Association of C2 and CFB Polymorphisms with Anterior Uveitis

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PURPOSE. Association of rs800292 (I62V) in the complement factor H (CFH) gene with anterior uveitis (AU) was identified in our previous study. We proceeded to investigate whether polymorphisms of two tightly linked genes in the complement pathway, complement component 2 (C2) and complement factor B (CFB), are associated with AU.

METHODS. Five single-nucleotide polymorphisms (SNPs), rs1048709, rs537160, rs4151657, rs2072653 in CFB, and rs3020644 in C2, were examined using genotyping assays in 98 Chinese AU patients and 291 unrelated controls. Adjustments and stratifications were given for sex, clinical manifestations, and HLA-B27 status.

RESULTS. There were significant increases in the frequency of A allele and AA homozygosity for CFB-rs1048709 in AU patients compared with that of controls (P value after Bonferroni correction [Pcorr] = 2.67 × 10⁻⁴, Pcorr = 0.001, respectively). No association was found between AU and the other four SNPs after adjustment for multiple testing. Logistic regression analysis showed none of the 5 SNPs had significant interaction with sex. Stratified analyses showed that only rs1048709 was significantly associated with AU in HLA-B27-positive patients but not in HLA-B27-negative patients. No association was found in the 5 tested SNPs with clinical manifestations. A haplotype block across CFB (AATA) was significantly predisposed to AU with increased risk of 1.97 (Pcorr = 0.0005). Additive effect of CFB-rs1048709 and CFH-rs800292 was identified with an odds ratio of 7.48.

CONCLUSIONS. Our results revealed an association between AU and CFB-rs1048709. The influence on AU might differ depending on HLA-B27 status. The joint effect in CFB and CFH strengthens the concept that the complement system plays an important role in the pathogenesis of AU. (Invest Ophthalmol Vis Sci. 2012;53:4969–4974) DOI:10.1167/iovs.12-9478

Uveitis is a general term for intraocular inflammatory disorders of the uveal tract; it has been described as anterior, intermediate, posterior, and panuveitis anatomically. Anterior uveitis (AU) is the most common form and accounts for approximately 75% of all cases. AU may be idiopathic and associated with other systemic diseases such as ankylosing spondylitis, Behçet’s disease, juvenile idiopathic arthritis, and inflammatory bowel disease. Infection is also one important cause. AU may ultimately develop into vision loss secondary to cataract, glaucoma, cystoid macular edema, and epiretinal membrane.

Although the exact pathogenesis of AU is unclear, it is an immune system-mediated disorder and is regulated by various endogenous immunological factors. AU can be triggered by genetic predisposition coupled with environmental factors.

Until now, the most common and strongest association of AU has been identified with human leukocyte antigen (HLA)-B27, the major histocompatibility complex type I gene. Meanwhile, many non-HLA gene variants, such as chemokines and antioxidant enzyme gene polymorphisms, also play important roles in the pathogenesis of AU. Complement system is a key innate factor for immune defense and modulates various immune and inflammatory responses. Several studies have demonstrated that the activation of complement system is critical for the development of autoimmune anterior uveitis (EAU), and depletion of the host’s complement system could result in complete inhibition of EAAU.

Recently, we identified the association between CFH (I62V) and AU and thus revealed the genetic association of complement system with uveitis. The complement system can be divided into classical, lectin, and alternative pathways triggered by different stimuli. CFH is one of the key regulators in the alternative pathway; complement via this pathway is under strict control. Disruption of the balance of complement activation and regulation results in harmful effects and leads to immune system-related diseases. CFH binds to complement component 3b (C3b) and reduces activation of the alternative pathway C3-convertase (C3bBb). CFB is paralogous with C2; both are control factors in the complement system. Especially for CFH, an opponent of CFH in an alternative pathway for the same binding site in C3b regulates the activation of complement. A recent study indicated that EAAU was completely inhibited in rats injected with CFB antibody via suppressing the alternative pathway. The present study aimed to investigate the association of polymorphisms in C2 and CFB genes with AU.

METHODS

Study Design and Subjects

The study protocol was approved by the Ethics Committee on Human Research, the Chinese University of Hong Kong. All procedures were conducted according to the tenets of the Declaration of Helsinki. Informed consent was obtained from all study subjects after explanation of the nature of the study. All patients underwent a detailed ocular examination and clinical information was collected.
including visual acuity, intraocular pressure, clinical features, sex, medical history such as systemic diseases, age at onset, laterality, complications, and pattern of AU (acute, recurrent, or chronic). All patients were recruited during the active phase of uveitis and were followed for at least 3 months after recruitment. The definition of uveitis was based on the Standardization Uveitis Nomenclature (SUN) classification.15 Acute AU was defined as AU that resolved completely within 3 months; chronic AU as AU not fully resolved within 3 months; recurrent AU as the development of AU more than once.

Table 1. Comparison of Genotype and Allele Frequencies of C2-CFB Polymorphisms in Patients with AU and Control Subjects

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Designation</th>
<th>AU (n = 196)</th>
<th>Control (n = 582)</th>
<th>P Value (Pcorr)</th>
<th>Odds Ratio (95% CI)</th>
<th>AU (n = 98)</th>
<th>Control (n = 291)</th>
<th>P Value (Pcorr)</th>
<th>Odds Ratio (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>C2</td>
<td>rs3020644</td>
<td>G &gt; A</td>
<td>110(56.1)</td>
<td>280(48.1)</td>
<td>0.052§ (0.26)</td>
<td>1.38 (1.0–1.91)</td>
<td>AA</td>
<td>35(35.7)</td>
<td>67(23.0)</td>
</tr>
<tr>
<td></td>
<td>Promoter</td>
<td>G</td>
<td>86(43.9)</td>
<td>302(51.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>rs1048709</td>
<td>G &gt; A</td>
<td>85(43.4)</td>
<td>162(27.8)</td>
<td>5.34×10^−5§ (2.67×10^−4)</td>
<td>1.99 (1.42–2.78)</td>
<td>AA</td>
<td>19(19.4)</td>
<td>19(6.5)</td>
</tr>
<tr>
<td></td>
<td>Exon3(R150R)</td>
<td>G</td>
<td>111(56.6)</td>
<td>420(72.2)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>rs537160</td>
<td>G &gt; A</td>
<td>108(55.1)</td>
<td>280(48.1)</td>
<td>0.09§ (0.96–1.83)</td>
<td>1.32 (0.96–1.83)</td>
<td>AA</td>
<td>28(28.6)</td>
<td>63(21.6)</td>
</tr>
<tr>
<td></td>
<td>IVS7</td>
<td>G</td>
<td>88(44.9)</td>
<td>302(51.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs4151657</td>
<td>T &gt; C</td>
<td>42(21.4)</td>
<td>146(25.1)</td>
<td>0.3§ (0.83–1.81)</td>
<td>1.23 (0.83–1.81)</td>
<td>CC</td>
<td>6(6.1)</td>
<td>16(5.5)</td>
</tr>
<tr>
<td></td>
<td>IVS10</td>
<td>T</td>
<td>154(78.6)</td>
<td>456(74.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2072653</td>
<td>A &gt; G</td>
<td>71(36.2)</td>
<td>254(43.6)</td>
<td>0.069§ (0.98–1.90)</td>
<td>1.36 (0.98–1.90)</td>
<td>CC</td>
<td>6(6.1)</td>
<td>16(5.5)</td>
</tr>
<tr>
<td></td>
<td>IVS17</td>
<td>A</td>
<td>125(63.8)</td>
<td>328(56.4)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data are the number of subjects (% of the total group).
§ χ² test.
* Fisher's exact test.
† P value for dominant model.
‡ P value for recessive model.

Han Chinese population (minor allele frequency ≥15%). These SNPs were assessed by TaqMan genotyping assays with a Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol. All PCR amplifications were performed with the following thermal cycling conditions: 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds, and 62°C for 15 minutes. The HLA-B27 allele was detected by nested PCR as described by Konno et al.16 Genotypes were read by using Prism 7000 SDS version 1.1 software (Applied Biosystems).

Statistical Analysis

Hardy-Weinberg equilibrium was tested by χ² test for genotype frequencies of the SNPs in control group. Allelic and genotypic frequencies between cases and controls were compared by χ² test or Fisher’s exact test. Dominant and recessive models were applied to investigate the disease association with regard to the minor allele (A for rs3020644, A for rs1048709, A for rs537160, C for rs4151657, and G for rs2072653). Stratified analyses based on clinical manifestations and HLA-B27 status were also performed. Logistic regression analysis was applied to adjust the association of these SNPs with sex. Pairwise linkage disequilibrium (LD [D’]) between polymorphisms and expectation maximization (EM)-based haplotype association analysis were performed with Haplovier (ver. 4.2) software. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. A P value of <0.05 was considered statistically significant. P values were corrected by using a Bonferroni test for multiple comparisons (α = total number of SNPs).

DNA Extraction and Genotyping

Venous blood was obtained from each study subject, and genomic DNA was extracted with a DNA blood kit (QIAamp; Qiagen, Hilden, Germany). Five single-nucleotide polymorphisms (SNPs), C2-rs3020644, CFB-rs1048709, rs537160, rs4151657, and rs2072653 across the C2-CFB region, including upstream and downstream, were selected by using HapMap (http://hapmap.ncbi.nlm.nih.gov/) for the
**RESULTS**

**Patient Demographics**

A total of 98 AU patients were recruited, including 45 (45.9%) males and 53 (54.1%) females and 47 (48.0%) unilateral and 51 (52.0%) bilateral patients. The mean age was 49.7 ± standard deviation of the patients was 49.7 ± 16.0 years, range 11 to 87 years. Acute AU occurred in 92 (95.9%) patients, of whom 59 (60.2%) had recurrent episodes of AU and 6 (6.1%) developed chronic AU after acute episodes. Systemic diseases associated with AU patients included ankylosing spondylitis (n = 19, 19.4%), 1 case (1.0%) each of psoriasis, systemic lupus erythematosus, ulcerative colitis, and interstitial nephritis.

**Associations between SNPs and AU**

All 5 SNPs followed Hardy-Weinberg equilibrium in controls. Significant association was detected at CFB-rs1048709, where there was a significant increase in the frequencies of A allele and AA homozygosity and decrease in the frequency of GG homozygosity in AU patients compared with that in controls (Pcorr = 2.67 × 10^-4, OR = 1.99, 95% CI = 1.42–2.78; Pcorr = 0.001, OR = 3.44, 95% CI = 1.74–6.82; and Pcorr = 2.14, 95% CI = 1.32–3.45, respectively). There was a significant increase in the frequency of AA homozygosity for C2-rs3020644 and decrease in the frequency of GG homozygosity for CFB-rs2072653 in AU patients compared with controls (Pcorr = 0.01, OR = 1.86, 95% CI = 1.13–3.05; and Pcorr = 0.029, OR = 0.44, 95% CI = 0.21–0.94, respectively), but these two associations lost significance after adjustment for multiple testing (Pcorr = 0.065 and Pcorr = 0.15, respectively). No significant differences in genotypic or allelic frequencies were observed for either CFB-rs557160 or CFB-rs4151657 SNPs between AU patients and controls (Table 1). As sex-specific genetic differences were found between AU and CFB-rs800292 in our previous study, sex ratio adjustments were performed by logistic regression analysis. The association did not alter between AU and all 5 SNPs after adjusting for sex (data not shown). No differences in sex susceptibility were found between AU and C2-CFB polymorphisms.

**Associations between SNPs and AU Stratified by HLA-B27 Status and Clinical Features**

Among the 98 AU patients, 42 (42.9%) were HLA-B27-positive AU patients, significantly higher proportions of A allele and AA homozygosity and a lower proportion of GG homozygosity in AU patients compared with those in controls (Pcorr = 7.30 × 10^-3, OR = 2.72 (95% CI, 1.71–4.33); Pcorr = 0.005, OR = 4.47 (95% CI, 1.75–8.21); and Pcorr = 0.0018, OR = 4.47 (95% CI, 1.92–10.46), respectively). No significant differences in genotypic or allelic frequencies were observed for either HLA-B27-negative AU patients and controls (Table 1). As sex-specific genetic differences were found between AU and HLA-B27-negative AU patients, sex ratio adjustments were performed by logistic regression analysis. The association did not alter between AU and all 5 SNPs after adjusting for sex (data not shown). No differences in sex susceptibility were found between AU and C2-CFB polymorphisms.
controls (Table 2). For the HLA-B27-negative AU patients, only one modest association was found in CFB-rs1048709 with higher proportions of A allele and AA homozygosity, but the differences lost significance after correction (P = 0.04, P_corr = 0.2; and P = 0.028, P_corr = 0.14, respectively (Table 3). No significant differences were found in allelic and genotypic frequencies among AU patients stratified by either laterality or recurrence status (data not shown).

**Linkage Disequilibrium and Haplotype Association Analysis**

Pairwise LD analysis revealed extensive LD throughout the CFB gene. One haplotype block was detected, including all 4 SNPs in CFB (rs1048709G > A, rs537160G > A, rs4151657T > C, and rs2072633A > G) (Fig. 1). Haplotype AATA conferred a 1.97-fold significantly increased risk of AU (P = 8.92 × 10^{-5}, permutation P = 0.0005) (Table 4).

**Table 4.** Haplotype Analysis of C2-CFB Polymorphisms between Cases and Controls

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case</th>
<th>Control</th>
<th>P Value (P_corr)</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AATA</td>
<td>0.309</td>
<td>0.421</td>
<td>0.272</td>
<td>8.92 × 10^{-5}</td>
<td>0.0005</td>
</tr>
<tr>
<td>GGCC</td>
<td>0.242</td>
<td>0.214</td>
<td>0.251</td>
<td>0.3009</td>
<td>NS</td>
</tr>
<tr>
<td>GATA</td>
<td>0.188</td>
<td>0.130</td>
<td>0.208</td>
<td>0.0157</td>
<td>NS</td>
</tr>
<tr>
<td>GGTG</td>
<td>0.168</td>
<td>0.141</td>
<td>0.177</td>
<td>0.2372</td>
<td>NS</td>
</tr>
<tr>
<td>GGTA</td>
<td>0.083</td>
<td>0.081</td>
<td>0.084</td>
<td>0.9094</td>
<td>NS</td>
</tr>
</tbody>
</table>

Interaction Analysis between CFH-rs800292 and CFB-rs1048709 in AU

Combined effects of CFH-rs800292 and CFB-rs1048709 were assessed, and corresponding ORs of AU for each possible combination of the genotypes of the two variants were estimated (Table 5a, b). ORs were compared with the baseline genotype of the two genes. The frequency of the homozygous risk genotypes at both loci was 5.3 lower in controls (2.1%) than in AU patients (11.2%, P = 0.005) (Table 5a). A joint disease OR of 7.48 in individuals with homozygous risk alleles at both loci was observed compared with the baseline nonrisk genotypes (Fig. 2).

**Discussion**

This is the first genetic study examining the association of variants in C2 and CFB genes with uveitis. Our results showed...
that CFB-rs1048709 is significantly associated with AU. CFB is paralogous with C2. These genes are located in tandem in the major histocompatibility complex class III region, a cluster on chromosome 6p21 with respect to infection and autoimmunity.17 Moreover, C2 and CFB are involved in the activation of classical complement pathway and the alternative complement pathway, respectively. CFB levels were significantly up-regulated in sera of uveitis patients. Treatment with CFB antibodies resulted in suppression of EAAU in animals.14,18 In addition, CFB gene polymorphisms are associated with multiple inflammatory diseases, such as age-related macular degeneration (AMD), lupus, and atypical hemolytic-uremic syndrome.19–21 Our results provide additional evidence for the involvement of complement system in ocular inflammatory disease-uveitis, especially CFB. Meanwhile, there are limitations in the present study: the relatively small sample size may lower statistical power. Some of the modest associations such as CFB-rs537160 and -rs2072653 could not remain statistically significant after adjustment for multiple testing. Additionally, these variants in CFB represent either synonymous substitutions (rs1048709 R150R) or intronic SNPs (rs537160, rs4151657, and rs2072653). Currently, there is no information on its biological functions. These polymorphisms may be linked with an undiscovered but biologically relevant structural variant in this region. Also, synonymous or intronic regulation could be involved in gene transcription or tissue specificity of gene expression. Thus, similar to the impaired CFH-mediated complement inhibition conferring AU risk, decreased complement activation by CFB might protect from AU.22 Further investigations of more polymorphisms in C2-CFB genes, using a larger cohort and in other ethnic groups, could help to consolidate the findings and identify more genetic associations. Also, comprehensive evaluation of this region by extensive resequencing to uncover unknown variation is worthwhile.

In addition to the association of CFB polymorphism with AU, our results also showed that AA homozygosity at C2-rs3020644 might be associated with increased risk of AU, although the association was modest and could not be obtained after adjustment for multiple testing (Table 1). C2-rs3020644 is located at the promoter that regulates gene expression and could be a susceptibility factor to AU. As a key component in classical complement pathway, the probable genetic association between C2 polymorphism and AU suggests the participation of each complement activation pathway in uveitis.

Sex susceptibility of CFH (I62V) in AU was found in our previous study.10 In this study, logistic regression analysis showed none of the 5 SNPs had significant interaction with sex, indicating that sex factor in C2 and CFB may not confer analogous effect as CFH. In addition, for HLA-B27-positive patients, significant association was identified with CFB-rs1048709, where there were significant increased frequencies of A allele and AA homozygosity in HLA-B27-positive patients compared with controls (Table 2), while for HLA-B27-negative patients, such association could not be detected after correction for multiple testing. Our findings suggested that the influence of C2 and CFB polymorphisms on AU differed depending on HLA-B27 status, inconsistent with results of our previous study of CFH.18 The exact reason for this discrepancy is unclear, further indicating the complexity of immune regulation in uveitis.6–10 Moreover, based on our previous study,6 only 6% of control subjects are HLA-B27 positive, therefore we did not perform stratified analysis of the controls based on HLA-B27 positivity due to the small sample size. To further clarify the association between CFB-rs1048709 and AU, under the condition of HLA-B27, formal hypothesis testing was performed (Table 3).

Table 5. Interaction Analysis between CFH-rs800292 and CFB-rs1048709 in AU

<table>
<thead>
<tr>
<th>Genotype Distribution</th>
<th>Genotype at CFH-rs800292</th>
<th>Genotype at CFB-rs1048709</th>
<th>b. Joint Odds Ratios and 95% Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 240)</td>
<td>AU (n = 98)</td>
<td>CFH-rs800292</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
</tr>
<tr>
<td>GG</td>
<td>17 (7.1%)</td>
<td>55 (22.1%)</td>
<td>55 (22.9%)</td>
</tr>
<tr>
<td>AG</td>
<td>19 (7.9%)</td>
<td>47 (19.6%)</td>
<td>34 (14.2%)</td>
</tr>
<tr>
<td>AA</td>
<td>3 (1.5%)</td>
<td>53 (22.1%)</td>
<td>5 (2.1%)</td>
</tr>
</tbody>
</table>

Data in a are numbers (%). Data in b are OR (95% CI).
also performed by comparing the allele and genotype frequency of rs1048709 in AU patients with or without HLA-B27. Our results showed that no significant difference was found between allele frequency and HLA-B27 positivity ($P = 0.06$). Also, no significant different was found between genotypic frequency and HLA-B27 positivity ($P = 0.117$). The nonsignificant results suggested no association between HLA-B27 and rs1048709 in our study cohort. While the $P$ value was marginal, the results should be interpreted cautiously, and further investigations are still needed. Meanwhile, stratified analyses according to recurrence and laterality status of AU patients showed that clinical severity did not affect the association of these SNPs on AU.

We identified a novel risk haplotype block across the CFB gene. This haplotype (AATA), defined by all the risk alleles of SNPs in CFB, conferred a 1.97-fold increased susceptibility to AU. In this block, 4 SNPs were in high LD with each other ($r^2 = 0.94$). Studies also showed that these 4 SNPs were shared in the C2-CFB region, including the functional variants (L9H and R32Q) in CFB and a joint effect between CFB and rs1048709 with AU (joint OR $\frac{GG}{AG}$ for several immunologic or inflammatory diseases, such as AMD and Lupus, $\frac{AG}{AA}$ was identified. Genetic influences on AU may be affected by HLA-B27 status.

**Acknowledgments**

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**References**