Polymorphisms in Inflammatory and Immune Response Genes Associated with Cerebral Cavernous Malformation Type 1 Severity

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Key Words
Cerebral cavernous malformation · CCM1 disease severity · Intracerebral hemorrhage · Brain lesion count · Inflammation and immune response modifier genes

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 or grouped by sub-pathways or whole pathways. Results: At baseline, 30.3% of CCM1-CHM subjects had ICH, with a mean ± standard deviation (SD) of 60.1 ± 115.0 (range 0–713) for total lesions and 4.9 ± 8.7 (range 0–104) 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 ≤ 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...
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, for example, 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 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 and 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 the accuracy of measurements decreases as lesion size becomes smaller than slice thickness for T2-weighted images (around 5 mm). Gradient-recall sequences did have thinner slice thickness but are unreliable for the 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, Wisc., USA) 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 analyses.
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 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, IGL, LOC390714, MS4A1 and SDC1) as inflammatory or immune cells are present in human CCM lesions [4, 6, 11]; (6) 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, IGL, LOC390714, MS4A1 and SDC1) as inflammatory or immune cells are present in human CCM lesions [4, 6, 11]; (7) genes with altered expression in human CCM lesions (CD247, CD3G, CD68, CD200, GUSP11, HLA-DRB1, IGH, IGL, LOC390714, MS4A1 and SDC1) as inflammatory or immune cells are present in human CCM lesions [4, 6, 11].

**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 the 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 genetic 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 minor allele frequency (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 estimated 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 13–63% 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 aver-

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender, n (%)</td>
<td>64 (34.0)</td>
</tr>
<tr>
<td>Family size (affected members), n (%)</td>
<td></td>
</tr>
<tr>
<td>1 member</td>
<td>54 (55.1)</td>
</tr>
<tr>
<td>2 members</td>
<td>23 (23.5)</td>
</tr>
<tr>
<td>3–8 members</td>
<td>21 (21.4)</td>
</tr>
<tr>
<td>Age at enrollment, years</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>39.0±19.5</td>
</tr>
<tr>
<td>Range</td>
<td>6.6–84.9</td>
</tr>
<tr>
<td>History of hemorrhage, n (%)</td>
<td>57 (30.3)</td>
</tr>
<tr>
<td>Total lesion count</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>60.1±15.0</td>
</tr>
<tr>
<td>Range</td>
<td>0–713</td>
</tr>
<tr>
<td>Large lesion count</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.9±8.7</td>
</tr>
<tr>
<td>Range</td>
<td>0–104</td>
</tr>
</tbody>
</table>
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 ≤ 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 gen-

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**Table 2. Heritability estimates of markers of CCM1 disease severity**

<table>
<thead>
<tr>
<th></th>
<th>SNP-based analysis</th>
<th>Family-based approach</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>heritability estimate, % (SE)</td>
<td>p</td>
</tr>
<tr>
<td>ICH</td>
<td>20 (31)</td>
<td>0.30</td>
</tr>
<tr>
<td>Total lesion count</td>
<td>81 (17)</td>
<td>2 × 10⁻⁶</td>
</tr>
<tr>
<td>Large lesion count</td>
<td>48 (19)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Table 3. Genetic variants associated with ICH**

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Minor allele</th>
<th>MAF</th>
<th>χ²</th>
<th>p</th>
<th>p*</th>
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<tbody>
<tr>
<td>Cytokine signaling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs315947</td>
<td>A</td>
<td>0.26</td>
<td>8.25</td>
<td>0.004</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>rs928940</td>
<td>G</td>
<td>0.14</td>
<td>7.86</td>
<td>0.005</td>
<td>0.076</td>
</tr>
<tr>
<td>IL4</td>
<td>rs9327638</td>
<td>A</td>
<td>0.20</td>
<td>7.09</td>
<td>0.008</td>
<td>0.18</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>rs17025785</td>
<td>C</td>
<td>0.42</td>
<td>9.03</td>
<td>0.003</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>rs9823731</td>
<td>A</td>
<td>0.36</td>
<td>6.88</td>
<td>0.009</td>
<td>0.41</td>
</tr>
<tr>
<td>NFkB signaling</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CHUK</td>
<td>rs4919435</td>
<td>T</td>
<td>0.07</td>
<td>5.73</td>
<td>0.017</td>
<td>0.13</td>
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<tr>
<td>Selenoproteins</td>
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<td></td>
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<tr>
<td>SELS</td>
<td>rs4965815</td>
<td>T</td>
<td>0.06</td>
<td>6.90</td>
<td>0.009</td>
<td>0.069</td>
</tr>
<tr>
<td>Immune response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3G</td>
<td>rs11216856</td>
<td>T</td>
<td>0.31</td>
<td>6.56</td>
<td>0.010</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>rs55847330</td>
<td>T</td>
<td>0.14</td>
<td>6.70</td>
<td>0.010</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>rs88002270</td>
<td>T</td>
<td>0.03</td>
<td>6.72</td>
<td>0.009</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>rs10854762</td>
<td>A</td>
<td>0.22</td>
<td>6.52</td>
<td>0.011</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>rs5322749</td>
<td>G</td>
<td>0.14</td>
<td>5.83</td>
<td>0.016</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>rs5757039</td>
<td>T</td>
<td>0.10</td>
<td>5.76</td>
<td>0.016</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table gives the Chi-squared (χ²) from a Cochran-Mantel-Haenszel test, and p values. p values in bold are considered statistically significant (p ≤ 0.017). *p values adjusted for Bonferroni correction (for the number of variants tested within candidate genes).
Inflammatory and Immune Response Polymorphisms and CCM1 Severity

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 the 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).

Table 4. Genetic variants associated with lesion counts

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Minor allele</th>
<th>MAF</th>
<th>Total lesion count</th>
<th>Large lesion count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PI (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Cytokine signaling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$IL4$</td>
<td>rs9327638</td>
<td>A</td>
<td>0.20</td>
<td>1.21 (0.90–1.61)</td>
<td>0.28</td>
</tr>
<tr>
<td>$IL5$</td>
<td>rs194395</td>
<td>T</td>
<td>0.22</td>
<td>0.68 (0.52–0.90)</td>
<td>0.016</td>
</tr>
<tr>
<td>$IL6R$</td>
<td>rs10072700</td>
<td>C</td>
<td>0.20</td>
<td>1.45 (1.11–1.90)</td>
<td>0.017</td>
</tr>
<tr>
<td>$IL12RB1$</td>
<td>rs374326</td>
<td>C</td>
<td>0.35</td>
<td>1.40 (1.11–1.75)</td>
<td>0.016</td>
</tr>
<tr>
<td>$IL18R1$</td>
<td>rs3732126</td>
<td>C</td>
<td>0.14</td>
<td>1.56 (1.15–2.12)</td>
<td>0.016</td>
</tr>
<tr>
<td>$TGFB2$</td>
<td>rs12491780</td>
<td>T</td>
<td>0.27</td>
<td>0.80 (0.63–1.02)</td>
<td>0.11</td>
</tr>
<tr>
<td>$CD14$</td>
<td>rs778588</td>
<td>C</td>
<td>0.27</td>
<td>1.51 (1.19–1.90)</td>
<td>0.003</td>
</tr>
<tr>
<td>$CD3G$</td>
<td>rs3181261</td>
<td>T</td>
<td>0.08</td>
<td>1.40 (0.90–2.17)</td>
<td>0.16</td>
</tr>
<tr>
<td>$CD68$</td>
<td>rs9901675</td>
<td>A</td>
<td>0.07</td>
<td>0.57 (0.38–0.84)</td>
<td>0.009</td>
</tr>
<tr>
<td>$IGH$</td>
<td>rs57767447</td>
<td>T</td>
<td>0.15</td>
<td>1.76 (1.31–2.36)</td>
<td>0.003</td>
</tr>
<tr>
<td>$IGL$</td>
<td>rs987710</td>
<td>G</td>
<td>0.29</td>
<td>0.70 (0.55–0.89)</td>
<td>0.011</td>
</tr>
<tr>
<td>Eicosanoid signaling</td>
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<tr>
<td>$COX-2$</td>
<td>rs689462</td>
<td>G</td>
<td>0.08</td>
<td>2.02 (1.32–3.09)</td>
<td>0.012</td>
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<tr>
<td>Extracellular pattern recognition</td>
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</tr>
<tr>
<td>$MSR1$</td>
<td>rs62489577</td>
<td>C</td>
<td>0.04</td>
<td>0.47 (0.27–0.79)</td>
<td>0.013</td>
</tr>
<tr>
<td>$TLR4$</td>
<td>rs10759930</td>
<td>T</td>
<td>0.37</td>
<td>1.72 (1.36–2.18)</td>
<td>0.0002</td>
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<tr>
<td>$TLR6$</td>
<td>rs73811240</td>
<td>A</td>
<td>0.17</td>
<td>0.69 (0.53–0.89)</td>
<td>0.015</td>
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<tr>
<td>Immune response</td>
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<tr>
<td>$CD14$</td>
<td>rs778588</td>
<td>C</td>
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<td>$CD3G$</td>
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<td>$IGL$</td>
<td>rs987710</td>
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<td>0.29</td>
<td>0.70 (0.55–0.89)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

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 values adjusted for Bonferroni correction.
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 the 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 the 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 rs7785888 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 reported 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 TGFBI 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 (63–81%), 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 BVCM 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.
Inflammatory and Immune Response Polymorphisms and CCM1 Severity

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