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Cerebral cavernous malformation (CCM) is a vascular malformation causing neurological problems, such as headaches, seizures, focal neurological deficits, and cerebral haemorrhages. CCMs can occur sporadically or as an autosomal dominant condition with variable expression and incomplete penetrance. Familial forms have been linked to three chromosomal loci, and loss of function mutations have been identified in the KRIT1/CCM1, MGC4607/CCM2, and PDCD10/CCM3 genes. Recently, many new pieces of data have been added to the CCM puzzle. It has been shown that the three CCM genes are expressed in neurones rather than in blood vessels. The interaction between CCM1 and CCM2, which was expected on the basis of their structure, has also been proven, suggesting a common functional pathway. Finally, in a large series of KRIT1 mutation carriers, clinical and neuroradiological features have been characterised. These data should lead to more appropriate follow up, treatment, and genetic counselling. The recent developments will also help to elucidate the precise pathogenic mechanisms leading to CCM, contributing to a better understanding of normal and pathological angiogenesis and to the development of targeted treatment.

Abbreviations: CCM, cerebral cavernous malformation

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ADVANCEMENTS REGARDING KRIT1
Clinical developments
As expected on the basis of linkage, a mutation in KRIT1 was found in approximately 40% of familial CCM cases. So far about 100 distinct mutations have been reported; however, until recently the associated phenotype has been poorly described. Denier et al carried out a detailed clinical, neuroradiological, and molecular analysis of 64 KRIT1 families. Nearly half the patients had their first neurological symptoms before 25 years of age, which is earlier than in sporadic CCM. In their cohort, the maximum clinical penetrance was about 62% on the basis of 202 KRIT1 mutation carriers (table 1). This percentage is smaller than the clinical penetrance previously associated with the CCM1 locus (88%), and highlights the importance of large clinical studies to allow precise genetic counselling. This discrepancy could be explained by the size of the cohort (64 v 8 families) but also by the age limit of the KRIT1 mutation carriers included (10 v 20 years), older patients being more susceptible to developing symptoms than younger ones. Denier et al also showed that the radiological penetrance is incomplete, as at least five asymptomatic mutation carriers—aged from 27 to 48 years—did not show any cerebral lesion on highly sensitive gradient echo MRI sequences. In addition, at least four patients had only one lesion on gradient echo MRI sequences, showing that in some patients the familial nature of the lesion on the basis of multiplicity could be overlooked.

New insights into the expression and function of KRIT1 protein
During recent years, many studies have attempted to identify the function of KRIT1 and the mechanism through which it causes CCM. Despite the vascular nature of CCMs, KRIT1 mRNA and protein have been detected in astrocytes, neurons, and various epithelial cells in adults. Importantly, KRIT1 protein, but not mRNA, was also detected in vascular endothelial cells during early angiogenesis, as well as in capillaries and arterioles of several human adult organs. Krit1 was also shown to co-localise with microtubules in bovine endothelial cells. However, the Krit1 polyclonal antibody used, detected a much smaller protein (58–60 kDa) than predicted (78–84 kDa). Another antibody has since identified the predicted size protein on western blot, but the primary location of Krit1 awaits elucidation.

Murine embryos lacking the Cm1 gene suggested an essential role of Krit1 in arterial morphogenesis and identity. The homozygous mutant embryos died in mid-gestation and the vascular defect was associated with downregulation of artery specific markers: Efnb2, Dll4, and Notch4. This suggests that the starting point of the development of CCM could be defective arterialisation. This is surprising, as CCMs are slow flow lesions. However, it could be that Krit1 is important for arterial differentiation, and if deficient leads by default to venous identity, with abnormal morphology. If so, Krit1 could also be involved in the pathogenic pathways leading to arteriovenous malformations.

Krit1 does not have exactly the same effect in mouse as in man. Analysis of 20 Cm1−/− mice did not show any cavernous malformation. In contrast to man, an additional deficit may be needed, as shown by Cm1/Trp53 double mutant mice. Cerebral vascular malformations, presenting features reminiscent of cavernous malformation but also of venous malformation and capillary telangiectasia, were observed in 55% of the Cm1−/−Trp53−/− mice. As neither Trp53−/− nor Trp53+/− mice present vascular lesions, the p53 tumour suppressor gene is not enough to cause them, but suggests that cell cycle regulators can act as modifiers in the pathogenesis of cerebrovascular malformations.

The initially reported interaction between KRIT1 and RAP1a has not been confirmed. Instead, an interaction with integrin cytoplasmic domain associated protein-1a (ICAP1α) was identified (fig 2). The latter is known to participate in integrin β1 mediated cell adhesion and migration. The interactions of β1 integrin and KRIT1 with ICAP1α occur similarly, through a NPxY motif/PTB domain, which suggests that integrin signalling plays a role in CCM pathogenesis. ICAP1α and KRIT1 possess a functional nuclear localisation sequence and both of them have the capacity to shuttle between the cytoplasm and the nucleus (fig 2).

Moreover,
ICAP1α is able to sequester KRIT1 in the nucleus. It seems that KRIT1 acts as an intracellular signalling molecule through extracellular/adhesion signals, which may be important for the activation of differentiation programmes that determine arterial identity.

IDENTIFICATION OF THE CCM2 GENE

Two teams independently identified \textit{MGC4607} (malcavernin) to be the \textit{CCM2} gene (table 1). They used two different approaches: sequencing of positional candidate genes and loss of heterozygosity mapping, respectively. After exclusion of mutations in the \textit{KRIT1} gene, Liquori and co-workers identified eight different mutations in nine of 27 patients, and Denier \textit{et al} in 10 of 30 patients. This corresponds to the expected frequency of about 20%, based on previous linkage data. All but one mutation provoked either a premature termination codon or a deletion of the first exon, suggesting loss of function. In one case, a missense mutation, L198R, was reported. Verlaan \textit{et al} did not identify any mutation in \textit{MGC4607} in a cohort of 31 sporadic patients lacking \textit{KRIT1} mutation: 21 with a single and 10 with multiple malformations.

The \textit{CCM2} gene has several orthologues in various vertebrates, but no parologue. Northern blot analysis showed ubiquitous expression in human adult tissues (MTC1 panel, Clontech), similar to \textit{KRIT1}. In situ hybridisation studies have shown Ccm2 mRNA expression in neurons\textsuperscript{15} and astrocytes,\textsuperscript{16} similar to Ccm1 expression at embryonic and adult stages. Ccm2 expression was also transiently observed in meningeal and parenchymal cerebral vessels.

Interestingly, the CCM2 protein (malcavernin) contains a phosphotyrosine binding (PTB) domain, similar to ICAP1α, suggesting an interaction between KRIT1 and malcavernin, and a common functional pathway. Indeed, Zawistowski \textit{et al} showed that KRIT1 and malcavernin interact and that KRIT1 acts as an intracellular signalling molecule through extracellular/adhesion signals, which may be important for the activation of differentiation programmes that determine arterial identity.

IDENTIFICATION OF THE CCM3 GENE

The latest breakthrough in unravelling CCM pathogenesis was the identification of \textit{PDCD10} as the \textit{CCM3} gene (table 1). Bergametti and coworkers again used loss of heterozygosity mapping, proving the efficiency of this method in loss of function based disorders. They found seven distinct mutations including one large deletion in eight of 20 families studied. As point/small mutations in the \textit{KRIT1} and the
MGCG4607 genes had already been excluded, a larger proportion of mutations in the PDCD10 gene was expected, according to previous linkage data. Similarly, two other groups identified PDCD10 mutations in a minority of CCM families lacking KRIT1 and MGCG4607 mutations: two of 15 and three out of 29, respectively. Moreover, in the study undertaken by Liquori and co-workers, in one large CCM3 locus linked family, the PDCD10 gene was excluded by a recombination event and these investigators suggested the existence of a fourth CCM gene mapping near or within chromosome 3q26.3–3q27.2.

As in the case of the MGCG4607 gene, no paralogue was identified for PDCD10 in the human genome. Instead, database searches revealed several highly conserved orthologues in vertebrates and invertebrates, highlighting an evolutionary conservation. Like KRIT1 and malcavernin, PDCD10 is ubiquitously expressed on the basis of northern database searches revealed several highly conserved orthologue genes. As with Ccm1 and Ccm2, in situ hybridisation showed Ccm3 mRNA expression in neuronal cells at embryonic and adult stages. Similarly, its expression coincided with that of Ccm2 in meningeal and parenchymal cerebral vessels. Otherwise little is known about this gene. Curiously, its expression was found to be upregulated in the human myeloid cell line TF-1, upon induction of apoptosis. As the CCM 1, 2, and 3 phenotypes seem to be very similar if not the same, and as the mRNA expression patterns overlap, PDCD10 most probably has a function closely linked to that of CCM1 and 2.

**FUTURE**

The identification of the three CCM genes represents an important step towards the elucidation of the molecular basis of CCM. In addition, large prospective studies of genotype–phenotype association can now be undertaken. This would allow evaluation of clinical penetrance, age at onset, frequency and severity of symptoms, evolution, and response to treatment, all pivotal factors for designing accurate patient care guidelines. Other interesting questions could be addressed. What is the frequency of de novo germline mutations? Is the KRIT1 associated hyperkeratotic cutaneous capillary-venous malformation also present in patients with mutations in the MGCG4607 or the PDCD10 genes, suggesting an important function in cutaneous angiogenesis for all three proteins? Is there a significant co-occurrence of CCMs with developmental venous anomalies as suggested, and if so, what is the basis for this? Do all CCM families have a mutation in one of the three known genes, or do others exist? Twelve families in the screening carried out by Bergametti et al did not harbour a mutation in any of the three CCM genes. Large deletions and non-sensitivity of the techniques used could explain this, but it also opens the door for the existence of a fourth gene, as recently suggested by Liquori et al. Another aspect for deeper investigation is the aetiology of sporadic CCM: are they caused by genetic or environmental factors or both? If genetic in origin, are the causative genes the same as in familial cases? Sporadic patients with multiples lesions seem to harbour KRIT1 mutations in approximately the same proportion as familial cases. These mutations are either de novo or inherited from an asymptomatic parent. If this is also true for malcavernin and PDCD10, multiple CCMs can be postulated to have a genetic aetiology. Thus sporadic cases with multiple lesions need to be considered as familial cases, which is of major importance for patient care and genetic counselling. In contrast, sporadic cases with only one malformation may indeed differ in aetiology, with no increase in risk for progeny. Based on these data, we suggest a clinico-genetic risk evaluation scheme (Fig 3).

**Figure 3** Scheme for evaluation of genetic risk.

The pathogenic mechanism leading from the heterozygous germline mutation to CCM formation is poorly understood. Two major hypotheses have been put forward: haploinsufficiency and paradigmatic inheritance. The latter has been favoured and could explain several CCM features: the localised nature and the number of lesions (usually one in sporadic cases versus multiple in familial cases) and the age at the first symptom (earlier in familial cases). Moreover, this mechanism has been shown to be true for a patient with another inherited multifocal vascular malformation, glomuvenous malformation. Gault and coworkers also identified two truncating biallelic mutations in KRIT1 in one CCM patient with multiple lesions, although two previous screens had failed to detect somatic mutations in 20 and 72 patients, respectively (including familial and sporadic ones). While the techniques used may not have been sensitive enough, other mechanisms are likely to contribute to CCM pathogenesis. One explanation for trans-heterozygosity, in which case a patient with a germline mutation in the CCM1 gene would have a somatic mutation in CCM2 or CCM3 gene and so forth. This could explain intrafamilial clinical variability. This can now be tested in CCM lesions, as well as in the Ccm1Trp53<sup>−/−</sup> murine model, but may need laser capture dissection to enrich the subpopulation of cells with the hit.

As patients with a familial CCM history present similar neurological symptoms, one could assume that the three CCM genes are involved in a common functional pathway. Indeed, it has been shown that KRIT1 interacts with malcavernin, and there is evidence that loss of this interaction contributes to the CCM pathogenesis. In this scenario, the CCM3 gene product, suspected to be involved in apoptosis, should be a member of this complex or play a role in a KRIT1/malcavernin pathway. As KRIT1 and malcavernin are suggested to be involved in β1 integrin signalling through ICAP1, with possible downstream signalling via p38MAPK, and as PDCD10 was identified as a gene involved in apoptosis, it may be that the CCM pathway functions in cell adhesion governed survival. If the CCM genes have such a role in vascular endothelial cells or neural cells or both, the enlarged endothelial lined cerebral vascular channels could result from inhibited apoptosis. This would be similar to what has been proposed for cutaneous venous malformations, which are caused by TIE-2 point mutations that lead to increased Akt activity.
As CCM1, CCM2, and CCM3 seem to be expressed in neurons rather than in blood vessels, the vascular phenotype should result from a defect in signalling between these two juxtaposed structures. Interestingly, it has been shown that vascular and neuronal development are closely linked with several proteins—for example, neuropilin and VEGF, having a functional role in both (reviewed by Carmeliet12). Moreover, some studies have shown the importance of neuronal invasion of primary capillary plexus for its proper remodelling and maturation (reviewed by Eichmann et al13). Supplementary in vitro and in vivo studies on the three CCM proteins are clearly needed: co-immunoprecipitation and co-localisation studies implicating CCM3, and the generation of conditional homozygous, heterozygous and compound heterozygous mutant mice. This should yield further fundamental insights, especially as to whether the primary defect is vascular or neuronal. Despite the lack of detailed mechanistic understanding of CCM formation, the important discoveries that have been made enable precise molecular diagnosis in patients with a family history or with multiple lesions, testing that should now be taken into clinical practice to allow more appropriate follow up, treatment, and genetic counselling.

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Conflicts of interest: none declared

REFERENCES


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