RESEARCH REPORT

MMP9 Gene Polymorphism is not Associated with Polypoidal Choroidal Vasculopathy and Neovascular Age-related Macular Degeneration in a Chinese Han Population

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ABSTRACT

Background: Recently, one of our studies has revealed that the serum matrix metalloproteinase 9 (MMP9) level is elevated in polypoidal choroidal vasculopathy (PCV) but not in age-related macular degeneration (AMD). Previous studies have demonstrated that abnormal extracellular matrix (ECM) metabolism plays an important role in the pathogenesis of AMD and PCV. MMP9 is an important regulating enzyme in ECM metabolism, and the MMP9 gene may be a candidate gene for the susceptibility of PCV and AMD. In this study, we aimed to investigate whether the MMP9 gene polymorphism is associated with PCV and neovascular AMD (nAMD) in a Chinese Han population.

Methods: We performed a case-control study in a Chinese Han population. Three tag single nucleotide polymorphisms (SNPs) (rs17576, rs3787268 and rs2274755) of the MMP9 gene were genotyped in 251 patients with PCV, 157 patients with nAMD, and 204 control individuals using the Multiplex SNaPshot system and the direct DNA sequencing technique. The three SNPs genotypes and allele frequencies in the PCV, nAMD and control groups were evaluated using PLINK software and binary logistic regression analysis.

Results: In the PCV, nAMD, and control groups, the minor allele frequencies were 0.2099, 0.2070 and 0.2108 for the rs17576 variant; 0.4442, 0.4522 and 0.4461 for the rs3787268 variant; and 0.1036, 0.1338 and 0.1225 for the rs2274755 variant, respectively. The three tag SNPs were not significantly associated with susceptibility to PCV (p = 0.9524, 0.9553, and 0.3672, respectively) or nAMD (p = 0.9015, 0.8692, and 0.6543, respectively). None of the p values for the additive, dominant, or recessive models were statistically significant in the PCV or nAMD group.

Conclusions: No evidence was found to support an association between the MMP9 gene variants and susceptibility to either nAMD or PCV in a Chinese Han population.

Keywords: Chinese Han population, matrix metalloproteinase 9, neovascular age-related macular degeneration, polypoidal choroidal vasculopathy, single nucleotide polymorphism

INTRODUCTION

Age-related macular degeneration (AMD) is a common cause of blindness in the elderly population in both developed and developing countries.1,2 Neovascular AMD (nAMD) is typically characterized by choroidal neovascularization (CNV) underlying the retina. Polypoidal choroidal vasculopathies (PCV) is a serosanguineous maculopathy with a higher incidence in Asian populations than in Caucasian populations.3-5 It is characterized by branching choroidal vascular networks with polyp-like terminal
The roles of genetic variants in AMD have only been described in detail in recent years. And it is well known that genetic variants may affect the protein expression levels. Recently, one of our studies revealed that the levels of serum matrix metalloproteinase 9 (MMP9), an extracellular matrix (ECM)-metabolizing enzyme, elevated in polypoidal choroidal vasculopathy (PCV) but not in AMD, suggesting that the two disorders may have different molecular mechanisms in ECM metabolism. It is unclear whether the differential expression of serum MMP9 level in PCV and nAMD is caused by differential variants of the MMP9 gene, and there is no report of studies on the association between MMP9 polymorphisms and PCV and AMD. Although some genetic studies have demonstrated that PCV and AMD share some common genetic background, including the CFH, ARMS2, and HTRA1 genes, the results of our previous study indicate that chromosome 9p21 polymorphism (rs10757278), which is involved in abnormal vascular remodeling and ECM metabolism, affects the susceptibility of PCV and nAMD differently. Therefore in this study, to compare PCV and nAMD, we genotyped the tag single nucleotide polymorphisms (SNPs) in the MMP9 gene, and aimed to investigate whether the MMP9 gene polymorphism is associated with PCV and nAMD in a Chinese Han population. Additionally, we tried to determine whether the two disorders show differences in the genetic predisposition of the MMP9 gene.

METHODS

Study Participants

All of the study participants were Chinese Han individuals who were recruited from the Zhongshan Ophthalmic Center of Sun Yat-sen University. The study protocol was approved by the institutional review board at the Zhongshan Ophthalmic Center of Sun Yat-sen University and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all of the study participants, who were fully informed about the purpose and procedures of this study.

All of the nAMD and PCV patients underwent ophthalmic examinations, which included visual acuity measurements, slit-lamp biomicroscopy, ophthalmoscopic exams, color fundus photographs, fluorescein angiography (FFA), and ICGA. The nAMD patients were diagnosed by the identification of CNV on FFA or ICGA, and the diagnosis of PCV was based on the presence of characteristic polypoidal lesions with or without a continuous branching choroidal vascular network on ICGA. All of the PCV patients enrolled in this study met the criteria that were proposed by the Japanese PCV Study Group. All diagnoses were made by at least two of the authors. The cases that were diagnosed as probable or having both nAMD and PCV in the same eye and those patients who had nAMD in one eye and PCV in the other eye were excluded. Patients with other neovascularized maculopathies, such as retinal angiomatous proliferation, idiopathic CNV, angiod streaks, pathological myopia, presumed ocular histoplasmosis, and other retinal or choroidal diseases that could account for CNV, were also excluded.

All of the control subjects were unrelated to the case subjects and were aged ≥50 years. These subjects underwent comprehensive ophthalmic examinations, and those with macular changes (such as drusen or pigment abnormalities), macular degeneration of any cause, or refracting media opacities preventing clear visualization of the fundus were excluded.

Single Nucleotide Polymorphism Selection

SNPs across the MMP9 gene in the international haplotype map (HapMap) for the Han Chinese in Beijing, China (CHB), were used to select tag SNPs. SNPs with a minor allele frequency greater than 5% were evaluated for linkage disequilibrium (LD) using Haplovie software 4.1. A minimum threshold value of 0.8 for the R^2 parameter was set in the Haplovie software. R^2 represents the multivariate coefficient of determination for all alleles that are to be captured. The selected SNPs and the SNPs that were captured by these SNPs are given in Supplementary Table 1 (online only).

SNP Genotyping

Genomic DNA was isolated from peripheral blood samples using the NucleoSpin® Blood XL kit (Macherey-Nagel GmbH & Co., KG Düren, Germany) as previously described. The three tag SNPs of the MMP9 gene (rs17576, rs3787268, and rs2274755) were genotyped using the Multiplex SNAPSHOT system and an ABI 3730XL Genetic Analyzer (Applied
Biosystems, Foster City, CA). Genotypes of the tag SNPs were determined using the GeneMapper software (Applied Biosystems, Foster City, CA). The sequences of the primers that were used for each tag SNP are provided in Supplementary Table 2 (online only). To confirm the accuracy of the Multiplex SNaPshot method, 10% of the samples (randomly selected) were analyzed by direct sequencing (Shanghai Generay Biotech Co., Ltd, China).

Statistics

The data were statistically analyzed using SPSS software (version 16.0, SPSS Inc, Chicago, Illinois, USA). A p value of less than 0.05 was considered statistically significant. The age and gender differences between case and control subjects were assessed using unpaired Student’s t-test for the means and the chi-square test for the proportions. Deviations from the Hardy–Weinberg equilibrium were tested using the exact test as implemented in the software package PLINK v1.07.27 Allele frequencies of the SNP in case and control subjects were determined using the chi-square test in PLINK. For calculations assuming the genotypic additive model, we used the logistic option in PLINK, which recommended a test based on logistic regression, and for calculations assuming the dominant and recessive model, we used the model option in PLINK, which recommended a chi-square test. The odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated relative to the minor allele and wild-type homozygotes. The post-hoc power was calculated with G-power 3.0 software28,29 using the following parameters: effect size = 0.20; α = 0.017; degree of freedom (Df) = 1 for the allelic frequencies, Df=2 for the genotype frequencies.

RESULTS

A total of 612 subjects consisting of 251 patients with PCV, 157 patients with nAMD, and 204 control individuals participated in this study. The baseline information (including gender and age) of the case and control subjects is presented in Table 1. The gender distribution and mean age in the nAMD and PCV groups were not significantly different from the control group.

The genotypes of the three tag SNPs were successfully determined by the SNaPshot method in all of the subjects and confirmed by direct sequencing. Our data did not show any significant deviations from the Hardy–Weinberg equilibrium in the case or control subjects (Table 2). The minor allele frequencies of the three SNPs in each group are summarized in Table 2. The three SNPs (rs17576, rs3787268 and rs2274755) did not exhibit an association with PCV (p = 0.9524, 0.9553, and 0.3672, respectively) or nAMD (p = 0.9015, 0.8692, and 0.6543, respectively). The genotype frequencies of the three SNPs in each group are summarized in Supplementary Table 3 (online only). None of the p values for the additive, dominant or recessive models were statistically significant in PCV or nAMD (Supplementary Table 3). The post-hoc power values for the allelic and genotype frequencies in the PCV and nAMD groups are presented in Tables 2 and Supplementary Table 3.

DISCUSSION

MMP9 (aka 92-kD type IV collagenase or gelatinase B) is a zinc-dependent endopeptidase belonging to a family of proteases known as metzincins, which play roles in degrading the ECM constituents.30 Abnormal MMP9 expression may lead to structural and functional changes in ECM.31

Previous studies have demonstrated that abnormal ECM metabolism plays an important role in the pathogenesis of PCV and AMD.32–34 Bruch’s membrane (BM) is an elastin- and collagen-rich ECM that is strategically located between the fenestrated choroidal capillaries and the retinal pigment epithelium (RPE) of the eye. Histopathological studies have shown that the ECM components (collagen layer and elastic layer) of BM change their thickness and integrity in AMD eyes; the diffuse and focal thickening of BM is considered a sign of early AMD,35 while the disruption and segmental thinning of BM can be observed at the site of CNV in nAMD.36–38 In addition, drusen are abnormal ECM deposits that are located between the RPE and BM. It is the main sign of early AMD. Soft and large drusen are risk factors for progression to advanced AMD.39 For PCV, histopathological findings from surgically excised human PCV specimens demonstrate notable arteriosclerotic changes in PCV lesions and disruption of the elastic layer within the wall of polypoidal vessels,34,40 which
TABLE 2. Association test for the minor allele frequency of the three SNPs in the PCV, nAMD, and control subjects.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position(bp)</th>
<th>Minor allele</th>
<th>Subjects</th>
<th>MAF</th>
<th>HWE</th>
<th>OR (95%CI)</th>
<th>p</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17576</td>
<td>44640225</td>
<td>G</td>
<td>PCV</td>
<td>0.2099</td>
<td>0.4459</td>
<td>0.9903 (0.7186–1.365)</td>
<td>0.9524</td>
<td>0.9699</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nAMD</td>
<td>0.2070</td>
<td>0.4762</td>
<td>0.9774 (0.6805–1.404)</td>
<td>0.9015</td>
<td>0.9212</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>0.2108</td>
<td>0.4027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3787268</td>
<td>44641731</td>
<td>A</td>
<td>PCV</td>
<td>0.4442</td>
<td>0.1613</td>
<td>0.9925 (0.7631–1.291)</td>
<td>0.9553</td>
<td>0.9699</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nAMD</td>
<td>0.4522</td>
<td>0.1462</td>
<td>1.0250 (0.7627–1.378)</td>
<td>0.8692</td>
<td>0.9212</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>0.4461</td>
<td>0.5706</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2274755</td>
<td>44639692</td>
<td>T</td>
<td>PCV</td>
<td>0.1036</td>
<td>0.3160</td>
<td>0.8274 (0.5478–1.250)</td>
<td>0.3672</td>
<td>0.9699</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nAMD</td>
<td>0.1338</td>
<td>0.3128</td>
<td>1.1060 (0.7124–1.716)</td>
<td>0.6543</td>
<td>0.9212</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>0.1225</td>
<td>1.0000</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; PCV, Polypoidal Choroidal Vasculopathy; nAMD, neovascular Age-related Macular Degeneration; MAF, minor allele frequency; HWE, p Value of Hardy-Weinberg equilibrium test; OR, odds ratio; 95%CI, 95% confidence interval.

*Minor allele was calculated based on all of the case and control subjects.

Our previous study found that the serum MMP9 levels were significantly elevated in PCV, also suggesting a role of abnormal ECM metabolism in PCV. We speculated that the association between serum MMP9 and PCV may largely attribute to its proteolytic effects on the ECM proteins (elastin, and collagen), permitting the breakdown of the basement membrane of choroidal vessels and BM. Such changes may contribute to weaken the vascular wall and cause vessel dilatation (forming polypoidal lesions). Particular interest has focused on MMP9 due to its ability to degrade basement membrane components, such as type IV collagen. Besides, MMP9 has additional inhibitory effects on the Ca2+-dependent mechanism of vascular smooth muscle (VSM) contraction, which induces blood vessel relaxation and progressive dilatation. Thus, the MMP9-induced inhibition of VSM contraction may function synergistically with its degradation of the ECM and thereby contribute to further weakening of the blood vessel wall and polypoidal lesion formation. Interestingly, a recent study reported that the MMP9 levels were significantly elevated in the aqueous humor of patients with nAMD and the macular thickness was significantly associated with the MMP9 concentration. Because genetic variants may affect the expression levels of proteins, the MMP9 gene may be a candidate gene for the susceptibility of PCV and nAMD. However, the results of our case-control study in a Chinese Han population demonstrate that the MMP9 gene polymorphism is not significantly associated with either PCV or nAMD, which is consistent with our previous genetic study that the TIMP3 gene polymorphism was not associated with nAMD and PCV in a Chinese Han population. TIMP3 is one of the inhibitors of MMP9, and is also involved in the regulation of ECM metabolism. Moreover, this study also indicated that the serum MMP9 level elevated in nAMD patients is not due to the MMP9 gene polymorphism, but may be due to the regulation of the MMP9 gene at the transcription and translation level or other reasons that require further study.

In this study, all of the subjects were genotyped at the same institution at the same time, minimizing any bias resulting from differences in the genotyping conditions. Furthermore, in an effort to validate the results of genotyping, two genotyping methods (the Multiplex SNaPshot method and direct sequencing) were used in this study. Calculations of our study’s statistical power revealed that our sample size was large enough to detect a gene-disease association with a power of greater than 86%. The failure of this study to demonstrate a significant association between the MMP9 gene polymorphism and PCV or nAMD might not reflect type II error due to the sample size. Although the current analyses have enough statistical power to assess the effect of the MMP9 gene polymorphism on the risk of PCV and nAMD, we also suggest that future studies use larger sample sizes to effectively test the association of the MMP9 gene variants with PCV and nAMD.

There are some limitations to our study. The participants were only drawn from the Chinese Han population, and only the MMP9 gene was genotyped, while other MMPs were excluded from the analysis. Due to the limitation of a relatively small sample size and only the Chinese Han population, our data cannot exclude the possibility that the MMP9 gene polymorphism influences the susceptibility to PCV and nAMD. Larger cohorts and other ethnic populations are required to investigate the association between the MMP9 gene polymorphism and PCV and nAMD.

In summary, in this study, we first investigated the association between MMP9 gene polymorphism and PCV and nAMD in a Chinese Han population, and found no evidence to support a link between the MMP9 gene variants and susceptibility to PCV or nAMD, suggesting that the MMP9 gene...
polymorphism may not play a significant role in the risk of developing PCV or nAMD in the Chinese Han population.

**DECLARATION OF INTEREST**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Supplementary Material Available Online
Supplementary Tables 1–3.