Familial cavernous malformations in a large French kindred: mapping of the gene to the CCM1 locus on chromosome 7q

L Notelet, F Chapon, S Khoury, K Vahedi, J P Chodkiewicz, P Courtheoux, M T Iba-Zizen, E A Cabanis, B Lechevalier, E Tournier-Lasserve, J P Houtteville

Abstract

***Objectives*—To characterise clinically a large French family affected with cerebral cavernomas and to check for linkage of this condition to chromosome 7.**

***Methods*—A family, originating from Normandy and in which five members had undergone surgery for cavernomas, was extended. All members older than 18 were studied clinically and by neuroimaging. Genetic linkage analysis was conducted using 11 polymorphic microsatellite markers located between D7S502 and D7S479.**

***Results*—The family included three generations. Among the 25 members investigated, 11 had an abnormal cerebral MRI, eight of them being symptomatic, and 12 were asymptomatic with a normal MRI. The status of the two remaining members could not be established on the basis of clinical and MRI data. The family reported shares some striking features with other previously linked families—namely, a high clinical penetrance and the presence of multiple lesions within most of the affected members. A lod score of 4.04 was obtained with marker D7S657 with no recombinant. Significant lod scores were also obtained with D7S524 (Zmax=3.32 at θ=0.00) and D7S630 (Zmax=3.44 at θ=0.00). These results establish linkage of the condition found in this family to chromosome 7. Haplotype analysis strongly suggests that the gene is telomeric to D7S802 and centromeric to D7S479.**

***Conclusions*—These data confirm linkage of cerebral cavernous malformations to chromosome 7 in a non-Hispanic family.**

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Laboratoire de Neuropathologie, CHRU de Caen, France
L Notelet
F Chapon
B Lechevalier

Inserm U 25, Faculté de Médecine de Necker, Paris, France
L Notelet
K Vahedi
E Tournier-Lasserve

Service de Neurochirurgie, CHRU de Caen, France
S Khoury
JP Houtteville

Service de Neurochirurgie, Centre hospitalier Sainte Anne, Paris, France
JP Chodkiewicz

Service d'imagerie, CHRU de Caen, France
P Courtheoux

Service d'imagerie par résonance magnétique, Höpital des Quinze-vingts, Paris, France
MT Iba-Zizen
EA Cabanis

Correspondence to:
Dr E Tournier-Lasserve, INSERM U25, Faculté de médecine Necker, 156 rue de Vaugirard, Paris, 75015, France.

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Intracranial cavernomas (or cavernous vascular malformations or cavernous haemangiomas) are so far considered to be hamartomas.[1] Their histological appearance is characteristic, with the juxtaposition of vascular capillary cavities without intervening brain parenchyma.[1 2] Vascular walls can be very thin, with a "lacework" appearance, or thicker, due to recurrent parietal thromboses.[1–3]

According to postmortem studies, the prevalence of cavernomas has been estimated as 0.5% in the general population.[4–6] Clinical onset usually occurs between the third and fourth decades of life,[2 7 8] but the first symptoms may appear at all ages.[9–11] Symptomatic cavernomas are rarely life threatening but, depending on their location, may cause severe clinical disability.[8 12] Clinical presentation includes epileptic seizures (30% to 50% of patients), focal neurological deficits (10 to 35%), headaches (20% to 30%), and recurrent haemorrhages, more often occult.[2 5 6 13–16]

Diagnosis is usually made on cerebral MRI, which is more sensitive than CT and shows, in typical cases, a heterogeneous core signal surrounded by a hypointense ring on T2 weighted images.[17 18]

The frequency of familial cerebral cavernous malformations has been estimated by some authors to be as high as 50% of clinical cases.[19 20] However, this frequency may have been overestimated due to collection biases, particularly in Hispanic-American families.[18–22] The pattern of inheritance is in most cases autosomal dominant with incomplete clinical penetrance. Cavernomas can be detected on cerebral MRI in clinically asymptomatic people who have transmitted the disease to their offspring.[19] Patients with cerebral cavernous malformations often have multiple localisations (up to 75% of patients), and these seem to be associated with earlier age of onset.[21 23–25]

Whereas an operative decision is widely accepted when cavernomas are symptomatic and easily accessible, it is still controversial when they are deep seated or located in functional brain areas.[5 7 14 18 26 27] The lack of knowledge about natural history of cerebral cavernomas makes therapeutical decisions often difficult.

Recently, Dubovsky *et al* mapped a gene responsible for cerebral cavernous malformations (CCM gene) in a large hispanic family to chromosome 7q11-q22, in a 33 cM interval bracketed by markers D7S502 and D7S479.[28] This genetic mapping has been confirmed in 10 additional families, seven of them being Hispanic-American and the other three white.[29–33] The analysis of two of these families allowed Günel *et al* to reduce the size of the mapping interval to 7 cM, the most likely interval being bracketed by ELN (the elastin gene) and D7S802.[29] Genetic mapping of the CCM gene centromeric to D7S802 was supported by one crossover event which occurred in an affected member. By contrast with these results, Johnson *et al* analysed addi-

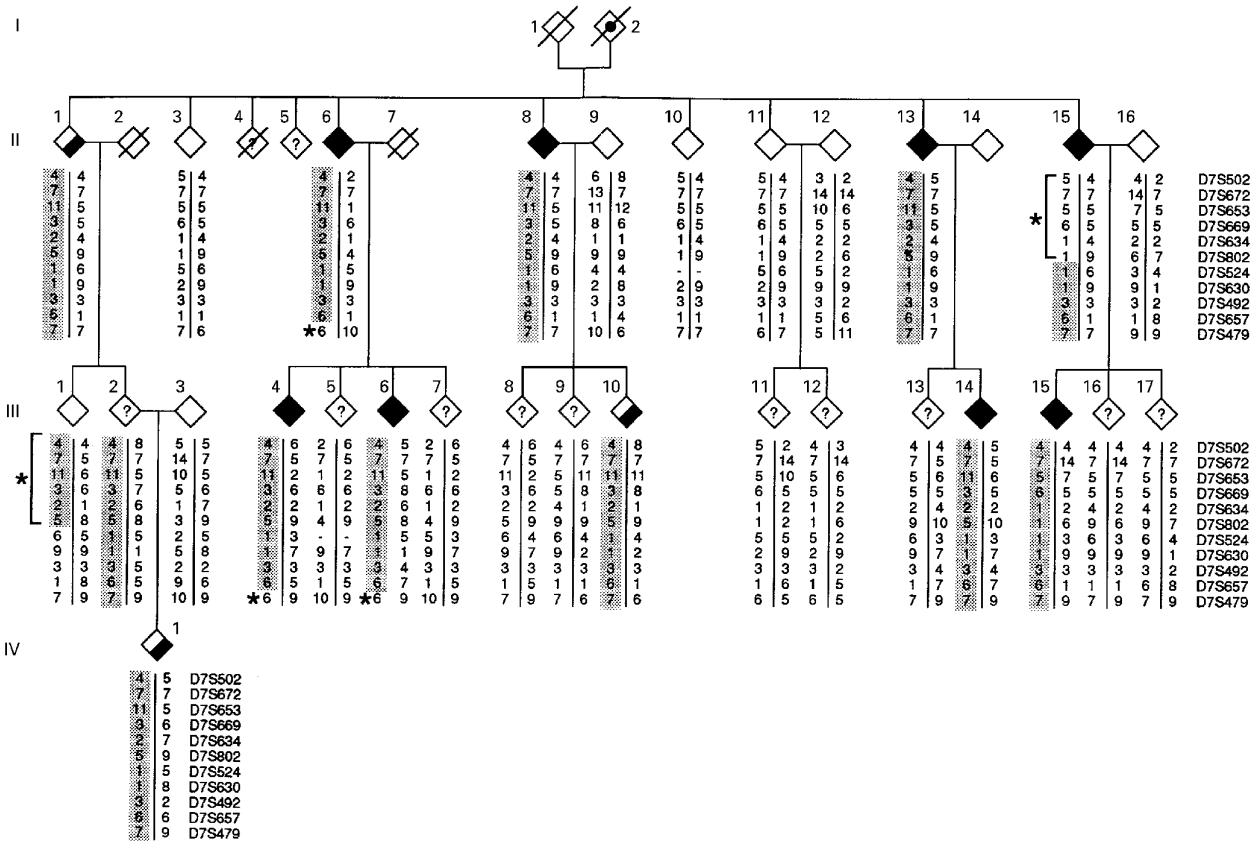

Figure 1 Pedigree and chromosome 7 haplotypes. The affected patients are represented by a filled symbol when they have neurological symptoms, by a filled circle when the symptoms are assumed on the basis of familial history, and by a half filled symbol when they have only an abnormal MRI. Open symbols represent unaffected subjects older than 45 years and question marks represent subjects younger than 45 years. The haplotypes obtained with D7S502, D7S672, D7S653, D7S669, D7S634, D7S802, D7S524, D7S630, D7S492, D7S657, and D7S479 are indicated. Three crossover events were seen in two clinically affected members (II-15, II-6) and one clinically asymptomatic member having a normal MRI and aged 49 (III-1). Haplotypes strongly suggest that the gene is telomeric to D7S802 and centromeric to D7S479.

tional families in which several crossover events strongly suggest that the gene causing cavernomas in these families is telomeric to D7S802 and resides within a 4 cM interval flanked by D7S2410 and D7S689.[31] Recently Günel *et al* excluded linkage to 7q in two non-Hispanic families and established the genetic heterogeneity of this condition.[33]

Herein we report the results of a clinical, neuroimaging, and genetic linkage analysis conducted in a large family originating from France. Haplotype analysis strongly suggests that the gene lies telomeric to D7S802, within an interval bracketed by D7S802 and D7S479.

Table 1 Clinical and MRI features of affected patients

Patients	*Clinical symptoms*	*Age of onset (y)*	*No of lesions (MRI)*	*Surgery age (y)*	*Location*	*Histology*
II-1	Healthy	—	> 1	—	—	—
II-6	Seizures: focal deficit	18	> 1	57		Cavernous angioma
II-8	Focal deficit	35	> 1	—	—	—
II-13	Seizures	18	> 1	—	—	—
II-15	Trigeminal neuralgia	47	> 1	—	—	—
III-4	Seizures	14	> 1	14	Parieto-occipital	Cavernous angioma
	Focal deficit	18		18	Pons	Complex vascular malformation
III-6	Seizures	20	1	20 and 32	Temporal	Cavernous angioma
III-10	Healthy	—	1	—	—	—
III-14	Haemorrhage	8	1	8	Frontal	Cavernous angioma
III-15	Haemorrhage	5	> 1	19	Thalamic	Complex vascular malformation
IV-3	Headaches	Childhood	> 1	—	—	—

Materials and methods

CLINICAL AND PATHOLOGICAL EVALUATION

The study protocol was approved by the Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale at the Université de Médecine de Caen. The family reported herein originates from Normandy (western France) and includes three generations (fig 1). Five members (II-6, III-4, III-6, III-14, III-15) had undergone surgery for cavernomas before this study was started. In four of them the diagnosis of cavernous angiomas was confirmed by pathological analysis. In the other, there was insufficient material for precise characterisation of the lesion.

Based on family history, member I-2 was said to have been operated on in 1955 for a cerebellar haemorrhage due to a vascular malformation.

Twenty five members were then studied clinically and by neuroimaging; MRI was performed using T1 weighted and first and second echo T2 weighted sequences. Cavernomas were identified using previously established criteria.[18 19] For pathological study, all specimens were processed using glutaraldehyde or formalin fixative, embedded in paraffin, and stained with haematoxylin and eosin, Gomori trichrome, and elastin stain.

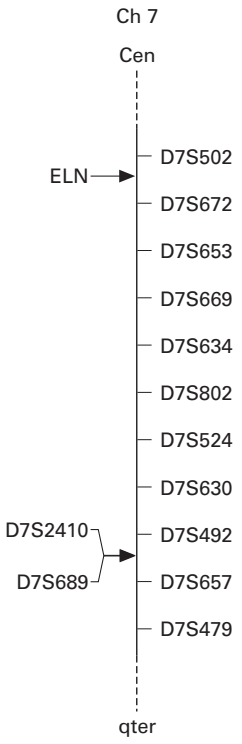

Figure 2 Genotypic regional map of chromosome 7. Critical markers used for linkage analysis are shown on the right side (genetic distances between these markers are indicated in the Genethon map (see Dib et al[40]). On the left are shown the respective positions of the elastin gene (ELN) and D7S2410 and D7S689.

GENOTYPING AND LINKAGE ANALYSIS

Markers

Eleven polymorphic microsatellite markers located between D7S502 and D7S479 were chosen from the Genethon linkage map, and CHLC linkage map on the basis of their informativity (fig 2): D7S502, D7S672, D7S653, D7S669, D7S634, D7S802, D7S524, D7S630, D7S492, D7S657, and D7S479.[35] All oligonucleotides sequences are available through the Genome Data Base (John Hopkins University, Baltimore, USA).

LINKAGE ANALYSIS

DNA was extracted from peripheral blood of all consenting members. Based on previous data suggesting the incomplete clinical penetrance of this condition, MRI was used to establish the status of a given member for linkage analysis. Members with an abnormal MRI were considered as affected, whether or not they were clinically symptomatic. Asymptomatic subjects having a normal MRI were considered as "healthy" when aged older than 45. Asymptomatic subjects with a normal MRI and younger than 45 years, were considered as having an unknown status.

Twenty five family members were studied, including 16 potentially informative meiosis, and 10 of unknown status. In addition, blood samples were taken from four spouses to analyse their offspring. Polymorphic genomic sequences were amplified by polymerase chain reaction as previously described.[36]

Linkage analysis was performed using the 5.1 version of the Linkage program package[37] using published allele frequencies from CEPH pedigrees. The most likely haplotypes were inferred for each person by minimising the number of crossover events in each sibship.

Table 2 Pairwise linkage data

	Recombination fraction							
Locus	*0.00*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*Z max*	*θ*
D7S502	−3.82	0.33	0.68	0.75	0.51	0.19	0.78	0.16
D7S669	−4.44	1.01	1.15	1.02	0.66	0.23	1.16	0.11
D7S634	−4.30	1.22	1.33	1.13	0.70	0.23	1.33	0.10
D7S802	−3.95	1.60	1.70	1.47	0.98	0.36	1.70	0.10
D7S524	3.32	3.02	2.70	2.01	1.25	0.45	3.32	0.00
D7S630	3.44	3.14	2.82	2.13	1.35	0.49	3.44	0.00
D7S657	4.04	3.69	3.33	2.53	1.64	0.64	4.04	0.00
D7S479	−4.33	0.24	0.46	0.48	0.32	0.12	0.51	0.15

Results

CLINICAL AND NEUROIMAGING INVESTIGATIONS

Among the 25 investigated members, 11 had an abnormal cerebral MRI highly suggestive of cavernomas (fig 3), eight of them being symptomatic. Five of these had undergone surgery. Table 1 shows the age of onset and clinical symptoms found in these patients. Twelve had a normal MRI and were totally asymptomatic. Four were older than 45 years, the oldest one, II-3, being 71. Among the eight asymptomatic subjects younger than 45, the youngest one was aged 18.

For subjects III-2 and III-17 it was not possible to be definitive. Member III-2, who has severe headaches had an abnormal MRI with a temporal cortical lesion presenting as an hypointensity on T2 weighted images including a punctate area of increased signal intensity. This image strongly suggested the diagnosis of either a cavernoma or a thrombosed angioma (fig 3E). This member has a son affected with two typical cavernoma lesions and was therefore considered as an obligate carrier. The MRI of subject III-17, aged 16 and totally asymptomatic, showed a right paraventricular lesion presenting as an hyposignal on T1 weighted images and an hypersignal on T2 weighted images without any change on a four year interval. This subject was considered as having an unknown status.

PATHOLOGICAL INVESTIGATIONS

Seven resected cerebral specimens from five patients (II-6, III-4, III-6, III-14, and III-15; and III-4 and III-6 both operated on twice) were available for neuropathological examination. Typical cavernoma lesions were found in patient II-6 (fig 4), and patients III-4, III-6, and III-14. In patient III-15, findings were consistent with a mixed vascular malformation including dilated vascular channels without intervening neural tissue as well as venous vessels.

One patient, II-15, presenting typical cavernomas on cerebral MRI, underwent dorsal surgery for a recurrent thoracic intercostal neuralgia. Interestingly, surgical investigation showed a vascular malformation in the lateros-pinal space at the T10 thoracic level strongly suggesting a venous angioma, confirmed by pathological examination.

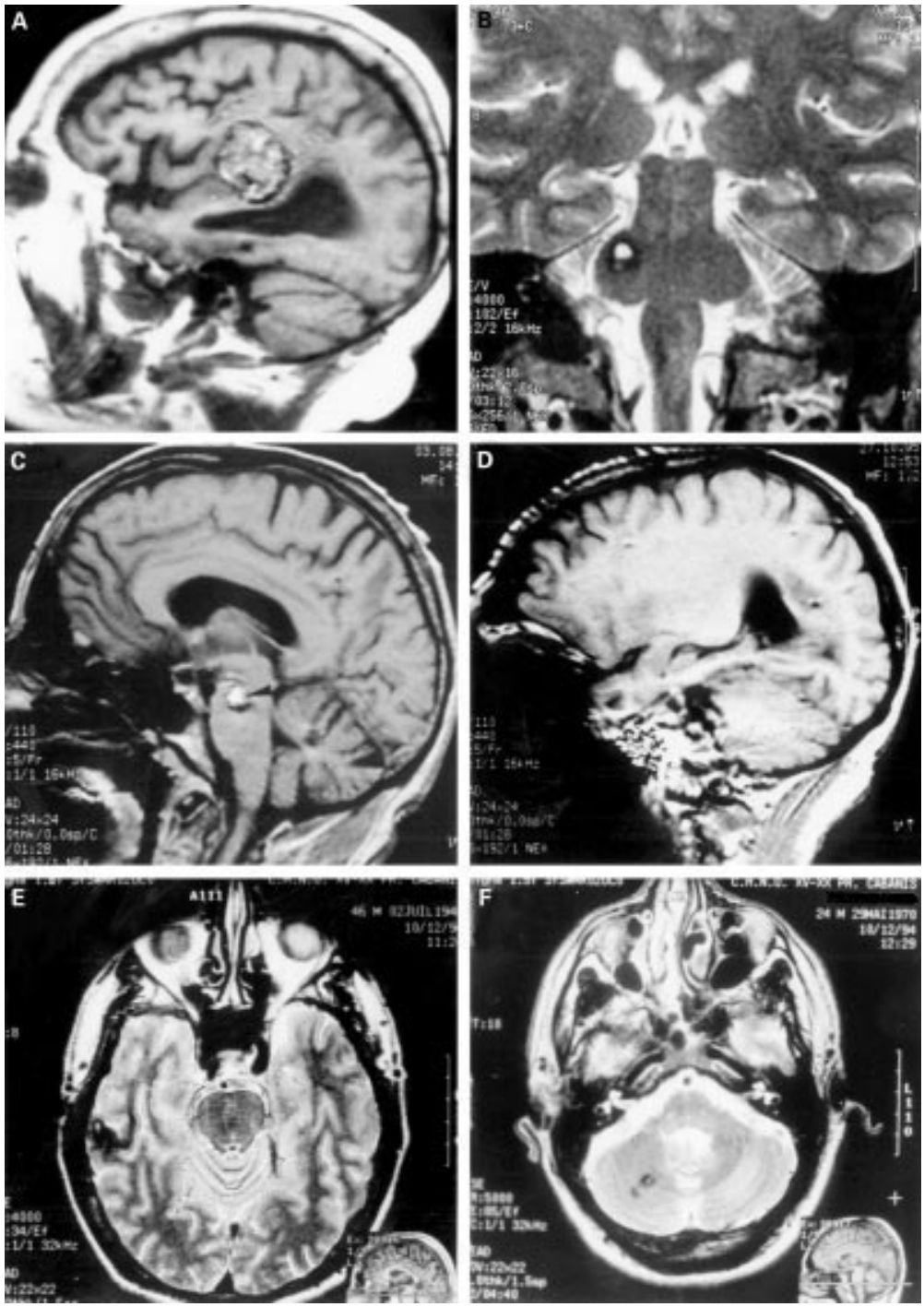

Figure 3 Typical features of cavernous angiomas on MRI from several affected members. (A) Patient II-6: sagittal T1 weighted image. Rolandic lesion with a reticulated core of mixed increased and decreased signal intensity surrounded by a rim of decreased signal. (B) Patient III-10: coronal T2 weighted section. Upper cerebellar pedoncle lesion. (C) Patient II-5: sagittal T1 weighted image. Pontine lesion. (D) Patient II-1: sagittal T1 weighted section. Multiple supratentorial hypointense and hyperintense small lesions. (E) Patient III-2: axial T2 weighted section. Small hyperintense area inside a right temporal hypointense lesion. (F) Patient IV-1: axial T2 weighted section. Lesion in the right cerebellar hemisphere.

LINKAGE DATA

Table 2 shows the two point linkage data. Significant lod scores were obtained for markers D7S657 (LS=4.04 at θ=0.00) and D7S630 (LS=3.44 at θ=0.00). No recombinant was found with these two markers. Positive lod scores were also obtained for several additional closely linked markers (table 2). These data establish linkage of the condition present in this family to chromosome 7.

The analysis of inherited haplotypes shows three crossover events (fig 1) strongly suggesting that the CCM1 gene is flanked proximally by marker D7S802 and distally by marker D7S479. Two recombinants were found with D7S802 (III-1, II-15). One of them, III-1, is a 49 year old asymptomatic person with a normal MRI. The second one, II-15, is a clinically affected patient with multiple cavernoma lesions on cerebral MRI. This patient has three

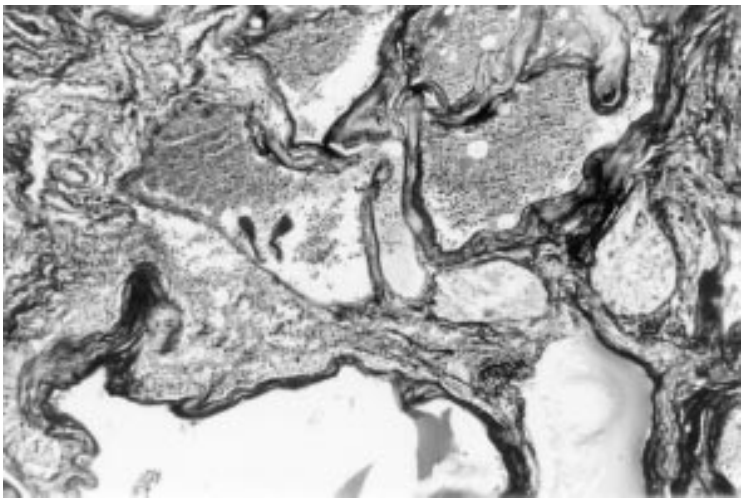

Figure 4 Patient II-6: histological appearance of lesion illustrated in fig 3A: juxtaposition of vascular cavities lined by a thin layer of endothelial cells and connective tisssue without intervening brain tissue. Modified Gomori trichrome stain. Originally × 32.

children, among whom one, III-15, had multiple typical cavernoma lesions on cerebral MRI. One crossover event was seen with marker D7S479; it occured in an affected patient, II-6, showing a typical cavernoma lesion on resected cerebral specimen.

These data support the most likely placement of the CCM1 gene between D7S802 and D7S479.

Discussion

Recently Dubovsky *et al* mapped a gene on chromosome 7 causing familial cavernomas within a Hispanic-American family.[28] This mapping was confirmed in seven other Hispanic-American families and three white families.[29–33] We report herein a large French kindred affected with familial cerebral cavernomas in which we established linkage to chromosome 7q.

It was first suggested on only a few families that cerebral cavernous malformations may be a genetically homogenous condition. Recently, Günel *et al* excluded linkage to 7q in two non-Hispanic families with cerebral cavernous malformations.[33] Clinical features of these families were similar to those found in families presenting linkage to 7q.

Interestingly, in the family reported here, two patients presenting multiple lesions highly suggestive of cavernomas on cerebral MRI and carrying the affected haplotype, presented other vascular malformations. Patient II-15 had a spinal venous angioma and his daughter, III-15, had a mixed vascular malformation. Such associations have been reported in the literature, raising the question of the nosological link between these various vascular malformations.[21 39] The availability of genetic markers which can identify, within a family with various vascular malformations, the members carrying the affected gene will in the very near future help to ascertain whether these various malformations are the result of the same genetic alteration.

Haplotype analysis in the French family strongly suggests that the CCM1 gene is most likely telomeric to D7S802 in agreement with the data of Johnson *et al*.[31] Based on one crossover event in an affected patient belonging to an Italian-American family, Günel *et al* suggested that the most likely placement of the gene would be centromeric to D7S802.[29] These apparent discrepancies could be explained in different ways, including, as suggested by the authors, the absence of linkage to chromosome 7 of the family. Another explanation could be that there is more than one CCM gene on 7q, which may have arisen by duplication. The analysis of additional families and particularly families of a large size is needed to reach a conclusion.

A founder effect has been found in Hispanic-American families, as confirmed by the recent studies of Günel *et al*.[31 34] The comparison of the size of the "affected" alleles segregating in our family with those previously reported does not show any common allele either with the Hispanic-American or white families previously reported (data not shown). However, additional families are currently being analysed to search for a linkage disequilibrium within families of French ancestry.

The analysis of additional families will also help in positional cloning of the CCM1 gene which is ongoing in several teams and should provide a useful tool for direct genotypic diagnosis as well as a better understanding of this condition in the future.

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