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Mapping a gene causing cerebral cavernous malformation to 7q11.2–q21

(genetics/linkage analysis/vascular malformation/brain)

MURAT GÜNEL*†‡, ISSAM A. AWAD†, JOHN ANSON§, AND RICHARD P. LIFTON*¶‖**

*Howard Hughes Medical Institute and Boyer Center for Molecular Medicine, †Section of Neurosurgery and Departments of ‡Cell Biology, ¶Medicine, and ‖Genetics, Yale University School of Medicine, New Haven, CT 06510; and §Division of Neurosurgery, University of New Mexico Hospital, Albuquerque, NM 87131

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ABSTRACT **Cerebral cavernous malformation is a common disease of the brain vasculature of unknown cause characterized by dilated thin-walled sinusoidal vessels (caverns); these lesions cause varying clinical presentations which include headache, seizure, and hemorrhagic stroke. This disorder is frequently familial, with autosomal dominant inheritance. Using a general linkage approach in two extended cavernous malformation kindreds, we have identified linkage of this trait to chromosome 7q11.2–q21. Multipoint linkage analysis yields a peak logarithm of odds (lod) score of 6.88 with zero recombination with locus *D7S669* and localizes the gene to a 7-cM region in the interval between loci *ELN* and *D7S802*.**

Vascular malformations are a heterogeneous group of disorders that affect an estimated 0.1–4% of the population (1, 2). Cerebral cavernous malformation (CCM) (also known as angiomas, cavernous angiomas, cavernous hemangiomas, cavernous angiomatomas, or cavernomas) accounts for 10–20% of all vascular malformations (1, 3). These lesions are characterized by collections of large, abnormal vascular spaces without intervening brain parenchyma (2). These thin-walled vascular channels or caverns are lined by endothelium and vary widely in size (Fig. 1). There are no identifiable mature vessel-wall elements within lesions, and there is almost always evidence of prior hemorrhage characterized by hemosiderin accumulation. Although solitary lesions are most commonly found, multiple lesions have been reported in 33% of the sporadic cases and in 50–73% of the familial cases (3–6). The size of these lesions varies from millimeters to centimeters, with a mean size at diagnosis of about 2 cm (1, 7). Cavernous malformation lesions are dynamic in time; subjects have been shown to acquire new lesions, and lesions have also been shown to undergo spontaneous involution. The variable behavior of these lesions is believed to account for the variable clinical course of affected subjects (6, 8–10).

Clinical sequelae of these lesions are variable. Patients frequently are asymptomatic (40–70%) (3, 11, 12); symptomatic subjects typically present between ages 20 and 40, although symptoms can begin at any age (6, 7, 13). Patients commonly present with seizures (7, 14), brain hemorrhage (3, 6, 11, 13–17), focal neurological deficits (6, 11–13, 15), or headaches. Risk of bleeding from these lesions is in part dependent on age and the prior occurrence of hemorrhage from the same lesion (15, 18). Diagnosis is usually made by MRI of the brain, which reveals a characteristic image of heterogeneous signal intensity surrounded by a dark ring attributable to hemosiderin deposition (Fig. 1) (8, 19). Treatment options range from observation in asymptomatic subjects, to pharmacologic therapy in subjects with seizures, and surgical excision of accessible lesions (11).

While the pathogenesis of CCM is unknown, 30–50% of patients with cavernous malformation have been found to have one or more relatives with the disease (3, 4, 6, 17) and several large kindreds supporting autosomal dominant inheritance of the trait have been reported (3, 17, 20, 21). These observations raise the possibility of using genetic approaches to define the pathogenesis of this disorder. We have used linkage with anonymous genetic markers to search for the location of mutations causing CCM in two extended kindreds. We report herein linkage of a gene responsible for CCM to a 7-cM interval of chromosome 7q11.2–21.

MATERIALS AND METHODS

Family and DNA Studies. Genomic DNA was prepared from venous blood of members of K2008 and 2015 by standard methods (22) after obtaining informed consent from family members. Di- and tetranucleotide repeat polymorphic markers distributed at 20-cM intervals throughout the human genome were selected for genotyping. One primer of each primer pair was end-labeled by incubation in the presence of [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs). PCR was performed in 10-μl volumes in the presence of 50 mM KCl/1.5 mM $MgCl_2$/5 mM Tris·HCl, pH 8.3/125 μM dNTPs/0.01% gelatin/0.5 unit *Taq* DNA polymerase (Perkin–Elmer)/0.5 μM each primer/100 ng of human genomic DNA. After an initial denaturation step at 94°C for 5 min, PCR was conducted for 35 cycles with denaturation at 94°C for 30 seconds, annealing at temperatures ranging from 53°C to 58°C for 30 sec, and extension at 72°C for 45 sec. Optimal annealing temperatures were determined individually. Genotypes were analyzed by denaturation of the PCR products by addition of 10 μl of formamide and heating to 94°C for 5 min, followed by electrophoresis on standard DNA-sequencing gels and autoradiography. Genotypes were read blinded to affection status.

Analysis of Linkage. Analysis of linkage was performed by using the LINKAGE software package (Version 5.1) (23). Pairwise and multipoint logarithm of odds (lod) scores were calculated by using loci of known order on 7q. In addition, *D7S802* (GenBank data base accession no. 43794) was localized on the human genetic map by linkage as described in *Results*. Linkage analysis was performed for each family assuming cavernous malformation to be an autosomal dominant disorder with a gene frequency of 0.001, a penetrance of 95%, and a phenocopy prevalence of 0.001. Penetrance was prospectively specified to be less than 100% on the basis of the

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Abbreviations: CCM, cerebral cavernous malformation; MRI, magnetic resonance imaging; lod, logarithm of odds.

**To whom reprint requests should be addressed at: Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06510.

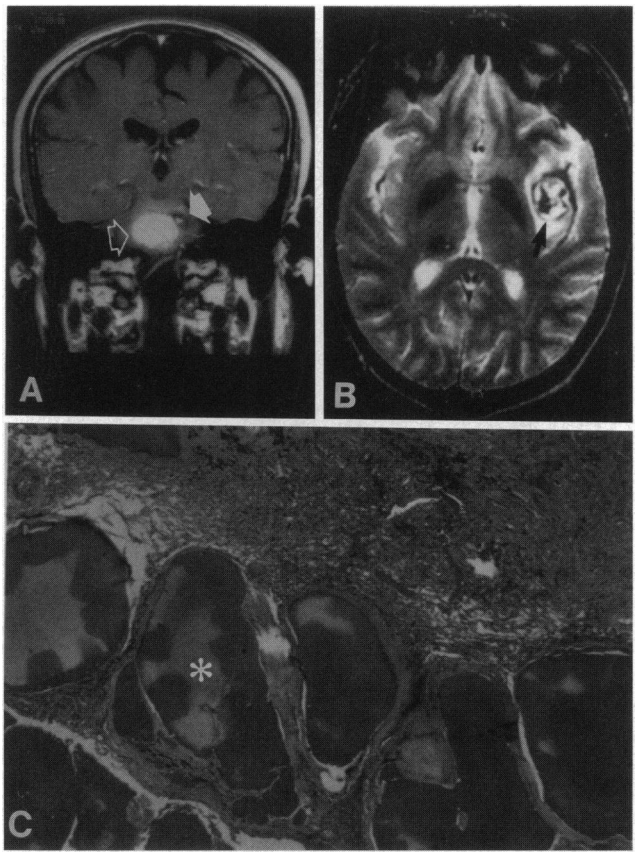

FIG. 1. Radiological and histological features of CCM. (*A*) Sagittal T1-weighted magnetic resonance imaging (MRI) image of the brain of individual III-9 in K2015. The CCM is marked by the large white arrow, which reveals a small malformation with high intensity signal (white) surrounded by low intensity signal (black) caused by the typical hemosiderin ring. The open white arrow on the left side indicates the site of catastrophic hemorrhage into the brain stem caused by the malformation. (*B*) Axial T2-weighted MRI image of the brain of individual III-10 in K2015. This image also demonstrates the typical appearance of CCM on MRI scanning, with the black arrow indicating a large lesion of heterogeneous signal intensity surrounded by a black ring indicative of hemosiderin deposition from prior hemorrhage. (*C*) Histological section of a typical CCM. This figure demonstrates the abnormally dilated sinusoidal vascular channels called caverns, which are diagnostic for this disorder. One of the dilated vascular channels is marked with an asterisk. These caverns are found without any intervening normal vessel wall elements or neural tissue. Areas of thrombosis can be seen within caverns while dense gliosis and evidence of remote hemorrhage are evident in the surrounding brain tissue. (Hematoxylin/eosin stain; ×400.)

observation of asymptomatic obligate carries in these kindreds, as well as others (6); the proportion of at-risk subjects found to be affected in these kindreds approached the Mendelian ratio of 0.5, leading to a high prospective estimate of penetrance. Overlapping four-point linkage analyses were performed with the number of alleles at each locus reduced to permit efficient computation, and lod scores at each map location were calculated. Analysis was performed on a SunSparc 20 workstation with 128 million bytes of random access memory.

RESULTS

Cavernous Malformation Kindreds. Two multiplex CCM kindreds were identified from the records of IAA and JA and characterized; all phenotypic assignments were performed prospectively (Fig. 2 *A* and *B*). K2008 and K2015 are of Hispanic and European descent, respectively. Cavernous malformation subjects in both kindreds were frequently severely affected, including eight subjects having sudden death or profound neurologic deficit from documented or presumed cerebral hemorrhage before the age of 30. No clinical abnormalities other than those related to the central nervous system have been noted in these families.

Living subjects with a definitive diagnosis of CCM by MRI scanning ($n = 7$) or pathological diagnosis of surgically resected tissue ($n = 9$) were classified as affected. MRI scans were considered diagnostic on the basis of T1- and T2-weighted images of the brain showing vascular lesions of heterogeneous signal intensity typically surrounded by a hypointense ring due to hemosiderin deposition (Fig. 1). All patients diagnosed by MRI in this study had multiple lesions. At-risk subjects older than age 20 with no neurologic symptoms or signs and no affected offspring ($n = 18$) were classified as unaffected. Three deceased genetically at-risk subjects (K2008 II-2, K2008 II-3, and K2015 II-1) with two or more definitively affected offspring were classified as obligate carriers and affected; two at-risk living asymptomatic subjects (K2008 III-19 and K2015 II-3) with two or more affected offspring were classified as obligate carriers and affected; and one at-risk symptomatic subject (K2015 II-5) with three affected offspring was classified as affected. Because patients with CCM commonly present with clinical signs after age 20, we prospectively classified asymptomatic subjects younger than age 20 with either no MRI or a negative MRI scan as phenotype unknown for the purpose of linkage analysis. Individuals IV-4 in K2008 and IV-1 in K2015 are asymptomatic at ages 8 and 18 years, respectively, and were consequently classified as phenotype unknown. Finally, individual III-5 in pedigree K2015 is an adult reported to have hemangiomas affecting her sinuses, liver, and genitourinary tract (features not typically associated with cavernous malformation); however, she has no neurologic signs or symptoms and has had normal computerized tomography of the head; she was classified as phenotype unknown.

Linkage of Cavernous Malformation to 7q. Highly polymorphic di- and tetranucleotide repeat markers at loci spaced at approximately 20-cM intervals were typed in the collected families as described in *Materials and Methods.* Evidence for linkage was analyzed by both pairwise and multipoint analyses under a prospectively specified model of the trait locus specifying autosomal dominant inheritance, a mutant gene frequency of 0.001, 95% penetrance, and a phenocopy prevalence of 0.001. A strongly positive lod score was obtained with locus *D7S802*, which is located on 7q (Table 1). Additional 7q markers were then genotyped in these families and pairwise lod scores were calculated, which confirmed linkage to a cluster of markers on proximal 7q (Table 1 and Fig. 3). The combined lod score for both families reached a maximum value of 6.16 at a recombination fraction of zero with marker *D7S669.* Additional loci proximal and distal to those shown give strongly negative lod scores (data not shown).

Multipoint Linkage Analysis. To more precisely locate the cavernous malformation gene on 7q, we performed multipoint linkage analysis, comparing the segregation of cavernous malformation to the segregation of markers of known map order. Markers whose orders were known from prior genetic maps (24, 25) were included in the analysis (Fig. 3). In addition, the position of *D7S802* relative to other loci was determined by linkage analysis in Center for the Study of Human Polymorphism and disease kindreds; *D7S802* (previous known to lie approximately 5 cM centromeric of *D7S820*) was found to map in the interval flanked by *D7S669* and *D7S804* with odds of greater than 1000:1 in favor of this interval over the next most likely interval, with a maximum likelihood location 3 cM telomeric to *D7S669* (data not shown).

Multipoint linkage analysis revealed a peak lod score of 6.88 for linkage of CCM to *D7S669* at a recombination fraction of

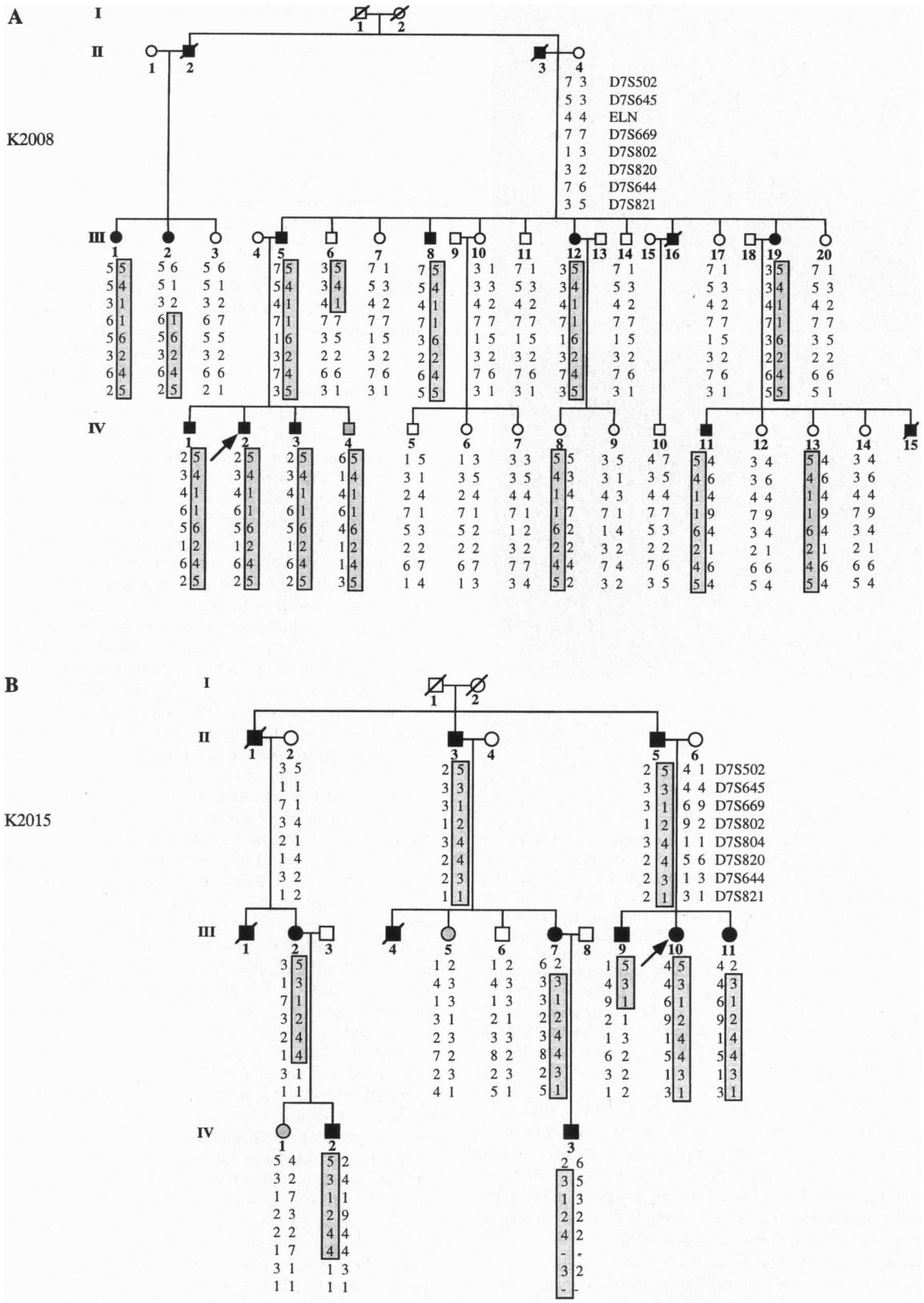

FIG. 2. Kindreds with CCM. The family relationships within kindreds 2008 (*A*) and 2015 (*B*) are shown. Individuals classified as affected with CCM are indicated by filled symbols; individuals classified as unaffected are indicated by unfilled symbols; individuals classified as phenotype unknown are indicated by stippled symbols; and index cases are indicated by arrows. Below each symbol, genotypes of loci on proximal 7q which were informative for linkage in each kindred are shown. These loci are presented in their mapped order on 7q, with the most centromeric locus at the top. In each kindred, the portion of the inferred ancestral haplotype segregating with the CCM locus which has been inherited by each subject is enclosed by a box and shaded, permitting inference of the location of recombinants on these chromosomes.

zero (lod scores in K2008 and K2015 of 5.03 and 1.85, respectively), odds of 7.59×10^6:1 in favor of linkage (Fig. 4). This analysis localized the *CCM* gene in these kindreds to the interval between *ELN* and *D7S802*, an approximately 9-cM

Table 1. Pairwise analysis of linkage of cavernous malformation and loci on chromosome 7q

Locus	Recombination fraction					
	0.00	0.05	0.1	0.2	0.3	0.4
D7S502	−1.38	1.74	2.32	2.37	1.80	0.90
K2008	1.80	2.37	2.52	2.31	1.71	0.85
K2015	−3.18	−0.63	−0.20	0.06	0.09	0.05
D7S645	1.40	3.16	3.26	2.84	2.04	1.00
K2008	0.62	2.47	2.66	2.42	1.79	0.89
K2015	0.78	0.69	0.60	0.42	0.25	0.10
ELN	1.13	2.74	2.79	2.36	1.64	0.76
K2008	0.78	2.44	2.54	2.21	1.57	0.74
K2015	0.35	0.30	0.25	0.15	0.07	0.02
D7S669	6.16	5.90	5.44	4.26	2.85	1.31
K2008	5.06	4.82	4.45	3.52	2.41	1.15
K2015	1.10	1.08	0.99	0.74	0.44	0.16
D7S802	3.10	4.66	4.58	3.78	2.60	1.21
K2008	3.85	3.92	3.74	3.07	2.15	1.04
K2015	−0.75	0.74	0.84	0.71	0.45	0.17
D7S804	1.34	2.75	2.73	2.20	1.40	0.53
K2008	1.86	1.88	1.77	1.39	0.87	0.30
K2015	−0.52	0.87	0.96	0.81	0.53	0.23
D7S820	−0.73	0.95	1.13	1.03	0.71	0.32
K2008	0.11	0.30	0.38	0.39	0.32	0.18
K2015	−0.84	0.65	0.75	0.64	0.39	0.14
D7S644	−0.55	2.48	2.82	2.64	1.97	0.98
K2008	4.09	4.01	3.77	3.05	2.12	1.02
K2015	−4.64	−1.53	−0.95	−0.41	−0.15	−0.04
D7S821	2.33	3.73	3.65	3.01	2.10	1.01
K2008	4.21	4.07	3.80	3.05	2.12	1.02
K2015	−1.88	−0.34	−0.15	−0.04	−0.02	−0.01

Markers are listed in their chromosomal map order on 7q with the most centromeric marker at the top. The lod scores for each kindred and the two kindreds together are shown.

interval, with odds of greater than 1000:1 favoring location in this interval; the 95% confidence interval (as approximated by the lod −1 support interval) localizes the cavernous malformation locus to a 7-cM region within this interval. Changing the affection status of obligate carriers from affected to affection status unknown had a negligible effect on the pairwise and multipoint lod scores. Evidence of linkage to this same chromosomal position was robust to reduction in estimates of penetrance and to use of only affected pedigree members in linkage analysis (data not shown).

Analysis of critical recombinants strongly supports localization of the *CCM* locus within the specified interval. In K2015, affected individual III-9 has inherited the linked haplotype from *D7S502* to *D7S669*; loci telomeric to *D7S669* are derived from the unlinked chromosome and place the *CCM* gene centromeric to *D7S802* (Fig. 2*B*). This recombinant individual is a severely affected 50-year-old male, with multiple intracranial hemorrhages resulting in paraplegia and impaired cognition; he has a diagnostic MRI scan (Fig. 1*A*), as well as diagnostic surgical pathology. An alternative but less likely explanation to this subject being recombinant would be that *CCM* in this kindred is unlinked to 7q (likelihood ratio of linked:unlinked = 76:1). In K2008, affected individual III-2 has inherited the linked haplotype from *D7S669* to *D7S821* and shows recombination in the interval between *ELN* and *D7S669* (Fig. 2*A*). This recombinant places the disease locus telomeric of *ELN*. This individual is a severely affected 56-year-old female symptomatic from age 11, with multiple intracranial hemorrhages leaving her paraplegic and aphasic; she too has a diagnostic MRI scan.

DISCUSSION

We have demonstrated linkage of a mutation causing CCM to the proximal region of chromosome 7q. The evidence for linkage is very strong in the two affected families studied, with a combined lod score of 6.88 at a recombination fraction of zero with locus *D7S669*. Multipoint analysis has localized the *CCM* gene to a 7-cM region of 7q. Since *in situ* hybridization of yeast artificial chromosomes containing loci proximal and distal to *D7S669* map respectively to 7q11.2 and 7q21 (25), we assign the *CCM* gene in these kindreds to 7q11.2–q21. These results represent a first step toward identification of the underlying mutations which cause CCM.

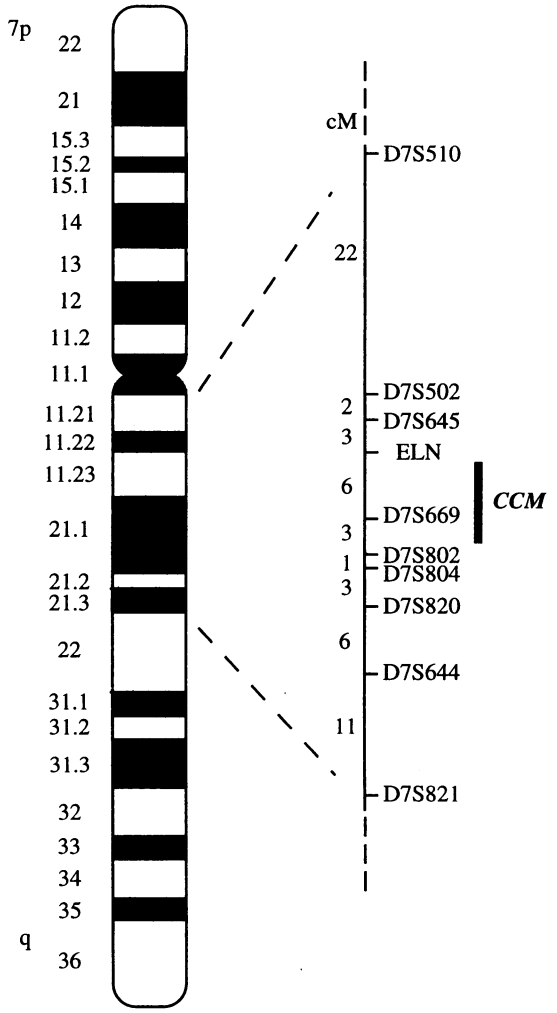

FIG. 3. Chromosome 7 ideogram with map of loci on 7q. Loci on 7q which were used in this study are shown in their map order; the sex-averaged distance in centimorgans between adjacent loci is shown. The map location of the *CCM* locus is indicated.

A potential candidate gene, CD36 (glycoprotein IV), has been assigned by *in situ* hybridization to 7q11.2 (26); however, its precise location in this region is uncertain. The encoded product of this gene is expressed in both endothelial cells and platelets and is believed to be involved in platelet interaction with collagen (27); this gene product has been proposed to mediate the adhesion of *Plasmodium falciparum* to cerebral blood vessels (28). Further work will be required to determine whether CD36 lies in the completely linked interval and assess its potential role in the pathogenesis of CCM. Mutations in elastin (*ELN*) have been shown to cause another vascular disease, supravalvular aortic stenosis (29); mutations in this gene would appear to be excluded by linkage analysis.

Identification of linkage permits assessment of penetrance of CCM in these families. Between the two families studied, there are seven living subjects who are asymptomatic who carry the linked haplotype and are older than age 20. Three of these

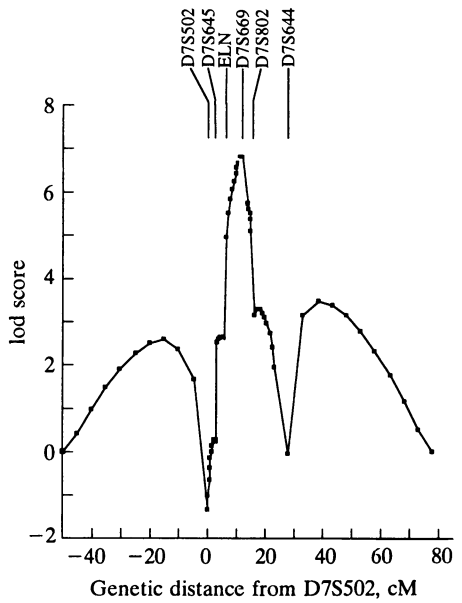

FIG. 4. Multipoint linkage analysis of *CCM* and loci on 7q. The location of each locus used for multipoint analysis is indicated, and the lod score for linkage of *CCM* to each position on the map is shown. The maximum lod score is 6.88 at zero recombination with *D7S669*, with the disease locus lying in the interval between *ELN* and *D7S802*.

have had positive MRI scans diagnostic for cavernous malformation, while two (K2008 III-19 and K2015 IV-8) have had negative MRI scans at ages 43 and 32, respectively. These findings demonstrate the incomplete penetrance of this trait and indicate the difficulty of providing concrete prognostic information on the basis of genotype. These families were selected for study because of their high penetrance; it is important to point out that other families with inherited CCM may commonly have lower penetrance.

Vascular malformations of the brain are grouped into four categories: arteriovenous malformations (AVM), venous malformations, capillary telengiectasias, and cavernous malformations (1). The AVMs are rarely familial or multiple in the same subject; however, this is the case in hereditary hemorrhagic telangiectasia (HHT), an autosomal-dominant disease associated with vascular malformations of the skin, lung, gastrointestinal tract, and brain (30). Some cases of HHT have recently been shown to be due to mutations in the gene on chromosome 9 encoding endoglin, a transforming growth factor β-binding cell surface protein of uncertain function (31). It will be interesting to determine whether the gene(s) responsible for CCM prove to be related to the pathway implicated by endoglin mutations in HHT.

CCM was thought to be uncommon prior to the advent of MRI imaging, but is now recognized to be relatively common with a prevalence of up to 0.5% in MRI and autopsy series (1, 11, 13, 32). Similarly, the inherited nature of these lesions is being recognized more frequently with the screening of asymptomatic family members of index cases. The true proportion of genetic cases remains unclear, with estimates of 30–50% (3, 4, 6, 17). Identification of the gene(s) underlying inherited CCM will permit assessment of whether all CCM is genetic and determination of whether incomplete and variable penetrance in different families is due to specific allelic effects, inheritance of genetic modifiers at other loci, or environmental effects. Moreover, we anticipate that characterization of the molecular basis of CCM will provide insights into the pathophysiology of this disorder, leading to approaches to identification of genetically susceptible subjects, as well as to therapies to prevent either development of lesions or the adverse sequelae of lesion growth and hemorrhage.

Note Added in Proof. After submission of this manuscript, Dubovsky *et al.* (33) reported a lod score of 4.2 for linkage of CCM to a 33-cM region of chromosome 7 which includes the interval reported here.

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