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Polymorphisms in inflammatory and immune response genes associated with cerebral cavernous malformation type 1 severity

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Running head: Inflammatory and immune response polymorphisms and CCM1 severity

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ABSTRACT

Background: Familial cerebral cavernous malformation type 1 (CCM1) is an autosomal dominant disease caused by mutations in the Krev Interaction Trapped 1 (*KRIT1/CCM1*) gene, and characterized by multiple brain lesions that often result in intracerebral hemorrhage (ICH), seizures, and neurological deficits. Carriers of the same genetic mutation can present with variable symptoms and severity of disease, suggesting the influence of modifier factors. Evidence is emerging that inflammation and immune response play a role in the pathogenesis of CCM. The purpose of this study was to investigate whether common variants in inflammatory and immune response genes influence the severity of familial CCM1 disease, as manifested by ICH and greater brain lesion count.

Methods: Hispanic CCM1 patients (n=188) harboring the founder Q455X 'common Hispanic mutation' (CHM) in the *KRIT1* gene were analyzed at baseline. Participants were enrolled between June 2010 and March 2014 either through the Brain Vascular Malformation Consortium (BVMC) study or through the Angioma Alliance organization. Clinical assessment and cerebral susceptibility-weighted magnetic resonance imaging were performed to determine ICH as well as total and large (≥5 mm in diameter) lesion counts. Samples were genotyped on the Affymetrix Axiom Genome-Wide LAT1 Human Array. We analyzed 830 variants in 56 inflammatory and immune response genes for association with ICH and residuals of log-transformed total or large lesion count adjusted for age at enrollment and gender. Variants were analyzed individually, grouped by sub-pathways or whole pathway.

Results: At baseline, 30.3% of CCM1-CHM subjects had ICH, with a mean ± standard deviation (SD) of 60.1 ± 115.0 (range 0 to 713) for total lesions and 4.9 ± 8.7 (range 0 to 104) for large lesions. The heritability estimates explained by all autosomal variants were 0.20 (SE=0.31), 0.81 (SE=0.17) and 0.48 (SE=0.19), for ICH, total lesion count and large lesion count, respectively. *TGFBR2* rs9823731 was significantly associated with ICH as well as with total and large lesion counts ($P \leq 0.017$). Further, *IL-4* rs9327638, *CD14* rs778588, *IL-6R* rs114660934 and *MSR1* rs62489577 were associated with two markers of disease severity. Finally, the whole pathway was associated with total lesion count (P=0.005) with *TLR-4 rs10759930*, *CD14* rs778588, *IL-6R* rs114660934 and IGH rs57767447 mainly bearing this association. Eicosanoid signaling, extracellular pattern recognition and immune response sub-pathways were also associated with total lesion count.

Conclusions: These results suggest that polymorphisms in inflammatory and immune response pathways contribute to variability in CCM1 disease severity and might be used as predictors of disease severity. In particular, *TGFBR2* rs9823731 was associated with all three markers of CCM1 disease severity tested, suggesting that TGFBR2 might be a key participant in the mechanism underlying CCM1 disease severity and phenotype variability. However, further longitudinal studies in larger sample sizes are needed to confirm these findings.

Introduction

Familial cerebral cavernous malformations (CCM) are characterized by multiple lesions consisting of thin-walled leaky capillaries, which can lead to intracerebral hemorrhage (ICH), seizures, and neurological deficits. Familial CCM type 1 (CCM1) is an autosomal dominant disease caused by mutations in the Krev Interaction Trapped 1 (*KRIT1*) gene. Familial CCM1 patients with the same genetic mutation can present with variable symptoms and disease severity even among members of the same family [1-3]. The factors underlying this variability are poorly understood, but may include genetic modifiers or epidemiological factors, e.g., obesity or hypertension [1].

Several studies have implicated dysregulated inflammatory and immune responses in vascular malformation pathogenesis, including CCM [4-7]. Inflammatory and immune cells such as monocytes, macrophages, B and T cells, are present in human CCM lesions, particularly in response to acute bleeding [6-8], as well as in mouse models of CCM [9, 10]. Further, gene expression arrays have revealed a number of immunoglobulin genes and markers of immune cells with altered expression in human CCM tissue [4, 11]. Common functional polymorphisms in inflammatory cytokine genes have been associated with ICH in other brain vascular diseases, including arteriovenous malformations (AVM) [12-16] and intracranial aneurysms [17].

Therefore, we hypothesized that common genetic variation in inflammatory and immune response genes would influence disease severity in CCM1, as manifested by an increased risk of ICH or greater total or large lesion count, in a cohort of Hispanic CCM1 subjects.

Methods

Study Population

The study sample comprised 188 CCM1 subjects, all confirmed carriers of the Common Hispanic Mutation (CHM) in *KRIT1* (Q455X, rs267607203) by genetic testing as previously described [1], and with both genotype and phenotype data available. Subjects were recruited from two sources: (a) 182 participants enrolled between June 2010 and March 2014 through the Brain Vascular Malformation Consortium (BVMC) study at the University of New Mexico (UNM); and (b) 6 participants enrolled through the Angioma Alliance patient advocacy group's DNA & Tissue Bank study. All data, including DNA, imaging, and clinical data, were de-identified prior to analysis. The study was approved by the local institutional review boards at UNM, University of California, San Francisco (UCSF), and Quorum IRB (Angioma Alliance), and by the National Institutes of Neurological Disorders and Stroke (NINDS). Written informed consent was obtained from all participants.

Phenotyping

Clinical assessment of each participant was conducted to obtain information on presenting symptoms leading to CCM diagnosis using standardized guidelines [18]. MRI was performed at study enrollment using a volume T1 acquisition (MPRAGE, 1-mm slice reconstruction) and axial TSE T2, T2 gradient recall, susceptibility-weighted, and FLAIR sequences. Lesion counting was based on concurrent evaluation of axial susceptibility-weighted imaging, which is a volume acquisition, with 1.5-mm reconstructed images and axial T2 gradient echo, 3-mm images. Large lesions were

defined as those with a maximum diameter of 5 mm or greater on TSE T2 images. CCM lesions less than 5 mm in size mostly represent hemosiderin-only signal. These were not additionally measured because accuracy of measurements decreases as lesion size becomes smaller than slice thickness for T2-weighted images (around 5mm). Gradient-recall sequences did have thinner slice thickness but are unreliable for measurement of size because of well-recognized susceptibility effects that result in "blooming" in the apparent size. We analyzed three markers of CCM1 disease severity: history of ICH, total lesion count, and large lesion count.

Genotyping and Quality Control

Blood or saliva samples were collected and genomic DNA was extracted using standard protocols. Blood samples collected for the BVMC study were sent to the NINDS Repository at the Coriell Institute for Medical Research for DNA extraction and cell line immortalization. Blood samples collected from Angioma Alliance were sent to PreventionGenetics (Marshfield, WI) and saliva samples were sent directly to UCSF for DNA extraction. Samples were normalized, plated on two 96-well plates, and genotyped at the UCSF Genomics Core Facility using the Affymetrix Axiom® Genome-Wide LAT 1 (Axiom GW LAT) Human Array [19], which includes 817,810 single nucleotide polymorphisms (SNPs) and is optimized for genotyping Hispanic populations. The Affymetrix Genotyping Console (GTC) 4.1 Software package was used to generate quality control (QC) metrics and genotype calls. All samples had a genotyping call rate of 97% or greater, and the two Affymetrix Reference DNA controls were concordant. Genotype data were exported into PLINK software (v1.07) for further QC (sex check,

Mendelian errors and cryptic relatedness) and data analysis. Neither sex discordance nor Mendelian errors were identified.

Gene and Variant Selection

We selected 56 candidate genes that encode proteins functioning in inflammatory or immune response pathways, and prioritizing genes previously reported for other brain vascular malformations or implicated in CCM lesion biology. Our candidate gene list includes: 1) Inflammatory cytokines, as polymorphisms in notably IL1A, IL1B, IL1RN and TNF genes have been previously reported associated with phenotypes of brain vascular diseases, including brain arteriovenous malformations or intracranial aneurysms [12-17, 20]; 2) Transforming growth factor-β (TGF-β) and related genes that inhibit TGF-β signaling, reducing the number and size of lesions and vessel leakage in CCM1-deficient mice [10]; 3) Genes that encode proteins expressed or secreted by inflammatory or immune cells (T cells, B cells, monocytes and macrophages) and other related genes coding for proteins involved in immune cell development (i.e., CD14, IFNG, IL5, IL6R, IL8, IL18, IL18R1, IL12A, IL12B, IL2, IL17A, IL23A, NFKB1, MIF and MSR1) as inflammatory or immune cells are present in human CCM lesions [4, 6, 11]; 4) Toll-like receptors genes (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6 and TLR10) as well as COX-2 and Selenoprotein genes as encoded proteins have been reported to be essential in the pathogenesis of cerebral ischemia and the pathologic progression of the disease [21-26]; 5) Immunoglobulins and other related genes with altered expression in human CCM lesions (CD247, CD3G, CD68, CD200, GUSP11, HLA-DRB1, IGH, IGJ, IGL, LOC390714, MS4A1 and SDC1) [4, 11].These

genes were classified into the following biological sub-pathways [27, 28]: (a) cytokine signaling; (b) eicosanoid signaling; (c) extracellular pattern recognition; (d) NFKB signaling; (e) selenoproteins; and (f) immune response (see online **Suppl. Table 1**). Gene loci were defined as ± 5 kb upstream and downstream of the sequence using UCSC Genome Browser Assembly Feb. 2009 (GRCh37/hg19). Genotypes for 1,700 SNPs on the Axiom GW LAT Human Array within the candidate loci were extracted. We excluded 257 SNPs for the following reasons: genotype call rate <98%, a minor allele frequency (MAF) <1%, or deviation from Hardy-Weinberg equilibrium (P<0.001). After removing 613 SNPs that were in high linkage disequilibrium ($r^2 \geq 0.8$) using a 5-SNP sliding window approach in PLINK, 830 variants remained for association analysis. MAF of SNPs associated with CCM1 severity markers are reported in **Suppl. Table 2** and are compared with general populations from public databases: 1000 Genomes global population (phase 1 genotype data from 1094 worldwide individuals) and HapMap MEX (Mexican Ancestry in Los Angeles).

Statistical Analysis

Residuals of log-transformed total or large lesion count were obtained after adjustment for age at enrollment and gender (R v2.10.1 software). To identify genotypes (assuming an additive genetic model, i.e., 0, 1, or 2 copies of the minor allele) associated with ICH, we performed a DFAM family-based association test for disease traits (PLINK v1.07) to accommodate for different family structures, which uses a Cochran-Mantel-Haenszel test. To identify genotypes associated with residuals of log-total or large lesion count, we first performed linear regression analysis implemented

in the QFAM family-based association test for quantitative traits (PLINK v1.07) which uses between and within family permutation to account for differences in family structure. *P*-values were generated using 100,000 permutations. Variants with *P*-values ≤0.017 (0.050/3 markers of disease severity) in any outcome are reported. We also present more stringent multiple testing correction (Bonferroni adjustment for the number of variants tested within each candidate genes). SNP-based heritability estimates were obtained separately for ICH, total lesion count and large lesion count using the GCTA software [29], which computes the phenotypic variance explained by all analyzed SNPs in the genome by restricted maximum likelihood achieved using expectation maximization (REML). For quality control purposes, we restricted the analysis to autosomal SNPs with genotype call rate ≥98%, a MAF ≥1%, and in Hardy-Weinberg equilibrium (*P*≥0.001). As related individuals were present in our sample and this can bias heritability estimates from GCTA [30-33], we also estimate heritability using a family-based approach in SOLAR v7.2.5 software [34]. To analyze the whole set of SNPs together, or sets of SNPs grouped by sub-pathways, we used the set-based test in PLINK v1.07 which takes account of the LD between the SNPs and corrects *P*-values for the multiple SNPs tested within a set. Power calculations were performed using QUANTO software (http://hydra.usc.edu/gxe/). With 188 CCM1-CHM subjects, we have over 80% power to detect an odds ratio (OR) between 2.2 and 5.2 for ICH and a 13 to 63% difference in lesion count when the MAF varies between 0.05 and 0.50.

Results

Participant Characteristics

Table 1 shows the descriptive statistics of the 188 CCM1-CHM subjects included in this study. The mean age at enrollment was 39.03 ± 19.5 years and the majority were female (66.0%). In our sample, 30.3% of CCM1-CHM subjects had a history of ICH. At baseline, the average number of lesions observed was 60.1 ± 115.1 (range from 0 to 713) and the average number of large lesions was 4.9 ± 8.7 (range from 0 to 104).

Estimation of Heritability

SNP-based analysis resulted in a heritability estimate of 0.20 (SE=0.31), 0.81 (SE=0.17) and 0.48 (SE=0.19), for ICH, total lesion count and large lesion count, respectively. The family-based analysis, yielded similar heritability estimates (**Table 2**).

Association with ICH

We first evaluated whether common variants in inflammatory and immune response genes were associated with ICH. We found that 7 variants in 5 inflammatory genes (*IL-1RN*, *IL-4*, *TGFBR2*, *CHUK* and *SELS*) and 6 variants in 3 immune response genes (*CD3G*, *IGH* and *IGL*) were significantly associated with ICH ($P \leq 0.017$) (**Table 3**). No association remained significant after adjusting for the number of variants tested within candidate genes (**Table 3).**

Association with Total and Large Lesion Counts

We also evaluated whether common variants in inflammatory and immune response genes were associated with total and large lesion counts in CCM1-CHM subjects. Interestingly, the *IL-4* rs9327638 and *TGFBR2* rs9823731 polymorphisms reported above and associated with ICH were also significantly associated with total and/or large lesion counts, independent of age and gender (**Table 4**). Further, 3 variants (*IL-6R* rs114660934, *MSR1* rs62489577 and *CD14* rs778588) were significantly associated with both total and large lesion counts (**Table 4**). An additional 9 variants in 8 inflammatory genes and 5 variants in 4 immune response genes were significantly associated with either total or large lesion count. Associations between total lesion count and *TLR-4* rs10759930 and *CD14* rs778588 remained significant after correcting for the number of variants tested per gene (**Table 4**).

As a sensitivity analysis, we further adjusted analyses for presence of co-morbidities which might influence inflammatory and immune responses, including obesity, diabetes, hyperlipidemia, hypertension, auto-immune disorders and cancer. None of these co-morbidities were associated with large lesion count or history of ICH in any of the analyses, except obesity which was significantly associated with fewer total lesions (37% less lesions; P=0.005), as previously reported [1]. Genetic association results did not change whether we included these covariates in the model or not (data not shown).

Association of the Whole Pathway and Sub-pathways with CCM1 severity

Finally, to determine the impact of the whole inflammatory and immune response pathway, as well as the sub-pathways on CCM1 severity, we performed a set-based

analysis. Taken together, the whole pathway was significantly associated with total lesion count (P=0.005); this association was driven notably by IL-6R rs114660934 and CD14 rs778588 (above mentioned associated with both total and large lesion counts), as well as TLR-4 rs10759930 and IGH rs57767447. Two sub-pathways (eicosanoid signaling and extracellular pattern recognition) were significantly associated with total lesion count (P=0.006) as well as immune response sub-pathway which was nominally associated (P=0.033) (see online **Suppl. Table 3**).

Discussion

We provide the first report of associations between common genetic variation, and markers of CCM disease severity. Our results show that variants in the inflammatory and immune response pathways analyzed individually and as a set may be associated with disease severity in Hispanic CCM1-CHM subjects. Specifically, *TGFBR2* rs9823731 was associated with all 3 markers of CCM1 disease severity examined: history of ICH, total lesion count and large lesion count. Given the role of TGFBR2 as a receptor protein that binds TGF-β, this finding supports the implication of TGF-β signaling in the onset and progression of CCM disease. Recently, Maddaluno et al [10] reported that inhibition of TGF-β signaling reduces the number and size of lesions and vessel leakage in CCM1-deficient mice. Thus, TGFBR2 might be a key participant in the mechanism underlying CCM disease severity and phenotype variability. Further, *IL-6R* rs114660934 and *CD14* rs778588 seem to be important genetic modifiers of CCM1 disease severity as those SNPs 1) were significantly associated with total and large lesion counts, and 2) drove the association of the whole pathway with total lesion count in addition to *TLR-4* rs10759930, and IGH rs57767447.

Our findings extend previous studies implicating immune response in CCM pathogenesis. Shenkar et al reported that immunoglobulin heavy and light chain genes were upregulated with up to 20-fold change in human CCM lesions in comparison to brain AVM and normal superficial temporal arteries [4, 11]. Interestingly, we found that 3 common variants in the immunoglobulin heavy locus (*IGH*) and 5 common variants in the immunoglobulin lambda light chain locus (*IGL*) were associated with either ICH or total lesions. It is noteworthy that *IGH* and *IGL* are both markers of B cells, and

histology studies have shown the presence of B cells within quiescent CCM lesions as well as within aggressive ones (characterized notably by new hemorrhage) [4-7].

Our findings also extend previous studies suggesting that inflammatory cytokine genes are involved in brain vascular disease pathogenesis. As similarly observed in brain AVM patients [14], we found that polymorphisms in *IL-1RN* were significantly associated with ICH in CCM1-CHM subjects. Further, we also report associations with additional inflammatory cytokines and their receptors, notably, *IL-6R,* and *TGFBR2*. Similarly, polymorphisms in *IL-6R* and *TGFBR2* genes have been associated with other vascular diseases, such as abdominal aortic aneurysm [35, 36]. Multiple genetic polymorphisms in inflammatory cytokines have been reported to act as modifying factors in numerous diseases. For example, polymorphisms in *TGFB1* modify the severity of pulmonary disease in patients with cystic fibrosis [37], while functional polymorphisms in *IL-4* and *IL-10* may predict evolution and functional outcome of ischemic stroke [38].

We also for the first time provide heritability estimates for the three markers of CCM1 disease severity using two different methods (SNP-based and family-based approaches). Similar heritability estimates were produced by both methods and the total lesion count phenotype had the highest heritability estimate (63 to 81%), suggesting that this marker of CCM1 disease severity is the most likely to be affected by genetic modifiers which can be discovered by association studies. However, the presence of relatedness in our sample might have led to an overestimation of heritability, as previously described [30-33]. Our estimate of heritability for ICH risk (20%) is similar to

that reported in non-CCM cohorts (29% in unrelated subjects) [39], and suggests that ICH risk may be more strongly influenced by environmental factors than genetic effects.

A limitation of the current study was the relatively small sample size for association studies. In view of this limitation, we restricted the number of candidate genes; however, other genes in inflammatory and immune response pathways might be important to explore in future larger genetic studies. Nevertheless, we were able to detect a polymorphism (*TGFBR2* rs9823731) consistently associated with all three markers of CCM1 disease severity tested. Additionally, this was a cross-sectional analysis of baseline findings and cannot directly address whether these genetic variants are associated with CCM1 disease progression. The BVMC study is continuing follow-up in the cohort, and it will be interesting to determine if these genetic variants predict risk of ICH or increase in lesion counts in longitudinal analysis. The main strength of the study is the unique population of well-characterized familial CCM1 subjects all sharing an identical genetic mutation, which allows for the evaluation of genotype-phenotype associations without confounding by CCM mutation type.

In conclusion, these results suggest that common genetic variation in inflammatory and immune response pathways may influence familial CCM1 disease severity, and warrant replication in other CCM cohorts and further investigation into the precise mechanism of how those pathways are involved. A better understanding of the natural history of the disease, including risk factors for disease severity and phenotype variability, is essential to improve knowledge of the mechanisms involved in CCM pathogenesis that may lead to new therapies.

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TABLES

Table 1. Clinical characteristics of the 188 Hispanic CCM1-CHM subjects

Characteristics		**Values**
Male Gender	**N (%)**	64 (34.0)
Family Size (affected members)	**N (%)** 1 member 2 members 3 – 8 members	 54 (55.1) 23 (23.5) 21 (21.4)
Age at enrollment (years)	**Mean ± SD** **Range**	39.0 ± 19.5 6.6 – 84.9
History of Hemorrhage	**N (%)**	57 (30.3)
Total Lesion Count	**Mean ± SD** **Range**	60.1 ± 115.0 0 – 713
Large Lesion Count	**Mean ± SD** **Range**	4.9 ± 8.7 0 – 104

Table 2. Heritability estimates of markers of CCM1 disease severity

	SNP-Based Analysis		**Family-Based Approach**	
	Heritability Estimate (%, SE)	***P***	**Heritability Estimate (%, SE)**	***P***
ICH	20 (31)	0.30	21 (38)	0.28
Total Lesion Count	81 (17)	**$2x10^{-6}$**	63 (21)	**0.0018**
Large Lesion Count	48 (19)	**0.004**	30 (21)	0.059

Table 3. Genetic variants associated with ICH

Gene	SNP	Minor	MAF	χ^2	*P*	**P*
Cytokine signaling						
IL1RN	rs315947	A	0.26	8.25	**0.004**	0.061
	rs928940	G	0.14	7.86	**0.005**	0.076
IL4	rs9327638	A	0.20	7.09	**0.008**	0.18
TGFBR2	rs17025785	C	0.42	9.03	**0.003**	0.12
	rs9823731	A	0.36	6.88	**0.009**	0.41
NFKB signaling						
CHUK	rs4919435	T	0.07	5.73	**0.017**	0.13
Selenoproteins						
SELS	rs4965815	T	0.06	6.90	**0.009**	0.069
Immune Response						
CD3G	rs11216856	T	0.31	6.56	**0.010**	0.052
IGH	rs55847330	T	0.14	6.70	**0.010**	0.68
IGL	rs6002270	T	0.03	6.72	**0.009**	1.0
	rs10854762	A	0.22	6.52	**0.011**	1.0
	rs9622749	G	0.14	5.83	**0.016**	1.0
	rs5757039	T	0.10	5.76	**0.016**	1.0

Table gives the Chi-squared (χ^2) from a Cochran-Mantel-Haesnzel test, and *P*-values. *P*-values in bold are considered statistically significant ($P \leq 0.017$).

**P* are *P*-values adjusted for Bonferroni correction (for the number of variants tested within candidate genes).

Table 4. Genetic variants associated with lesion counts

				Total Lesion Count			Large Lesion Count		
Gene	**SNP**	**Minor**	**MAF**	**PI (95%C.I.)**	***P***	****P***	**PI (95%C.I.)**	***P***	****P***
Cytokine signaling									
IL4	rs9327638	A	0.20	1.21 (0.90-1.61)	0.28	1.0	1.38 (1.14-1.67)	**0.005**	0.025
	rs194395	T	0.22	0.68 (0.52-0.90)	**0.016**	0.36	0.81 (0.67-0.97)	0.048	0.24
IL5	rs10072700	C	0.20	1.45 (1.11-1.90)	**0.017**	0.58	1.15 (0.95-1.38)	0.20	1.0
IL6R	rs114660934	A	0.02	4.44 (2.10-9.37)	**0.004**	0.057	2.53 (1.53-4.17)	**0.004**	0.059
IL12RB1	rs374326	C	0.35	1.40 (1.11-1.75)	**0.016**	0.062	1.18 (1.01-1.37)	0.077	0.31
IL18R1	rs3732126	C	0.14	1.56 (1.15-2.12)	**0.016**	0.19	1.01 (0.82-1.25)	0.93	1.0
TGFBR2	rs12491780	T	0.27	0.80 (0.63-1.02)	0.11	1.0	0.81 (0.69-0.95)	**0.015**	0.71
	rs11924422	C	0.36	1.41 (1.13-1.76)	**0.007**	0.32	1.21 (1.05-1.41)	0.023	1.0
	rs9823731	A	0.36	1.39 (1.12-1.73)	**0.011**	0.53	1.26 (1.09-1.45)	**0.009**	0.42
Eicosanoid signaling									
COX-2	rs689462	G	0.08	2.02 (1.32-3.09)	**0.012**	0.058	1.29 (0.97-1.72)	0.15	0.74
Extracellular pattern recognition									
MSR1	rs62489577	C	0.04	0.47 (0.27-0.79)	**0.013**	0.46	0.61 (0.43-0.87)	**0.012**	0.45
TLR4	rs10759930	T	0.37	1.72 (1.36-2.18)	**0.0002**	**0.003**	1.16 (0.99-1.37)	0.12	1.0
TLR6	rs73811240	A	0.17	0.69 (0.53-0.89)	**0.015**	0.16	0.86 (0.72-1.02)	0.13	1.0
Immune Response									
CD14	rs778588	C	0.27	1.51 (1.19-1.90)	**0.003**	**0.009**	1.24 (1.06-1.45)	**0.017**	0.051
CD3G	rs3181261	T	0.08	1.40 (0.90-2.17)	0.16	0.82	1.47 (1.10-1.97)	**0.012**	0.061
CD68	rs9901675	A	0.07	0.57 (0.38-0.84)	**0.009**	0.026	0.72 (0.55-0.93)	0.021	0.063
IGH	rs57767447	T	0.15	1.76 (1.31-2.36)	**0.003**	0.18	1.27 (1.04-1.55)	0.045	1.0
	rs10147756	A	0.11	0.56 (0.40-0.79)	**0.006**	0.40	0.82 (0.65-1.04)	0.14	1.0
IGL	rs987710	G	0.29	0.70 (0.55-0.89)	**0.011**	1.0	0.91 (0.77-1.07)	0.30	1.0

Table gives proportional increase (PI, or decrease if less than 1) in either total or large lesion count, along with 95% confidence intervals and *P*-values.

P-values in bold are considered statistically significant (*P*≤0.017).

**P* are *P*-values adjusted for Bonferroni correction.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Candidate inflammatory and immune response genes

Classification	Gene	Full Name	Chr	Size (bp)	N SNPs Analyzed
Cytokine signaling	***IL1A***	interleukin-1, alpha proprotein	2	11,479	4
	IL1B	interleukin-1, beta proprotein	2	7,020	9
	IL1RN	interleukin 1 receptor antagonist	2	16,124	15
	IL2	interleukin 2	4	5,025	7
	IL4	interleukin 4	5	200,150	23
	IL5	interleukin 5	5	200,053	34
	IL6	interleukin-6 precursor	7	4,855	7
	IL6R	interleukin-6 receptor isoform 1	1	64,258	15
	IL8	interleukin 8	4	3,211	4
	IL10	interleukin-10 precursor	1	4,892	4
	IL12A	interleukin 12A	3	7,184	7
	IL12B	interleukin 12B	5	15,691	10
	IL12RB1	interleukin 12 receptor, beta 1	19	16,326	4
	IL12RB2	interleukin 12 receptor, beta 2	1	89,537	29
	IL17A	interleukin 17A	6	4,252	12
	IL18	interleukin 18	11	20,867	3
	IL18R1	interleukin 18 receptor 1	2	36,121	12
	IL23A	interleukin 23, alpha subunit p19	12	1,532	1
	IFNG	interferon, gamma	12	4,972	4
	IFNGR1	interferon gamma receptor 1	6	21,947	11
	IFNGR2	interferon gamma receptor 2	21	34,627	6
	MIC1	growth differentiation factor 15	19	3,019	6
	MIF	macrophage migration inhibitory	22	845	12
	TGFB1	transforming growth factor, beta 1	19	23,020	6
	TGFBR1	transforming growth factor, beta	9	49,062	8
	TGFBR2	transforming growth factor, beta	3	87,640	47
	TNF	tumor necrosis factor alpha	6	6,236	12
Eicosanoid signaling	***COX-2***	prostaglandin-endoperoxide synthase 2	1	8,616	5
Extracellular pattern	***MSR1***	macrophage scavenger receptor 1	8	84,914	36
	TLR1	toll-like receptor 1	4	8,537	6
	TLR2	toll-like receptor 2	4	21,802	10
	TLR3	toll-like receptor 3	4	15,944	17
	TLR4	toll-like receptor 4	9	13,317	18

recognition	***TLR5***	toll-like receptor 5	1	33,877	12
	TLR6	toll-like receptor 6	4	33,110	11
	TLR10	toll-like receptor 10	4	10,752	11
NFKB signaling	***CHUK***	conserved helix-loop-helix	10	41,221	8
	IKBKB	inhibitor of kappa light polypeptide	8	61,352	11
	NFKB1	nuclear factor of kappa light	4	115,974	14
	NFKBIA	nuclear factor of kappa light	14	3,245	17
	RELA	v-rel avian reticuloendotheliosis	11	9,377	3
Selenoproteins	***SELS***	selenoprotein S	15	6,634	8
	SEP15	15 kDa selenoprotein	1	51,980	7
Immune response	***CD14***	CD14 molecule	5	1,973	3
	CD3G	T-cell surface glycoprotein CD3	11	9,439	5
	CD68	macrosialin precursor	17	2,625	3
	CD200	OX-2 membrane glycoprotein	3	29,743	11
	CD247	T-cell surface glycoprotein CD3	1	87,971	45
	GUSP11	glucuronidase, beta pseudogene 11	22	78,936	8
	HLA-DRB1	major histocompatibility complex,	6	11,067	7
	IGH	immunoglobulin heavy locus	14	1,255,437	71
	IGJ	immunoglobulin J chain precursor	4	11,091	5
	IGL	immunoglobulin lambda locus	22	884,611	153
	LOC390714	immunoglobulin heavy chain	16	13,406	1
	MS4A1	membrane-spanning 4-domains,	11	14,944	6
	SDC1	syndecan 1 (CD138)	2	24,370	6

Supplementary Table 2. Comparison of minor allele frequency (MAF) of SNPs associated with CCM1 disease severity between CCM1-CHM patients and control subjects from public databases (1000 Genomes - HapMap).

Gene	SNP	Minor Allele	MAF (CCM1-CHM Subjects)	Global MAF (1000 Genomes)	MAF (HapMap MEX)
Cytokine signaling					
IL1RN	rs315947	A	0.26	0.40	0.22
	rs928940	G	0.14	0.32	0.15
IL4	rs9327638	A	0.20	0.09	NA
	rs194395	T	0.22	0.34	0.24
IL5	rs1007270	C	0.20	0.15	0.20
IL6R	rs1146609	A	0.02	0.02	NA
IL12RB1	rs374326	C	0.35	0.19	0.33
IL18R1	rs3732126	C	0.14	0.24	0.24
TGFBR2	rs1249178	T	0.27	0.22	NA
	rs1192442	C	0.36	0.37	0.36
	rs1702578	C	0.42	0.44	0.49
	rs9823731	A	0.36	0.28	NA
Eicosanoid signaling					
COX-2	rs689462	G	0.08	0.08	0.03
Extracellular pattern recognition					
MSR1	rs6248957	C	0.04	0.03	NA
TLR4	rs1075993	T	0.37	0.39	0.43
TLR6	rs7381124	A	0.17	0.08	NA
NFKB signaling					
CHUK	rs4919435	T	0.07	0.06	NA
Selenoproteins					
SELS	rs4965815	T	0.06	0.11	0.23

Immune Response					
CD14	rs778588	C	0.27	0.27	0.19
CD3G	rs1121685	T	0.31	0.38	NA
	rs3181261	T	0.08	0.09	0.03
CD68	rs9901675	A	0.07	0.06	0.04
IGH	rs5776744	T	0.15	0.12	NA
	rs1014775	A	0.11	0.04	NA
	rs5584733	T	0.14	0.17	NA
IGL	rs987710	G	0.29	0.38	0.22
	rs6002270	T	0.03	0.12	NA
	rs1085476	A	0.22	0.32	NA
	rs9622749	G	0.14	0.13	NA
	rs5757039	T	0.10	0.09	NA

Supplementary Table 3. Association of the whole pathway, and sub-pathways of inflammatory and immune response with CCM1 disease severity.

		***P*-value**		
SNP set	**N SNPs**	**ICH**	**Total Lesions**	**Large Lesions**
Inflammatory/Immune response	1149	0.36	**0.005**	0.41
● Cytokine signaling	506	0.36	0.12	0.13
● *Eicosanoid signaling*	6	1	**0.006**	1
● *Extracellular pattern recognition*	172	0.39	**0.006**	0.55
● *NFKB signaling*	60	0.64	0.70	0.18
● *Selenoproteins*	20	0.46	0.49	1
● *Immune response*	385	0.21	0.033	0.74

P-values are corrected for the multiple SNPs within a set (taking account of the LD between these SNPs). *P*-value in bold are considered statistically significant ($P \leq 0.017$).