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ARTICLE

Spectrum and expression analysis of *KRIT1* mutations in 121 consecutive and unrelated patients with Cerebral Cavernous Malformations

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Cerebral Cavernous Malformations (CCM/MIM 604214) are vascular malformations characterised by abnormally enlarged capillary cavities without intervening brain parenchyma. Clinical manifestations include seizures, cerebral haemorrhages and focal neurological deficits. They occur as a sporadic or autosomal dominant condition. Most often, sporadic cases have only one lesion and familial cases are characterised by a high frequency of multiple lesions. Three CCM loci were previously mapped on 7q (CCM1), 7p (CCM2) and 3q (CCM3) and CCM1 gene was identified as coding Krit1, a protein of unknown function, which was shown initially to interact in yeast two hybrid assays with Rap1A, a small ras GTPase and more recently to Icap1α, a modulator of β1 integrin signal transduction. Herein, we screened *KRIT1* gene in 121 unrelated, consecutively recruited, CCM probands having at least one affected relative and/or showing multiple lesions on cerebral MRI. Fifty-two of these probands (43%) were shown to carry a *KRIT1* mutation. Forty-two distinct mutations were identified including six recurrent ones. Three-quarters of these mutations were located in the C-terminal half of the gene, mostly within exons 13, 15 and 17. All of them are predicted to lead to a premature stop codon. No missense mutation was identified. The only two nucleotide substitutions predicted to be missense mutations led in fact to an abnormal splicing and a premature stop codon. Altogether these data suggest that *KRIT1* mRNA decay due to the presence of premature stop codons and Krit1 haploinsufficiency may be the underlying mechanism of CCM.
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Introduction

Cerebral Cavernous Malformations (CCM/OMIM 116860) are vascular malformations, mostly located in the central nervous system and characterised by abnormally enlarged capillary cavities without intervening brain parenchyma.[1] Most common symptoms include seizures, cerebral haemorrhages and focal neurological deficits. From large series based on necropsy and/or magnetic resonance imaging, their prevalence in the general population has been estimated to be close to 0.1–0.5%.[2]

Cavernous angiomas can occur as a sporadic or autosomal dominant condition with incomplete penetrance; the frequency of the latter has been estimated as high as 50% in Hispano-American CCM patients[3] and is most likely close to 10–20% in Caucasians (Tournier-Lasserve, unpublished data). Sporadic cases most often harbour only one CCM lesion. On the contrary, familial CCM are characterised by a high frequency of multiple lesions, whose

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number is strongly correlated to patients' age suggesting a dynamic nature of these lesions.[4] 'Sporadic' cases harbouring multiple lesions have been shown to be in reality affected by an hereditary form of the disease since 75% of them have an asymptomatic parent with CCM lesions on brain MRI, due to incomplete clinical penetrance of this condition.[4] *De novo* mutation of *KRIT1*/CCM1 gene has also been reported in one CCM patient with multiple lesions.[5]

Three CCM loci had been previously identified on 7q (CCM1), 7p (CCM2) and 3q (CCM3).[6,7] Recently, CCM1 was identified as the *KRIT1* gene.[8,9] *KRIT1*, a protein of unknown function so far, was previously identified through a yeast two-hybrid screen designed to identify proteins interacting with Rap1A, a small Ras like GTPase protein.[10] *KRIT1* encodes a 736 amino-acids protein containing four ankyrin domains, a FERM domain and a C-terminal portion interacting with Rap1A. Comprehensive analysis of *KRIT1* has been precluded because the full genomic sequence has been available only very recently.[11–14] Initially, *KRIT1* gene was reported to include 12 coding exons. Screening of these 12 exons led to the identification of germline mutations leading to a premature stop codon or splice mutations whose consequences were not investigated at the cDNA level.[8–9,14–18] Recently, eight additional exons have been described among which four coding exons. This novel N-terminal region of krit1 has been shown very recently to contain an NPXY motif required for interaction of krit1 with icap1α, a modulator of β1 integrin signal transduction.[19,20] So far, screening of these four novel exons of *KRIT1* has been performed only in few families.[11,14] Almost none of these mutations has been studied at the cDNA level and none of them at the protein level due to unvailability of Krit1 specific antibodies.

In the present study, we searched for *KRIT1* mutations in a panel of 121 unrelated, consecutively recruited, CCM probands. Our aims were to estimate the implication of *KRIT1* mutations in CCM pathogenesis, to evaluate the distribution and nature of *KRIT1* mutations and to investigate the consequences of these mutations on *KRIT1* mRNA.

Patients, material and methods

Patients

Twenty-eight French University Neurosurgery Centres participated in this study which was approved by the local ethics comittee. A total of 121 unrelated clinically affected CCM probands were consecutively enrolled in this study. All these patients were known to have at least one affected relative and/or multiple cavernous angiomas on cerebral MRI.

CCM diagnosis was based on cerebral Magnetic Resonance Imaging and/or pathological analysis as described previously; detailed clinical and MRI informations on 57 of these 121 families have been reported elsewhere previously.[4] A mutation was previously identified in 12 of them.[8] Detailed clinical and neuroimaging informations were collected for all patients through direct interview and reviewing of medical charts.

Genomic DNA screening

Genomic DNA from each proband was extracted from peripheral blood using standard procedures. Genomic DNA from 100 unrelated healthy French Caucasian individuals was available as a control group. We screened genomic DNA of all 121 probands using SSCP.[21] All 16 coding exons were amplified with a set of 19 primers (sequences and PCR conditions on Table 1A). PCR products were electrophoresed on 10% acrylamide-bisacrylamide 37.5 : 1 gels with 10% glycerol at 4°C on a Mighty small II apparatus at a constant current of 35 mA. Patients' conformers were compared to those of an unrelated healthy individual. Amplimers showing an atypical SSCP banding pattern were directly sequenced (ABI377, Perkin Elmer). Sensitivity of this SSCP procedure was first compared to direct sequence analysis in a panel of 22 unrelated probands belonging to KRIT1 most likely linked families. SSCP analysis run with these conditions (10% glycerol at 4°C) has detected within this panel all 19 mutations and sequence variants identified by sequence analysis. These SSCP conditions were therefore used for the screening of all remaining probands.

Numbering of nucleotides is according to the full length *KRIT1* cDNA (accession number AF296765), beginning nucleotidic numbering at the A of the ATG initiator codon. Mutations were described as recommended by the Ad Hoc Committee on Mutation Nomenclature.[22]

cDNA preparation, cloning and analysis

Total RNA was extracted from patients lymphoblastoid EBV cell lines or peripheral blood phytohemagglutinin stimulated lymphoblasts and used as samples for cDNA synthesis with oligo dT_{15} primers and 200 U of Moloney murine leukemia virus (M-MLV) reverse transcriptase (Life Technologies®) according to standard procedures.[8] In order to study cDNA consequences of genomic DNA variants, RT–PCR amplifications were analysed for all probands in whom cDNA were available. cDNAs were amplified with sets of primers located two exons upstream and dowstream of the exon or intron where was located the sequence variant in order to check for aberrant splicing events (sequences and PCR conditions on Table 1B). RT–PCR products from patients and healthy controls were run on 2.5% Agarose TBE gels. Fragments were sequenced either directly or after cloning using the pGEM®-T Easy Vector Systems (Promega®, Madison, USA).

Results

Genomic DNA mutations

Likely disease causing mutations were identified in a total of 52 probands out of the whole panel of 121 consecutive probands (Table 2 and Figure 1). Mutations were observed in all parts of the gene but were significantly more often located within the second half of the gene, most of them

Table 1 *KRIT1* primers and conditions for the polymerase chain reactions

A. Primers for genomic DNA analysis

Exon	*Forward primer*	*Reverse primer*	*Fragment size*	*Annealing temperature*
Exon 4	CTTGCCATCAAATGTGTC	CAGAATGACATTCAATGG	210	55
Exon 5	TTTTAAGCTTTCTCTTAATG	AGAATCTTTCTCCACAAG	247	50
Exon 6	GCTGCCTTTTAAAGTATCTG	CCTTTACTTAGCATCTAAAG	210	55
Exon 7	GATGAAAACATGCAGATG	TTCTCAAAGTGTCTGTATC	282	50
Exon 8 a	CACTTTCGAATGGCTACTTC	CCTGATTCAGTAGCATATGC	157	55
b	GAGCGGATAAAAACTAAT	GAGCTAAAATTCATTCAA	208	50
Exon 9	GCTCTTAATGGGTTTTTG	AGCAATGTGGAGTAAAAC	183	50
Exon 10	GTCCTGTTGTATTTTTCA	TTTGGAATGAGAACAGTC	275	50
Exon 11	AGATGATCTTTTTAGGTAAG	TTACTTGTTATTCACTGCTT	275	55
Exon 12	ATGTAATGCCTTTTTTCC	ATGCCTGGCTCTAACTAT	181	55
Exon 13	TAAAGCACATGAAGTTGAAG	AGTTGTATTTTCTACCAACC	319	55
Exon 14 a	TTCAGTACAGAAGTGCAGAC	TCTCACATCTCTTCTTAG	228	55
b	TTGCAACATGTTCGTGAC	TTTCTGCCTCTAGTGCTTAC	204	55
Exon 15	TGTAGCCTAATAACCAAA	AGCATAGCACAAGACCAT	263	50
Exon 16	AATTACGTTACTGAAAGC	TGTCTTTTCATTTCTATT	213	50
Exon 17 a	TGGTACATTTTCCTTTCA	CTTTATGATTGCTGGGGC	201	50
b	ATATTTACAAAGGCAAGC	TGACATGATTGGTAAAAA	179	50
Exon 18	GGTGAAGTTTTTAATATG	CAATAGTTTATGAAGTCC	205	50
Exon 19	AATAGATAGGGAACTGCC	GTGGCTTGAGTAACAGTT	184	55

B. primers for cDNA analysis

Amplified cDNA fragment	*Forward primer*	*Reverse primer*	*Fragment size*	*Annealing temperature*
Exon 4 – exon 10	ATACTGCCAGTCTCAATTCTC	AGCATGCATAATGAATGG	933	50
Exon 8 – exon 12	CTAGAGAAAGCAGTACC	GGTTTGTTAATTGCTTCC	590	50
Exon 10 – exon 14	CCTGTGAAGGAGATTCAG	TCTCACATCTCTTCTTAG	652	50
Exon 12 – exon 14	TATAACAGACCAACAAGG	TCTCACATCTCTTCTTAG	391	50
Exon 12 – exon 17	TATAACAGACCAACAAGG	CTTTATGATTGCTGGGGC	815	50
Exon 12 – exon 19	TATAACAGACCAACAAGG	GTGGCTTGAGTAACAGTT	1189	50
Exon 14 – exon 17	TTGCAACATGTTCGTGAC	CTTTATGATTGCTGGGGC	523	50
Exon 14 – exon 19	TTGCAACATGTTCGTGAC	GTGGCTTGAGTAACAGTT	852	50

PCR was carried out under standard conditions: 94°C for 3 min, 30 cycles of amplification at 94°C for 30 s, specific annealing temperature for 30 s and 72°C for 30 s, and a final extension 10 min at 72°C, with a thermocycler Applied Biosystem 9700.

being located within exons 13, 15 and 17 (56% of the mutations are located within one of these three exons which span 24% of the coding sequence). Thirty five per cent of them were exonic nucleotide substitutions leading in most cases to non sense codons, 48% were small exonic insertions/deletions, 17% were intronic nucleotide substitutions or deletions (Table 2).

Among the 13 non sense mutations, two were recurrent, R179X and Q455X. Q455X, which was previously identified as a strongly recurrent mutation within CCM hispano-American families, was detected in two distinct families with respective French and Italian backgrounds. A haplotype analysis was performed only within the French pedigree (ped 040) with markers D7S1813, D7S646, D7S558 and D7S689 and did not detect the Hispano-American founder haplotype. Twenty-five probands carried small exonic deletions or insertions leading to a frameshift and premature stop codon; most of them occured in short stretches of repeated sequences. Nine probands were shown to carry an intronic mutation located at canonical splice sites and/or leading to aberrantly spliced transcripts (see below). In addition, we identified in three probands two nucleotide subtitutions located within the coding sequence (pedigree 005 : exon 17/GCA 1943 GTA; pedigrees 028 and 054 : exon 15/GCT 1579 ACT).

Expression analysis of the mutations at the cDNA level

A total of 30 probands, in whom either an intronic or exonic genomic DNA mutation was identified, were screened, whatever the nature of the mutation, in order to search for aberrant splicing events and screen for the presence of the mutated transcript (Table 2, Figure 2).

Twelve probands carrying a non sense mutation were tested at the cDNA level: in all cases the mutated transcript was detected on sequencing chromatograms, although in most cases its abundance was lower than that of the wild type (as judged on the respective heights of sequence chromatograms peaks). We did not detect any aberrantly spliced transcript in the cDNA of those patients.

cDNA was available for two of the probands carrying canonical splice site mutations IVS7-2 A to G and IVS12-1 G to A; those mutations were shown to result respectively in exon 8 skipping (ped 125) and to a 10 bp exonic deletion within exon 13 (ped 136). Only one aberrant transcript was identified for each mutant. Both of these splicing events led to a premature stop codon. The recurrent 4 bp deletion

Table 2 Nature, location and RNA repercussions of the 52 CCM related KRIT1 mutations

Ped	*Location*	*Genomic DNA*	*mRNA*	*Putative protein*	*Comments*
Nonsense Mutations					
060	Exon 6	Substitution 268 C→T	CGA 90 TGA	R90X	–
033	Exon 8	Substitution 535 C→T	CGA 179 TGA[b]	R179X	–
059	Exon 8	Substitution 535 C→T	CGA 179 TGA[b]	R179X	–
152	Exon 8	Substitution 690 C→G	TAC 230 TAG	Y230X	–
013	Exon 9	Substitution 805 G→T	GAA 269 TAA[b]	E269X	–
124	Exon 9	Substitution 817 A→T	AGA 273 TGA	R273X	–
019	Exon 10	Substitution 858 G→A[a]	TGG 286 TGA[b]	W286X	–
041	Exon 12	Substitution 1211 G→A[a]	TGG 404 TAG[b]	W404X	–
015	Exon 13	Substitution 1267 C→T	CGA 423 TGA[b]	R423X	–
040	Exon 13	Substitution 1363 C→T	CAA 455 TAA[b]	Q455X	–
126	Exon 13	Substitution 1363 C→T	CAA 455 TAA[b]	Q455X	–
003	Exon 13	Substitution 1391 G→A	TGG 464 TAG[b]	W464X	–
009	Exon 15	Substitution 1708 A→T	AAA 570 TAA[b]	K570X	–
010	Exon 17	Substitution 1879 C→T[a]	CAG 627 TAG[b]	Q627X	–
092	Exon 17	Substitution 1905 T→G	TAT 635 TAG[b]	Y635X	–
Exonic insertions and deletions leading to frameshift (fs) and premature termination codon					
075	Exon 5	143-144 ins A	Frameshift	48fs63X	$A_{(8\ mer)}$nt repeat[c]
102	Exon 5	151-154 del AAAG	Frameshift	50fs63X	tandem AAAG repeat
018	Exon 9	843-844 ins C[a]	Frameshift[b]	281fs289X	–
025	Exon 11	1034 del A[a]	Frameshift[b]	344fs351X	tandem GAAG repeat[c]
119	Exon 11	1060 del C	Frameshift	353fs370X	–
012	Exon 12	1197-99 del CAA with ins AG	Frameshift[b]	398fs411X	tandem CAAA repeat[c]
138	Exon 12	1202-1205 del AAAA	Frameshift	400fs414X	–
027	Exon 12	1229 del T[a]	Frameshift[b]	409fs411X	tandem TTG repeat[c]
081	Exon 12	1237-1240 del GAAG	Frameshift[b]	412fs435X	tandem GAAG repeat[c]
042	Exon 13	1277-1280 del GAAT[a]	Frameshift	427fs435X	–
074	Exon 13	1360-1363 del TCTC	Frameshift	453fs493X	$TC_{(4\ mer)}$ repeat[c]
008	Exon 13	1362-1363 del TC	Frameshift[b]	454fs478X	$TC_{(4\ mer)}$ repeat[c]
050	Exon 13	1362-1363 del TC	Frameshift	–	–
145	Exon 13	1362-1363 del TC	Frameshift	–	–
132	Exon 14	1527-1528 del AA	Frameshift	509fs518X	tandem AAG repeat[c]
035	Exon 15	1608-1633 del 26 nt[a]	Frameshift[b]	535fs539X	–
135	Exon 15	1702 del A	Frameshift[b]	567fs576X	–
068	Exon 15	1710 del A	Frameshift	569fs576X	–
051	Exon 15	1715 del A	Frameshift	571fs576X	tandem CAAG repeat[c]
034	Exon 15	1719 del A	Frameshift[b]	572fs576X	tandem CAAG repeat[c]
058	Exon 17	1867-1868 ins C[a]	Frameshift	622fs654X	–
098	Exon 17	1885 del T	Frameshift	628fs660X	–
006	Exon 17	1940 del A[a]	Frameshift	646fs660X	$A_{(3\ mer)}$ nt repeat[c]
118	Exon 17	1970-1971 ins C	Frameshift	657fs675X	$C_{(3\ mer)}$ nt repeat[c]
061	Exon 17	2002-2003 ins C	Frameshift	667fs675X	–
Intronic or exonic mutations leading to aberrant splicing					
125	Intron 7	IVS 7−2 A→G	Exon 8 deletion[b]	161fs163X	–
014	Intron 10	IVS 10−15 T→A	Cryptic intronic acceptor splicee site (AG IVS10-15-14) →13 intronic bp insertion[b]	329fs339X	–
089	Intron 10	IVS 10−1 G→C	–	–	–
136	Intron 12	IVS 12−1 G→A	Cryptic exonic acceptor splice site (nt AG 1263-64) →10 exonic bp deletion[b]	418fs433X	–
028	Exon 15	Substitution GCT 1579 ACT	Cryptic exonic acceptor splice site (nt AG 1602-03) →44 exonic bp deletion[b]	521fs527X	–
054	Exon 15	Substitution GCT 1579 ACT	Cryptic exonic acceptor splice site (nt AG 1606-07) →40 exonic bp deletion[b]	521fs523X	–
134	Intron 15	IVS 15 del (+4→+7) (AGTA)	Exon 15 deletion[b]	521fs522X	–
109	Intron 15	IVS 15 del (+4→+7) (AGTA)	–	521fs522X	–
113	Intron 15	IVS 15 del (+4→+7) (AGTA)	–		
005	Exon 17	Substitution GCA 1943 GTA[a]	Cryptic exonic donor splice site (nt GT 1942-43) →84 bp deletion in frame[b]	In frame 28 aa del (648→675)	–

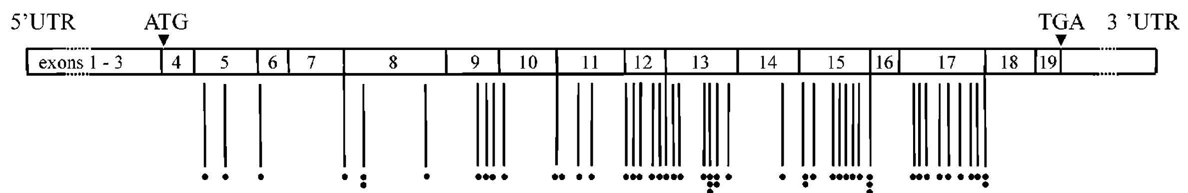
Continued

Table 2 *(Continued)*

Ped	*Location*	*Genomic DNA*	*mRNA*	*Putative protein*	*Comments*
011	Intron 17	IVS 17−12 A→G[a]	Exon 18 deletion[b]	In frame 39 aa del (676→714)	–
097	Intron 17	IVS 17−12 A→G	Exon 18 deletion[b]	In frame 39 aa del (676→714)	–

[a]Previously reported mutations (Laberge *et al*, 1999), [b]RT–PCR products were analysed. In all cases, analysed mutant was detected, [c]Location of the mutation as regard to short tandem repeats or repeat stretches.

cDNA :



Protein :

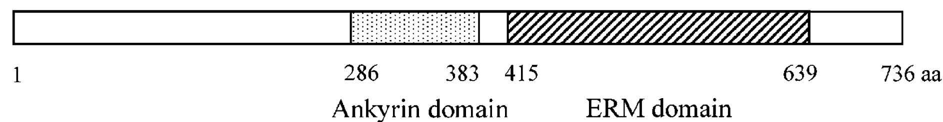


Figure 1 Location of Krit1 mutations.

located within intron 15 (IVS15 +4→+7 del AGTA) led to an aberrant transcript deleted of exon 15 and a premature stop codon. The substitution located within IVS10 (−15 T to A) created a new splice site, and led to a transcript with a 13 bp insertion and a premature stop codon. All these aberrant transcripts were detected on RT–PCR cDNA sequence analysis although less abundant than the wild-type ones. Two probands (pedigree 011 and 097) share a common A to G substitution at position −12 within intron 17; this mutation was absent in 200 control chromosomes and led to a transcript in which exon 18 has been spliced. Quite interestingly, this abnormal transcript does not harbour a premature stop codon and would lead to an in frame deletion leading to a protein deleted of 39 aa; it was never detected in normal controls.

We identified in three probands two nucleotide subtitutions located within the coding sequence (pedigree 005 : exon 17/GCA 1943 GTA; pedigrees 028 and 054: exon 15/GCT 1579 ACT). Those mutations, if translated, would theoretically lead to a putative protein carrying a missense mutation. When analysing the cDNA of these patients, we showed that these 'missense' mutations led in fact to aberrant transcripts due to destabilisation of preexisting splice sites and utilisation of a cryptic splice site or a newly created site (Figure 2). The aberrant transcript resulting from mutation GCA 1943 GTA, created an acceptor splice site (GT) and led to an 84 bp in frame deletion (Figure 2). This mutation was absent in 200 control chromosomes. This transcript was as abundant as the WT transcript based on chromatograms peak intensity as well as cloning data. Sequence analysis of RT–PCR products in this patient did not detect any normally spliced mature transcript carrying the 'missense' mutation GCA 1943 GTA. The GCT 1579 ACT nt substitution destabilized the WT acceptor splice site of exon 15 leading to the use of a cryptic exonic splice site located downstream of the mutation. Sequence analysis of subcloned RT–PCR detected both wild type transcripts and shorter transcripts due to abnormal splicing events occuring at nucleotides AG 1602–03 (pedigree 028) or 1606–07 (pedigree 054). These abnormal transcripts were not detected in healthy controls. We did not detect any normal sized transcript carrying the expected 'missense' mutation/GCT 1579 ACT.

KRIT1 polymorphisms

Five other nucleotide substitutions were considered as being most likely polymorphisms based on the fact that they have been observed in both patients and controls or they have been detected in individuals in whom a deleterious mutation has been identified. Three of them were intronic and two exonic. None of them led to an aminoacid substitution. Among these polymorphisms, two (IVS15 −53 C>G; nt 1809 wobble T>C (His603His) in exon 16) were

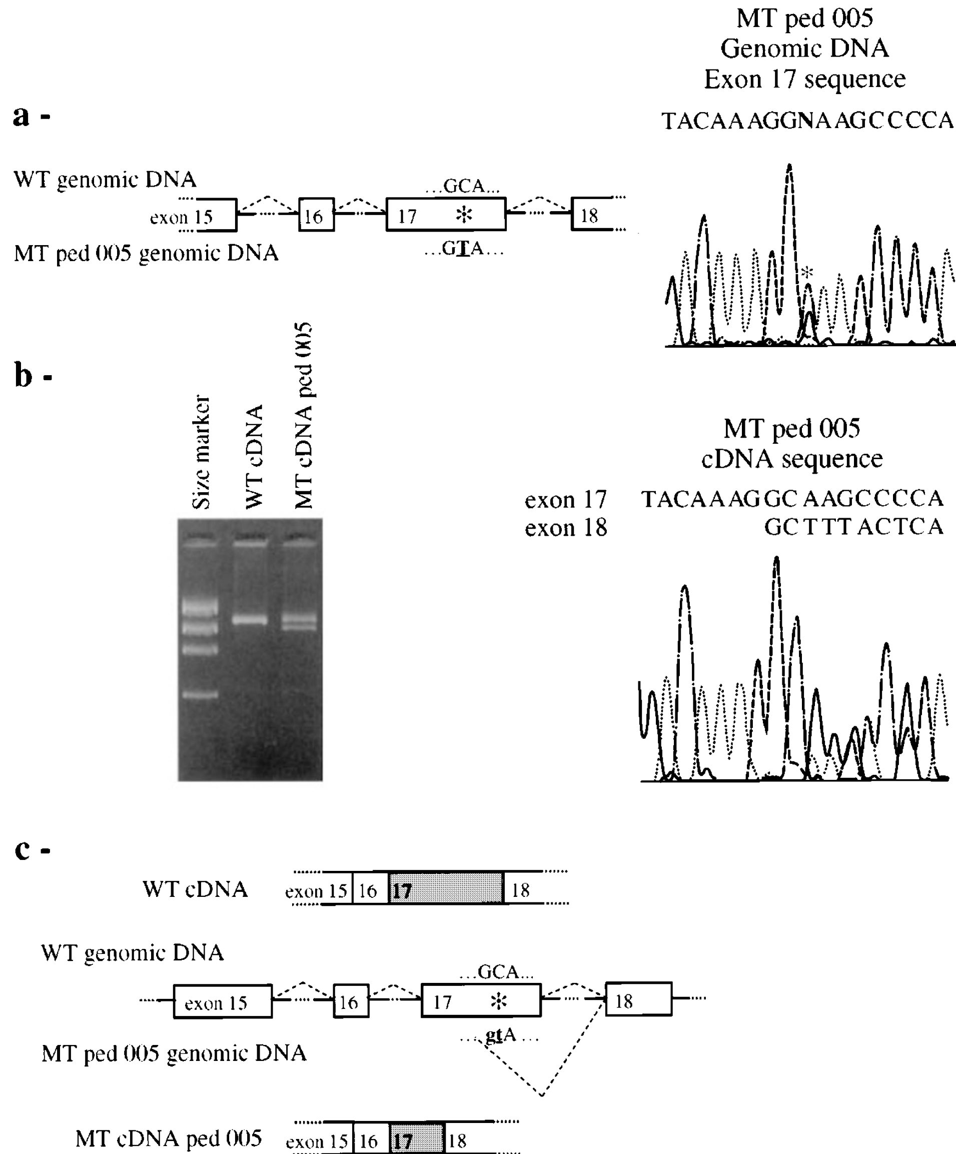


Figure 2 Aberrant splicing resulting from exon 17 nucleotide substitution nt 1943 GCA to GTA in pedigree 005. *Top panel*: Left/wild type (WT) and mutated (MT) genomic DNA. *Position of the mutation. Right/genomic DNA sequence chromatogram. Proband 005 is heterozygous for the mutation. *Middle panel*: Left/WT and MT electrophoresed cDNA RT–PCR products. Two distinct bands are identified for proband 005. Right/Proband 005 cDNA sequence chromatogram. The mutation causes partial skipping of exon 17 leading to a 84 bp in frame deletion. *Bottom panel*: Use of a new acceptor splice site resulting from nt 1943 C to T mutation.

rare nucleotide substitutions; each of them was detected only in one CCM proband in whom a deleterious mutation has been later on identified (ped 010 and ped 135).

A silent substitution at nt 1980 (G>T //Val 660 Val) was detected in an heterozygous state in 25% of our genotyped population (AA: 72%//AG: 25%//GG: 3%) and was also detected in control individuals.

The last two detected variants were nucleotide substitutions located within intronic sequences: IVS7+65 G/C and IVS10+63 G/C. They have been isolated both in CCM patients and in control individuals. Respective allelic frequencies checked on 100 individuals were

— IVS7+65 G/C: GG: 74%; GC: 22%; CC: 4%
— IVS10+63 G/C: GG: 28%; GC: 45%; CC: 27%

Discussion

We showed herein that 52 out of 121 consecutively recruited CCM patients having an affected relative and/or showing multiple CCM lesions harbour a *KRIT1* mutation. All these

mutations lead to a premature termination codon through non sense or frameshift mutations except for two of them which lead to small in frame deletions of the C-terminal part of the gene. No missense mutation was identified. Quite strikingly, cDNA analysis showed that the two sole exonic nucleotide substitutions identified did not lead to transcripts carrying the expected 'missense' mutation but to aberrantly spliced transcripts with a premature stop codon.

These data suggest that *CCM1/KRIT1* may account for more than 40% of genetic forms of CCM and are in agreement with multilocus linkage data from others.[7] However, this proportion is most likely underestimated for several reasons. Based on the strategy that we have used, we may have missed deletions of *KRIT1* (however, *KRIT1* deletions have not been reported so far), and we may also have missed *KRIT1* point mutations due to incomplete SSCP sensitivity. Therefore, this 43% proportion of *KRIT1* positive families should be, in our opinion, considered as a minimum estimate.

The most important result is the stereotyped nature of the mutations on the putative Krit1 protein since almost all of them lead to premature stop codons. It is indeed quite striking that the only two nucleotide substitutions that would be predicted to lead to missense mutations led in fact to aberrantly spliced KRIT1 transcripts and not to any transcript that would be translated with a missense mutation; similar unexpected aberrant splicing has been reported previously for exonic nonsense or missense mutations in BRCA1 and other genes.[23] Non sense mRNA decay (NMD) as a consequence of premature stop codons has been shown to underlie haploinsufficiency in many disorders.[24–27] Based on the data reported herein, a putative loss of function of Krit1 through NMD would be a likely mechanism of CCM. However, it would be important, in order to draw a firm conclusion, to check for the presence or the absence of a Krit1 truncated protein, once Krit1 specific antibodies will be available.

This latter information will be quite interesting as regard to recently published data on the interaction of Krit1 and Icap1α.[19,20] This interaction required a NPXY motif located within the N-term region of Krit1 at aa residues 191–194 (exon 8). The C-terminal region of *KRIT1* has been reported to interact with rap1A.[10] This interaction with rap1A was not detected by Zhang *et al* when using the full length krit1 protein; however, a weak interaction was detected when using a Krit1 protein truncated of its N-term. All these data strongly suggest that complex mechanisms may regulate the effects of Krit1 and it would be important to sort out if CCM mutations lead to non sense mRNA decay and absence of the mutated protein or if Krit1 truncated proteins may be present in some CCM patients.

At last, detailed clinical, MRI analysis and genotyping of all consenting symptomatic or asymptomatic at risk individuals will allow in the future to estimate in a sufficient number of individuals the penetrance in families of probands with *KRIT1* mutations and to delineate potential genotype-to-phenotype correlations within *KRIT1* positive families. Serial follow up of such a genotyped cohort will also help to delineate the natural history of this disease in both groups.

Main clinical investigators

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