

Case report

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Variable expression of cerebral cavernous malformations in carriers of a premature termination codon in exon 17 of the *Krit1* gene

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Abstract

Background: Cerebral cavernous malformations (CCM) present as either sporadic or autosomal dominant conditions with incomplete penetrance of symptoms. Differences in genetic and environmental factors might be minimized among first-degree relatives. We therefore studied clinical expression in a family with several affected members.

Methods: We studied a three-generation family with the onset of CCM as a cerebral haemorrhage in the younger (four-year-old) sibling. Identification and enumeration of CCMs were performed in T2-weighted or gradient-echo MRIs of the whole brains. Genetic analysis comprised SCCP, sequencing and restriction polymorphism of the *Krit1* gene in the proband and at risk relatives.

Results: The phenotypes of cerebral cavernous malformations (CCMs) in carriers of *Krit1* mutations were very variable. We identified a novel frameshift mutation caused by a 1902A insertion in exon 17 of the *Krit1* gene, which leads to a premature TAA triplet and predicts the truncating phenotype Y634X. A very striking finding was the absence of both clinical symptoms and CCMs in the eldest sibling harbouring the 1902insA.

Conclusions: Patients in this family, harbouring the same mutation, illustrate the very variable clinical and radiological expression of a *Krit1* mutation. The early and critical onset in the proband contrasts with minor clinical findings in affected relatives. This consideration is important in genetic counselling.

Background

Cerebral cavernous malformations (CCM) are enlarged capillary cavities without alterations in the surrounding brain parenchyma. Studies based on necropsy and magnetic resonance imaging indicate a prevalence in the general population close to 0.1–0.5 percent. CCMs present as

either sporadic or autosomal dominant conditions with incomplete penetrance of symptoms such as epilepsy, headaches, seizures and cerebral haemorrhage. Neuroimaging penetrance of CCMs is much higher than clinical penetrance and almost 60% of at-risk relatives are symptom-free in spite of having MRI lesions [1]. Sporadic CCM

is characterised by a single cavernous angioma, whereas images of multiple lesions are found in inherited CCM [1].

Mutations causing premature termination triplets in *CCM1*, the gene encoding the Krit1 protein, cause hereditary cavernous angiomas [2,3], and a germline mutation in the *KRIT1* gene has been reported to cause multiple lesions in one CCM patient [4]. A great variety of mutations have been described [5], including a redundant mutation in Hispano-Mexican families corresponding to a chromosomal haplotype that is not shared by patients of Spanish extraction [6].

The Krit1 protein was identified by its interaction with Rap1A, a small Ras like GTPase [7]. Four novel coding exons and other non-coding exons have been described by computational and experimental analyses [8,9]. *KRIT1* encodes a 736 amino-acids protein containing four ankyrin domains, a FERM domain and a C-terminal portion that interacts with Rap1A. The four novel exons encode an N-terminal region containing an NPXY motif that is required for interaction of with icap1 α , a modulator of β 1 integrin [10,11].

We report a mutation causing a premature termination triplet in exon 17 of the Krit1 gene in a family with diagnoses of cerebral cavernous angioma. The early onset in the proband contrasts with minor symptoms in relatives who are carriers of the mutation. Particularly striking is the absence of CCMs in the eldest sibling.

Methods

Patients

The onset of the disease in this family was a cerebral haemorrhage in the younger sibling, the 4-year-old

proband. The family comprises a three-generation pedigree with minor neurological symptoms in the antecedents. Antecedents were assessed by a retrospective clinical study that revealed three asymptomatic siblings and non-specific symptoms in other affected relatives (his sister and grandmother) who complained about headaches. Identification and enumeration of CCMs were performed in T2-weighted or gradient-echo MRIs of the whole brains.

Genetic analysis

CCM1 haplotypes of individuals of the CVE10 family were analyzed using the protocols and primers of a set of polymorphic microsatellite markers spanning the *CCM1* interval, as previously described [12].

A fragment of the Krit1 gene containing exon 17 was PCR-amplified with forward (5'TGGTACATTTTCCTTCA) and reverse (5'TGACATGATTGGTAAAAA) primers. The nomenclature of the cDNA refers to the full length *KRIT1* cDNA in GenBank (accession number AF296765). Genomic DNA was initially screened by analysis of SSCP. PCR fragments were denatured at 95°C for 5 min, ice chilled, and separated by electrophoresis in 10% acrylamide-bisacrylamide (37.5:1) gels containing 10% glycerol without urea.

Sequencing of exon 17 was carried out in genomic DNA by the terminal dideoxynucleotide method. Automated sequence analyses of both the sense and antisense strands were done with 5'-labeled IRD800 primers according to the protocols of the SequiTherm kit (Epicentre Technologies, Madison, Wisconsin). Polyacrylamide gel electrophoresis and analysis of the sequencing fragments were performed in a LI-COR DNA4000 sequencer with the software provided by the manufacturer (LI-COR Inc, Lincoln, Nebraska, Canada).

Table 1: Location and number of cerebral malformations. MRIs were obtained with echo-gradient sequences except the T2-weighted images of patient I-1. Individual II-5 is the proband's uncle, not shown in figure 1, who harbours the 1902insA mutation. MRIs of sibling III-2 were repeatedly negative and therefore he was excluded from the table.

	I-1	II-1	II-5	III-3	III-4	III-5	%
Age	82	46	52	15	11	4	
Onset age	67	---	39	---	9	4	
Symptoms	Headache	None	Hemorrhage	none	Headache	Hemorrhage	
Total	34	51	37	5	9	8	100
Frontal	8	21	14	1	1	1	32
Parietal	10	12	10	1	1	2	25
Temporal	4	8	6	2	2	4	18
Occipital	5	5	3	0	4	0	12
Diencephalon	0	1	1	1	0	0	2
Midbrain	1	0	0	0	0	0	0.7
Pons	0	1	1	0	1	0	2
Medulla	0	1	0	0	0	0	0.7
Cervical	6	2	2	0	0	1	7.6

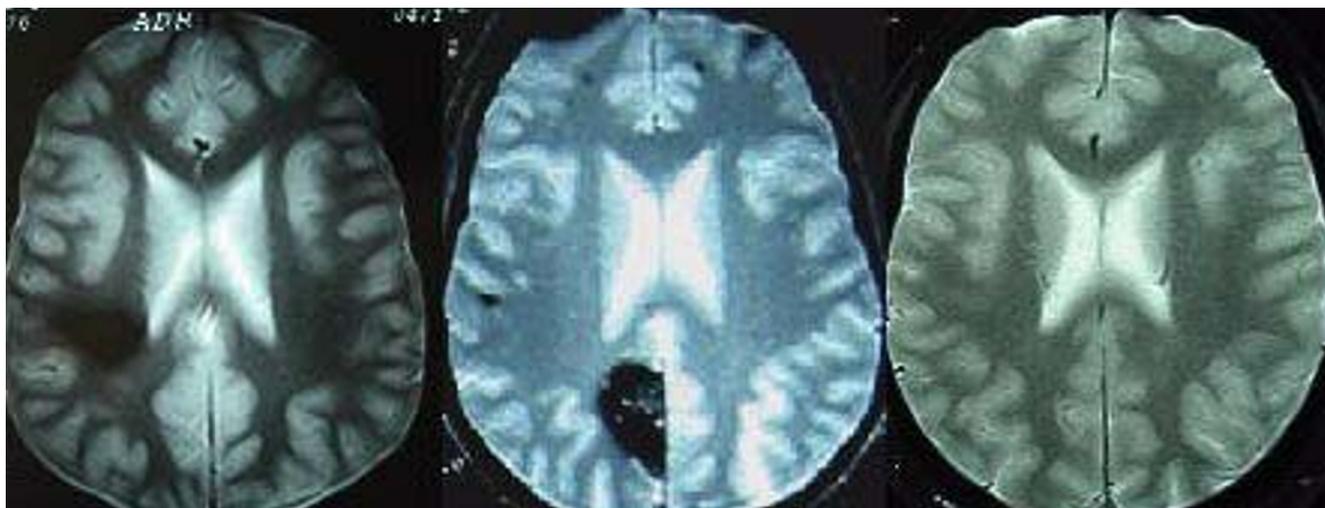


Figure 1
MRIs of CCMs. The echo gradient sequences of the proband (left), grandmother (middle) and the older brother (right) harbouring the Y634X mutation (III-2 in figure 3). Note the typical images of cavernous malformation and the absence of lesions in III-2, the asymptomatic carrier of the 1902insA.

Results

The onset of cerebral hemorrhage in the 4-year-old proband was the main clinical finding in the CVE10 family. The retrospective study of siblings and parents showed minor symptoms though one sibling had epilepsy. The clinical study was completed with MRI scans of consenting symptomatic and asymptomatic at-risk first-degree relatives. Multiple CCMs were observed in the MRIs of the grandmother, mother and two of the siblings and therefore familial characteristic was confirmed (see table 1 and figure 1 for details of number and locations of CCMs). The number of CCMs in patients of this pedigree was related to age with a ratio around 0.9 (range 0.3–2) and no significant differences in location were observed. Comparison of CCM locations is weakened because there were far fewer malformations in the younger patients (table 1).

Haplotyping of markers of the locus demonstrated segregation linkage of this family to CCM1. The affected individuals, i.e. those with CCMs in MRIs, shared the same grandmother's chromosome. It is worth noting that the asymptomatic sibling III-2 shares the linked haplotype, without apparent recombination, but his MRI showed no CCMs. Sequencing of the forward and reverse strands of the proband demonstrated an exonic A/T insertion at position 1902 leading to a premature TAA termination codon and the predicted phenotype Y634X. The 1902A insertion and the ambiguities of the overlapping strands were identified in both the sense and antisense strands. The nucleotide insertion at this position originates a TTAA

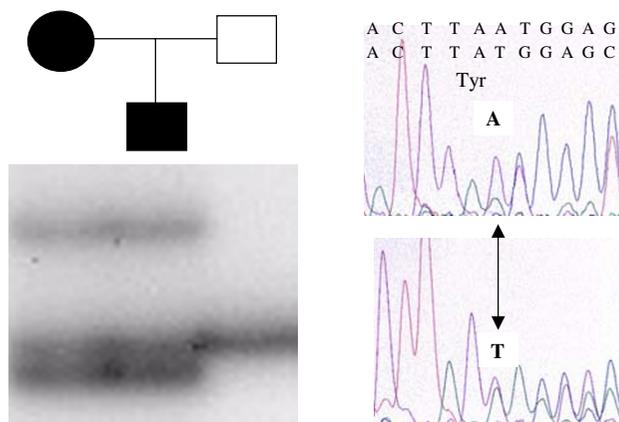


Figure 2
Left panel, SCCP of the proband and parents of CVE10 PCR was performed with the reaction mixture supplemented with [α^{32} P]-dCTP. Aliquots of the product were run in acrylamide gels (see Methods) and revealed by auto-radiography. *Right panel, chromatogram of the 1902insA.* Traces of the sense (upper) and antisense (lower) DNA strands of the proband. The wild type and mutated nucleotide sequences and the aminoacid change are indicated over the sense strand. The sequence shows the site of the 1902insA mutation (see arrow) and the frameshift. The ambiguities are caused by the overlapping bands of the wild type and mutated strands due to the nucleotide insertion. The stop TAA triplet predicts a truncating protein with the changed phenotype Y634X.

I-1		I-2		II-1		II-2		III-1		III-2		III-3		III-4		III-5	
221	219	221	219	221	221	219	221	221	221	221	219	221	219	221	219	221	219
127	127	127	127	127	127	127	125	127	125	127	127	127	127	127	127	127	127
129	133	139	139	129	139	133	129	139	129	129	133	129	133	129	133	129	133
197	185	185	197	197	185	183	183	185	183	197	183	197	183	197	183	197	183
107	107	103	103	107	103	103	103	103	103	107	103	107	103	107	103	107	103
127	127	131	129	127	131	127	127	131	127	127	127	127	127	127	127	127	127
275	273	273	275	275	273	273	273	273	273	275	273	275	273	275	273	275	273
149	147	151	151	149	151	151	145	151	145	149	151	149	151	149	151	149	151

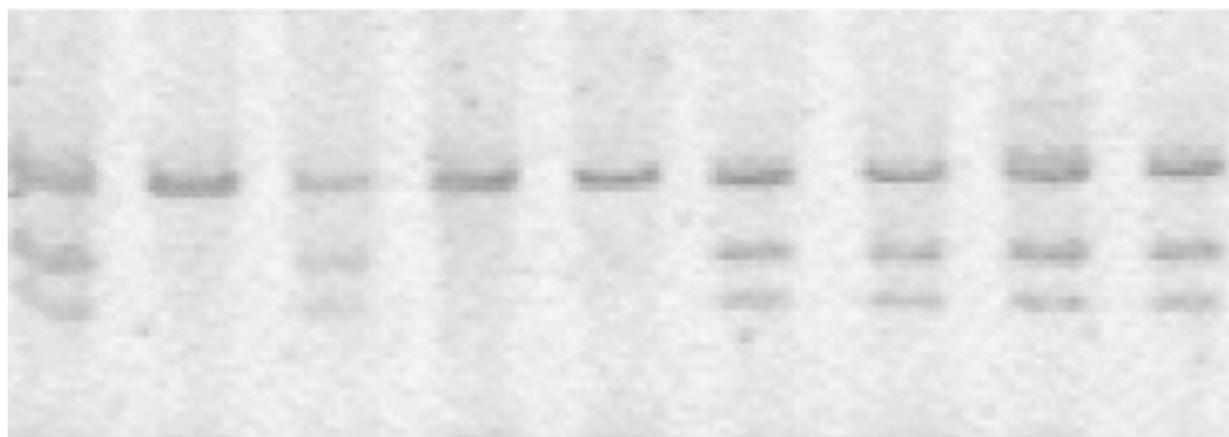
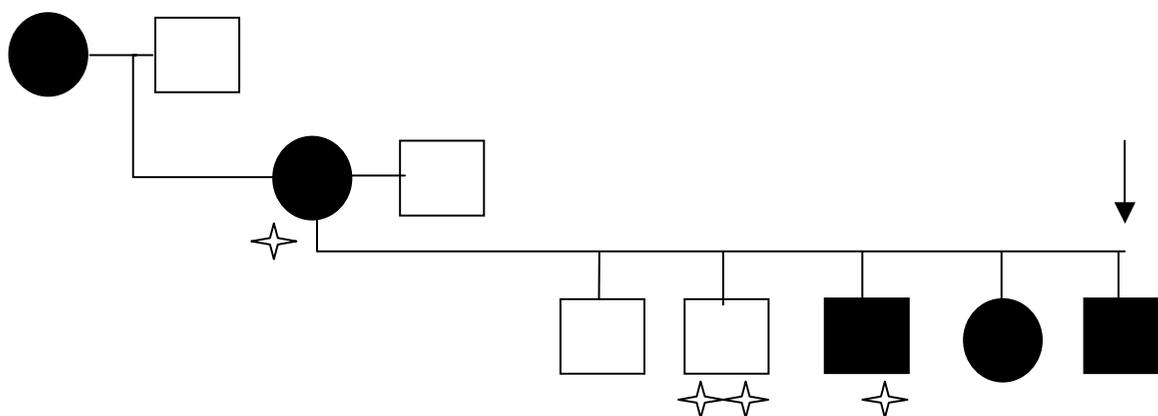


Figure 3

Haplotypes and restriction fragment polymorphism (RFLP). Haplotypes were analysed using the markers surrounding CCMI as described in Methods. The markers from top to bottom were: D7S2409, D7S1813, D7S1789, D7S646, D7S558, D7S689, D7S652 and D7S492. Exon 17 was amplified with the primers and aliquots of the PCR were digested overnight at 37°C with MseI. The fragments were separated in an 8% polyacrylamide gel containing urea and, after staining with ethidium bromide, the gel was photographed under UV. The undigested product is 297 pb long and the fragments separated from the TTAA restriction site are 158 and 139 pb. Therefore, individuals harbouring the I902insA mutation have three fragments of 297, 158 and 139 bp. Filled symbols in the pedigree refer to patients with CCMs in the echo-gradient MRIs. Arrow indicates the proband. Stars refer to asymptomatic carriers of the mutation. The double stair denotes the asymptomatic carrier with MRIs free of CCMs

restriction. This is recognised by the MseI endonuclease that splits the 297 bp PCR product into 158 and 139 bp. The RFLP of exon 17 in affected CV10E individuals demonstrated a heterozygous polymorphism of 297, 158 and 139 bp. Here again, the second brother (individual III-2 in the pedigree) had the mutated polymorphism although his MRI was free of CCMs. There is a complete coincidence of the CCM-linked haplotype with the mutated RFLP in this pedigree (see figure 3). Analysis of the chromosomes of 80 healthy individuals (not shown) coincided with the wild type RFLP.

Discussion

Hereditary forms of CCM, in contrast to sporadic forms that are characterized by single lesions, present as multiple malformations and, frequently, two or more relatives are affected [1]. This family illustrates the very variable clinical phenotype of cavernous malformations since the proband is the younger sibling suffering a cerebral hemorrhage. This early and critical onset contrasts with the minor symptoms observed in the affected relatives, even in the 82-year-old grandmother, notwithstanding the multiple cerebral lesions. However, the most striking finding was that repetitive studies of brain images of the III-2 sibling, who is 18 years old, demonstrated no malformations in spite of the presence of the 1902A insertion. The absence of cavernous malformations in echo-gradient sequences in this sibling was confirmed by MRIs of the spinal cord and the genetic status was corroborated by the inheritance of the haplotype harbouring the mutation and its illustration with MseI restriction polymorphisms. Nonetheless small CCMs, undetectable even on gradient echo sequences, could still be present in the eldest sibling and might develop later.

Several mutations in exon 17 of the Krit1 gene associated with CCMs have been described [5]. The Y634X mutation modifies the FERM sequence in the last residues (IPTYGAA), which are replaced by YPT*WSS, indicating the potential pathogenic mechanism of the FERM domain. The report of a novel N-terminal region in Krit1 containing an NPXY motif interacting with icap1 α , a modulator of β 1 integrin, and the presence of a FERM domain in Krit1, have suggested that this protein might be involved in bi-directional signaling between the integrin and the cytoskeleton [11].

Penetrance of Krit1 mutations has not been properly studied but significant locus-specific differences in penetrance have been identified. The penetrance of clinical CCMs among apparent carriers in kindreds linked to CCM1, CCM2 and CCM3 have been estimated as 88, 100 and 63% respectively [13]. Individuals usually become symptomatic between 20 and 40 years of age, although clinical signs have been described in all age groups [1,5].

Asymptomatic carriers are probably not an unusual finding, but the demonstration of the mutation is necessary to raise this conclusion and for genetic counselling. CCMs may develop *in utero* decades before any clinical presentation [3] but it is possible that penetrance varies among families with particular mutations at each gene. Small cavernomas could be present in the eldest sibling of the family studied here and may develop later. Nonetheless, the variable clinical and radiological traits in affected individuals of the same family exclude a direct correlation between clinical expression and genotype. The affected siblings, in addition to the Y634X mutated phenotype, share the same paternal chromosome, so a variable genetic background, at least in the vicinity of CCM1, can be excluded.

Assuming a pathogenic mechanism similar to the double-hit hypothesis of tumorigenesis and given the multiplicity of lesions, a very potent susceptibility to genetic or environmental factors is required to cause the multiplicity of somatic events. Differences in genetic and environmental factors are presumably minimal among first-degree relatives and therefore cannot easily explain the variable clinical and radiological expression. In addition to genetic and environmental causes, epigenetic factors and other mechanisms, i.e. non-sense mRNA decay, can be considered in the pathogenesis of CCM.

Conclusions

Patients in this family, harbouring the same mutation and chromosomes, are illustrative of the very variable clinical and radiological expression of a Krit1 mutation. The early and critical onset in the proband contrasts with minor clinical findings in the affected relatives. This consideration is important in genetic counselling.

Competing interests

None declared.

Authors' contributions

ML conceived and coordinated this study, analysed the DNA sequences, designed the RFLP analysis and drafted the manuscript. AFC carried out the SCCP and sequencing reactions. FS carried out the haplotypes and the restriction polymorphisms. JMG-M, MAG and GI contributed and coordinated the clinical and MRI studies of patients.

All authors read and approved the final manuscript

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