The Involvement of Complement Factor B and Complement Component C2 in an Indian Cohort with Age-Related Macular Degeneration

Inderjeet Kaur, Saritha Katta, Rajeev K. Reddy, Raja Narayanan, Annie Mathai, Ajit B. Majji, and Subhabrata Chakrabarti

PURPOSE. Genes involved in the complement cascade such as complement factor B (CFB) and complement component C2 have been implicated in age-related macular degeneration (AMD) worldwide. In continuation of the analysis of CFB and LOC387715/HTRA1, this study was conducted to gain understanding of the role of CFB and C2 in an Indian AMD cohort.

METHODS. Single nucleotide polymorphisms in CFB and C2 were screened in a cohort of clinically well-characterized patients with AMD (n = 177) and unaffected normal control subjects (n = 175). Screening was accomplished by a combination of customized genotyping followed by validation through resequencing. In addition, genotyping of two CFB variants (rs12614 and rs641153) that were in close proximity had to be resolved by resequencing. Estimates of allele and genotype frequencies, odds ratios, Hardy-Weinberg equilibrium, linkage disequilibrium (LD), and haplotype frequencies were also performed.

RESULTS. Three SNPs in C2 (rs547154 [IVS10]; P = 5.4 × 10^-11 and CFB (rs641153 [R32Q]; P = 2.2 × 10^-7 and rs2072633 [IVS17]; P = 2.0 × 10^-5) were strongly associated with reduced risk of AMD. The rs547154 and rs641153 were in strong LD (D’ = 0.90, 95% CI = 0.81–0.96) and a protective haplotype T-A was observed (OR = 0.10, 95% CI = 0.05–0.20). LD was moderate (D’ = 0.77, 95% CI = 0.67–0.85) between the rs547154 and the rs2072633 SNPs, and the haplotype T-T generated with these SNPs was relatively less protective (OR = 0.28, 95% CI = 0.18–0.44).

CONCLUSIONS. The results of the present study provide an independent validation of the association of rs547154 (C2) and rs641153 (CFB) SNPs with reduced risk of AMD in an Indian cohort. (Invest Ophthalmol Vis Sci. 2010;51:59–63) DOI: 10.1167/iovs.09-4135
**METHODS**

**Clinical Characterization of the Cases and Controls**

The study conformed to the tenets of the Declaration of Helsinki, and prior approval was obtained from the Institutional Review Board of the L.V. Prasad Eye Institute. A cohort of 512 consecutive subjects included patients with AMD ($n = 262$) and ethnically matched normal controls ($n = 250$) drawn from the same geographic region of habitat. Clinical characterization of these cases and controls along with the details of clinical examinations have already been described in our earlier publications in this journal.25,26 AMD in each subject was independently diagnosed by two retina specialists with previously laid inclusion and exclusion criteria. Written informed consent was obtained from all the subjects before their enrollment in the study.

**Selection of Variants (SNPs) in the Candidate Genes**

We chose multiple arrays of SNPs in each gene for genotyping. The selection criteria were based on previous evidence of association of these SNPs with AMD. We then chose additional flanking SNPs to the associated SNPs, to enlarge the genomic region being screened. Based on these criteria, we chose a set of 12 SNPs that included the associated SNPs, to enlarge the genomic region being screened. Based on these criteria, we chose a set of 12 SNPs that included the CFB gene, we could accommodate only one of the SNPs harboring codon 32 (i.e., rs12614 [R32W]) in the assay (GoldenGate Assay; Illumina). Hence the other SNP rs641153 (R32Q) at this codon was screened by resequencing with appropriate primers by using dye termination chemistry on an automated DNA sequencer (Big Dye Termination on a 3130xl sequencer; ABI, Foster City, CA) according to the manufacturer’s guidelines.

**Validation of SNPs**

Apart from replication of the samples in the same and different plates in the assay, resequencing was performed to validate these SNPs in a subset of samples (BigDye Chemistry; ABI) on an automated DNA sequencer (3130xl; ABI). To validate the three associated SNPs, further resequencing was done in the remaining cohort as well as in the previously assayed subjects. There was total concordance between the genotype calls obtained (Bead Analysis software; Illumina) and the SNP sequencing data.

**Statistical Analysis**

Allele and genotype frequencies were estimated by the gene-counting method and their significance was calculated by $\chi^2$ statistics. Odds ratios (ORs) were computed to assess the odds of the associated alleles and genotypes. Hardy-Weinberg equilibrium was calculated and haplotype frequencies were estimated with Haploview software (ver. 4.0) which uses the EM algorithm.27 Permutation tests were performed to assess the extent of association of individual SNPs and haplotype blocks. LD analysis between the SNPs was analyzed using the LD plot function of the software.

**RESULTS**

Initially, all the 12 SNPs in CFB and C2 were screened in a cohort of 352 subjects (177 AMD cases and 175 controls) by a
Table 3. Estimated C2/CFB Haplotype Frequencies

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>% Cases</th>
<th>% Controls</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9332739</td>
<td>rs547154</td>
<td>rs4151667</td>
<td>rs12614</td>
<td>rs641153</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>T</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>G</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>T</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>x</td>
<td>G</td>
<td>x</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>x</td>
<td>T</td>
<td>x</td>
<td>x</td>
<td>A</td>
</tr>
<tr>
<td>x</td>
<td>G</td>
<td>x</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>x</td>
<td>x</td>
<td>G</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>x</td>
<td>T</td>
<td>x</td>
<td>x</td>
<td>A</td>
</tr>
<tr>
<td>x</td>
<td>x</td>
<td>T</td>
<td>x</td>
<td>G</td>
</tr>
<tr>
<td>x</td>
<td>G</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>x</td>
<td>T</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>x</td>
<td>G</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

The bold rows indicate haplotypes based on the combinations of the minor alleles of three strongly associated SNPs in C2 (rs547154) and CFB (rs641153 and rs2072633).
Table 4. Distribution of Minor Allele Frequencies and ORs of rs547154, rs641153, and rs2072633 across Different Populations Worldwide

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. (900, 400)</td>
<td>—</td>
<td>8.45 × 10⁻⁸</td>
<td>0.44</td>
<td>—</td>
<td>—</td>
<td>6.43 × 10⁻⁹</td>
<td>0.32</td>
<td>—</td>
<td>—</td>
<td>0.044</td>
<td>0.36</td>
<td>—</td>
</tr>
<tr>
<td>U.S. (698, 2824)</td>
<td>0.050</td>
<td>0.11</td>
<td>2.6</td>
<td>—</td>
<td>0.05</td>
<td>0.10</td>
<td>2.3 × 10⁻⁵</td>
<td>—</td>
<td>0.45</td>
<td>0.46</td>
<td>0.592</td>
<td>—</td>
</tr>
<tr>
<td>U.S. (187, 168)</td>
<td>0.025</td>
<td>0.096</td>
<td>0.00011</td>
<td>—</td>
<td>—</td>
<td>0.00008</td>
<td>0.40</td>
<td>—</td>
<td>0.33</td>
<td>0.393</td>
<td>0.093</td>
<td>—</td>
</tr>
<tr>
<td>U.K. (518, 243)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.05</td>
<td>0.12</td>
<td>0.50</td>
<td>—</td>
<td>0.30</td>
<td>0.44</td>
<td>0.05</td>
<td>1.27</td>
</tr>
<tr>
<td>Australia (560, 204)</td>
<td>0.055</td>
<td>0.117</td>
<td>9.1 × 10⁻⁵</td>
<td>—</td>
<td>0.055</td>
<td>0.118</td>
<td>7.0 × 10⁻⁵</td>
<td>—</td>
<td>0.459</td>
<td>0.43</td>
<td>0.57</td>
<td>—</td>
</tr>
<tr>
<td>India (177, 175)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.085</td>
<td>0.274</td>
<td>5.4 × 10⁻¹¹</td>
<td>0.24</td>
<td>0.077</td>
<td>0.232</td>
<td>7.2 × 10⁻⁷</td>
<td>0.28</td>
</tr>
<tr>
<td>(Present Study)</td>
<td>0.085</td>
<td>0.274</td>
<td>5.4 × 10⁻¹¹</td>
<td>0.24</td>
<td>0.077</td>
<td>0.232</td>
<td>7.2 × 10⁻⁷</td>
<td>0.28</td>
<td>0.077</td>
<td>0.232</td>
<td>7.2 × 10⁻⁷</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>0.085</td>
<td>0.274</td>
<td>5.4 × 10⁻¹¹</td>
<td>0.24</td>
<td>0.077</td>
<td>0.232</td>
<td>7.2 × 10⁻⁷</td>
<td>0.28</td>
<td>0.077</td>
<td>0.232</td>
<td>7.2 × 10⁻⁷</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* Includes case-control data only.

Recent evidences by Montes et al. have suggested a functional basis of protection for the rs641153 (R32Q) SNP in AMD. The association of AMD with CFB abnormalities strongly suggests that the problem is unregulated activation of the alternative pathway, in which CFB is a critical factor. The authors have convincingly demonstrated the underlying mechanistic details of the rs641153 SNP in regulating the activity of the alternate complement pathway that may lead to a reduced risk of AMD. But, since the association of the CFB (rs641153) is directly linked to C2 (rs547154), it would also be interesting to know the combined functional effect of both these SNPs involving the classic pathway.

In summary, we have provided an independent replication of the association of C2 and CFB in an Indian AMD cohort. These results mimic our previous associations with respect to CFB and LOC387715 variants, wherein, our data resembled a similar genetic profile as observed in Caucasian populations. Haplotype analysis further refined the region of association harboring the rs547154 and rs641153 SNPs in C2 and CFB. Although our sample sizes were relatively smaller than those from Caucasian populations, these associations underscore the role of C2 and CFB SNPs in AMD pathogenesis and could be used for risk assessment in the Indian cohort.

Acknowledgments

The authors thank all the patients and volunteers for their participation in the study; Nazimul Hussain, Anjli Hussain, Taraprasad Das, Subhadra Jalali, and Avinash Pathangay for providing us some of the initial patient samples; and Kollu N. Rao and Avid Hussain for technical support.

References


