Cerebral cavernous malformations (CCM) are vascular malformations that can occur as a sporadic or a familial autosomal dominant disorder. Clinical and cerebral MRI data on large series of patients with a genetic form of the disease are now available. In addition, three CCM genes have been identified: CCM1/KRIT1, CCM2/MGC4607, and CCM3/PDCD10. These recent developments in clinical and molecular genetics have given us useful information about clinical care and genetic counselling and have broadened our understanding of the mechanisms of this disorder.

Introduction
Cerebral cavernous malformations (CCM) are vascular malformations that are histologically characterised by abnormally enlarged capillary cavities without intervening brain parenchyma. From large series studies involving post-mortem examinations or MRI of patients, the prevalence of CCM in the general population has been estimated as 0·1–0·5%; most of these malformations were located in the CNS.

Both sporadic and familial forms have been identified. The pattern of inheritance of the familial form is autosomal dominant. The proportion of familial cases has been estimated to be as high as 50% in Hispanic–American patients and close to 10–40% in other populations. Familial cases are characterised by the presence of multiple lesions, whereas sporadic cases usually have a single lesion.

Recent developments in molecular genetics have given us useful information for the clinical management of patients with CCM and their relatives and clues to the mechanisms of this disorder, which will be the focus of this Review.

Familial CCM: clinical and MRI features
Families with several clinically affected patients were first reported by Kufs and colleagues in 1928. Thereafter, several additional studies were published but each of them included families with very limited neuroradiological data until Rigamonti and colleagues, in 1988, characterised clinical and MRI features of familial CCM in a series of 13 Hispanic–American families—a population in which a strong founder effect was later shown. Clinical and MRI features of the first large series of consecutive white, non-Hispanic–American patients were reported in 1998. The recent discovery of CCM genes allowed the identification of mutation carriers and the full description of this disorder.

Clinical symptoms
In most cases, cavernous malformations are located within the brain. About 60% of mutation carriers are symptomatic. Symptomatic individuals typically have generalised or focal seizures, cerebral haemorrhages, focal neurological deficits, and headaches. The average age of onset is about 30 years but symptoms can also start in early infancy and in elderly people. At clinical onset, symptoms frequently include seizures (45%) and cerebral haemorrhage (41%). Haemorrhage occurs at an earlier age than non-haemorrhagic symptoms. Spinal intramedullary cavernous malformations are rarely encountered but their exact frequency is unknown. The malformations can be extra or intramedullar, extradural or intradural, and patients present with progressive or acute neurological symptoms, the latter being associated with acute haemorrhage.

Extra neurological symptoms are detected in a small proportion of patients with familial CCM. Retinal and cutaneous cavernomas are among the most common extraneurological locations. Retinal cavernomas are rare and occur in about 5% in those with familial CCM; they are unilateral, generally stable, and are usually asymptomatic. Retinal haemorrhage is not common. Diagnosis is based on fundus examination and does not require fluorescein angiography (figure 1). Retinal cavernomas have been reported in the absence of any associated brain CCM lesion. Distinct ocular lesions, such as choroidal haemangiomas, have recently been described in association with familial CCM. Multiple cutaneous lesions, including bluish nodules, cherry angiomas, capillary vascular anomalies, and eruptive multiple angiokeratomas, have also been described in association with CCM. Hyperkeratotic cutaneous capillary venous malformations (HCCVM) are the most specific type of lesion; they are congenital, stable, and in most cases involve a single lesion located on the limbs (figure 2). These HCCVM malformations have been described in association with familial CCM.
reported only in familial CCM, and never in the general population or in sporadic CCM. In addition to these retinal and cutaneous CCM lesions, other organs may very occasionally be affected.32

Neuroradiological features
MRI is the most sensitive investigation for CCM lesions. A specific CCM neuroradiological classification for four types of lesions has been proposed.35 Type 1 lesions present as hyperintense signals on T1 and T2 sequences. Type 2 lesions—which appear as mixed hyperintense and hypointense signals, surrounded by a rim of hypointense signal—are the most typical CCM lesions. Other MRI patterns include hypointense signals on T1 and T2 sequences (type 3 lesions) and hypointense signals detected only on gradient-echo sequences (type 4 lesions). Type 1 lesions indicate acute haemorrhage, whereas type 2 lesions indicate acute and chronic haemorrhage or calcifications. Type 3 and type 4 lesions are asymptomatic. Type 4 lesions may correspond to precursors of mature cavernous angiomas.36 Type 2 lesions are strongly suggestive of CCM lesions. However, in some cases diagnosis can be difficult with haemorrhagic metastases, particularly in the presence of contrast enhancement, surrounding oedema, rapid recurrent haemorrhage, and when lesions increase in size. If there is doubt over diagnosis, surgical removal of the lesions could be considered. Biopsy is not recommended because of the haemorrhagic risk. T2 and gradient-echo magnetic resonance hypointense signals indicate microbleeds and are not specific to CCM. They may be encountered in various cerebral small vessel diseases (amyloid angiopathy, atherosclerosis, CADASIL) and are almost invariably associated with leucoaraisis.

The presence of multiple lesions on cerebral MRI is one of the main features of symptomatic and asymptomatic mutation carriers.4,12,13 Gradient-echo sequences are much more sensitive than turbo spin echo (TSE) sequences in establishing the presence of multiple lesions (figure 3). Three times more lesions are detected on gradient echo than on TSE sequences in a given mutation carrier.13 About 20% of mutation carriers have only one lesion on both T1 and T2 sequences and among them, 75% have multiple lesions on gradient-echo sequences.36 These data emphasise the need for a systematic use of gradient-echo sequences to investigate patients suspected to have CCM. These data also suggest that the hereditary nature of the disorder can be overlooked in a few patients who present as sporadic cases with a unique lesion.

Some mutation carriers may not have any lesions on both TSE and gradient-echo MRI sequences, even at an advanced age.14,37 Together, these data also establish that not only clinical but also radiological penetrance is incomplete—precluding the use of cerebral MRI to firmly establish a non-carrier status even at an adult age and even when using highly sensitive gradient-echo sequences.

Evolution and prognosis
Familial CCM is a disorder that has a high tendency to evolve over time as shown by the strong association between patients’ age and the number of lesions.30 The appearance of new CCM lesions is a hallmark of the familial form of the disease and indicates the dynamic features of this disorder.30,35,38 The highly dynamic nature of CCM lesions is also indicated on MRI sequences by changes in size and signal intensity of cavernomas and occurrence of haemorrhages. The annual haemorrhagic event rate is estimated at 2·5% per lesion.35,39,40

Prognosis of familial cavernomas is not well known. Both the number of patients included and the duration of follow-up were limited in the various studies done so far.12,35,39,40 In a cross-sectional study, most of the patients with cerebral haemorrhage presented with only one symptomatic haemorrhage and the remaining patients presented with two to four hemorrhagic events with an average interval between two events close to 37 months (range: 3–138 months).12,41 All these studies strongly suggest that the number of clinical events and functional outcome are not associated with the number of lesions, but rather with the location of lesions, with brainstem and basal ganglia lesions leading to a particularly severe outcome.
In addition to a brainstem location, other factors may increase the haemorrhagic risk, including a previous history of haemorrhage, pregnancy, or anticoagulant treatment. Haemorrhagic risk and treatment strategies during pregnancy and delivery are not clearly defined, but there is growing evidence that pregnancy increases the haemorrhagic risk. Patients with small supratentorial lesions and no recent clinical haemorrhage usually have a normal pregnancy and normal delivery. However, large lesions and brainstem or basal ganglia lesions or a recent symptomatic haemorrhage favour a caesarean section delivery, or may even contraindicate pregnancy. The counselling of women about pregnancy and delivery is difficult and requires a multidisciplinary approach. Recurrent symptomatic haemorrhages after changes in contraceptive treatment have been reported in one case; however, large series studies are needed to assess the impact of hormonal treatment.

Anticoagulant treatment, including prophylactic therapy, can induce haemorrhage even at low doses. It is unknown whether antiplatelet drugs increase the hemorrhagic risk. Influence of cranial trauma on CCM evolution is also not yet known.

Altogether and despite the fact that prospective studies enrolling a large number of patients for a long period of time are not available so far, the available data suggest that the long-term prognosis is quite favourable with a preserved autonomy in 80% of cases except for those with brainstem cavernomas.

“Sporadic” cases with multiple lesions

Sporadic cases usually show a single lesion on both TSE and gradient-echo sequences, are not inherited, and do not carry a CCM gene mutation. However, some cases have multiple MRI lesions although they do not have any known clinically affected relative and present therefore as sporadic cases. Combined use of clinical and MRI screening together with molecular testing has helped to clarify the situation in these cases.

Clinical and neuroradiological analyses done before the identification of CCM genes in a series of 22 consecutive sporadic cases with multiple lesions showed that 75% of them were affected with a hereditary form of the disease with incomplete penetrance because one of their two asymptomatic parents showed lesions on MRI. These patients are true familial genetic cases.

What about the 25% remaining patients whose biological parents had a normal TSE/gradient-echo brain MRI? Some of these patients were affected by a hereditary form of the disease as they were shown to carry a de novo mutation in one of the three CCM genes. These patients are therefore true genetic cases and their offspring have a 50% chance of inheriting the disorder. In some other of these sporadic cases with multiple lesions, screening of all three CCM genes did not detect any mutation. The multiplicity of their lesions strongly suggests, however, that these patients were affected by a genetic form of the disease. There may be several hypotheses to explain the absence of any detected mutation. Some cases may involve a somatic mosaicism of a de novo mutation that occurred during gestation and is not detectable in DNA extracted from peripheral blood cells. This concept is important for genetic counselling. Other hypotheses include the possibility of mutations located outside CCM coding exons, large deletions or duplications which would not have been detected by the strategies used so far, and finally, the possibility of an as-yet unidentified gene.

Altogether, these data strongly suggest that the vast majority, if not all, of these sporadic cases with multiple lesions are true genetic cases and should be managed in the same way as familial cases.

Genetics and CCM

Pattern of inheritance

The pattern of inheritance in CCM is autosomal dominant with an incomplete clinical and neuroradiological penetrance. In a genetic linkage analysis of the known CCM loci in 20 families Craig and colleagues have estimated the clinical penetrance at 88% in CCM1 families, 100% in CCM2, and 63% in CCM3 families. However, a recent analysis done in a large series of families segregating a Krit1/CCM1 mutation showed a clinical penetrance of about 60%. Additional studies done on large series of CCM2 and CCM3 families are now needed to precisely estimate the penetrance in those families. De novo mutations have been reported for all three genes. They may be more common in CCM3 patients.

Molecular genetics data

Genetic linkage analyses mapped three CCM loci to chromosome 7q (CCM1), 7p (CCM2), and 3q (CCM3). A strong founder effect has been reported in Hispanic–American patients with CCM, with most families linked to the CCM1 locus. All three genes lying at these loci have now been identified (figure 4).

The CCM1 gene contains 16 coding exons that encode Krit1—a 736 amino-acid protein containing three ankyrin domains and one FERM domain. So far, more than 90 distinct CCM1 mutations have been published. CCM1 mutations are highly stereotyped as all lead to a premature termination codon. These data strongly suggest that a loss of function, through mRNA decay of the mutated allele, is the most likely pathophysiological mechanism involved in patients with the CCM1 mutation.

CCM2, a ten-exon gene, encodes the MGC4607 protein, also called malcavernin, which contains a phosphotyrosin binding domain. CCM3 has very recently been identified. This seven-exon gene encodes PDCD10, a protein without any known conserved functional domain that may be involved in apoptosis. Most CCM2 and CCM3 mutations reported so far lead to a premature termination
Molecular screening of coding exons of these three genes in 163 white non-Hispanic–American patients with CCM with multiple lesions and/or an affected CCM relative has led to the identification of the causative mutation in 78% of CCM cases. In the remaining 22% of cases, no point mutation was detected, which suggests the existence of either undetected deletions or mutations or the existence of other CCM genes. The mutation detection rate was substantially higher in patients with an affected relative (96%) than in sporadic cases with multiple lesions (57%). Among the 78% of mutation carriers, 53% had a mutation in CCM1, 15% in CCM2, and 10% in CCM3. The 10% proportion of patients with a CCM3 mutation was much lower than the proportion expected from linkage data.

Interestingly, Liquori and colleagues recently showed in a series of families without any point mutation in any of the three CCM genes a recurrent large deletion of CCM2, which accounted for CCMs in 14 of the 25 families investigated. Detailed analyses of clinical and radiological features of CCM1, CCM2, and CCM3 patients has been reported. Genotype–phenotype correlation analysis strongly suggests that CCM3 mutations may confer a higher risk for cerebral haemorrhage during childhood. With regard to sporadic cases with a unique lesion on both T2 and gradient-echo cerebral MRI, no mutation was detected in the reported series. The combination of these data with those obtained from familial CCM strongly suggests that sporadic cases with a unique lesion do not carry any germ line CCM mutation.

On the basis of the autosomal dominant pattern of inheritance of CCM and the presence of multiple lesions in familial CCM contrasting with the detection of a single lesion in non-hereditary cavernous angiomas, it has been proposed that a Knudson’s two-hit mechanism might be involved in CCM pathophysiology as reported previously in other hamartomatous disorders. According to this hypothesis, CCM formation would require a complete loss, within affected cells, of the two alleles of a given CCM gene. Loss of one of the alleles (first hit) would be the result of a germ line mutation and loss of the second allele (second hit) will occur somatically within the brain. Gault and colleagues recently reported a biallelic CCM1 germ line and somatic truncating mutations in a CCM lesion, which supported this two-hit mechanism in the formation of lesions at least in patients with the CCM1 mutation.

**Genetic counselling**

The identification of CCM genes means that molecular genetic screening is technically possible for this disorder. When screening all three genes in a CCM proband, sensitivity of genetic screening is around 96% in patients with an affected relative, and 57% in sporadic cases with multiple lesions. Once the mutation has been identified within a proband, sensitivity is 100% when screening the relatives of the patient. The main questions now are: how can molecular genetics screening affect the clinical care of patients and relatives with CCM? Can it be useful to screen for these three CCM genes, and, if so, in which patients or family members? And what are the respective indications of MRI and genetic screening in asymptomatic individuals? Genetic screening should have a balanced risk–benefit ratio and this may vary depending on the situation of the individual being investigated. On the basis of clinical, MRI, and molecular genetic data, the panel 1 should provide some clear indications of the clinical management of CCM patients.

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**Panel 1: Clinical management**

**Asymptomatic**
- MRI survey every 1 or 2 years
- In cases of progressive enlargement of a lesion, discuss surgery

**Symptomatic**
- Haemorrhage: surgery, when feasible
- Medication-responsive epilepsy: medical follow-up
- Refractory epilepsy: consider surgery, discuss with caution
- Radiosurgical treatments

**Pregnancy**
- Small lesions, supratentorial location, absence of recent clinical haemorrhage: normal pregnancy and normal delivery
- Large lesions, brainstem or basal ganglia location, recent symptomatic hemorrhage: caesarean section may be indicated/pregnancy may be contraindicated

**Spinal cord location**
- Asymptomatic: medical survey
- Symptomatic: discuss surgery

**Medications contraindicated**
- Anticoagulant treatment: contraindicated
- Antiplatelet drugs: no data

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**CCM1: KRIT1**
- NH2-Ankyrin-FERM-COOH

**CCM2: MGC4607 (Malcavernin, OSM)**
- NH2-PTB-COOH

**CCM3: PDCD10**
- NH2-COOH

**Figure 4:** Proteins encoded by the three CCM genes: KRIT1 (CCM1), MGC4607 (CCM2), and PDCD10 (CCM3).

NPXY = motif interacting with ICAP1 protein. FERM = Protein 4·1, radixin, moesin domain. PTB = phospho, tyrosin-binding domain.
following algorithm may be proposed to facilitate molecular screening decisions.

**Sporadic cases with a unique lesion on T2 and gradient-echo MRI sequences**

The combination of having no affected relative and a unique lesion on gradient-echo sequences renders the risk of having a mutation extremely low. Genetic screening is not indicated in those cases.

**Symptomatic cases with multiple CCM lesions and at least one affected relative**

The genetic nature of the disease is already known and screening will not carry additional information for the care of the patient himself. Unless genetic counselling is being considered, molecular genetic screening is not currently useful for the clinical care of the patient.

**Symptomatic sporadic cases with multiple CCM lesions**

In these cases, the disease is most likely to have a genetic cause. Screening for CCM genes may identify a mutation in around 50% of cases. This information will not change the patient’s clinical care but may be useful for genetic counselling. At this point, however, the patient has to be aware that a negative test does not exclude a genetic cause.

**Asymptomatic individuals with a known affected relative with multiple CCM lesions**

The first step will be to draw the genealogical tree of the family to check whether this individual is at risk depending on the link with the proband. In most cases, neurosurgeons recommend presymptomatic screening and MRI follow-up in positive asymptomatic individuals. However, presymptomatic screening should always be done with great caution because its benefits are unclear. Asymptomatic, adult individuals should be clearly informed in order to make their decision about whether to have presymptomatic screening. Presymptomatic screening can be done using MRI or molecular screening. Sensitivity of MRI, when including a gradient-echo sequence, is very high. However, a negative MRI can be observed in a small proportion of patients who may later develop CCM lesions. Therefore, in order to firmly exclude risk for the disorder, a molecular test has to be done if MRI is negative. Alternatively, presymptomatic screening can be done with molecular screening as a first step. In order to do so, the mutation should have been identified first in a symptomatic patient within the family. When negative, the test allows exclusion of risk of being a carrier and there is no need for an MRI. When positive, MRI is recommended. The choice to use MRI or molecular screening as a first step depends on the availability of the information on the mutation present in the family as well as practical local availability of genetic testing. With regard to ethical considerations, presymptomatic MRI screening should be considered as the equivalent of presymptomatic molecular screening.

**Asymptomatic child with possible risk for the disease**

In the case of a parental request for screening an asymptomatic child who has an affected first degree relative, even more caution should be taken because psychological issues may be more important than in adult individuals.

**Prenatal diagnosis request**

Experience shows that prenatal diagnosis requests for CCM are quite rare but may be encountered in families in which several patients have had severe symptoms. Prenatal diagnosis may be of benefit in such families. In all cases, information should be given to the parents before they make any final decisions. This information should include the fact that 50% of mutation carriers will probably not develop clinical symptoms, that clinical onset occurs in most patients between ages 20 and 40 years, and that several therapeutic approaches are available.

**CCM pathogenesis**

The mechanisms involved in cerebral blood vessel angiogenesis during development are still poorly defined, therefore hampering the understanding of the mechanisms that lead to cerebrovascular malformations. The identification of the CCM genes has broadened understanding of the mechanisms underlying CCM and recent studies have unravelled some of the function of these genes.

In situ hybridization studies showed that the three CCM genes have very similar patterns of expression within the CNS, with a strong expression of CCM mRNAs transcripts in neuronal cells and no detectable or barely detectable expression within the cerebral vessels during development and adult life. The endogenous CCM1 protein expression pattern is more controversial. Gunel and colleagues reported that CCM1 is expressed in neurons and astrocytes but also in cerebral endothelial cells. The authors also reported that CCM1 is associated with microtubules, which is contrary to recently published data that showed that CCM1 is expressed in nuclear and cytoplasmic compartments. These discrepancies may be associated with the specificity of antibodies and will have to be solved before we can draw any conclusions about the CCM1 protein expression pattern.

More information on CCM came from the identification of proteins that interact with the various CCM proteins. CCM1 has been shown to interact with rap1A, a small Ras GTPase, Icap1, a modulator of integrin β1, and more recently with nexin I7, and the CCM2 protein itself. The interaction between CCM1 and Icap1 has been confirmed by two studies and is of great interest with regard to the well-known role of integrin β1 in angiogenesis.

CCM2, also called malcavernin and OSM, has been shown to be a scaffold protein that interacts with kinases involved in the p38 mitogen activated kinase pathway. CCM2 also interacts with actin and Rac1, a small GTPase...
critical for local actin organisation at cell surface structures. The p38 pathway, which is involved in several environmental stresses also plays a major part in angiogenesis. A recent in vitro analysis has shown that CCM1 and CCM2 proteins interact. When overexpressed, CCM2 is capable of sequestering CCM1 within the cytoplasm and forming a molecular complex with MEKK3, a kinase involved in the activation of the p38 alpha pathway. Altogether, these data suggest that CCM1 and CCM2 are in a complex that probably involves MAPK and the p38 pathway. Whether CCM3 is also a member of this complex, and the mechanisms, and cells in which a perturbation of the p38 pathway may lead to CCM vascular lesions still need to be determined.

Very recent in vivo experiments shed some light on the roles of CCM1 and CCM2 in cardiovascular development. Inactivation of CCM1 leads to early death of murine homozygous embryos at midgestation. An arterial defect that includes both dilation and constriction of the aorta and some of its branches, a failure of recruitment of arterial vascular smooth muscle cells, and an abnormal differentiation of arteries were detected in those embryos, which strongly suggests that CCM1 is involved in arterial morphogenesis and differentiation during early embryonic life. Inactivation of CCM1 and CCM2 with the morpholino strategy in zebrafish also leads to an early death of the fish embryo with massive dilation of the heart. This massive enlargement is indicative of an inability of the endocardium to instruct myocardial muscle to thicken. These in vivo data provided important information on the role of CCM1 and CCM2 in large arteries and heart development, the latter being quite unexpected. However, the early death of murine embryos, at a time when cerebral blood vessels have not yet developed, precluded any analysis of the effect of CCM1 on cerebral blood vessel development. CCM2 inactivation also leads to an early death in the mouse embryo. These data emphasise the need to develop conditional mutants in which inactivation of CCM genes will be restricted to the CNS with the use of various tissue or cell-specific promoters.

Conclusions and future directions
The recent identification of the three CCM genes is an important step towards understanding the mechanisms of this disorder. These genes have helped to clarify several features of the disorder, including its incomplete clinical and MRI penetrance, as well as the molecular basis of sporadic cases with multiple lesions. Additional large series studies will now be needed to further assess genotype–phenotype correlations, particularly the prognosis, in relation to the nature of the mutated gene. Several questions have to be addressed. What is the nature of the molecular anomaly in familial CCM cases in whom no mutation has been detected? Are there additional as-yet unidentified CCM genes? Do some patients have mosaicism of a de novo mutation? Are there modifying genes that can explain the intrafamilial clinical variability?

In addition to these questions, one main challenge will be to use the molecular data to understand the mechanisms of this disorder. Several mutant mouse models are currently made that should allow the analysis of the consequences of the inactivation of any of the three CCM genes in specific compartments of the CNS. These mouse studies will be invaluable to understand the mechanisms that lead to these malformations and to assess therapeutic approaches.

Conflicts of interest
We have no conflicts of interest.

Contributors
PL participated in the layout and literature search, wrote sections on clinical and neurological data, and contributed to the figures. CD contributed to the writing of sections on genetic data and contributed figures. FB contributed to the writing of sections on genetic data in the first draft. ETL coordinated the project, drafted the layout, did the literature search, contributed to the writing of sections on clinical and molecular genetics data, and figures, and edited the final version.

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Review