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REVIEW

Cerebral cavernous malformation: new molecular and clinical insights

N Revencu, M Vikkula

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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.

See end of article for authors' affiliations

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Cerebral cavernous malformation (CCM; OMIM 116860) is a vascular malformation characterised by closely clustered enlarged capillary-like channels with a single layer of endothelium without intervening brain parenchyma.[1] CCMs are mostly located in the brain, but are also observed in the spinal cord, retina, and as hyperkeratotic cutaneous capillary-venous malformations on the skin.[2 3] The prevalence is about 0.5%, based on cerebral magnetic resonance imaging (MRI) and necropsy studies of large cohorts of patients.[4 5] However, the clinical prevalence is much lower, as only 20–30% of individuals are symptomatic,[4] usually between the third and the fifth decade of life. The main symptoms are headaches, seizures, focal neurological deficits, and cerebral haemorrhages.

Ultrastructurally, these lesions consist of endothelium lined vascular sinusoids embedded in a dense collagenous matrix. They are characterised by the absence or abnormality of blood–brain barrier components.[6–8] The tight junctions between the endothelial cells are poorly formed or absent, with gaps observed between the cells. No astrocytic foot and no normal nervous tissue is present within the lesion, and pericytes are rare.

Both sporadic and familial forms of CCM have been identified. In the familial form inheritance is autosomal dominant with incomplete penetrance and variable expression.[9 10] The proportion of familial cases is estimated to be 50% in Hispanic American patients of Mexican descent,[11] but seems to be less in other populations.[12] Often, sporadic cases are characterised by the presence of one lesion, whereas in familial CCM, multiple lesions are present and their number is strongly correlated with the patients' age (fig 1).[9 11] In the past, it was considered that up to 31% of familial CCM cases had only one lesion (as reviewed by Siegel[10]), but this was probably an overestimation because of the relative insensitivity of the radiological techniques used. The dynamic nature of the inherited lesions has also been emphasised.[13] The diameter of lesions ranges from a few millimetres to several centimetres, and new lesions appear at a rate of 0.2 to 0.4 lesions per patient-year. The prospective haemorrhage rate is of 3.1%, and the new onset seizure rate is 2.4% per patient-year.[14]

Although rare, in populations other than Hispanic Americans, familial cases allow us to unravel pathogenic molecular mechanisms. Indeed, by linkage analysis, three genetic loci have been implicated, on chromosomal arms 7q (CCM1), 7p (CCM2), and 3q (CCM3) (table 1).[15 16] The symptoms seem to be similar, yet the clinical penetrance was estimated at 88, 100 and 63% in the three loci, respectively.[16] The first break through led to the identification of *KRIT1* (Krev Interaction Trapped1) as the *CCM1* gene.[17 18] It encodes a protein containing known protein–protein interaction domains: four ankyrin repeats, a C-terminal FERM domain and one NPxY motif. Although without known function, KRIT1 was thought to be an intracellular effector owing to its structure and its initial identification by yeast two-hybrid screen as a KREV1/RAP1a interactor.[19] Characterisation of KRIT1 expression during early angiogenesis and in human adult organs, and demonstration of a role in arterial morphogenesis and identity in mice lacking *Ccm1*, have started to unravel KRIT1 function.[20–23] In addition, the identification of the *CCM2*[24 25] and *CCM3* genes[26] has paved the way toward understanding the molecular pathways involved in CCM formation.

Abbreviations: CCM, cerebral cavernous malformation

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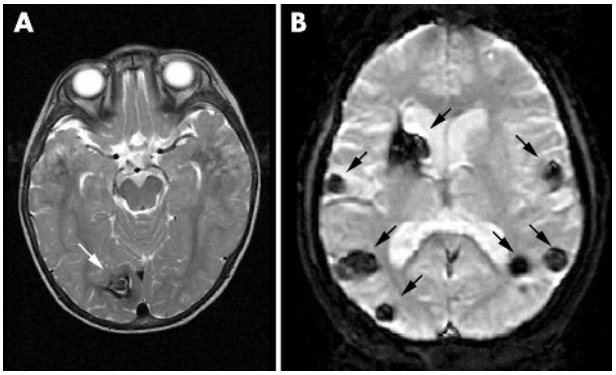

Figure 1 (A) Axial T2 weighted magnetic resonance imaging sequence; solitary lesion (white arrow) in the right occipital lobe in a 3 year old boy. (B) Axial T2 weighted gradient echo sequence; multiple supratentorial cerebral cavernous malformations (black arrows) in a young adult.

ADVANCEMENTS REGARDING KRIT1

Clinical developments

As expected on the basis of linkage,[16] 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.[27] 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 (table1). This percentage is smaller than the clinical penetrance previously associated with the CCM1 locus (88%),[16] 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, neurones, and various epithelial cells in adults.[20 22] 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.[20–22] 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).[28] Another antibody has since identified the predicted size protein on western blot, but the primary location of Krit1 awaits elucidation.[29]

Murine embryos lacking the *Ccm1* gene suggested an essential role of Krit1 in *arterial* morphogenesis and identity.[23] 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.[30] 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 $Ccm1^{+/-}$ mice did not show any cavernous malformation.[30] In contrast to man, an additional deficit may be needed, as shown by *Ccm1/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 $Ccm1^{+/-}Trp53^{-/-}$ 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-1α (ICAP1α) was identified (fig 2).[31 32] 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).[29 33] Moreover,

Table 1 Genes involved in familial cerebral cavernous malformations (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM)

	CCM1	CCM2	CCM3
Locus	7q11–q22	7p15–13	3q25.2–q27
Gene	*KRIT1*	*MGC4607*(malcavernin)	*PDCD10*
OMIM	604214	603284	603285
Mutation type	Loss of function	Loss of function?	Loss of function?
Second hit/trans-heterozygosity	1 Patient ?	? ?	? ?
Molecular function	Modulator of ICAP1α, malcavernin interaction, association with microtubules?	KRIT1 interaction, scaffold for MEKK3	?
Cellular/tissue function	Cell adhesion/migration, arterial morphogenesis/identity	Osmoregulation ?	Apoptosis?
Clinical penetrance	62–88%	?	?
References	12–15, 20, 24, 26, 28, 29, 40	13, 21, 22, 26	13, 23, 26

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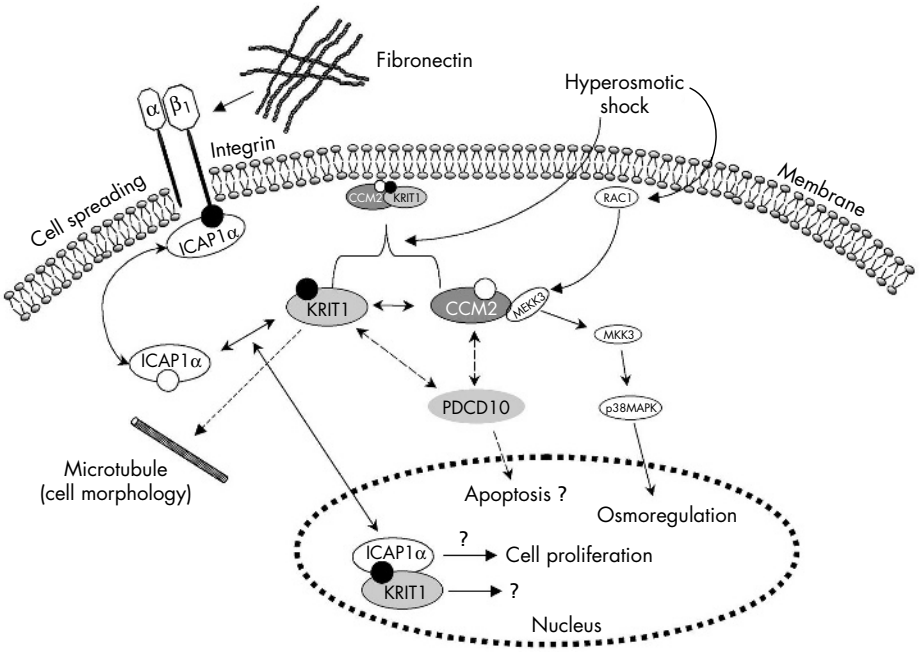

Figure 2 Schematic presentation of molecular pathways involving the CCM proteins. ● NPxY motif; ○ phosphotyrosine binding (PTB) domain; dashed lines, hypothetical interactions. ICAP1α interacts with the β1 integrin cytoplasmic domain and controls cell spreading/cell proliferation on fibronectin. It was suggested that KRIT1 could be a modulator of this pathway. KRIT1/CCM2/ICAP1α can form a ternary complex. ICAP1α and KRIT1 can go to the nucleus. It is not known if they go separately or together. Hyperosmotic environment stimulates in mammalian cells the Rac1-OSM(CCM2)-MEKK3-MKK3-p38MAPK pathway leading to osmoregulation. KRIT1/CCM2/MEKK3 form a ternary complex.

ICAP1α is able to sequester KRIT1 in the nucleus.[29] 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 *MGC4607* (malcavernin) to be the *CCM2* gene (table 1).[24 25] They used two different approaches: sequencing of positional candidate genes and loss of heterozygosity mapping, respectively. After exclusion of mutations in the *KRIT1* gene, Liquori and co-workers identified eight different mutations in nine of 27 patients, and Denier *et al* in 10 of 30 patients. This corresponds to the expected frequency of about 20%, based on previous linkage data.[16] 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 *et al* did not identify any mutation in MGC4607 in a cohort of 31 sporadic patients lacking KRIT1 mutation: 21 with a single and 10 with multiple malformations.[34]

The *CCM2* gene has several orthologues in various vertebrates, but no paralogue. Northern blot analysis showed ubiquitous expression in human adult tissues (MTC1 panel, Clontech), similar to KRIT1. In situ hybridisation studies have shown Ccm2 mRNA expression in neurones[35 36] and astrocytes,[37] 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 *et al* showed that KRIT1 and malcavernin interact and that malcavernin is capable of sequestering KRIT1 in the cytoplasm (fig 2).[29] The only *CCM2* missense mutation reported so far—L198R, located in the PTB domain—was able to inhibit this interaction. This suggests that loss of the KRIT1–malcavernin interaction could contribute to CCM pathogenesis, possibly by its regulation of KRIT1 shuttling to and from the nucleus. Compared with ICAP1α, the NPxY motif was not critical for the KRIT1–malcavernin interaction. Moreover, KRIT1, malcavernin, and ICAP1α were found in a ternary complex, suggesting that other interaction sites exist.

The murine orthologue of malcavernin, OSM (osmosensing scaffold for Mekk3), was shown to modulate the Mekk3 dependent p38Mapk activation induced by hyperosmotic shock (fig 2).[38] Knockdown of endogenous *CCM2* in HEK293 cells resulted in marked inhibition of sorbitol mediated p38 activation, which was confirmed in murine $Ccm2^{+/-}$ embryonic fibroblasts.[29 38] As KRIT1 was identified by immunoprecipitation in a ternary complex with malcavernin and MEKK3, this suggests a possible function of the CCM1/2 complex in p38MAPK activation.[29] Moreover, upon sorbitol treatment the CCM1/2 complex was shifted from the cytoplasm to the cell periphery. It is well known that PTB domain containing proteins have the capacity to bind phospholipids in addition to peptides, and preliminary experiments suggest that OSM would be able to do this as well.[39] It could be that this pathway also lies downstream of integrin mediated activation. Interestingly, mice lacking Mekk3 or p38α Map kinase have significant defects in placental angiogenesis and in blood vessel development, in particular in the head region.[40 41]

IDENTIFICATION OF THE *CCM3* GENE

The latest breakthrough in unravelling CCM pathogenesis was the identification of *PDCD10* as the *CCM3* gene (table 1).[26] 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 *KRIT1* and the

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MGC4607 genes had already been excluded, a larger proportion of mutations in the *PDCD10* gene was expected, according to previous linkage data.[16] Similarly, two other groups identified *PDCD10* mutations in a minority of CCM families lacking *KRIT1* and *MGC4607* mutations: two of 15 and three out of 29, respectively.[42 43] 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 *MGC4607* 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 blot analysis. As with Ccm1 and Ccm2, in situ hybridisation showed Ccm3 mRNA expression in neuronal cells at embryonic and adult stages.[35] 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.[44] 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 percentage of familial CCM and 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 *MGC4607* 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,[45] 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.[26] 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*.[43] 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 the familial forms? Sporadic patients with multiples lesions seem to harbour *KRIT1* mutations in approximately the same proportion as familial cases.[46 47] 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).

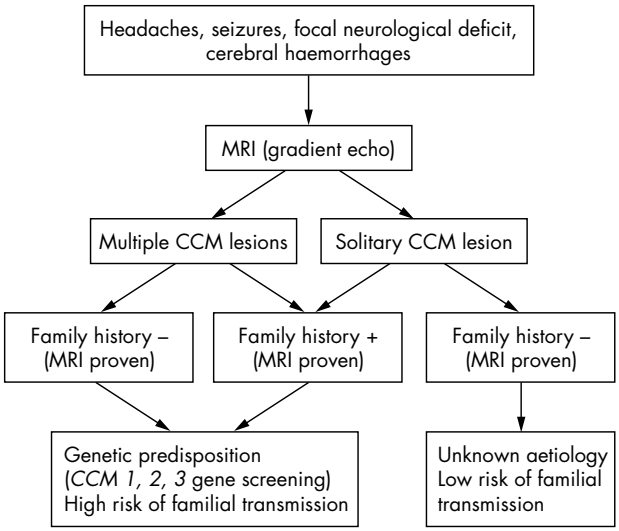

Figure 3 Scheme for evaluation of genetic risk.

The pathogenic mechanism leading from the heterozygous germline mutation to CCM formation is poorly understood. Two major hypotheses have been put forward: haploinsufficiency and paradominant 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.[48] 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).[49–51] While the techniques used may not have been sensitive enough, other mechanisms are likely to contribute to CCM pathogenesis.[52] One explanation is 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 $Ccm1^{+/-}Trp53^{-/-}$ murine model, but may need laser capture dissection to enrich the subpopulation of cells with second 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.[29] 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.[53 54]

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As CCM1, CCM2, and CCM3 seem to be expressed in neurones 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 Carmeliet[55]). Moreover, some studies have shown the importance of neuronal invasion of primary capillary plexus for its proper remodelling and maturation (reviewed by Eichmann *et al*[56]). 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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