Association of Genetic Variation on Chromosome 9p21 with Polypoidal Choroidal Vasculopathy and Neovascular Age-Related Macular Degeneration

Xiongze Zhang, Feng Wen, Chengguo Zuo, Meng Li, Hui Chen, and Kunfang Wu

PURPOSE. Polypoidal choroidal vasculopathy (PCV) contains aneurismatic morphologic and histopathologic feature and it is considered to be a possible distinct entity from neovascular age-related macular degeneration (AMD). In this study, the association of identified risk variants for intracranial aneurysm on chromosome 9p21 with PCV and neovascular AMD in a Chinese Han population was investigated.

METHODS. The authors genotyped rs1333040 and rs10757278 on 9p21 in 177 PCV patients, 131 neovascular AMD patients, and 182 controls using a genotyping method and direct DNA sequencing. Allele and genotypes frequencies in the PCV and neovascular AMD groups were compared with controls using a free open-source software and binary logistic regression analysis.

RESULTS. Rs1333040 was not associated with PCV or neovascular AMD. Rs10757278 was significantly associated with PCV [risk allele: A, P (allelic) = 0.014; odds ratio = 1.44; 95% confidence interval, 1.08–1.94], but not associated with neovascular AMD. After adjusting for sex, age, smoking status, history of hypertension, type 2 diabetes, and coronary artery disease, the odds ratio for homozygous carriers of rs10757278-A was 2.10 (95% confidence interval, 1.14–3.85) for PCV.

CONCLUSIONS. The rs10757278 on chromosome 9p21 is significantly associated with the risk of PCV but not with neovascular AMD in the Chinese Han population. (Invest Ophthalmol Vis Sci. 2011;52:8063–8067) DOI:10.1167/iovs.11-7820

Polypoidal choroidal vasculopathy (PCV) is a form of serous-sanguineous maculopathy found in a significant proportion of Asian patients.1–5 It is characterized by an abnormal choroidal vascular network with characteristic aneurismatic dilations at the border of the vascular network.4–6 Patients with PCV frequently suffer multiple recurrent serous and hemorrhagic detachments of the retinal pigment epithelium (RPE).4 Indocyanine green angiography (ICGA) can be used to make a definitive diagnosis of PCV. The widespread availability of ICGA has helped us to recognize PCV as a possible distinct clinical entity from age-related macular degeneration (AMD) both clinically and demographically.4,7 An advanced stage of AMD is characterized by choroidal neovascularization (neovascular AMD, nAMD) or geographic atrophy. The choroidal neovascularization of nAMD appears in the macular area leading to subretinal hemorrhages, serous retinal detachments, fibrous scars and/or RPE atrophy.9 There are two different perspectives about the nature of PCV lesions regarding the variants of choroidal neovascularization9,10 and abnormalities of the inner choroidal vessels.11–12 However, the differences between the pathogeneses of PCV and nAMD are still unknown.

The dilations of PCV are seen as reddish-orange lesions ophthalmoscopically, and they are clearly visible as aneurismatic hyperfluorescence during the early-phase of ICGA.13,14 Pulsation of the vascular dilations is a unique and important characteristic of PCV.12,13,15,16 The pulsatile movement is also observed in some arterial aneurysms, including retinal macroaneurysms.17,18 Histopathologic findings demonstrate notable sclerotic changes in PCV lesions and disruption of the elastic layer within the wall of polypoidal vessels,11,19 which is known to decrease the elasticity and strength of the vascular wall leading to aneurysm formation.20,21 Moreover, the elastin gene has also been reported to be associated with both intracranial aneurysm22 and PCV.23 Though the precise etiology of PCV is still undetermined, the aneurismatic morphologic and histopathologic features of PCV suggest its possible association with aneurysm. Intracranial aneurysm and PCV both originate from branches of the internal carotid artery and are likely to share some predisposing factors. The chromosome 9p21 is well known to be associated with coronary artery disease24–26 and type 2 diabetes.27–29 Recently, two single nucleotide polymorphisms (SNPs) on 9p21 (rs1333040 and rs10757278) associated with intracranial aneurysm were identified by a multigenome-wide association study.30 The association of intracranial aneurysm with rs10757278-G was replicable in many populations,31–33 while the association of intracranial aneurysm with rs1333040-T was confirmed in Japanese34 and other populations32 as well. We hypothesized that these two SNPs might play a role in the susceptibility of PCV.

In this study, we genotyped rs1333040 and rs10757278 on 9p21 and analyzed the associations between these two SNPs and PCV in a Chinese Han population. Additionally, for comparison of PCV and nAMD, we also analyzed the associations between the two SNPs and nAMD.

METHODS

Study Population

All case and control subjects were Chinese Han individuals recruited from the Zhongshan Ophthalmic Center. The study protocol was approved by the institutional review board at the Zhongshan Ophthalmic Center of Sun Yat-sen University and was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all study subjects that provided medical information and
were classified as stage 4 by the Rotterdam Study classification. Neovascularization by fluorescein angiography and ICGA. All nAMD cases were excluded because they were difficult to distinguish from AMD cases. The diagnosis of nAMD was based on identification of characteristic polypoidal choroidal vascular dilations with or without branching inner choroidal vessels on ICGA. Thus, all PCV patients enrolled in this study met the criteria of definite cases of PCV, as proposed by the Japanese Study Group of PCV. Cases diagnosed as probable were excluded because they were difficult to distinguish from AMD cases. The diagnosis of nAMD was based on identification of choroidal neovascularization by fluorescein angiography and ICGA. All nAMD cases were classified as stage 4 by the Rotterdam Study classification. Geographic atrophy, another advanced form of AMD, was excluded from this study. Moreover, patients with other neovascularized maculopathies, such as pathologic myopia, angioid streaks, presumed ocular histoplasmosis, and retinal angiomatous proliferation, were excluded.

All control subjects were aged ≥ 50 years. They all underwent ophthalmic examinations, including visual acuity measurements, slit-lamp biomicroscopy, ophthalmoscope examination, and 50° color fundus photography. Those with macular degeneration of any cause, macular changes (such as drusen or pigment abnormalities), or media opacities preventing clear visualization of the macula were excluded from the study.

Genotyping
Collection of peripheral blood samples and extraction of genomic DNA were performed as previously described. We genotyped rs133040 and rs10757278 on 9p21 with a genotyping system (Multiplex SNaPshot; Applied Biosystems, Foster City, CA) using a genetic analyzer (ABI 3730XL; Applied Biosystems). Genotypes of the SNPs were determined using software (Genemapper v4.1; Applied Biosystems). The sequences of primers used for each SNP are shown in Table 1. To confirm the accuracy of the genetic analysis method (Multiplex SNaPshot), randomly selected subjects (10% of all samples) were analyzed by direct sequencing (Shanghai Generay Biotech Co., Ltd., China). All the primers used for direct sequencing are available on request.

Statistical Analysis
Differences in baseline characteristics between cases and controls were assessed using the unpaired Student’s t-test for means and χ² tests for proportions, with statistical software (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL). Deviations from the Hardy-Weinberg equilibrium were tested using the exact test implemented in the open-source software package (PLINK v1.07; http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). The minor allele frequency was calculated based on all the case and control subjects. Allele frequencies between cases and controls were evaluated for each SNP using the χ² test (implemented in PLINK). For the genotypic additive model we used the logistic option (in PLINK), which provided a test based on logistic regression; for the dominant and recessive model we used the model option (in PLINK), which provided a χ² test. The odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated relative to the major allele and the wild type homozygote. Binary logistic regression analysis in computer software (SPSS 13.0 for Windows; SPSS Inc.) was used to estimate the adjusted genotype-specific odds ratio and 95% CIs with adjustment for sex, age, smoking status, history of hypertension, type 2 diabetes, and coronary artery disease. Power calculation of single association was performed using software (PGA: Power for Genetic Association Analyses). The Bonferroni correction was used to control inflation of the type I error rate, where a value of \( P < 0.025 \) was considered statistically significant.

RESULTS
A total of 490 subjects were enrolled in this study, including 177 patients with PCV, 131 patients with nAMD, and 182 control individuals. The baseline characteristics of the study groups, including sex, age, smoking status, history of hypertension, type 2 diabetes, and coronary artery disease are summarized in Table 2. The mean age of the PCV group (65 ± 8.45 years) was significantly lower than the control group (68 ± 9.17 years) \((P < 0.001)\). The proportion of subjects with a history of hypertension in the nAMD group (33.6%) was significantly higher than that in the control group (20.9%) \((P = 0.012)\). Meanwhile, the proportion of subjects with a history of coronary artery disease in the nAMD group (8.4%) was significantly higher than the control group (1.6%) \((P = 0.004)\). Other baseline characteristics assessed in the PCV group and nAMD group showed no statistical differences when compared with the control group.

Genotyping call rates were 100% for both SNPs across patients and controls. Genotypes of the replicate samples were confirmed by direct sequencing with a consensus rate of 100%.

Table 1. Primers for SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primers F</th>
<th>Extension Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs133040</td>
<td>F: TCA TCA AAG AGA CAC GAG GAG</td>
<td>TTTTTTTTT TAGG GTC AAG GTTA AGA ATG</td>
</tr>
<tr>
<td>rs10757278</td>
<td>F: TAG TGG AAG ACG TGA ACC GGC</td>
<td>TTTTTTTTTTTTTTGG GTT GGTC ATTC CGG TA</td>
</tr>
</tbody>
</table>

F, Forward; R, Reverse.

Table 2. Baseline Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n = 182)</th>
<th>PCV (n = 177)*</th>
<th>nAMD (n = 131)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, n (%)</td>
<td>69 (37.9)</td>
<td>60 (33.9)†</td>
<td>47 (35.9)†</td>
</tr>
<tr>
<td>Age, mean ± SD (range), y</td>
<td>68 ± 9.17 (50–87)</td>
<td>65 ± 8.45 (42–85)‡</td>
<td>67 ± 9.46 (46–84)†</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>62 (34.1)</td>
<td>71 (40.1)†</td>
<td>48 (36.6)†</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>38 (20.9)</td>
<td>48 (27.1)†</td>
<td>44 (33.6)‡</td>
</tr>
<tr>
<td>Type 2 diabetes, n (%)</td>
<td>10 (5.5)</td>
<td>11 (6.2)†</td>
<td>12 (9.2)‡</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>5 (1.6)</td>
<td>8 (4.5)†</td>
<td>11 (8.4)‡</td>
</tr>
</tbody>
</table>

*P values derived from t-tests for means and χ² tests for proportions compared with control. † Not significant compared with control.‡ P < 0.010.§ P < 0.025.
The genotyping data of rs1333040 and rs10757278 on chromosome 9p21 did not show any significant deviations from Hardy-Weinberg equilibrium in either group (Table 3). Rs10757278 was found to be significantly associated with PCV in a codominant model and a recessive model (genotypic) $P$-value of 0.016 and 0.018, respectively, but not in a dominant model (genotypic) $P$ = 0.108). After adjusting for sex, age, smoking status, history of hypertension, type 2 diabetes, and coronary artery disease, we estimated the odds ratios for homozygous and heterozygous carriers of the risk allele A of rs10757278 to be, respectively, 2.10 [95% CI, 1.14–3.85; $P$ = 0.017] and 1.17 [95% CI, 0.70–1.96; $P$ = 0.560] for PCV. Rs10757278 in nAMD and rs1333040 in nAMD have common associations with the complement factor $H$ ($CFH$) and the HtrA serine peptidase $1$ ($HTRA1$) genes, which suggests some genetic similarity between these two entities. Given the differences in the clinical presentation between PCV and nAMD, several studies focused on determining other genetic components of these two entities to find out if these two different phenotypes can be attributed to differences in genetic components that may reveal different underlying pathogenic mechanisms. In our previous study, we investigated the serpin peptidase inhibitor, clade G, member 1 ($SERPING1$) gene in PCV. The $SERPING1$ gene, whose protein product is a key regulator of the classic complement pathway, was reported to be associated with AMD.

PCV and nAMD have common associations with the complement factor $H$ ($CFH$) and the HtrA serine peptidase $1$ ($HTRA1$) genes, which suggests some genetic similarity between these two entities. Given the differences in the clinical presentation between PCV and nAMD, several studies focused on determining other genetic components of these two entities to find out if these two different phenotypes can be attributed to differences in genetic components that may reveal different underlying pathogenic mechanisms. In our previous study, we investigated the serpin peptidase inhibitor, clade G, member 1 ($SERPING1$) gene in PCV. The $SERPING1$ gene, whose protein product is a key regulator of the classic complement pathway, was reported to be associated with AMD.

We did not find any association between its four tag SNPs and PCV in the Chinese Han population. Complement factor $2$ ($C2$) and factor $B$ ($CFB$) genes also have associations with nAMD, but not with PCV in Chinese population. These findings may indicate a different role for the complement system in pathogenesis of PCV and nAMD. The elastin gene, as mentioned in previous studies, has been investigated for its role in AMD.

### Table 3. Association between Minor Allele Frequency of Variants on 9p21 and PCV and nAMD

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (bp)</th>
<th>Population</th>
<th>Minor Allele$^*$</th>
<th>MAF</th>
<th>HWE</th>
<th>OR (95% CI)</th>
<th>P Allelic</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1333040</td>
<td>2207340</td>
<td>Control</td>
<td>C</td>
<td>0.297</td>
<td>0.213</td>
<td>1.26 (0.92–1.73)</td>
<td>0.146</td>
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<tr>
<td></td>
<td></td>
<td>PCV</td>
<td>A</td>
<td>0.344</td>
<td>0.563</td>
<td>1.24 (0.88–1.74)</td>
<td>0.215</td>
</tr>
<tr>
<td>rs10757278</td>
<td>22114477</td>
<td>Control</td>
<td>A</td>
<td>0.457</td>
<td>0.881</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCV</td>
<td>A</td>
<td>0.528</td>
<td>0.452</td>
<td>1.44 (1.08–1.94)</td>
<td>0.014</td>
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<tr>
<td></td>
<td></td>
<td>nAMD</td>
<td>A</td>
<td>0.500</td>
<td>1.000</td>
<td>1.29 (0.94–1.77)</td>
<td>0.118</td>
</tr>
</tbody>
</table>

* The minor allele was calculated based on all case and control subjects.

### Table 4. Association between Genotype of Variants on 9p21 and PCV and nAMD

<table>
<thead>
<tr>
<th>Disease</th>
<th>SNP</th>
<th>Genotype</th>
<th>Genotype Distribution (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted $P^*$</th>
<th>Statistical Power (%)$^†$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td>rs1333040</td>
<td>Additive</td>
<td>TT</td>
<td>47.3</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>6.6</td>
<td>2.08 (0.96–4.67)</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>26.1</td>
<td>1.06 (0.69–1.65)</td>
<td>0.779</td>
</tr>
<tr>
<td></td>
<td>rs10757278</td>
<td>Additive</td>
<td>GG</td>
<td>31.3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>50.0</td>
<td>1.24 (0.75–2.04)</td>
<td>0.400</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>18.7</td>
<td>2.08 (1.15–3.74)</td>
<td>0.015</td>
</tr>
<tr>
<td>nAMD</td>
<td>rs1333040</td>
<td>Additive</td>
<td>TT</td>
<td>47.3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>6.6</td>
<td>2.10 (1.14–3.85)</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>rs10757278</td>
<td>Additive</td>
<td>GG</td>
<td>31.3</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>50.0</td>
<td>1.24 (0.72–2.10)</td>
<td>0.441</td>
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<tr>
<td>nAMD</td>
<td>rs10757278</td>
<td>Additive</td>
<td>GG</td>
<td>31.3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>50.0</td>
<td>1.24 (0.72–2.10)</td>
<td>0.441</td>
</tr>
</tbody>
</table>

$^*$ Adjusted OR (95% CI) and $P$ value were computed with binary logistic regression analysis by adjusting for sex, age, smoking status, history of hypertension, type 2 diabetes, and coronary artery disease.

† Statistical power of single association was calculated with false positive rate of 5%, disease prevalence of 0.56% for nAMD and 0.14% for PCV, given minor allele frequencies and sample sizes, and genotype relative risks of 1.01 and 2.00 in a codominant model with 2 df using software (Power for Genetic Association Analyses).$^{38}$

§ Compared with major allele homozygote.

$^\dagger$ P trend.
above, is associated with both intracranial aneurysm and PCV, but it is not associated with nAMD. In this Chinese Han population study, we identified the association between rs10757278 on 9p21 and PCV, and we demonstrated that rs10757278, another genetic variant strongly associated with intracranial aneurysm, affected the susceptibility of PCV and nAMD differently. The result that rs10757278 was not associated with nAMD in our study is consistent with the AMD genetic study by Haas et al. in a Caucasian Austrian population. Combined with the aneurysmal morphologic and histopathologic features of PCV lesions, our results provide new evidence to bring the nature of PCV a little closer to that of intracranial aneurysm from the basis of nAMD. The genome-wide association studies for AMD have led to substantial discoveries, but PCV is not isolated from these cohorts by ICGA and investigated separately. It will help rectify the classification bias of association results and explain the different pathogenic mechanisms to treat PCV and nAMD separately, especially in Asian cohorts.

PCV is a common disease in the elderly Chinese population, amounting to 24.5% of patients suspected of having nAMD. We estimated the OR for homozygous carriers of rs10757278A to be moderate at 2.10 for PCV. However, because the risk allele is very common in the Chinese Han population, it contributes substantially to the susceptibility of PCV. Despite these results, it seems that rs10757278 is not the driving force behind PCV. The HapMap data demonstrate that the frequencies of rs10757278A are 53% and 50% in Han Chinese in Beijing (CHB) and United States residents with ancestry from northern and western Europe (CEU), respectively. However, PCV is relatively uncommon in Americans, only amounting to 7.8% of patients suspected of having nAMD. Although the association of rs10757278 and PCV in Caucasians is unknown, the ethnic difference of PCV incidence may not be interpreted by the rs10757278 variant.

Besides the PCV association shown in this study and intracranial aneurysm mentioned above, rs10757278 on the 9p21 interval is also reported to be associated with coronary artery disease, ischemic stroke, and abdominal aortic aneurysm. Coronary artery disease, ischemic stroke, and abdominal aortic aneurysm are closely related to atherosclerosis, which is characterized by accumulation of lipids, inflammatory cells, calcium, and necrosis within the walls of large and medium sized arteries, forming atherosclerotic plaque. The plaques may then encroach the lumen of arteries gradually and slow down the blood flow, or the plaques may rupture, forming a thrombus that suddenly blocks arterial blood flow. The arterial wall may also develop degenerative changes, leading to localized dilatation or aneurysm. However, there is no evidence that PCV and intracranial aneurysm have a relationship with atherosclerosis. PCV and intracranial aneurysm are somewhat similar histopathologically. Intracranial aneurysm, located at an arterial bifurcation, has a thin media or none at all, and the internal elastic lamina is either absent or severely fragmented. This PCV, which mostly involves choroidal arteries, is characterized by the disrupted inner elastic layer in the sclerotic arteriolar wall. Helgadottir et al. studied the effects of the rs10757278 variant on five arterial diseases, including coronary artery disease, abdominal aortic aneurysm, intracranial aneurysm, peripheral arterial disease, and large artery atherosclerotic or cardiogenic stroke combined, indicating that the variant had a role in a pathophysiological component common to these arterial phenotypes. This component might involve abnormal vascular remodeling and/or repair rather than atherosclerosis. Chromosome 9p21 “risk” interval is devoid of protein-coding genes. It does overlap a large noncoding RNA termed antisense noncoding RNA, in the INK locus. The 9p21 interval lies adjacent to a cluster of cell-cycle regulating genes, including cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A and CDKN2B). Visel et al. deleted a 70 kb non-coding region on mouse chromosome 4, which is orthologous to the human 9p21 risk interval, and observed markedly decreased expression levels of CDKN2A and CDKN2B in the mutant mice and doubling of the proliferative capacity of mutant aortic smooth muscle cells in culture. These results indicate that the 9p21 risk interval regulates vascular cell proliferation and senescence, and it confirms the presumption put forth in the study by Helgadottir et al., implying that the 9p21 risk interval, including rs10757278, is pivotal for vascular remodeling and/or repair. Thus, the sequence variant rs10757278 on 9p21 may function as a genetic determinant of the tissue response to unfavorable conditions that impact choroidal vasculature, attributing to the development of abnormal choroidal vascular network and aneurismal dilations presented in PCV. Our results also suggest that the PCV pathogenesis might be partially different from nAMD.

Intriguingly, the risk variant in our study was tagged by allele A of the SNP rs10757278 for PCV, rather than allele G known for the other associated vascular diseases previously mentioned. This suggests that PCV and the other vascular diseases may be situated at opposite sides within the same pathophysiological process influenced by rs10757278. In our study, the patients with PCV were not susceptible to coronary artery disease. Furthermore, variants on 9p21 are reported to increase platelet aggregation on coronary artery disease and stroke. However, PCVs with severe thrombocytopenia have been reported. The lower abdominal aorta, coronary arteries, and the circle of Willis, where abdominal aortic aneurysm, coronary artery disease, and intracranial aneurysm occur, respectively, are all large and medium sized arteries. However, PCV involves choroidal arteries and even capillaries. It is unknown whether the allele A and allele G of rs10757278 might produce the different biological effects on the vessels of certain sizes separately. Therefore, the dual role for rs10757278 on 9p21 in modulating susceptibility to vascular diseases serves to demonstrate the biological plausibility of the genetic associations uncovered here.

The major limitation of this study is the relatively small sample size and lack of replication group. Extended cohorts with both PCV and nAMD patients offering higher statistical power will be necessary to confirm our association results. Another limitation of this study is the lack of complete survey of all tag SNPs in the 9p21 interval. Thorough investigations on 9p21 polymorphisms in PCV and nAMD susceptibility will also be required.

In summary, we investigated rs1333040 and rs10757278, two intracranial aneurysm-associated SNPs on chromosome 9p21 in PCV and nAMD. We determined that rs1333040 was not associated with PCV or nAMD. We also determined that rs10757278 was significantly associated with PCV, but not associated with nAMD. It indicates that chromosome 9p21 might affect the susceptibility of PCV and nAMD differently. Allele A of rs10757278, rather than allele G, was shown to confer risk of PCV. Further studies with larger sample sizes are needed to confirm the association results and such an effort should also focus on a comprehensive understanding of the influence of genetic variant of 9p21 on the susceptibility of PCV and other vascular diseases.

References


