RESEARCH

Refined Localization of the Cerebral Cavernous Malformation Gene (*CCM1*) to a 4-cM Interval of Chromosome 7q Contained in a Well-defined YAC Contig

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Cerebral cavernous malformations (CCM) are vascular lesions present in some 20 million people worldwide that are responsible for seizures, migraine, hemorrhage, and other neurologic problems. Familial cases of CCM can be inherited as an autosomal dominant disorder with variable expression. A gene for CCM (*CCM1*) was recently mapped to a 33-cM segment of chromosome 7q in a large Hispanic family (Dubovsky et al. 1995). Here, the collection of several new short tandem repeat polymorphisms (STRPs) within the region of interest on 7q and the refinement of the marker order in this region using both linkage analysis in CEPH families and especially YAC-based STS content mapping are described. Affected members of three Hispanic families share allele haplotypes indicating a common ancestral mutation within these families. Using the shared haplotype information along with analysis of crossovers in affected individuals from both the Hispanic and Caucasian families, the region likely to contain the *CCM1* gene has been reduced to a 4-cM segment of 7q between D7S2410 and D7S689. All markers within the refined chromosomal segment were located on a single YAC contig estimated to be ~2 Mb in size. Four potential candidate genes have been mapped to this region.

Cerebral cavernous malformations (CCM) (also called cerebral cavernous angiomas or cavernous hemangiomas) are vascular malformations consisting of large, closely clustered enlarged capillary channels (caverns) with a single layer of en-

dothelium and without the normal intervening neural tissue or brain parenchyma. The walls of CCM are devoid of any smooth muscle and elastic tissue. Microscopic hemorrhage, gliosis, and calcification are common (Voight and Yasargil 1976; Simard et al. 1986; Villani et al. 1989). CCM can range in size from a few millimeters to several centimeters in diameter.

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CCM represent 5%–15% of all cerebral vascular malformations. Affected individuals may have single or multiple lesions, usually in the cerebral hemispheres. Magnetic resonance (MR) imaging is exquisitely sensitive to the presence of hemosiderin and the other breakdown products of blood that characterize these lesions, leading to a highly distinctive appearance that is diagnostic for CCM in the majority of cases (Rigamonti et al. 1987; Zabramski et al. 1994). Two large retrospective reviews of >22,000 MR studies have yielded an incidence rate for cavernous malformations of between 0.39% and 0.47% in the general population (Curling, 1991; Robinson 1991). This rate is nearly identical to that reported in several autopsy series that included neuropathologic examinations (McCormick 1984; Otten et al. 1989; Curling et al. 1991; Robinson et al. 1991; Zabramski et al. 1994), and represents between 18 and 22 million people affected worldwide.

CCM have been reported in infants and children, but the majority of patients present with symptoms between the second and fifth decades (Curling et al. 1991; Robinson et al. 1991; Zabramski et al. 1994). Of patients who are MR imaging positive, 15%–20% can remain asymptomatic throughout their lives (Curling et al. 1991; Robinson et al. 1991). Others experience a number of serious neurological problems resulting from intracranial hemorrhage and mass effects, with focal and generalized epileptic seizures being the most common presenting symptom (40%–70%), followed by focal neurological deficits (35%–50%) and nonspecific headache pain (10%–30%) (Voight and Yasargil 1976; Simard et al. 1986; Rigamonti et al. 1988; Villani et al. 1989; Curling et al. 1991; Robinson et al. 1991; Zabramski et al. 1994). CCM can also lead to death as a result of cerebrovascular accident (Hayman et al. 1982; Gil-Nagel et al. 1995).

CCM occur in two forms: (1) a spontaneous form in which patients usually present with a single lesion and no family history of neurological disease; and (2) a familial form characterized by multiple lesions and a strong family history of seizures. The familial form of cavernous malformations (FCCM) is inherited as an autosomal dominant disorder with variable expression (Hayman et al. 1982; Dobyns et al. 1987; Rigamonti et al. 1988; Allard et al. 1989; Malik et al. 1992; Zabramski et al. 1994).

A gene for CCM (*CCM1*) was mapped by our group to a 33-centimorgan (cM) segment of chro-

mosome 7q in a large Hispanic family (Dubovsky et al. 1995). Marchuk and co-workers (1995) demonstrated linkage for CCM to a slightly broader, overlapping 41-cM segment of 7q in two additional families, one Hispanic and one Caucasian. Linkage to this same region of 7q has also been confirmed in another large Caucasian kindred (Gil-Nagel et al. 1995b). In this family, the gene could be localized to the 15-cM segment of 7q bounded by D7S660 and D7S558. Finally, Günel and colleagues (1995) recently reported linkage to a broad region of 7q in two additional families, one Hispanic and the other Caucasian of European decent.

Using analysis of crossovers in both affected and unaffected individuals along with common haplotype analysis of affected members from three Hispanic families, we have been able to refine the region likely to contain the *CCM1* gene to a 2-megabase pair (Mb) segment of 7q between D7S2410 and D7S689.

RESULTS

Linkage Mapping of 7q Polymorphic Markers

A sex-equal linkage map for the region of interest

Figure 1 Sex equal linkage map for the region of interest on chromosome 7q. Map distances are listed in cM. Several of the markers could not be completely ordered relative to the others. They are shown at *right* with the best single-crossover positions indicated.

Table 1. STSs in the YAC Contig Encompassing the CCM1 Critical Region

<u>STS Name</u>	<u>Alias</u>	<u>Locus</u>	<u>Source</u>	<u>PCR Primers</u>	<u>Size (bp)</u>	<u>GDB ID</u>
sWSS2102		D7S2651	YAC End	CATTTCTAAAAGCGTTTC TAAGTAATCACCAAGACC	201	G00-626-855
sWSS1326		D7S2658	YAC End	CAATAACTCTTGGATTGG GAATTAGTGAAGTTGAAGG	135	G00-626-840
sWSS2637		D7S2659	YAC Insert	CACATAAGATGCTTCACC GAGAATACAGTGTTATTTCC	126	G00-626-864
sWSS2967		D7S2660	YAC End	TATTGGGAACACAGAGAG ATAGCACTGTTTTACCACATC	103	G00-626-876
sWSS1228	AFM158xa1	D7S492	Genetic Marker	GTGTATCCAGAATCTCAG GGCTCTGCTCCATCTTCATA	231	G00-307-750
sWSS2073		D7S2661	YAC End	AGGTTGTATGTCTCTATG AATAAGAGAGAAGACGG	134	G00-626-852
sWSS893		D7S2662	Lambda Clone	AGTAATCCTTGGAGCTTG GCTATGAAGGCTTTTAAAC	125	G00-626-897
sWSS3044	AFMa218ze5	D7S2410	Genetic Marker	TATAGTGCCAACATCTCC CCAAGACATTTCAGATTTTTC	75	G00-626-882
sWSS1818		D7S2663	YAC End	AATCCTAAGTGTCTAGTG TTTTCTGGCATAGTGTTG	228	G00-626-846
sWSS370		D7S1426	M13 Clone	ATGAGGGTTAGGTTCTTG ATGGAGAGGTTGAAGTTG	131	G00-269-209
sWSS1813		D7S2664	YAC End	TGACTTTGTAGTGTAGCC GTATTGACCTTCACCTTG	109	G00-626-843
sWSS1968		D7S2665	YAC End	TTTCAGTGTGTTACAAGG TGAGTTCTGGAATATAGG	96	G00-626-849
sWSS409		D7S1431	Lambda Clone	AATCAGCATTTCCAGGTC GGCTAAATAGTCCCTAAG	113	G00-269-224
sWSS2703		D7S2652	YAC End	CATTAAAGTAACTGGAATGG ACTTCAGGAAAATCTCTATTGG	148	G00-626-870
sWSS532		D7S1539	Lambda Clone	CATAGCCAGGATATAAAC TTGAGCATCTCTAGGAAC	104	G00-626-894
sWSS3041	AFM359tf1	D7S2408	Genetic Marker	ACGGTTTACTCATTCTGG GAACATTTCTCTTGCTGG	162	G00-626-879
sWSS2344	AFMa202xa5	D7S2409	Genetic Marker	CACAATGTAAGGTGATAG TTTTTCCCTCATGCTTC	141	G00-626-858
sWSS3004	ATA24A12	D7S1813	Genetic Marker	AAGTGCACCCAGCTCCAG CCTTCTCAGGCTATATTTAGTTAGC	143	G00-364-997
sWSS3071		D7S2653	YAC End	GTCTCATTTGTTCACTGG TAAGGTTTAGATACAGAAAGGG	135	G00-626-885
sWSS2694		D7S2654	YAC End	ACTTTTAACTGGATCTC TTTCTCTAGTAGCCTG	190	G00-626-867
sWSS2683	ACT3E08	D7S1789	Genetic Marker	GAAAACAGTGATAGGAACCTGC ATTCACCCTGCTCTCTAGGG	131	G00-364-138
sWSS1708	EST00272	D7S535E	EST	AGTGGTCACTATCTAACTGG GATTTCAGAATTACTAAGCCG	67	G00-189-116
sWSS1727	EST00631	D7S548E	EST	CATGTATACTGGGGAGTATG TCAAAGTAGACTTACATCAG	68	G00-190-983

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STS Name	Alias	Locus	Source	PCR Primers	Size (bp)	GDB ID
sWSS1725	EST00979	D7S539E	EST	GGTTTGGGATAATTTTCCTTC GTTACATTCTGGGTTAGTAT	132	G00-188-919
sWSS3105	CDK6	CDK6	Gene	CGGAGAACACCCCTTGGTG GAGCCTGTCCAGAAGACAGC	105	G00-626-888
sWSS1376	AFM240ve3	D7S646	Genetic Marker	CTATTTCTTTTCTTGACTGG CATTTTAACTGCTCAGAC	97	G00-307-806
sWSS2533		D7S2655	YAC End	GGATTTTACGTGGAATGG GTGGAGCAATAAAGGTAAG	145	G00-626-861
sWSS462		D7S2656	Lambda Clone	GATGGTTGTAGATGTGTAG TGTTTTAAAGCACAACAGCC	72	G00-626-891
sWSS2689	MFD267	D7S558	Genetic Marker	CCCTGCCTCTAAAATTATAC GGAATCTGGTAGACTGGTTT	95	G00-195-021
sWSS1132	AFM333wf5	D7S689	Genetic Marker	CCTCAACCTGAATCTCACATC CAATGGAGCCAGACTCTGT	131	G00-626-837
sWSS2717		D7S2657	YAC End	GCAACTTTGTATAATCCC AGAAAACCTCAGACCTCAG	111	G00-626-873
sWSS1096	AFM263yd9	D7S657	Genetic Marker	GTCACAGCACAGTTTTTGG GTCAAGTAGAGATTGAGATTCC	246	G00-626-834
sWSS2011	WI-1840	D7S1762	Random Clone	TCACCTAGGGAGGTCGCTAA TGCGATAGTCTTATAATTCTTCATGG	204	G00-354-938
sWSS1091	AFM254xd5	D7S652	Genetic Marker	GGGCTTGTTCATTACACGTTG CATGATTTTTGGCACAGAATGTTAG	275	G00-626-831

The 34 chromosome 7-specific STSs mapped to the YAC contig shown in Fig. 2 are listed. In each case, the designated sWSS name, relevant alias, GDB-assigned locus name, STS source, PCR primer sequences, STS size, and GDB identification (ID) number are provided. The sources of STSs are as follows: YAC End [isolated insert end of a YAC (Green 1993)], YAC Insert (random segment from a YAC insert), Genetic Marker (STRP), λ Clone [random chromosome 7-specific λ Clone (Green et al. 1991; Green 1993)], M13 Clone [random M13 clone derived from a flow-sorted chromosome 7 library (Green et al. 1991; Green 1993)], EST (expressed sequence tag-specific STS), Gene (gene-specific STS), and Random Clone (random human genomic STS mapped to chromosome 7). Note that for some genetic marker-specific STSs, the PCR primers used for identifying YACs (listed here) are different from those used for performing genotype analysis, because the detection of YACs containing a genetic marker does not require amplification of the polymorphic tract itself.

is shown in Figure 1. The map was based on typing of the short tandem repeat polymorphic markers through eight large CEPH families. In each case, pairwise marker order was established on the basis of at least one clear recombination event. Because of the limited number of recombination events in the eight families, several of the markers could not be completely ordered relative to the others. These markers are shown on the right with the best single-crossover positions indicated. The dinucleotide short tandem repeat polymorphism (STRP) at locus D7S652, for example, was placed on the map distal to D7S1813 but proximal to D7S1820.

Physical Mapping of the *CCM1* Critical Region

As part of a global effort to construct a physical

map of human chromosome 7 (Green et al. 1991, 1994, 1995), a yeast artificial chromosome (YAC)-based sequence-tagged site (STS)-content map spanning the *CCM1* critical region was generated. A collection of YACs highly enriched for chromosome 7 (Green et al. 1995) was screened using 34 STS-specific polymerase chain reaction (PCR) assays. Information on the 34 STSs assigned to YAC clones is provided in Table 1, with additional information such as PCR reaction conditions and complete DNA sequence available from GenBank and/or the Genome Data Base (GDB). Among the STSs were 11 corresponding to genetic markers, 13 derived from YACs (in particular, 12 from YAC insert ends), 6 developed from random chromosome 7 sequences, and 4 corresponding to expressed sequence tag (EST) or gene sequences. On the basis of analysis of the result-

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ing STS content data using the program SEGMAP (Green and Green 1991; C.L. Magness and P. Green, unpubl.), the YAC contig shown in Figure 2 was deduced. This contig contained 92 YACs and provided a unique order for 30 of the 34 STSs. Original library positions of YACs derived from total human genomic libraries are listed in Table 2. Orientation of the contig relative to the centromere and telomere was determined through linkage mapping (see above) and radiation hybrid mapping (E.D. Green and D.R. Cox, unpubl.) of a subset of the STSs. Note that there was redundant YAC-based connectivity throughout the contig (i.e., there were two or more YACs connecting each adjacent pair of STSs), lending strong support for the relative order of STSs. The order of markers determined from the YAC contig was in complete agreement with the Centre d'Etude du Polymorphisme Humain (CEPH) family linkage map (Fig. 1). Three of the YACs in the contig (yWSS1257, yWSS1852, and yWSS4093) were found by fluorescence in situ hybridization (FISH) analysis to map within 7q21–q22 (Green et al. 1994), indicating that the *CCM1* gene probably resides within this cytogenetic region.

CCM Families (FCCM)

A total of five families with a history of cavernous malformation were examined for this study, two Caucasian and three of Hispanic origin. In-

formation documenting linkage to chromosome 7q in most of these families has been published previously (Dubovsky et al. 1995; Gil-Nagel et al. 1995a,b; Marchuk et al. 1995). One additional unpublished Hispanic family designated FCCM300 was also used. The pedigree for this family is presented in Figure 3. DNA was collected from eight affected and seven unaffected members of this family spanning three generations. All diagnoses in these individuals were confirmed with MR imaging.

DNA from the five families used in the study was analyzed initially using some 30 STRPs covering a 15-cM region on chromosome 7q roughly spanning the interval from D7S440 (Mfd50) proximally to D7S1820 (GATA26D09) distally (see Dubovsky et al. 1995). Pairwise maximum lod scores for the families are displayed in Table 3. Because of limited kindred size, not all of the pairwise lod scores were in excess of 3.0, but all were at least consistent with linkage to 7q.

Shared Haplotypes

Within each individual family, affected individuals shared broad haplotypes (Table 4). There is little overlap in haplotype between the two Caucasian families, FCCM1 and FCCM20, or between these families and the Hispanic families. However, the three Hispanic kindred, FCCM10, FCCM200, and FCCM300, shared alleles from

Figure 2 YAC contig containing the *CCM1* critical region. The YAC-based STS content map of the *CCM1* critical region of chromosome 7 is depicted, as deduced by SEGMAP/version 3.36 (Green and Green 1991; C.L. Magness and P. Green, unpubl.). The 34 STSs (see Table 1) mapped to the YAC clones are listed along the top. Of these STSs, 30 could be uniquely ordered based on the STS content of the YACs, with the groups of STSs not ordered relative to one another indicated by horizontal brackets above the STS names. Arrows indicate the positions of the two genetic markers [D7S2410 (sWSS3044) and D7S689 (sWSS1132)] that represent the flanking boundaries of the *CCM1* critical region, as defined in this study. Also shown are the predicted positions of the centromere (CEN) and 7q telomere (TEL) relative to the ends of the contig. Each of the 92 YAC clones is depicted by a horizontal bar, with its name given at left and estimated YAC size (in kb, measured by pulsed-field gel electrophoresis) provided in parenthesis. The presence of an STS in a YAC is indicated by a closed circle at the appropriate position. When an STS corresponds to the insert end of a YAC, a square is placed around the corresponding circle, both along the top (near the STS name) and at the end of the YAC from which it was derived. For the eight YACs at the bottom (below the horizontal broken line), one or more STS(s) expected to be present (based on the established STS order) was not detected as assessed by testing the individual YACs with the corresponding STS-specific PCR assays at least twice, and these are depicted as open circles at the appropriate positions. A subset of the YACs was isolated from a human–hamster hybrid cell-derived library (Green et al. 1995), with their original names as indicated. The remaining YACs were isolated from total human genomic libraries, and their original library locations are provided in Table 4. Boxes are placed around the names of the three YACs (yWSS1257, yWSS1852, and yWSS4093) that were found by FISH analysis to map to 7q21–q22. The contig is displayed in its uncomputed form, where YAC sizes are not used to estimate clone overlaps or STS spacing and all of the STSs are spaced in an equidistant fashion. In the computed form, where YAC sizes are used to estimate the relative distance separating each pair of adjacent STSs as well as the extent of clone overlaps, the total YAC contig appears to span just over 3.8 Mb.

Table 2. Original Well Locations of YACs Derived from Total Human Genomic Libraries

yWSS Name	Library	Location	yWSS Name	Library	Location
yWSS301	WU	A133B8	yWSS4721	CEPH	888G09
yWSS302	WU	A133C4	yWSS4727	CEPH	890C02
yWSS303	WU	A171H5	yWSS4793	CEPH	908G08
yWSS311	WU	D23F4	yWSS4835	CEPH	918E08
yWSS312	WU	D78F7	yWSS4843	CEPH	919G04
yWSS2611	CEPH	763A04	yWSS4849	CEPH	921G08
yWSS2694	CEPH	774F01	yWSS4853	CEPH	923D10
yWSS2806	CEPH	784A08	yWSS4855	CEPH	923H12
yWSS2807	CEPH	784A11	yWSS4864	CEPH	925E10
yWSS3222	CEPH	850G03	yWSS4898	CEPH	931G04
yWSS4312	ICI	I66H1	yWSS4926	CEPH	937C10
yWSS4313	ICI	I149D1	yWSS4985	CEPH	947A11
yWSS4314	ICI	I323E10	yWSS4987	CEPH	947G12
yWSS4315	ICI	I335A8	yWSS5018	CEPH	952D01
yWSS4331	ICI	I66H1	yWSS5035	CEPH	956B05
yWSS4343	CEPH	740G04	yWSS5051	CEPH	960E10
yWSS4358	CEPH	740E03	yWSS5104	CEPH	977H12
yWSS4385	CEPH	750F01	yWSS5127	CEPH	743B02
yWSS4389	CEPH	751G05	yWSS5145	CEPH	820H08
yWSS4433	CEPH	764G11	yWSS5157	CEPH	881H05
yWSS4441	CEPH	766G11	yWSS5160	CEPH	894E11
yWSS4448	CEPH	769D05	yWSS5164	CEPH	904G08
yWSS4494	CEPH	787D08	yWSS5213	CEPH	664F06
yWSS4501	CEPH	791A02	yWSS5214	CEPH	965E12
yWSS4513	CEPH	794F06	yWSS5261	CEPH	732E11
yWSS4546	CEPH	807E11	yWSS5307	CEPH	722D09
yWSS4573	CEPH	818D02	yWSS5310	CEPH	734D05
yWSS4598	CEPH	843B02	yWSS5318	CEPH	669C02
yWSS4633	CEPH	855E06	yWSS5345	CEPH	825H3
yWSS4681	CEPH	871D08	yWSS5361	CEPH	966F4
yWSS4709	CEPH	885F02	yWSS5362	CEPH	966G8
yWSS4711	CEPH	885G06			

A subset of the YACs depicted in Fig. 2 were isolated from total human genomic libraries constructed at Washington University (Burke et al. 1987; Brownstein et al. 1989; Burke and Olson, 1991), CEPH (Albertsen et al. 1990; Dausset et al. 1992), or ICI (Anand et al. 1989, 1990). To facilitate cross-correlation of these latter YACs, each yWSS name is listed along with the original library and precise well location from which the corresponding clone was isolated. The remaining YACs depicted in Fig. 2 were isolated from a human-hamster hybrid cell-derived library (Green et al. 1995), with their original names and library locations as indicated in the contig.

several adjacent markers. All three Hispanic families shared a segment of conserved haplotype distal to marker D7S630.

The proximal end of this "Hispanic interval" is well defined by at least three independent allelic variations; at D7S2417, FCCM10 showed a 6-base shift compared with the other two Hispanic families, and at D7S2485, FCCM10 showed an 8-base shift. Finally, at D7S630, FCCM300

showed a 12-base shift from the allele size seen in the other two Hispanic families.

Recombination Events

Key recombination events within three of the families are shown in Figure 4. In family FCCM1, individual 20, an affected female, was recombinant with respect to her affected mother between markers D7S2410 and D7S2408. The crossover seen in this individual effectively defined the proximal boundary of the interval. From marker D7S2410 proximally, all the alleles were derived from the affected mother's unaffected chromosome. The phase of the maternal alleles was firmly established from analysis of the grandparental genotypes. The presence of two affected sibs who shared the mother's affected haplotype (individuals 19 and 21) was additional evidence for an informative recombination in individual 20.

The distal boundary of the *CCM1* interval was defined by a crossover in individual 16 from family FCCM200. Individual 16, an affected female, was recombinant with respect to her affected mother between markers D7S1813 and D7S689. Centromeric to D7S1813, the alleles were unambiguously from the maternal affected chromosome. Again, the affected grandmother helped to establish phase. Note that markers at the three loci immediately proximal to D7S689 (D7S558, D7S646, and D7S1789) were uninformative in this crossover.

Two crossovers in unaffected individuals provided additional support for the location of *CCM1*. A crossover in individual 11 from FCCM300 was located between loci D7S630 and D7S2408. Centromeric to D7S630, the alleles were clearly derived from the affected maternal chromosome, supporting the localization of *CCM1* distal to D7S630. Another unaffected individual from FCCM300, individual 15, was recombinant at D7S652. Centromeric to D7S652,

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Figure 3 Pedigree for cavernous malformation family FCCM300. FCCM300 is of Hispanic origin and was collected at the Barrow Neurological Institute, Phoenix, AZ. DNA was collected from individuals 1 through 15. (●, ■) Affected individuals confirmed by MR imaging studies. (○, □) Unaffected individuals also confirmed by MR imaging studies. Symbols with a dot (individuals 17 and 23) represent obligate carriers.

the alleles were derived from the unaffected parental chromosome, putting *CCM1* proximal to this locus. Even though penetrance of CCM is incomplete, the interval defined by crossovers in the unaffected individuals was still completely consistent with that defined in the affected individuals.

In addition to the recombinations shown in Figure 4, one crossover in FCCM200 and one in FCCM300 placed the *CCM1* gene distal to D7S669 (afm286xf9) (data not shown; for linkage maps, see Gyapay et al. 1994; Dubovsky et al. 1995). Finally, another two crossovers in affected sibs from FCCM1 placed the gene distal to D7S524 (248ta5).

Whereas the contig shown in Figure 2 was deduced by SEGMAP without consideration of YAC sizes (thereby displaying STSs equidistant from one another), a similar analysis of the data by SEGMAP that accounted for YAC sizes suggests that the region covered by the contig spans

just over 3.8 Mb. On the basis of this analysis, the interval between the key genetic markers D7S2410 (sWSS3044) and D7S689 (sWSS1132) is estimated to be just over 2 Mb. Taken together, the genetic and physical mapping results presented here delimit the *CCM1* critical region to a 4-cM, 2-Mb interval of human chromosome 7q21–q22 that is contained within a highly redundant set of overlapping YAC clones.

DISCUSSION

The smallest interval shared by all affected individuals that can be defined by analysis of the crossovers in the families examined encompassed the polymorphic markers at loci D7S2410, D7S2408, D7S2409, D7S1813, D7S1789, D7S646, D7S558, and D7S689, a span of ~4 cM based on the linkage map or ~2 Mb based on the physical map. Additionally, the interval defined by crossovers in unaffected individuals is completely consistent with that defined in the affected individuals. Finally, the region of conserved haplotype shared by affected individuals in the three Hispanic families, which is independent of the observed crossovers, overlapped the interval of interest defined by these crossovers.

Within the most likely interval are four potential candidate genes, three expressed sequence tags of unknown function (EST00272, EST00631, and EST00979) and the cyclin-dependent kinase gene *CDK6* (Bullrich et al. 1995). None of these genes are intuitively strong candidates for *CCM1*, but their location makes them of interest. Potential candidate genes defined in earlier papers, including genes encoding elastin, CD36, the mul-

Table 3. Comparison of the Maximum lod Scores for CCM Families

Family	STRP/ marker	Max. lod score	θ	Ethnicity	Reference
FCCM-1	D7S558	5.24	0.12	Caucasian	A. Gil-Nagel et al. (in prep.)
FCCM-10	D7S502	3.04	0.00	Hispanic	Marchuk et al. (1995)
FCCM-20	D7S479	0.99	0.03	Caucasian	Marchuk et al. (1995)
FCCM-200	D7S804	4.19	0.00	Hispanic	Dubovsky et al. (1995)
FCCM-300	D7S1813	2.53	0.00	Hispanic	unpublished

The maximum lod score and θ value, as well as the STRP locus where that lod score was obtained, are shown for each of the previously published CCM families used in this study (Dubovsky et al. 1995; Gil-Nagel et al. 1995b; Marchuk et al. 1995). Calculation of the lod score for the new family FCCM300 was done as described previously (Dubovsky et al. 1995).

Table 4. Shared Haplotypes Among CCM Families

cM	STRP/ marker	Caucasian		Hispanic			Shared Hispanic haplotype
		FCCM-1	FCCM-20	FCCM-10	FCCM-200	FCCM-300	
0	D7S2417	170	170	170	164	164	
	D7S2481	205	205	205	205	205	
	D7S2485	92	92	92	100	100	
	D7S630	220	202	218	218	206	
1.5	D7S492	147	147	151	151	151	151
2.2	D7S2410	265	269	279	279	279	279
5	D7S2408	198	198	198	198	198	198
	D7S2409	102	102	98	98	98	98
	D7S1813	131	137	137	137	137	137
	D7S1789	131	131	137	137	137	137
6.2	D7S646	183	189	185	185	185	185
	D7S558	103	111	107	107	107	107
	D7S689	133	129	129	129	129	129
	D7S657	250	260	246	246	246	246
7.3	D7S652	273	275	277	277	277	277
	D7S1820	252	252	252	252	252	252
7.8	COL1A2	277	277	273	273	273	273

The numbers in the first column represent the cumulative positions in cM for markers on the linkage map (see Fig. 1). Other values indicate the sizes in nucleotides of the alleles linked to the cavernous malformation mutation (i.e., "affected alleles") in the affected members of the indicated families. Allele size was determined by comparison to amplified DNA from CEPH family members 1331-01 and 1331-02. Marker order was determined by both linkage and physical mapping (Figs. 1 and 2).

tiple drug resistance proteins 1 and 3, and collagen 1A2, have all been eliminated from consideration by the results presented here, because each of these genes has been definitively mapped to a YAC contig other than the one shown in Figure 2 (E.D. Green, unpubl.). A search of the portions of mouse chromosomes 5 and 6 syntenic to human 7q revealed no seizure or vascular deformation genes nor any obvious candidate genes for cavernous malformations within the relevant intervals (Grzeschik et al. 1994).

Günel and co-workers (1995) recently described two families with a history of cavernous malformations. In their Hispanic family, two independent crossovers clearly indicated that

CCM1 lies distal to the elastin locus. These data were consistent with our localization of *CCM1* to a more distal segment of 7q. In their other family, a Caucasian kindred of European descent, crossovers in two affected individuals placed *CCM1* proximal to D7S644 (afm234xc7) and D7S802 (Mfd340). Both of these loci are considerably proximal (4–9 cM) to the most likely location for *CCM1* described here. A possible explanation for this discrepancy (suggested by Günel et al. 1995) is that the genetic defect in their European CCM family is unlinked to 7q. The maximum pairwise lod score obtained for a 7q marker with this family was only 1.1; if confirmed, this would be the first example of locus heterogeneity for CCM.

Figure 4 Pedigree of selected members from FCCM1, FCCM200, and FCCM300 showing haplotypes for individuals with informative crossovers and their immediate family members. Arrows indicate the individuals with informative crossovers. The solid bar indicates those alleles that are linked to the cavernous malformation mutation (i.e., "affected alleles"). In the case of individuals with crossovers, the solid bar is used to depict only those alleles that can be unambiguously linked to the parental "affected" haplotype. The order of the chromosome 7q STRPs used to define this region (also displayed in a box in the lower right corner of this figure) was from top to bottom: D7S2417, D7S2481, D7S2485, D7S630, D7S492, D7S2410, D7S2408, D7S2409, D7S1813, D7S1789, D7S646, D7S558, D7S689, D7S657, D7S652, D7S1820, and COL1A2.

FINE MAPPING OF THE CAVERNOUS MALFORMATION GENE *CCM1*

Cavernous malformations affect tens of millions of people worldwide. With the advent of MR imaging, they are being increasingly recognized as a cause of seizures and other neurologic

impairments. Only 15%–20% of patients with MR-documented lesions remain asymptomatic throughout their lives. The presence of multiple lesions increases the risk of complications in the

Figure 4 (See facing page for legend.)

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familial form of this disease. In cases of familial epilepsy where there is an autosomal dominant pattern of inheritance, it will be important to exclude CCM as an etiologic cause. Identification of the *CCM1* gene may ultimately lead to significantly improved therapy for this disorder. Efforts are currently under way to collect additional CCM families, to further narrow the *CCM1* critical interval, and to determine all possible candidate genes in that region.

METHODS

YAC-based STS Content Mapping

STS-specific PCR assays were developed and optimized essentially as described (Green and Green 1991; Green et al.

1991, 1994; Green 1993). Each STS is named using the prefix sWSS followed by a unique number. For the genetic marker-specific STSs, the oligonucleotide primers used for testing YAC clones corresponded either to those employed for genotype analysis or those designed [most often with the computer program OSP (Hillier and Green 1991)] using the DNA sequence available in GenBank or provided by Weissenbach et al. (1992).

Most of the YACs used in this study were derived from a collection of clones highly enriched for human chromosome 7 [the chromosome 7 YAC resource (Green et al. 1995)] using a PCR-based screening strategy (Green and Olson 1990; Green et al. 1995). Among these YACs are clones derived from a chromosome 7-containing human-hamster hybrid cell line and clones isolated from total genomic libraries. Each YAC is named using the prefix yWSS followed by a unique number.

Phenotypic Evaluation of Family Members

Individual family members were considered affected if they demonstrated the positive presence of CCM on MR images whether they were symptomatic or not. Where possible, the results of the MR imaging were confirmed either at the time of surgery or at autopsy. Procedures for capturing and analyzing MR images have been described previously (Rigamonti et al. 1987; Zabramski et al. 1994). Some individuals were studied using cerebral angiography in addition to MR imaging.

Sample Collection and Genotyping

A detailed informed consent form approved by each participating institution's Institutional Review Board (IRB) was presented to all individuals participating in these studies. After obtaining informed consent, two 10-ml blood samples were drawn by venipuncture from the family members. Genomic DNA was extracted from the lymphocytes as described previously (Kurth et al. 1993). Genotyping with STRPs was completed using methods reported previously (Weber et al. 1993). Markers were developed

either at Genethon (Weissenbach et al. 1992; Gyapay et al. 1994; J. Weissenbach, pers. comm.), at Marshfield (Weber et al. 1990; Murray et al. 1994), or within the Cooperative Human Linkage Center (CHLC) (Murray et al. 1994). The marker at COL1A2 (collagen 1A2) locus was developed by Chi and colleagues (1992).

Linkage Mapping, Statistical Analysis, and Haplotype Analysis

Linkage maps were constructed using CEPH reference family genotyping data generated at Marshfield and data obtained from versions 6 and 7 of the CEPH data bases, as well as unpublished results (J. Weissenbach, pers. comm.). Maps were built using the program CRIMAP (Lander and Green 1987). Pairwise lod scores were computed using LINKAGE version 5.03b (Lathrop and Lalouel 1984). Analysis of haplotype was done both manually and using the Cyrillic software package (Cherwell Scientific).

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