79] conducted the development of heterozygous *Ccm1* mutations into mutant genetic backgrounds with increased somatic instability. One mechanism of introducing genetic instability is via the deletion of the p53 tumor suppressor gene. In many mouse models of disease, the addition of p53 mutations causes exacerbation of the phenotype of the mutated gene. The development of vascular malformations and hemangiosarcomas in the CNS due to polyoma virus infection in rats is mediated by the suppression of p53 activity associated with such a virus.[24] Additionally, the loss of the tumor suppressor gene *Trp53* (p53) leads to the development of CCM in a heterozygous mouse with a target mutation of *Ccm1*. However, no mutation in TP53 had been found while sequencing the DNA of human CCM specimens.[79] Indeed, the absence of CCM development in $Ccm1^{+/+}$ $Trp53^{-/-}$ mice also supports the notion that the isolated loss of p53 does not independently induce CCM.

$Ccm1^{+/-}$ individuals develop vascular malformations only when they are also homozygous for a loss of p53, which increases the rate of somatic mutations. $Ccm1^{-/-}$ $Trp53^{-/-}$ double-mutant mice exhibited cerebrovascular lesions in 55% of cases, whereas all other genotypes failed to show the phenotype.

The zebrafish dilated-heart mutants santa and valentine correspond to *CCM1* and *CCM2*, respectively.[67] Hogan et al.[45] found that the deletion of *ccm1* in this model expressed a phenotype similar to that of human and murine CCM. Vascular dilation was associated with the progressive spreading of endothelial cells and thinning of vessel walls during vascular morphogenesis, consisting of an alteration of cell shape but not of endothelial cell-cell adhesion, cell fate, or number. The *Ccm1* mutants, *ccm2* mutants, and *ccm1*/*ccm2* double mutants had indistinguishable vascular phenotypes, suggesting conservation of function. Finally, these authors corroborated that the CCM proteins regulate endothelial development.

Molecular Interaction of CCM Gene Products With Other Developmental Pathways

Molecular proteomic evidence suggests that the protein products of the 3 identified CCM genes in concert with other cellular machinery form ternary complexes that regulate key steps in blood vessel formation and maintenance. It is postulated that the inability to compose these protein complexes leads to the dysfunctional vascular cavities and pathology noted.

Krit1/*Rap1A*

The *CCM1* gene product, Krit1, interacts with the Ras-related protein 1A (Rap1a or Krev1), an evolutionarily conserved Ras-family GTPase encoded by the gene *RAP1A* through its C-terminal region.[78,85] One of the functions of Rap1A homologs in yeast and *Drosophila melanogaster* is to regulate GTPase signal transduction cascades participating in cell differentiation, morphogenesis, polarity, and cytoskeletal structure.[6,78,92] The Krit1 binds strongly to Rap1A. Accordingly, Serebriiskii et al.[92] initially suggested that Krit1 might help in localizing Rap1A to a proper cell compartment for function, given the common association of ankyrin repeat proteins with the cytoskeleton.[90] Indeed, several groups have identified high expression of Rap1A within vascular smooth muscle cells in various organs including the CNS[107] and have shown that structural changes altering this interaction results in an inability of endothelial cells to organize correctly and leads to the formation of dramatically enlarged blood vessels in mouse models.[44,108] Using anti-KRIT1 antibodies, Gunel et al.[39] demonstrated Krit1 expression within endothelial cells, in association with microtubules (tubulin). By interacting with microtubules and because of its particular distribution in cells during mitosis, the authors proposed that Krit1 could act in targeting microtubules. The initial stages of angiogenesis involve tube formation by endothelial cells and are modulated by cell intrinsic and extrinsic signals. The platelet endothelial cell adhesion molecule-1 (PECAM-1) is an important regulator of endothelial tubulogenesis and activates the GTPase activity of RAP1a, which ultimately interacts with the microtubule cytoskeleton.[39,82,92] Effector molecules such as Krit1 bind to Rap1A in endothelial cells, enhancing the stability of endothelial adherent junctions, and thus reducing vascular fragility.[22,111,113,114]

Krit1/*ICAP1α*/*SNX17*

The α isoform of the β1 ICAP-1 binds to Krit1 through its NPXY motif.[9] It is a modulator of β1 integrin

signal transduction and is involved in cell adhesion and migration. The binding of the α isoform of the β1 ICAP-1 to Krit1 inhibits its ability to bind to microtubules. As stated previously, Krit1 interaction with the microtubule machinery regulates cell adhesion and cell-cell junction formation. Interestingly, ICAP-1 also binds with the cytoplasmic tail of β1 integrin complex.[2] This complex is crucial in supporting endothelial cell proliferation for the formation of new vessels from vascular cores. The inability of Krit1 to bind with ICAP-1 results in ICAP-1's inability to initiate the β1 complex, and thus impairing capillary formation and/or maintenance, leading to vascular leaks.[40,41] Another player involved in this intricate protein machinery is the SNX17 protein (sorting nexin 17), a nonself-assembling and phosphatidylinositol 3-phosphate high affinity protein that interacts with ICAP-1.[14] Again, mutations that abolish ICAP-1/SNX17 interaction appear to have a similar phenotype preventing cytoskeletal recruitment. These data quickly begin to reveal a highly interconnected network of signaling events needed for the proper sprouting of new blood vessels. Loss of interaction with the cytoskeletal machinery appears to be an initial inciting event in the formation of CCM lesions (Fig. 4).

Krit1/CCM2

The critical role of Krit1 as an anchoring protein with the cytoskeletal matrix means that any deficiencies in interaction between this protein and other members of the skeletal machinery are likely to result in the development of a CCM phenotype. There is a critical interaction between the *CCM1* gene product and the CCM2/MEKK3 complex.[112] Note that *CCM2* is required as a scaffold for MEKK3-mediated p38 MAPK phosphorylation, while a mutation of the gene leads to a decrease in p38 MAPK activation, suggesting that *CCM2* may be part of a protein complex that regulates the p38 pathway. The *CCM1* binds to the CCM2/MEKK3 complex through their PTB domains. Mutation of the *CCM2/MGC4607* gene results in abnormal PTB domains, which blocks CCM1 binding to the CCM2/MGC4607 complex and thus affects regulation of the p38 MAPK pathway. It is hypothesized that CCM1/ICAP-1 binds with the CCM2/MEKK3 complex, acting as a nuclear-cytoplasmic shuttling agent, with CCM1/ICAP-1 localized in the nucleus, and then CCM2/MEKK3 signals it into the cytoplasm where they bind with one another to form a ternary complex that becomes a part of the signaling pathway for p38 MAPK.[112] Dysregulation of the p38 MAPK pathway, caused by an inability to form the ternary complex CCM1/CCM2/MEKK3, could contribute to the abnormal development of vessels in CCMs.

PDCD10/MST4

Proteomic studies have identified several critical components of the ternary complex needed for proper blood vessel sprouting. Among the players involved in the formation of the ternary complex, PDCD10, which is linked to *CCM3*, has been identified in association with several of the CCM cases. The PDCD10 gene product interacts with a member of the Ste20-related kinases, MST4. The over-expression of PDCD10 results in an increase in the MST4 kinase activity, which in turn results in increased cell proliferation.[66] It is postulated that the formation of the PDCD10/MST4 complex mediates cell

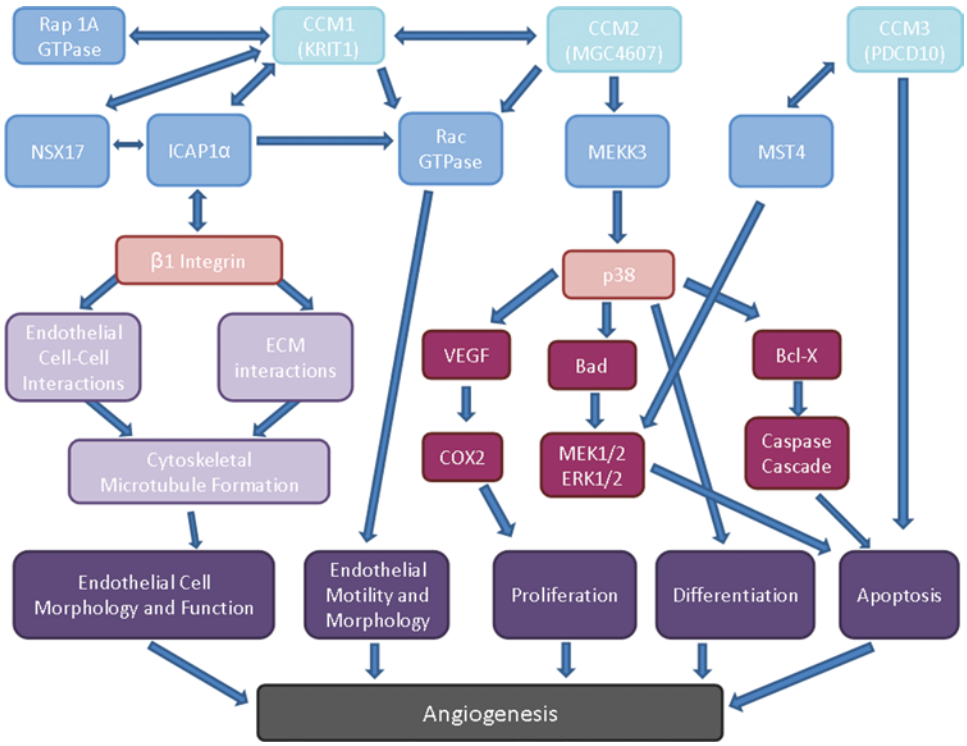

Fig. 4. Schematic depicting the role of CCM gene products in the organizing of sprouting vascular channels. Modified from Dashti et al.: **Neurosurg Focus 21 (1)**:E2, 2006.

growth by the modulation of the ERK-MAPK pathway. The ERK-MAPK pathway is very important in the regulation of cell proliferation and is involved in pathogenesis, suggesting that there may be a link between CCM pathogenesis and the ERK-MAPK pathway via the PDCD10/MST4 complex.[66]

CCM3/PDCD10

During the process of angiogenesis, PDCD10 is expressed in neocortex and ventricular and subventricular zones. Given the important role of this protein in apoptosis, it is interesting to postulate that the angiogenic machinery utilizes the protein product of this locus to prune newly developed blood vessels and that deficiencies in this pruning ability result in the formation of CCMs.

RhoA-ROCK Pathway

The 3 CCM protein products are crucial for the stabilization of the newly formed blood vessels during development. It has been shown that the CCM products interact with the RhoA-ROCK (Rho kinase) pathway, and those mutations in any of the CCM genes results in hyperactivation of the ROCK pathway. Note that RhoA activates ROCK (a putative RhoA effector), which leads to an increase in actin stress fiber (microtubules) production.[95] By increasing the myosin light chain phosphorylation, ROCK mediates the formation of microtubules. Phosphorylation of the myosin light chain allows the myosin cross-bridge to bind to the actin filaments, and thus increasing the cellular contraction rate. The resultant enhanced cell contractility can disrupt the cell-cell adhesion of the endothelial cells mediated via beta-catenin and vascular endothelial cadherin (adherin junctions) lining the vascular walls,[33] resulting in enlargement of the blood vessels and making it very difficult for them to restabilize, and thus leading to vascular leakage and initiating the cascade to CCM formation.[105]

Conclusions

Although the exact mechanism underlying the pathogenesis of CCMs is unknown, the study of families with autosomal dominant inherited and sporadic CCMs has shed light on the importance of the machinery involved in the proliferation and differentiation of angiogenic precursors and on members of the apoptotic machinery as key regulators of the pathogenesis of CCM. Despite the utility of animal models, they have inherent shortcomings such as low expression of the desired phenotype. The generation of an in vivo human model of the disease via the isolation of CCM progenitor cells and their transplantation into an immunodeficient animal, as has been recently described for hemangiomas,[36] may allow both the study of human forms of CCMs and a robust model for imaging and drug trials. Another exciting frontier of research is the sequencing of the entire genome of patients afflicted with CCM by using next-generation sequencing technology. Obtaining the full genomic profile of patients with multiple CCMs will provide further mechanistic clues about the role of other genes and gene regulatory machinery, such as small RNAs, in the pathogenesis of CCMs.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Cavalcanti, Kalani, Martirosyan. Acquisition of data: Cavalcanti, Kalani, Martirosyan, Eales. Analysis and interpretation of data: Cavalcanti, Kalani, Martirosyan. Drafting the article: Cavalcanti, Kalani. Critically revising the article: Preul, Cavalcanti, Kalani, Spetzler, Martirosyan. Reviewed final version of the manuscript and approved it for submission: Preul, Spetzler, Martirosyan. Administrative/technical/material support: Spetzler, Preul. Study supervision: Spetzler, Preul.

References

1. Abe M, Kjellberg RN, Adams RD: Clinical presentations of vascular malformations of the brain stem: comparison of angiographically positive and negative types. **J Neurol Neurosurg Psychiatry 52:**167–175, 1989
2. Béraud-Dufour S, Gautier R, Albiges-Rizo C, Chardin P, Faurobert E: Krit 1 interactions with microtubules and membranes are regulated by Rap1 and integrin cytoplasmic domain associated protein-1. **FEBS J 274:**5518–5532, 2007
3. Bergametti F, Denier C, Labauge P, Arnoult M, Boetto S, Clanet M, et al: Mutations within the programmed cell death 10 gene cause cerebral cavernous malformations. **Am J Hum Genet 76:**42–51, 2005
4. Bicknell JM: Familial cavernous angioma of the brain stem dominantly inherited in Hispanics. **Neurosurgery 24:**102–105, 1989
5. Bicknell JM, Carlow TJ, Kornfeld M, Stovring J, Turner P: Familial cavernous angiomas. **Arch Neurol 35:**746–749, 1978
6. Bos JL: All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. **EMBO J 17:** 6776–6782, 1998
7. Bouffard GG, Idol JR, Braden VV, Iyer LM, Cunningham AF, Weintraub LA, et al: A physical map of human chromosome 7: an integrated YAC contig map with average STS spacing of 79 kb. **Genome Res 7:**673–692, 1997
8. Cavé-Riant F, Denier C, Labauge P, Cécillon M, Maciazek J, Joutel A, et al: Spectrum and expression analysis of KRIT1 mutations in 121 consecutive and unrelated patients with cerebral cavernous malformations. **Eur J Hum Genet 10:**733–740, 2002
9. Chang DD, Wong C, Smith H, Liu J: ICAP-1, a novel beta1 integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of beta1 integrin. **J Cell Biol 138:**1149–1157, 1997
10. Chen L, Tanriover G, Yano H, Friedlander R, Louvi A, Gunel M: Apoptotic functions of PDCD10/CCM3, the gene mutated in cerebral cavernous malformation 3. **Stroke 40:**1474–1481, 2009
11. Clark JV: Familial occurrence of cavernous angiomata of the brain. **J Neurol Neurosurg Psychiatry 33:**871–876, 1970
12. Clatterbuck RE, Eberhart CG, Crain BJ, Rigamonti D: Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations. **J Neurol Neurosurg Psychiatry 71:**188–192, 2001
13. Craig HD, Günel M, Cepeda O, Johnson EW, Ptacek L, Steinberg GK, et al: Multilocus linkage identifies two new loci for a mendelian form of stroke, cerebral cavernous malformation, at 7p15-13 and 3q25.2-27. **Hum Mol Genet 7:**1851–1858, 1998
14. Czubayko M, Knauth P, Schlüter T, Florian V, Bohnensack R: Sorting nexin 17, a non-self-assembling and a PtdIns(3)P high class affinity protein, interacts with the cerebral cavernous

malformation related protein KRIT1. **Biochem Biophys Res Commun 345:**1264–1272, 2006
15. Davenport WJ, Siegel AM, Dichgans J, Drigo P, Mammi I, Pereda P, et al: CCM1 gene mutations in families segregating cerebral cavernous malformations. **Neurology 56:**540–543, 2001
16. Del Curling O Jr, Kelly DL Jr, Elster AD, Craven TE: An analysis of the natural history of cavernous angiomas. **J Neurosurg 75:**702–708, 1991
17. Denier C, Goutagny S, Labauge P, Krivosic V, Arnoult M, Cousin A, et al: Mutations within the MGC4607 gene cause cerebral cavernous malformations. **Am J Hum Genet 74:** 326–337, 2004
18. Denier C, Labauge P, Bergametti F, Marchelli F, Riant F, Arnoult M, et al: Genotype-phenotype correlations in cerebral cavernous malformations patients. **Ann Neurol 60:**550–556, 2006
19. Di Rocco C, Iannelli A, Tamburrini G: Cavernous angiomas of the brain stem in children. **Pediatr Neurosurg 27:**92–99, 1997
20. Dobyns WB, Michels VV, Groover RV, Mokri B, Trautmann JC, Forbes GS, et al: Familial cavernous malformations of the central nervous system and retina. **Ann Neurol 21:**578–583, 1987
21. Dubovsky J, Zabramski JM, Kurth J, Spetzler RF, Rich SS, Orr HT, et al: A gene responsible for cavernous malformations of the brain maps to chromosome 7q. **Hum Mol Genet 4:**453–458, 1995
22. Eerola I, McIntyre B, Vikkula M: Identification of eight novel 5′-exons in cerebral capillary malformation gene-1 (CCM1) encoding KRIT1. **Biochim Biophys Acta 1517**:464-467, 2001
23. Felbor U, Gaetzner S, Verlaan DJ, Vijzelaar R, Rouleau GA, Siegel AM: Large germline deletions and duplication in isolated cerebral cavernous malformation patients. **Neurogenetics 8:**149–153, 2007
24. Flocks JS, Weis TP, Kleinman DC, Kirsten WH: Dose-response studies to polyoma virus in rats. **J Natl Cancer Inst 35:**259–284, 1965
25. Fritschi JA, Reulen HJ, Spetzler RF, Zabramski JM: Cavernous malformations of the brain stem. A review of 139 cases. **Acta Neurochir (Wien) 130:**35–46, 1994
26. Gaetzner S, Stahl S, Sürücü O, Schaafhausen A, Halliger-Keller B, Bertalanffy H, et al: CCM1 gene deletion identified by MLPA in cerebral cavernous malformation. **Neurosurg Rev 30:**155–160, 2007
27. Gault J, Shenkar R, Recksiek P, Awad IA: Biallelic somatic and germ line CCM1 truncating mutations in a cerebral cavernous malformation lesion. **Stroke 36:**872–874, 2005
28. Gianfrancesco F, Esposito T, Penco S, Maglione V, Liquori CL, Patrosso MC, et al: ZPLD1 gene is disrupted in a patient with balanced translocation that exhibits cerebral cavernous malformations. **Neuroscience 155:**345–349, 2008
29. Gil-Nagel A, Dubovsky J, Wilcox KJ, Stewart JM, Anderson VE, Leppik IE, et al: Familial cerebral cavernous angioma: a gene localized to a 15-cM interval on chromosome 7q. **Ann Neurol 39:**807–810, 1996 (Erratum in **Ann Neurol 39:**480, 1996)
30. Gil-Nagel A, Wilcox KJ, Stewart JM, Anderson VE, Leppik IE, Rich SS: Familial cerebral cavernous angioma: clinical analysis of a family and phenotypic classification. **Epilepsy Res 21:**27–36, 1995
31. Giombini S, Morello G: Cavernous angiomas of the brain. Account of fourteen personal cases and review of the literature. **Acta Neurochir (Wien) 40:**61–82, 1978
32. Glading A, Han J, Stockton RA, Ginsberg MH: KRIT-1/CCM1 is a Rap1 effector that regulates endothelial cell cell junctions. **J Cell Biol 179:**247–254, 2007
33. Glading AJ, Ginsberg MH: Rap1 and its effector KRIT1/CCM1 regulate beta-catenin signaling. **Dis Model Mech 3:** 73–83, 2010
34. Green ED, Braden VV, Fulton RS, Lim R, Ueltzen MS, Peluso DC, et al: A human chromosome 7 yeast artificial chromosome (YAC) resource: construction, characterization, and screening. **Genomics 25:**170–183, 1995
35. Green ED, Green P: Sequence-tagged site (STS) content mapping of human chromosomes: theoretical considerations and early experiences. **PCR Methods Appl 1:**77–90, 1991
36. Greenberger S, Boscolo E, Adini I, Mulliken JB, Bischoff J: Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells. **N Engl J Med 362:**1005–1013, 2010
37. Günel M, Awad IA, Anson J, Lifton RP: Mapping a gene causing cerebral cavernous malformation to 7q11.2-q21. **Proc Natl Acad Sci U S A 92:**6620–6624, 1995
38. Gunel M, Awad IA, Finberg K, Anson JA, Steinberg GK, Batjer HH, et al: A founder mutation as a cause of cerebral cavernous malformation in Hispanic Americans. **N Engl J Med 334:**946–951, 1996
39. Gunel M, Laurans MS, Shin D, DiLuna ML, Voorhees J, Choate K, et al: KRIT1, a gene mutated in cerebral cavernous malformation, encodes a microtubule-associated protein. **Proc Natl Acad Sci U S A 99:**10677–10682, 2002
40. Guzeloglu-Kayisli O, Amankulor NM, Voorhees J, Luleci G, Lifton RP, Gunel M: KRIT1/cerebral cavernous malformation 1 protein localizes to vascular endothelium, astrocytes, and pyramidal cells of the adult human cerebral cortex. **Neurosurgery 54:**943–949, 2004
41. Guzeloglu-Kayisli O, Kayisli UA, Amankulor NM, Voorhees JR, Gokce O, DiLuna ML, et al: Krev1 interaction trapped-1/cerebral cavernous malformation-1 protein expression during early angiogenesis. **J Neurosurg 100 (5 Suppl Pediatrics):** 481–487, 2004
42. Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM: Expression and function of laminins in the embryonic and mature vasculature. **Physiol Rev 85:**979–1000, 2005
43. Hayman LA, Evans RA, Ferrell RE, Fahr LM, Ostrow P, Riccardi VM: Familial cavernous angiomas: natural history and genetic study over a 5-year period. **Am J Med Genet 11:**147–160, 1982
44. Henkemeyer M, Rossi DJ, Holmyard DP, Puri MC, Mbamalu G, Harpal K, et al: Vascular system defects and neuronal apoptosis in mice lacking ras GTPase-activating protein. **Nature 377:**695–701, 1995
45. Hogan BM, Bussmann J, Wolburg H, Schulte-Merker S: Ccm1 cell autonomously regulates endothelial cellular morphogenesis and vascular tubulogenesis in zebrafish. **Hum Mol Genet 17:**2424–2432, 2008
46. Johnson EW, Iyer LM, Rich SS, Orr HT, Gil-Nagel A, Kurth JH, et al: Refined localization of the cerebral cavernous malformation gene (CCM1) to a 4-cM interval of chromosome 7q contained in a well-defined YAC contig. **Genome Res 5:** 368–380, 1995
47. Kehrer-Sawatzki H, Wilda M, Braun VM, Richter HP, Hameister H: Mutation and expression analysis of the KRIT1 gene associated with cerebral cavernous malformations (CCM1). **Acta Neuropathol 104:**231–240, 2002
48. Kere J, Ruutu T, Davies KA, Roninson IB, Watkins PC, Winqvist R, et al: Chromosome 7 long arm deletion in myeloid disorders: a narrow breakpoint region in 7q22 defined by molecular mapping. **Blood 73:**230–234, 1989
49. Kidd HA, Cumings JN: Cerebral angiomata in an Icelandic family. **Lancet 1:**747, 1947
50. Knudson AG: Two genetic hits (more or less) to cancer. **Nat Rev Cancer 1:**157–162, 2001
51. Kondziolka D, Lunsford LD, Kestle JR: The natural history of cerebral cavernous malformations. **J Neurosurg 83:**820–824, 1995
52. Krebs LT, Xue Y, Norton CR, Shutter JR, Maguire M, Sundberg JP, et al: Notch signaling is essential for vascular morphogenesis in mice. **Genes Dev 14:**1343–1352, 2000

53. Kufs H: Über heredofamiliare Angiomatose des Gehirns und der Retina, ihre Beziehungen zueinander und zur Angiomatose der Haut. **Z Gesamte Neurol Psychiatr 113:**651–686, 1928
54. Kurth JH, Zabramski JM, Dubovsky J: Genetic linkage of the familial cavernous malformation (CM) gene to chromosome 7q. **Am J Hum Genet 55 (Suppl 3)**:A15, 1994 (http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=133322) [Accessed August 23, 2011]
55. Labauge P, Laberge S, Brunereau L, Levy C, Tournier-Lasserve E: Hereditary cerebral cavernous angiomas: clinical and genetic features in 57 French families. Société Française de Neurochirurgie. **Lancet 352:**1892–1897, 1998
56. Laberge S, Labauge P, Maréchal E, Maciazek J, Tournier-Lasserve E: Genetic heterogeneity and absence of founder effect in a series of 36 French cerebral cavernous angiomas families. **Eur J Hum Genet 7:**499–504, 1999
57. Laberge-le Couteulx S, Jung HH, Labauge P, Houtteville JP, Lescoat C, Cecillon M, et al: Truncating mutations in CCM1, encoding KRIT1, cause hereditary cavernous angiomas. **Nat Genet 23:**189–193, 1999
58. Liquori CL, Berg MJ, Siegel AM, Huang E, Zawistowski JS, Stoffer T, 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 73:** 1459–1464, 2003
59. Liquori CL, Berg MJ, Squitieri F, Leedom TP, Ptacek L, Johnson EW, et al: Deletions in CCM2 are a common cause of cerebral cavernous malformations. **Am J Hum Genet 80:**69–75, 2007
60. Liquori CL, Berg MJ, Squitieri F, Ottenbacher M, Sorlie M, Leedom TP, et al: Low frequency of PDCD10 mutations in a panel of CCM3 probands: potential for a fourth CCM locus. **Hum Mutat 27:**118, 2006
61. Liquori CL, Penco S, Gault J, Leedom TP, Tassi L, Esposito T, et al: Different spectra of genomic deletions within the CCM genes between Italian and American CCM patient cohorts. **Neurogenetics 9:**25–31, 2008
62. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al: Heart disease and stroke statistics—2010 update: a report from the American Heart Association. **Circulation 121:**e46–e215, 2010 (Erratum in **Circulation 121:** e260, 2010)
63. Lonjon M, Roche JL, George B, Mourier KL, Paquis P, Lot G, et al: [Intracranial cavernoma. 30 cases.] **Presse Med 22:** 990–994, 1993 (Fr)
64. Lucas M, Costa AF, García-Moreno JM, Solano F, Gamero MA, Izquierdo G: Variable expression of cerebral cavernous malformations in carriers of a premature termination codon in exon 17 of the Krit1 gene. **BMC Neurol 3:**5, 2003
65. Lucas M, Costa AF, Montori M, Solano F, Zayas MD, Izquierdo G: Germline mutations in the CCM1 gene, encoding Krit1, cause cerebral cavernous malformations. **Ann Neurol 49:**529–532, 2001
66. Ma X, Zhao H, Shan J, Long F, Chen Y, Chen Y, et al: PDCD10 interacts with Ste20-related kinase MST4 to promote cell growth and transformation via modulation of the ERK pathway. **Mol Biol Cell 18:**1965–1978, 2007
67. Mably JD, Chuang LP, Serluca FC, Mohideen MA, Chen JN, Fishman MC: Santa and valentine pattern concentric growth of cardiac myocardium in the zebrafish. **Development 133:**3139–3146, 2006
68. Marchuk DA, Gallione CJ, Morrison LA, Clericuzio CL, Hart BL, Kosofsky BE, et al: A locus for cerebral cavernous malformations maps to chromosome 7q in two families. **Genomics 28:**311–314, 1995
69. Mason I, Aase JM, Orrison WW, Wicks JD, Seigel RS, Bicknell JM: Familial cavernous angiomas of the brain in an Hispanic family. **Neurology 38:**324–326, 1988
70. Matos P, Skaug J, Marques B, Beck S, Veríssimo F, Gespach C, et al: Small GTPase Rac1: structure, localization, and expression of the human gene. **Biochem Biophys Res Commun 277:** 741–751, 2000
71. McCormick WF: The pathology of vascular ("arteriovenous") malformations. **J Neurosurg 24:**807–816, 1966
72. Michael JC, Levin PM: Multiple telangiectases of the brain: a discussion of hereditary factors in their development. **Arch Neurol Psychiatry 36:**514–529, 1936
73. Moriarity JL, Wetzel M, Clatterbuck RE, Javedan S, Sheppard JM, Hoenig-Rigamonti K, et al: The natural history of cavernous malformations: a prospective study of 68 patients. **Neurosurgery 44:**1166–1173, 1999
74. Moussa R, Harb A, Menassa L, Risk T, Nohra G, Samaha E, et al: [Etiologic spectrum of intracerebral hemorrhage in young patients.] **Neurochirurgie 52 (2–3 Pt 1):**105–109, 2006 (Fr)
75. Nibert M, Heim S: Uterine leiomyoma cytogenetics. **Genes Chromosomes Cancer 2:**3–13, 1990
76. Otten P, Pizzolato GP, Rilliet B, Berney J: [131 cases of cavernous angioma (cavernomas) of the CNS, discovered by retrospective analysis of 24,535 autopsies.] **Neurochirurgie 35:**82–83, 128–131, 1989 (Fr)
77. Pagenstecher A, Stahl S, Sure U, Felbor U: A two-hit mechanism causes cerebral cavernous malformations: complete inactivation of CCM1, CCM2 or CCM3 in affected endothelial cells. **Hum Mol Genet 18:**911–918, 2009
78. Pizon V, Chardin P, Lerosey I, Olofsson B, Tavitian A: Human cDNAs rap1 and rap2 homologous to the Drosophila gene Dras3 encode proteins closely related to ras in the 'effector' region. **Oncogene 3:**201–204, 1988
79. Plummer NW, Gallione CJ, Srinivasan S, Zawistowski JS, Louis DN, Marchuk DA: Loss of p53 sensitizes mice with a mutation in Ccm1 (KRIT1) to development of cerebral vascular malformations. **Am J Pathol 165:**1509–1518, 2004
80. Plummer NW, Squire TL, Srinivasan S, Huang E, Zawistowski JS, Matsunami H, et al: Neuronal expression of the Ccm2 gene in a new mouse model of cerebral cavernous malformations. **Mamm Genome 17:**119–128, 2006
81. Porter PJ, Willinsky RA, Harper W, Wallace MC: Cerebral cavernous malformations: natural history and prognosis after clinical deterioration with or without hemorrhage. **J Neurosurg 87:**190–197, 1997
82. Reedquist KA, Ross E, Koop EA, Wolthuis RM, Zwartkruis FJ, van Kooyk Y, et al: The small GTPase, Rap1, mediates CD31-induced integrin adhesion. **J Cell Biol 148:**1151–1158, 2000
83. Rigamonti D, Hadley MN, Drayer BP, Johnson PC, Hoenig-Rigamonti K, Knight JT, et al: Cerebral cavernous malformations. Incidence and familial occurrence. **N Engl J Med 319:** 343–347, 1988
84. Robinson JR, Awad IA, Little JR: Natural history of the cavernous angioma. **J Neurosurg 75:**709–714, 1991
85. Ruggieri R, Bender A, Matsui Y, Powers S, Takai Y, Pringle JR, et al: RSR1, a ras-like gene homologous to Krev-1 (smg21A/rap1A): role in the development of cell polarity and interactions with the Ras pathway in Saccharomyces cerevisiae. **Mol Cell Biol 12:**758–766, 1992
86. Russell DS, Rubinstein LJ: **Pathology of Tumours of the Nervous System**. London: E. Arnold, 1989, pp 730–736
87. Sahoo T, Goenaga-Diaz E, Serebriiskii IG, Thomas JW, Kotova E, Cuellar JG, et al: Computational and experimental analyses reveal previously undetected coding exons of the KRIT1 (CCM1) gene. **Genomics 71:**123–126, 2001
88. Sahoo T, Johnson EW, Thomas JW, Kuehl PM, Jones TL, Dokken CG, et al: Mutations in the gene encoding KRIT1, a Krev-1/rap1a binding protein, cause cerebral cavernous malformations (CCM1). **Hum Mol Genet 8:**2325–2333, 1999
89. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G: Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. **Nucleic Acids Res 30:**e57, 2002

90. Sedgwick SG, Smerdon SJ: The ankyrin repeat: a diversity of interactions on a common structural framework. **Trends Biochem Sci 24:**311–316, 1999
91. Seker A, Pricola KL, Guclu B, Ozturk AK, Louvi A, Gunel M: CCM2 expression parallels that of CCM1. **Stroke 37:**518–523, 2006
92. Serebriiskii I, Estojak J, Sonoda G, Testa JR, Golemis EA: Association of Krev-1/rap1a with Krit1, a novel ankyrin repeat-containing protein encoded by a gene mapping to 7q21-22. **Oncogene 15:**1043–1049, 1997
93. Simard JM, Garcia-Bengochea F, Ballinger WE Jr, Mickle JP, Quisling RG: Cavernous angioma: a review of 126 collected and 12 new clinical cases. **Neurosurgery 18:**162–172, 1986
94. Stahl S, Gaetzner S, Voss K, Brackertz B, Schleider E, Sürücü O, 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 29:**709–717, 2008
95. Stockton RA, Shenkar R, Awad IA, Ginsberg MH: Cerebral cavernous malformations proteins inhibit Rho kinase to stabilize vascular integrity. **J Exp Med 207:**881–896, 2010
96. Tanriover G, Boylan AJ, Diluna ML, Pricola KL, Louvi A, Gunel M: PDCD10, the gene mutated in cerebral cavernous malformation 3, is expressed in the neurovascular unit. **Neurosurgery 62:**930–938, 2008
97. Tomlinson FH, Houser OW, Scheithauer BW, Sundt TM Jr, Okazaki H, Parisi JE: Angiographically occult vascular malformations: a correlative study of features on magnetic resonance imaging and histological examination. **Neurosurgery 34:** 792–800, 1994
98. Uhlik MT, Abell AN, Johnson NL, Sun W, Cuevas BD, Lobel-Rice KE, et al: Rac-MEKK3-MKK3 scaffolding for p38 MAPK activation during hyperosmotic shock. **Nat Cell Biol 5:**1104–1110, 2003
99. Verlaan DJ, Siegel AM, Rouleau GA: Krit1 missense mutations lead to splicing errors in cerebral cavernous malformation. **Am J Hum Genet 70:**1564–1567, 2002
100. Voigt K, Yaşargil MG: Cerebral cavernous haemangiomas or cavernomas. Incidence, pathology, localization, diagnosis, clinical features and treatment. Review of the literature and report of an unusual case. **Neurochirurgia (Stuttg) 19:**59–68, 1976
101. Voss K, Stahl S, Schleider E, Ullrich S, Nickel J, Mueller TD, et al: CCM3 interacts with CCM2 indicating common pathogenesis for cerebral cavernous malformations. **Neurogenetics 8:**249–256, 2007
102. Wang HU, Chen ZF, Anderson DJ: Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. **Cell 93:**741–753, 1998
103. Wang Y, Liu H, Zhang Y, Ma D: cDNA cloning and expression of an apoptosis-related gene, humanTFAR15 gene. **Sci China C Life Sci 42:**323–329, 1999
104. Wernig F, Mayr M, Xu Q: Mechanical stretch-induced apoptosis in smooth muscle cells is mediated by beta1-integrin signaling pathways. **Hypertension 41:**903–911, 2003
105. Whitehead KJ, Chan AC, Navankasattusas S, Koh W, London NR, Ling J, et al: The cerebral cavernous malformation signaling pathway promotes vascular integrity via Rho GTPases. **Nat Med 15:**177–184, 2009 (Erratum in **Nat Med 15:**462, 2009)
106. Whitehead KJ, Plummer NW, Adams JA, Marchuk DA, Li DY: Ccm1 is required for arterial morphogenesis: implications for the etiology of human cavernous malformations. **Development 131:** 1437–1448, 2004
107. Wienecke R, Maize JC Jr, Reed JA, de Gunzburg J, Yeung RS, DeClue JE: Expression of the TSC2 product tuberin and its target Rap1 in normal human tissues. **Am J Pathol 150:**43–50, 1997
108. Wojnowski L, Zimmer AM, Beck TW, Hahn H, Bernal R, Rapp UR, et al: Endothelial apoptosis in Braf-deficient mice. **Nat Genet 16:**293–297, 1997
109. Wong JH, Awad IA, Kim JH: Ultrastructural pathological features of cerebrovascular malformations: a preliminary report. **Neurosurgery 46:**1454–1459, 2000
110. Zabramski JM, Wascher TM, Spetzler RF, Johnson B, Golfinos J, Drayer BP, et al: The natural history of familial cavernous malformations: results of an ongoing study. **J Neurosurg 80:** 422–432, 1994
111. Zawistowski JS, Serebriiskii IG, Lee MF, Golemis EA, Marchuk DA: KRIT1 association with the integrin-binding protein ICAP-1: a new direction in the elucidation of cerebral cavernous malformations (CCM1) pathogenesis. **Hum Mol Genet 11:** 389–396, 2002
112. Zawistowski JS, Stalheim L, Uhlik MT, Abell AN, Ancrile BB, Johnson GL, et al: CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis. **Hum Mol Genet 14:**2521–2531, 2005
113. Zhang J, Clatterbuck RE, Rigamonti D, Chang DD, Dietz HC: Interaction between krit1 and icap1alpha infers perturbation of integrin beta1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. **Hum Mol Genet 10:**2953–2960, 2001
114. Zhang J, Clatterbuck RE, Rigamonti D, Dietz HC: Cloning of the murine Krit1 cDNA reveals novel mammalian 5′ coding exons. **Genomics 70:**392–395, 2000

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