Significance of \( C2/CFB \) Variants in Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy in a Japanese Population


**Purpose.** To determine whether genetic variants in the complement component 2 and factor B gene (\( C2/CFB \)) locus are associated with the risk for typical age-related macular degeneration (AMD) or polypoidal choroidal vasculopathy (PCV) in a Japanese population.

**Methods.** Four single nucleotide polymorphisms (SNPs) were genotyped across the \( C2/CFB \) locus of patients with typical AMD \((n = 455)\) or PCV \((n = 581)\) and of 865 controls. Differences in the observed genotypic distribution between the case and control groups were tested by logistic regression analysis for age and sex adjustments. Significant associations were confirmed using a second control group of 336 cataract patients. A further model adjusting for age-related maculopathy susceptibility 2 \((ARMS2)\) A69S, complement factor H \((CFH)\) I62V, age, sex, and smoking status was performed, to confirm their independent association from other covariates.

**Results.** \( C2 \) rs547154 and \( CFB \) rs541862 were significantly associated with typical AMD and PCV in this Japanese sample \((P < 0.05)\). These two SNPs were also significantly associated with typical AMD and PCV in evaluation of the second control cohort \((P < 0.05)\). Furthermore, an independent association of \( C2/CFB \) variants was found for both typical AMD and PCV with age, sex, smoking, and genetic background of \( ARMS2 \) A69S and \( CFH \) I62V \((vs. \text{typical AMD}: P = 0.0073, \text{odds ratio [OR]} = 0.47; \text{vs. PCV}: P = 0.0083, \text{OR} = 0.53)\).

**Conclusions.** \( C2/CFB \) variants play a protective role in the risk of developing neovascular AMD and PCV in the Japanese. Investigative Ophthalmology & Visual Science, February 2012, Vol. 53, No. 2 DOI:10.1167/iovs.11-81488

An age-related macular degeneration (AMD) is the leading cause of visual impairment in the elderly and the most common cause of blindness in developed countries. Recent studies of the genetics of AMD have recognized it as a complex disease caused by the actions and interactions of several genes and environmental factors. The three major AMD-associated loci in Caucasians include (1) the age-related maculopathy susceptibility 2 and high-temperature requirement factor A1 \((ARMS2/HTRA1)\) gene \(5-7\) (2) the complement factor H \((CFH)\) gene \(5-7\) and (3) the complement component 2 and factor B \((C2/CFB)\) gene loci. Some studies have reported that inflammatory processes may play a central role in AMD by contributing to the formation of drusen, \(11-12\) with \( C2 \) and \( CFB \) being involved in initiation of the alternative complement cascade and activation of the classic component pathway, respectively. Numerous reports using Caucasian cohorts hold the consensus that genetic variants across \( C2/CFB \) are involved in protection against AMD. \(8,13-15\) However, all previous reports that evaluated populations in East Asia have shown an absence of association of \( C2/CFB \) variants in developing AMD. Thus, it was concluded that these variants are less likely to be associated with the development of AMD in Asians. \(16,17\)

Polypoidal choroidal vasculopathy (PCV) is clinically classified as a specific type of AMD and is usually diagnosed by indocyanine green angiography. \(18\) The incidence of PCV in Asian populations with neovascular AMD has been reported to be high, accounting for 54.7% of Japanese AMD patients and 24.5% of Chinese patients, compared with only 8% to 13% of Caucasians. \(19-21\) Previous studies revealed several genes that are susceptible to the development of PCV. \(22-24\) However, almost all reported genetic risk factors for developing PCV are identical with those for the development of AMD, which suggests that AMD and PCV share, at least in part, the same genetic background. In fact, PCV has many similarities with neovascular AMD, including demography, pathology, and manifestation. \(19,21,25\)

There have been studies in which the association between PCV and \( C2/CFB \) was evaluated in Asian populations, in a relatively smaller cohort size (165 participants by Lee et al. \(26\) and 313 participants by Kondo et al. \(27\)), but the results were negative, leading to the conclusion that pathobiological differences between PCV and neovascular AMD were present. However, these studies seemed to be underpowered, and recently, in a Caucasian cohort, Lima et al. \(28\) showed a positive associ-
Sex, $n$ (%)  
Men 330 (72.5) 420 (72.3) 161 (27.7)  
Women 125 (27.5) 200 (37.8) 319 (61.5)  
Smoking, $n$ (%)  
Never 151 (36.7) 200 (38.5) 454 (52.7)  
Ever 330 (72.5) 420 (72.3) 431 (49.8)  

**METHODS**

All procedures in this study adhered to the tenets of the Declaration of Helsinki, and the Ethics Committee of each institute involved approved the study protocols. All the patients were fully informed about the purpose and procedures of the study, with each patient providing written consent.

Four hundred and fifty-five patients with typical AMD (tAMD) and 581 patients with PCV were recruited from the Department of Ophthalmology at Kyoto University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. We used 865 healthy Japanese individuals, recruited from the Aichi Cancer Center Hospital, and Kobe City Medical Center General Hospital. We used 865 healthy Japanese individuals, recruited from the Aichi Cancer Center Research Institute, as control subjects. They were recruited from first-visit outpatients after it was confirmed that they did not have cancer according to the cancer registry, medical record, and self-report. We recruited them without ophthalmic data and evaluated them as general population controls. When we found a significant association in a studied variant using the general population controls, we confirmed the association using a second control group comprising 336 elderly individuals who had received cataract surgery without age-related maculopathy (ARM), recruited from the Department of Ophthalmology at Kyoto University Hospital, Osaka Eye Hospital, Japanese Red Cross Otsu Hospital, and Nagahama City Hospital. By fundoscopic examination, the cataract control samples were confirmed not to have any drusen or pigment change. The definitions of exudative AMD and ARM were based on those of the International Classification System for ARM and AMD. As proposed by the Japanese Study Group of Polypoidal Choroidal Vasculopathy, the diagnosis of PCV was based on indocyanine green angiography, which showed a branching vascular network terminating in polypoidal swelling. tAMD showed classic CNV, occult CNV, or both. Patients with the following status were excluded from the study subjects: (1) high myopia (spherical equivalent, $<-6.00$ D), (2) geographic atrophy or drusen only, (3) an eye with both typical choroidal neovascularization and polypoidal lesions, and (4) an old lesion without a clear diagnosis. All diagnoses were made by three retina specialists (KY, AT, and AO); a fourth specialist (NY) was consulted when the subtype classification could not be decided on by the initial three reviewers. All subjects in the present study were unrelated and of Japanese descent.

Information on smoking status was obtained via a self-report questionnaire, with the three categories of never smoker, former smoker, and current smoker. The never smokers were those who had smoked fewer than 100 cigarettes in the past, current smokers were those who had smoked in the past 1 year, and former smokers were those who had quit smoking more than 1 year earlier. As in our previous study, we combined the current smokers and the former smokers into ever smokers; thus, we analyzed the smoking status based on the two groups of never smokers and ever smokers.

We targeted $C2$ rs547154 (IVS10) and $CFB$ rs2072653 (IVS17), which have been described as having a positive association with the development of AMD in prior studies. We analyzed two additional single-nucleotide polymorphisms (SNPs) on $CFB$ (rs541862 and rs4151672) because they had relatively higher allele frequencies on the $C2/CFB$ locus. Genomic DNAs were prepared from peripheral blood by using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). All case samples and cataract samples were then genotyped (Taqman SNP assay with the PRISM 7700 system; Applied Biosystems, Inc. [ABI], Foster City, CA). Individuals recruited from the Aichi Cancer Center Research Institute were genotyped with another system (Human-Hap610 chips; Illumina Inc., San Diego, CA).

Deviations in genotype distributions from the Hardy-Weinberg equilibrium (HWE) were assessed with the HWE exact test. Statistical analyses for differences in the observed genotypic distribution were performed by logistic regression analysis for age and sex adjustments. Haploview software was used to perform haplotype analysis and to infer the linkage disequilibrium (LD) among the evaluated SNPs. $P$ value correction was performed with the Bonferroni method, using the ratio of the number of selected SNPs across a gene. $P < 0.05$ was considered statistically significant.

**RESULTS**

Demographics of the study population are shown in Table 1. Genotype and allele frequencies of the four SNPs were analyzed in the 455 patients with tAMD and 581 patients with PCV and compared with those of 865 healthy Japanese individuals. Details of allele frequencies and summary statistics are shown in Table 2. The genotyping of all evaluated SNPs was more than 98.2% successful, and the distributions of the genotypes for all study groups were in HWE $(P > 0.05)$. In logistic regression analyses adjusted for age and sex, $C2$ rs547154 and $CFB$ rs541862 were significantly associated with both tAMD

<table>
<thead>
<tr>
<th>Allele</th>
<th>tAMD $(n = 455)$</th>
<th>PCV $(n = 581)$</th>
<th>Control $(n = 865)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C2$</td>
<td>rs547154</td>
<td>T</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0018</td>
</tr>
<tr>
<td>CFB</td>
<td>rs541862</td>
<td>C</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td>CFB</td>
<td>rs2072653</td>
<td>A</td>
<td>0.436</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.487</td>
</tr>
<tr>
<td>CFB</td>
<td>rs4151672</td>
<td>T</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.845</td>
</tr>
</tbody>
</table>

* Adjusted for age and sex.
Because the age of the controls was definitely younger than that of the cases, to adjust for a birth cohort effect, differential survival, or survivorship, we also performed a stratification analysis using 420 controls aged 50 years or older. This stratified cohort included 212 (50.5%) men, 208 (49.5%) women, 223 (53.3%) never smokers, and 195 (46.7%) ever smokers. The mean age ± SD of the group was 62.68 ± 7.67 years. We found that the associations of C2 rs547154 and CFB rs541862 remained statistically significant in both tAMD (rs547154, P = 0.0048; rs541862, P = 0.0042) and PCV (rs547154, P = 0.0076; rs541862, P = 0.0075) in this stratification analysis, as well.

Next, we confirmed the positive associations using a second control cohort of 336 elderly cataract patients. The mean age ± SD of the cataract patients was 74.16 ± 8.42 years (range, 43–94), and 142 (42.3%) male and 194 (57.7%) female patients were included. Table 3 shows the result of this replication study using cataract patients aged 50 years or older. This stratified cohort included 212 (50.5%) men, 208 (49.5%) women, 223 (53.3%) never smokers, and 195 (46.7%) ever smokers. The mean age ± SD of the group was 62.68 ± 7.67 years. We found that the associations of C2 rs547154 and CFB rs541862 remained statistically significant in both tAMD (rs547154, P = 0.0048; rs541862, P = 0.0042) and PCV (rs547154, P = 0.0076; rs541862, P = 0.0075) in this stratification analysis, as well.

Finally, we conducted a logistic regression analysis that included the effects of the most robust Japanese AMD/PCV-associated variants, ARMS2 A69S (rs10490924) and CFH I62V (rs800292), as well as age, sex, and smoking status. Because C2 rs547154 and CFB rs541862 were in strong LD (pair-wise D' = 1.0 and r² = 1.0), we analyzed rs547154 as the representative SNP of the C2/CFB locus. Table 5 shows the result of this logistic regression analysis. C2/CFB rs547154 remained significant both in tAMD and PCV, even after including the effects of these covariates (vs. tAMD: P = 0.0073, OR = 0.47, 95% CI = 0.27–0.82; vs. PCV: P = 0.016, OR = 0.56, 95% CI = 0.38–0.84). After considering the effects of three major AMD-associated loci, we found that the effect of smoking was diminished in the risk for PCV (P = 0.292), and just a marginal association was found for tAMD (P = 0.0693).

**Table 3. Replication Study Using Cataract Patients**

<table>
<thead>
<tr>
<th>Cataract Patients</th>
<th>vs. tAMD (n = 455)</th>
<th>vs. PCV (n = 579)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor Allele</td>
<td>MAF</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>C2</td>
<td>rs547154 T</td>
<td>0.092</td>
</tr>
<tr>
<td>CFB</td>
<td>rs541862 C</td>
<td>0.091</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency in the cataract patients.

*Adjusted for age and sex.

(rs547154: P = 0.018, odds ratio [OR] = 0.57, 95% confidence interval [CI] = 0.35–0.91; rs541862: P = 0.016, OR = 0.56, 95% CI = 0.35–0.90) and PCV (rs547154: P = 0.0062, OR = 0.54, 95% CI = 0.35–0.84; rs541862: P = 0.0061, OR = 0.54, 95% CI = 0.35–0.84). These associations remained significant, even after a permutation procedure for multiple test correction (corrected P < 0.05). There was no SNP in the four tested SNPs across the C2/CFB locus, which showed a significant difference between tAMD and PCV.

DISCUSSION

The present study shows the significance of polymorphisms in C2 and CFB for development of tAMD and PCV in a relatively large sample of Japanese patients. As a result of comparing the genotypic distributions of C2/CFB variants in a sample of Japanese patients with tAMD (n = 455) or PCV (n = 581) and in two independent control groups (865 healthy Japanese individuals and 336 cataract patients), we found that C2 rs547154 (IVS10) and CFB rs541862 showed significant associations with the risk for both tAMD and PCV, with protective effects against the risk of the diseases.

Replication is the gold standard for assessing statistical results from genetic studies. However, a real result may fail to be replicated for numerous reasons, including inadequate sample size or variability in phenotype definitions across independent samples. Although numerous reports have shown a significant association between C2/CFB variants and AMD in Caucasians, all studies in Asians have been unable to replicate these results.16–17 However, in the present study, we clearly showed a significant association of C2/CFB variants for developing AMD in a Japanese cohort. Considering that minor allele frequency is similar between our cohort and previous reports, this discrepancy would be due to the small sample size used in the previous reports on Asian cohorts. Our study indicates that previous studies on the same subject did not reach statistical significance, and that large cohorts are needed to have enough statistical power to detect the association of the C2/CFB locus.

To date, all reports on Asian cohorts have shown a lack of association between C2/CFB polymorphism and PCV.26–27 However, recently, Lima et al.28 showed a positive association with PCV in Caucasians, even though incidence of PCV is lower in Caucasians than in Asians. In the present study,
C2/CFB variants were clearly associated with PCV. Our result is therefore in agreement with that of the Caucasian cohort used by Lima et al. Hence, our study also supports that there is no difference between tAMD and PCV in the role of C2/CFB for development of the disease.

In addition, we found the association of C2/CFB variants was unchanged, even when we adjusted for the effects of other established risk factors for AMD (age, sex, smoking, and a genetic background including ARMS2 A69S and CFH I62V). In this study, common genetic variations at all three loci were associated with PCV, similar to that already documented in AMD—that is, SNPs that conferred a higher risk or protection from the disease in AMD were associated with the same in PCV. Furthermore, logistic regression analysis revealed that the role of environmental factors (smoking) diminishes when the effects of the three major AMD-associated loci (ARMS2/HTRA1, CFH, and C2/CFB) are taken into consideration. This result indicates that genetic factors have an enormous influence on whether people develop AMD and/or PCV. Among all covariates, ARMS2/HTRA1 variants had the largest effect on the risk for tAMD (OR = 2.43), whereas sex had the largest effect on development of PCV (OR = 2.64). In a previous meta-analysis study, the prevalence of late AMD in Asian women was reported to be much lower than in Asian men; on the other hand it is said that those with PCV are predominately male. Considering the high prevalence of PCV in Asian populations, these results suggest that men would be more likely to develop PCV. In our study, genetic factors had important roles in the development of both tAMD and PCV. Thus, our results indicate that differences in sex would affect phenotypic differences in AMD.

In the present study, we evaluated different SNPs from those examined in the original study, because minor allele frequencies of the SNPs evaluated in Caucasians were extremely low in the Japanese. To confirm the association reported in Caucasians, we also genotyped C2 rs9332739, reported to be positive in the original study in cataract controls. However, there was no significant association in C2 rs9332739 for development of tAMD and PCV in our cohort, because of its low allele frequency (data not shown), and C2 rs9332739 did not have an impact on the result of haplotype analysis. We also grouped the current smokers and the former smokers into ever smokers, because this group had the highest tendency to develop PCV. However, smoking was not found to have a significant independent association with the development of either tAMD or PCV in this study. Considering that smoking status was obviously different between the cases and the controls, this association should reach statistical significance if the number of participants were increased. Another reason for the lack of association could be because of the heterogeneity of smoking status. As with the general trend, the former smokers were older than others, and more men than women had smoked in our cohort (data not shown). In addition, we could not exclude the possibility that there were interactions between genetic and environmental factors or between genes; several studies have reported the presence of interactions between ARMS2/HTRA1, CFH, and smoking in AMD and PCV. Hence, further studies should be performed to ascertain the effects of interaction of different risk factors in the development of disease, including AMD-associated genes and smoking.

Another limitation of the present study is the difference between the case and control samples. The control samples were definitely younger than those in the case group, which means that some of these young controls may develop AMD or PCV in the future. To exclude a potential confounder of genetic background in age, we confirmed that our results were unchanged, even after a stratification analysis adjusting for the difference in age. In addition, to avoid a sampling error, we performed a replication study using another control group of a much closer age to the cases (cataract patients without ARM) and found a significant association between C2/CFB variants and development of AMD/PCV. However, because the prevalence of late AMD in the Japanese population is reported to be 0.5%, the magnitude of the statistical bias of an association analysis should be negligible. In addition, considering that the case–control association analyses using such subjects are less apt to be statistically significant, our positive results should be acceptable.

Recently, subretinal drusenoid deposits, called reticular pseudodrusen, were differentiated from soft drusen with spectral domain optical coherence tomography (SD-OCT) and were reported to be associated with late AMD. We also evaluated whether C2/CFB variants are associated with developing reticular pseudodrusen in a small number of participants (n = 91) who had SD-OCT and autofluorescence imaging. However, we could not find a significant association between C2/CFB variants and the incidence of reticular pseudodrusen (data not shown). Further studies are needed to ascertain the association between C2/CFB variants and developing reticular pseudodrusen.

In conclusion, this study provides the first evidence that C2/CFB variants play a role in the risk of both neovascular AMD and PCV in Asians. Inflammation plays a central role in the pathobiology of AMD, with C2 and CFB both encoding regulatory proteins that activate the complement pathway. As the inhibition of CFB with a specific chemical binding entity has been suggested to be a viable approach for the treatment of neovascular AMD, our findings may suggest the potential effectiveness of such treatments by using anti-inflammatory agents, not only for AMD but also for PCV.

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References


