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REVIEW

Cerebral cavernous malformations: from molecular pathogenesis to genetic counselling and clinical management

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Cerebral cavernous (or capillary-venous) malformations (CCM) have a prevalence of about 0.1–0.5% in the general population. Genes mutated in CCM encode proteins that modulate junction formation between vascular endothelial cells. Mutations lead to the development of abnormal vascular structures. In this article, we review the clinical features, molecular and genetic basis of the disease, and management.
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INTRODUCTION

Cerebral cavernous (or capillary-venous) malformations (CCM; OMIM no. 116 860) are vascular malformations with a prevalence of 0.1–0.5% in the general population, with a familial incidence close to 20%.[1–3] CCM may occur sporadically, but most of the time it has an autosomal dominant inheritance pattern with variable expression and incomplete penetrance.[2–6] At least three genes have been associated with CCM: k-rev interaction trapped protein 1 (*KRIT1*) (*CCM1*; OMIM no. 604214), *MGC4607* (*CCM2*; OMIM no. 603284) and programmed cell death 10 (*PDCD10*) (*CCM3*; OMIM no. 603285). These genes encode proteins that are involved in junction formation between vascular endothelial cells. Mutations in the *CCM* genes, which are in general loss-of-function mutations, lead to the development of abnormal vascular structures characterized by thin-walled, dilated blood vessels with gaps between the endothelial cells.[1,7]

The underlying genetic mechanism in CCM is partially understood. Second-site genetic mutations have been proposed as one of the possible molecular mechanisms.[1,8]

A total of 9% of individuals were symptomatic before age 10 years, 62–72% between 10 and 40 years, and 19% after age 40 years.[9,10] Up to 25% of individuals with CCM remain symptom free throughout their lives.[11] This percentage may be an underestimate because many asymptomatic persons go unrecognized. Otten *et al*[12] reported an absence of symptoms in 90% of individuals with CCM ascertained in autopsy. Approximately 50–75% of persons with CCM become symptomatic. Affected individuals most often present with seizures (40–70%), focal neurologic deficits (35–50%), non-specific headaches (10–30%) and cerebral haemorrhage (41%).[9,11,13,14] In the most recent study, Denier *et al*[15] found seizures in 55%, focal neurological deficits in 9%, non-specific headaches in 4% and cerebral haemorrhage in 32%.

In most cases, cavernous malformations (or cavernomas) are located within the brain, but in a small proportion of patients with familial CCM, cavernomas may also be observed in the spinal cord, retina, skin or liver.[2,3,16] Retinal cavernomas occur in about 5% of patients with familial CCM. They are unilateral, generally stable and asymptomatic, and can be diagnosed by routine fundoscopy.[2,17] Cutaneous vascular malformations are seen in 9% of familial CCM patients. Three distinct major phenotypes were identified: hyperkeratotic cutaneous capillary-venous malformations (39%), strongly associated with a *KRIT1* mutation. Second, capillary malformations (34%) and finally, venous malformations (21%) mostly seen in patients with a *PDCD10* mutation. Patients with a *Malcavernin* mutation are possibly less prone for cutaneous vascular malformations.[2,16,18]

MOLECULAR AND GENETIC BASIS OF CCMs

Mutated genes and new loci

To date, three genes have been associated with the pathogenesis of CCM, including *KRIT1* (also known as *CCM1*) located on chromosome 7q11.2–21,[19,20] Malcavernin, murine OSM-osmosensing scaffold for MEKK3 (*MGC4607*, also known as *CCM2*) on chromosome 7p13[19,21] and *PDCD10* (also known as *CCM3*), originally identified as TF-1 cell apoptosis-related gene-15 (*TFAR15*) on chromosome 3q26.1 (Table 1).[19,22,23] In addition, there is at least one further – as yet unspecified – gene that can cause CCM, which has been mapped to chromosome 3q26.3–27.2. Gianfrancesco *et al*[25] reported the zona pellucida-like domain containing 1 gene as possible candidate. This gene is also located on the long arm of chromosome three centromeric of *PDCD10*.[3,24–26]

Distribution and frequency of gene mutations

Close to 100 mutations (88 germline mutations) have been identified in the *KRIT1* gene, representing about 40–53% of the CCM families. Mutations in the *MGC4607* gene may account for 15–20% of familial CCM cases.[3,7,26–28] The only missense mutation in the *MGC4607* gene

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Table 1 CCM protein interactions

Ligand	*Interacted protein*	*Possible function*
KRIT1 *(CCM1)*	Rap1a	Tumour suppressor gene
	ICAP1α	Cell adhesion
	Malcavernin	Cell spreading
	HEG1 receptor	Direction of endothelial cell migration
	Junctional proteins	
	(Plus end of) microtubules	
Malcavernin *(CCM2)*	KRIT1	Sequester KRIT1 to the cytosol
	PDCD10	Lumen formation
	Rac1	Vascular permeability
	Kinases involved in the p38 MAPK signalling cascade	Migration
PDCD10 *(CCM3)*	Malcavernin	Apoptosis
	STK24	Direction of endothelial cell migration
	STK25	
	MST4	

Abbreviations: CCM, cerebral cavernous (or capillary-venous) malformations; HEG1, heart of glass 1 receptor; ICAP1α, α isoform of the β_1-integrin regulator integrin cytoplasmic adaptor protein 1; KRIT1, k-rev interaction trapped protein 1; PDCD10, programmed cell death 10; STK24/25, serine/threonine protein kinase.

reported so far, is a leucine to arginine substitution at amino acid 198 (L198R), located in the phosphotyrosine-binding domain (PTB) of Malcavernin.[29] Approximately 10–40% of CCM families have been linked to the *PDCD10* gene.[26,28] With a single exception, mutations in the *PDCD10* gene are either truncating or large genomic deletions of the entire gene. The only known in-frame deletion of *PDCD10* is located in exon 5, encompassing amino acids L33–K50, encoding the serine/threonine kinase binding and phosphorylation domain .[22,23,30] In about 22% of CCM cases with multiple lesions no mutation is detected in the three *CCM* genes.[26] Although *de novo* mutations have been reported for all three *CCM* genes, they appear to be more common in the *PDCD10* gene.[2,31]

The proportion of familial cases has been estimated approximately at 20% in the general population, and estimated to be as high as 50% in Hispanic-American patients of Mexican descent. These families are all apparently related to the same founder mutation (Q455X) in the *KRIT1* gene.[2,3,5]

Genotype–phenotype relationship

CCM is an autosomal dominant disorder with a clinical penetrance of 88% in *CCM1* families, 100% in *CCM2* and 63% in *CCM3* families.[2,32]

Different explanations have been provided for the molecular pathogenesis of lesion formation in CCM. First, a Knudsonian two-hit mechanism might be involved. According to this mechanism, CCM formation would require a complete loss of the two alleles of a given *CCM* gene within affected cells. Loss of one of the alleles (first hit) would be the result of a germline mutation, whereas loss of the second allele (or second hit) will occur somatically. In this view, familial CCM exhibits an autosomal dominant mode of inheritance, but is likely recessive at the cellular level.[2,27,33] On the basis of animal, as well as human studies, evidence grows for the two-hit mechanism. For example, in Ccm heterozygous mice, homozygous knockout for *Msh2*, penetrance of CCM lesions has been increased. Even so, in surgically resected mature lesions from CCM patients, mutations have been found in both alleles.[34] Second, haploinsufficiency may also be an explanation in CCM pathophysiology. In this case, the patient has only a single functional copy of one of the *CCM* genes, due to mutational inactivation of the other. The single functional copy of the gene, however, does not result in sufficient protein for, for example, an adequate functional junction formation between endothelial cells, which in turn leads to the development of abnormal vascular structures. Third, paradominant inheritance might explain several CCM features. In paradominant inheritance, heterozygous individuals carrying a 'paradominant' mutation are phenotypically normal, but the trait only becomes manifest when a somatic mutation occurs during embryogenesis, giving rise to loss of heterozygosity and formation of a mutant cell population that is homozygous for the mutation. In addition, a second hit may be caused by environmental factors. The exposure of CCM mutated, presensitized microvascular regions to oxidative stress generated by endothelial nitric oxide synthase uncoupling and reactive oxygen species formation could lead to perivascular astrocytosis.[35–37] The localized nature and the number of lesions (usually a single one in sporadic cases versus multiple lesions in familial cases), as well as the age of first presentation of the phenotype being earlier in familial cases and fits in this type of inheritance. Finally, trans-heterozygosity, in which a patient has synergistic mutations in different genes of the CCM pathway (for example, a germline mutation in the *KRIT1* gene with an additional somatic mutation in the *MGC4607* or *PDCD10* gene), might also explain intrafamilial clinical variability. Indeed, it has been shown that a decrease in the *KRIT1*, *MGC4607* or *PDCD10* gene alone caused little or no effect independently, but when combined, resulted in very high incidence of intracranial haemorrhage.[1,3,27,38]

BIOLOGY OF CCMs

Protein function and expression pattern

The *KRIT1* gene contains 20 exons of which 16 encode a 736 amino acid protein containing three NPxY/F motifs and three ankyrin repeat domains at the N-terminus, and one C-terminal band 4.1 ezrin radixin moesin domain (FERM) found in exons 14–20 (Figure 1).[1,2,27,39–41] The NPxY/F motifs may be involved in dimerization either intramolecular folding of the KRIT1 protein, resulting in a closed and open conformation of KRIT1.[41] After that, the first NPxY/F motif interacts with the α-isoform of the β_1-integrin regulator integrin cytoplasmic adaptor protein 1 (ICAP1α). ICAP1α is a 200 amino acid protein containing a PTB domain and even as KRIT1 a nuclear localization signal motif in the N-terminus. There is evidence that both KRIT1 and ICAP1α can translocate into the nucleus, where they could cooperate in regulating gene expression. In particular, an open/closed conformation switch regulates KRIT1 nucleocytoplasmic shuttling and molecular interactions.[40,41] The ankyrin repeats in KRIT1 are thought to be involved in protein–protein interaction and have been found in many proteins. No partner interacting with KRIT1 ankyrin repeats has yet been found .[27,39,41] The FERM domain in KRIT1 is composed of three subdomains, F1–F3, arranged in cloverleaf shape. The F3 subdomain has a PTB-like domain, which recognizes the NPxY/F motif on the cytoplasmic tail of transmembrane receptors. Rap1a is also bind by the FERM domain, suggesting that KRIT1 may function as a scaffold for transmembrane receptors and Rap1a.[27,29,39,41]

The *MGC4607* gene contains 10 exons encoding Malcavernin, a 444 amino acid protein containing a PTB domain similar to that of ICAP1α. Malcavernin binds KRIT1 by the PTB domain and inhibits in this way nuclear translocation of the KRIT1–ICAP1α complex.[2,7,21,41]

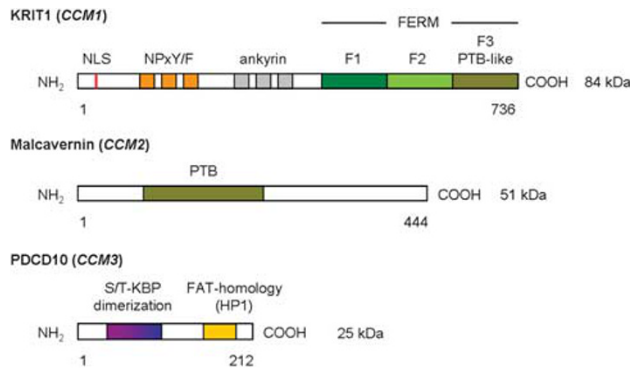

Figure 1 Functional domains of CCM proteins. Ankyrin, protein–protein interaction domain; FERM, four-point one ezrin radixin moesin domain; NLS, nuclear localization signal; NPxY/F, ICAP1α – Malcavernin binding site; PTB, phosphotyrosine-binding domain; S/T-KBP, serine/threonine kinase binding and phosphorylation domain.

The *PDCD10* gene contains seven exons encoding a 212 amino acid protein containing a dimerization domain at the N-terminus, and a C-terminal focal adhesion targeting-homology domain with a highly conserved HP1 surface.[42] Previous studies suggested also the presence of an N-terminal serine/threonine kinase binding and phosphorylation domain, which binds proteins of the germinal centre kinase III family (STK24, STK25, and Mst4).[2,30,43–46] The dimerization domain mediates dimerization of PDCD10. The Fat-homology domain is important for stabilization of the expressed PDCD10 protein and interacts with the PTB domain of Malcavernin and paxillin LD motifs.[42] PDCD10 also binds Ptdlns(3,4,5)P3 and functions in this way in the (PI3k–)PIP_3–PDPK1–Akt signalling pathway.[23,47] He *et al* suggested that the C-terminus of PDCD10 could be important in the stabilization of VEGFR2 signalling, which is crucial for vascular development.[23,48]

Despite the vascular nature of CCM, *in situ* hybridization studies have shown *KRIT1* mRNA and protein expression in astrocytes, neurons, and various epithelial cells. KRIT1 protein was also detected in vascular endothelial cells during early angiogenesis, localized in the cell–cell junctions.[3,49] Guzeloglu-Kayisli *et al*[50] demonstrate that KRIT1 is also present in endothelial cells and cells involved in the formation of the blood-brain barrier, which implicates an important role for KRIT1 in intercellular communication and adherence. *MGC4607* mRNA expression has been detected in neurons and astrocytes, as well as in cerebral vessels. *PDCD10* mRNA is expressed in neuronal cells at adult stages, but also during embryogenesis.[3,51]

Additional, neural expression of *KRIT1*, *MGC4607* and *PDCD10* imply that vascular malformations in CCM could also result from a defect in signalling between endothelial and neural cells, but it is still unclear whether the primary defect is of vascular or neuronal origin.[3] In spite of this, most research has been focused on endothelial cells.

Histology

The vessel wall in CCM is characterized by less and abnormal junction formation between endothelial cells. After that, the expression of intercellular junction proteins is increased to compensate for the loose of cell contacts. Another characteristic of CCM is the lack of subendothelial support in the vessel wall of CCM made visible by decline in the presence of perivascular supporting cells (pericytes) and deposition of a basal lamina with disorganized collagen bundles. In addition, the formation of microgaps at the interendothelial junction sites was observed using scanning electron microscopy.[52,53] Zhao *et al*[54] suggested that CCM may develop as a result of irregular organization of endothelial cells, as a consequence of an increased proliferation and migration potential of these cells. In line with this hypothesis, increased migratory and proliferatory endothelial cell function would indeed require reduced cell–cell contact and reduced presence of pericytes.

MOLECULAR PATHOGENESIS OF CCMs

At the molecular level CCM proteins regulate cell–cell adhesion (Figure 2a), cell polarity and most likely cell adhesion to the extracellular matrix (Figure 2b).[1,46,55]

Cell–cell adhesion

Initiation and maintenance of cell–cell adhesion require the assembly of adherence junctions. The formation of these adherence junctions is stimulated by Rap1a, which forms a complex with KRIT1. Rap1a recruits KRIT1 to the plasma membrane, where it binds to the heart of glass 1 (HEG1) receptor to form a ternary complex of HEG1, KRIT1 and Malcavernin.[19,41,56] HEG1 is a transmembrane protein, expressed specifically in the endothelium and endocardium. No binding ligand for HEG1 is currently known, although it has been suggested in previous studies that HEG1 may be involved in the Wnt/β-catenin signalling, possibly by binding KRIT1.[41,57–59] KRIT1 binds β-catenin and stimulate the association of β-catenin with vascular endothelial-cadherin, required for adherence junction formation.[60] *KRIT1* may also function as a tumour suppressor gene; KRIT1-β-catenin binding prevents β-catenin translocation to the nucleus where displacement of the transcriptional repressor Groucho from T-cell factor proteins by β-catenin would activate *Wnt* target gene expression.[55,61,62] However, β-catenin activity in the nucleus is also vital for the blood-brain barrier, as many regulatory proteins involved in its development are under Wnt/β-catenin control.[41,62]

Cell polarity

Adherence junctions also promote tight junction assembly. This takes place by the formation of a ternary complex of KRIT1, AF6/afadin and claudin-5.[55,63] Tight junctions may function as a physical barrier along the cell surface. As a consequence of asymmetrical distribution of proteins and lipids across this barrier, cell polarization takes place.[41,64] Cell polarity is important in the process of lumen formation.[41] Except tight junction formation, cell polarity is also established through a reshaping of the intracellular cytoskeleton organization. This is regulated by ROCK, a RhoA effector. Crose *et al*[65] showed that Malcavernin regulates RhoA protein level. Malcavernin binding of Smurf1 increases Smurf1-mediated degradation of RhoA. After that, Borikova *et al*[66] also showed that KRIT1 and PDCD10 in addition to Malcavernin are required for the regulation of RhoA protein levels. KRIT1 is a negative regulator of RhoA activity. The functional mechanism of KRIT1 is not yet totally known. In contrast, some aspects of PDCD10 inhibition of RhoA activation has been elucidated, as PDCD10 acts by stabilization of germinal centre kinase III proteins and subsequent activation of moesin, a RhoA inhibitor.[67–69] Loss of the CCM proteins results in an increase of RhoA activity and changes in regulation of ROCK and the cytoskeleton rigidity.

Cell adhesion to the extracellular matrix

β_1-integrin, essential for the control of the intracellular cytoskeleton organization, regulates endothelial cell adhesion to the extracellular matrix.[39,70] It is proposed that β_1-integrin signal to CDC42 and Rac1.[41,71,72] Both are required for the induction of vacuole and

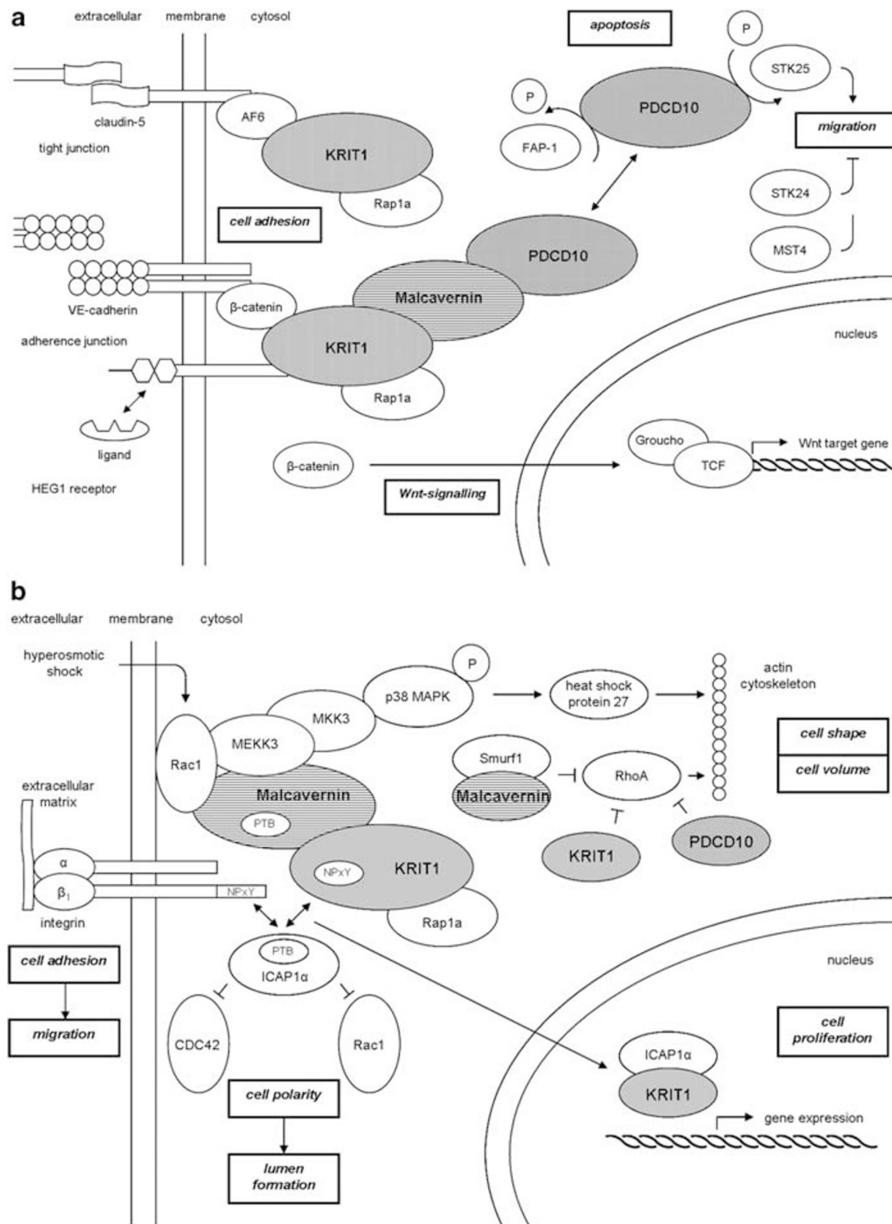

Figure 2 Integrin signalling pathway. (**a**) Cell–cell adhesion. HEG1 receptor, heart of glass type 1 receptor; KRIT1, k-rev interaction trapped protein 1; PDCD10, programmed cell death 10; STK24/25, serine/threonine protein kinase; TCF, T-cell factor protein. (—|) inhibition; (→) stimulation; (↔) interaction. (**b**) Cell-extracellular matrix adhesion. ICAP1α, α isoform of the β_1-integrin regulator integrin cytoplasmic adaptor protein 1; KRIT1, k-rev interaction trapped protein 1; PDCD10, programmed cell death 10; PTB, phosphotyrosine-binding domain. (—|) inhibition; (→) stimulation; (↔) interaction.

lumen formation in vascular endothelial cells.[39,43,73,74] In addition, β_1-integrin also promotes blood vessel maturation by stimulating the adhesion of mural cells to endothelial cells.[41] β_1-integrin function is inhibited by binding of ICAP1α. KRIT1 competes with β_1-integrin for binding to ICAP1α, suggesting that KRIT1 may regulate the ICAP1α inhibitory effect on β_1-integrin.[7,27–29,39,41]

Cell adhesion to the extracellular matrix induces formation of focal adhesion sites in which plaque proteins, such as vinculin and paxillin, provide a bridge between β-integrins and the actin cytoskeleton. Subsequent activation of signalling cascades, regulated by focal adhesion kinase, promote actin cytoskeleton plasticity.[75] Malcavernin has been shown to be capable to regulate actin cytoskeleton plasticity. In response to hyperosmotic shock, restoration of cell volume and cell shape is regulated by the p38 MAPK signalling cascade, controlled by Malcavernin. Malcavernin acts as a scaffold protein for Rac1 and the upstream kinases MEKK3 and MKK3. The p38 MAPK signalling pathway leads to the activation of heat shock protein 27, which in turn activates actin polymerization and stabilization.[29,41,55,73,76]

CLINICAL MANAGEMENT OF CCMs

Genetic counselling and molecular diagnosis

To estimate the genetic risk of CCM, three key points are essential (Figure 3):[3]

- a detailed three-generation family tree with specific enquiry about seizures, cerebral haemorrhages, focal neurological deficits and (recurrent) headaches.

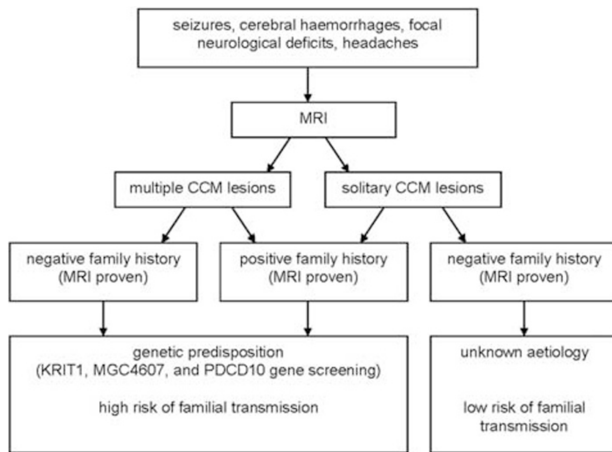

Figure 3 Scheme for work-up of CCM patients at clinical presentation. Most patients (50–80%) with CCM are sporadic without a known family history of CCM. Solitary CCM lesions may be found in 8–19% of familial cases and roughly 75% of sporadic cases. Multiple lesions are indicative of familial forms of CCM.[27]

- MRI of the brain to differentiate between solitary or multiple CCM lesions.
- age of onset.

Genetic testing for *KRIT1*, *MGC4607* and *PDCD10* can confirm the clinical diagnosis in patients, and enables predictive and prenatal testing.

The yield of mutation screening in CCM depends on family history. If only a single lesion can be detected, familial transmission is extremely rare. In contrast, sporadic cases with multiple cerebral lesions are most likely to have a genetic cause and need to be considered as familial cases. In these cases, genetic screening of all three *CCM* genes is indicated. The sensitivity of this screen is estimated to be 57%; therefore, the patient should be aware that a negative test does not exclude a genetic cause.[2,3] The explanation for a negative test may be a somatic mosaicism of a *de novo* mutation during gestation, which is not always detectable in DNA extracted from peripheral mononuclear blood cells. Also additional mutations outside the CCM coding exons may account for altered transcription of CCM associated proteins and fail to be detected by conventional gene mapping techniques.[2]

In familial cases, sensitivity of genetic screening of all three *CCM* genes in a CCM proband with an affected relative is 96%. Once the mutation has been identified in a proband, sensitivity of screening of the relatives of this particular patient is 100%.[2] Genetic counselling is important to help patients and relatives to come to an informed choice.

When mutation screening is negative, predictive testing of relatives is not an option, which precludes the need for a magnetic resonance imaging (MRI). When mutation screening is positive, an additional MRI would be recommended. Although the sensitivity of MRI is very high, MRI as an initial screening test does not exclude a predisposition for CCM, as the disease may be in its latent phase, devoid of CNS lesions.[2,27]

Predictive testing of minors should not be performed, given the possible psychological and socio-economic consequences of genetic testing, late onset, and reduced penetrance.[2]

Prenatal diagnosis and pregnancy

Prenatal diagnosis or pre-implantation genetic diagnosis is technically feasible in known familial mutations. Decisions about termination of pregnancy in case of familial mutation detection in a foetus might be difficult, because of reduced penetrance and late onset of symptoms. There is no contra-indication for pregnancy and normal delivery in patients with identified small lesions, without recent clinical signs of haemorrhage. Large lesions or recent symptomatic haemorrhages are a relative contraindication for pregnancy. In case of pregnancy, caesarean section should then be considered.[2,27]

Clinical management of CCMs

Clinical monitoring of CCM depends on the presence of clinical manifestations. In asymptomatic individuals with an increased risk of CCM, a MRI analysis every 1 or 2 years should be considered. In our hospital MRI will be performed in carriers or at-risk persons. Only if neurological problems arise or increase, MRI will be repeated. The indication for surgery should be discussed individually with the patient in an experienced neurosurgical centre. Thereby, patients clinical course in combination with MRI characteristics of the CCM lesion, such as localization, size or new haemorrhage, are important factors for the decision of surgery. In case of deep-seated or brainstem lesions, surgery is associated with a morbidity rate of 30–70% and a mortality rate of 2%. Stereotactic radiosurgery for these lesions remains controversial.[77–79]

Medical treatment consists of inhibition of RhoA by simvastatin, or its effector protein ROCK by fasudil. Also cyclic adenosine monophosphate-elevating drugs should be considered. All of them stabilize CCM lesions by improving vascular integrity.[66,79–82] Preventing progression of CCM lesions could be reached by sorafenib, an anti-angiogenic drug, targeting VEGF receptors and ERK signalling, which is enhanced in the endothelium of CCM lesions.[83,84] Treatment with antiplatelet drugs should be avoided, whereas anticoagulation with coumadin derivatives is contra-indicated .[2,19,53]

Prognosis of CCMs

The long-term prognosis of familial CCM is not well known, but the available data suggest that it is quite favourable after (surgical) treatment.

MRI identified new lesions appear at a rate of 0.2–0.4 lesions per patient year. The new onset seizure rate is 2.4% per patient year and the haemorrhage rate is 3.1%.[2,3,10,27,85]

CONCLUSIONS

The pathogenesis of CCM remains to date incompletely clarified. One theory is a perturbed relationship between adhesion and migration of endothelial precursor cells during the formation of the primary vascular plexus. Initiation, guidance and termination of migration are precisely regulated by interaction with the extracellular matrix and neighbouring cells. Adhesion and migration are linked by the CCM pathway proteins. CCM complex components function as bridging molecules between junctional and cytoplasmic proteins. Loss-of-function of one of the CCM proteins leads to a decrease in adhesion. This theory is mainly based on research performed in endothelial cells. Additional studies to the effect of interaction between neural and endothelial cells are necessary, as it remains unclear whether the primary defect of CCM is of vascular or neuronal origin.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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