INTRODUCTION

Age-related macular degeneration (AMD) has been shown to be a leading cause of blindness and visual impairment in an older Caucasian population. Neovascular AMD (nAMD) accounts for 80% of the severe visual loss associated with AMD. Secondary to choroidal neovascularization (CNV), nAMD is characterized by subretinal hemorrhage, fibrotic scarring, serous retinal pigment epithelium (RPE) detachment, and rapid loss of central vision.

Polypoidal choroidal vasculopathy (PCV), a hemorrhagic macular disorder characterized by posterior bleeding syndrome and recurrent sero-sanguineous RPE detachment, has been reported in Asian patients. Polypoidal choroidal vasculopathy is typified by a branched vascular network and lesions with polyp-like structural dilations at their borders. It has been proposed that PCV represents a variant of CNV. Thus, CNV is most likely one of the major underlying causes of the visual loss associated with nAMD and PCV.

Recent studies demonstrated that angiogenesis is controlled by a local net balance between factors that either stimulate or inhibit vessel growth in the normal eye. Several studies have established the presence of angiogenic growth factors—vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2)—in surgically removed human CNV membranes and in experimentally induced CNV in animals. Pigment epithelium-derived factor (PEDF), endostatin, and thrombospondin (TSP) are endogenous anti-angiogenic factors that have been suggested to contribute to vascular quiescence. The antagonistic relationship between PEDF and VEGF is key for development of CNV.

ABSTRACT

Purpose: To investigate whether Met72Thr (rs1136287), a common single nucleotide polymorphism (SNP) variant of the pigment epithelium-derived factor (PEDF) gene, is associated with neovascular age-related macular degeneration (nAMD) or polypoidal choroidal vasculopathy (PCV) in a Han Chinese cohort.

Methods: We genotyped Met72Thr (rs1136287) in persons of Han Chinese descent: 177 PCV patients, 131 nAMD patients, and 182 control persons. Genotyping was accomplished using the Multiplex SNaPshot system and by direct DNA sequencing. Genotypes and allele frequencies of patients and controls were evaluated for the SNP using PLINK software.

Results: The minor allele frequency of the PEDF Met72Thr variant did not differ significantly between either PCV or nAMD and the control group: p = 0.3822 and p = 0.9822, respectively. The p-values for the additive, dominant, and recessive models were not statistically significant for PCV or nAMD.

Conclusions: No evidence was found to support a role for the Met72Thr variant in susceptibility to either PCV or nAMD in a Han Chinese cohort.

Keywords: Polypoidal choroidal vasculopathy, Neovascular age-related macular degeneration, Pigment epithelium-derived factor, Han Chinese population, polymorphisms

Lack of Association with PEDF Met72Thr Variant in Neovascular Age-related Macular Degeneration and Polypoidal Choroidal Vasculopathy in a Han Chinese Population

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ORIGINAL ARTICLE
PEDF, which was initially identified as a potential neurotrophic factor, is a member of the serine protease inhibitor superfamily. It is also an inhibitor of angiogenesis and a regulator of CNV. PEDF is localized in many tissues, e.g., retina (interphotoreceptor matrix), vitreous, and aqueous humor. In the retina, PEDF inhibits VEGF-induced proliferation and migration of endothelial cells, as well as blocks VEGF-induced vascular permeability. Furthermore, PEDF is a potent anti-angiogenic protein in humans. Holekamp et al. found decreased vitreous levels of PEDF associated with CNV in AMD patients, indicating that eyes with CNV might possess angiogenic activity. The use of PEDF as a potential therapeutic agent for CNV has been explored in animal models.

In 2005, Yamagishi et al. hypothesized that Met72Thr (rs1136287), a PEDF gene single nucleotide polymorphism (SNP), is associated with AMD. Later, Lin et al. reported Met72Thr (rs1136287) to be associated with exudative AMD in a Taiwanese Chinese population—odds ratio (OR) of 3.9. However, such an association was not found in a European or a different Chinese cohort. Similarly, Bessho et al. investigated the association of Met72Thr (rs1136287) with PCV and nAMD in a Japanese cohort, but no significant associations were found. The studies of Lin et al. and Qu Y et al. of cohorts of the Chinese population did not include indocyanine green angiography (ICGA), which would delineate individuals with PCV so that they could be excluded from the study. Therefore, since ICGA is the only way to obtain clear images for classification of PCV lesions, these cohorts might have included patients with PCV. Several gene polymorphism studies have suggested differences in the genetic determinants of PCV and nAMD.

Until now, the association between Met72Thr and PCV and nAMD in the Chinese population has not been investigated. The goal of this study was to determine whether Met72Thr (rs1136287) is associated with PCV and nAMD in a Han Chinese cohort.

**MATERIALS AND METHODS**

**Study Participants**

A total of 490 persons participated in this study: 177 PCV patients, 131 nAMD patients, and 182 control persons. All study participants were of Han Chinese descent and were recruited from the Zhongshan Ophthalmic Center of Sun Yat-sen University, Guangzhou, China. All procedures conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants. Demographic details of the study population are presented in Table 1.

All nAMD and PCV patients underwent comprehensive ophthalmic examinations, including visual acuity measurements, slit-lamp biomicroscopy, color fundus photography, fundus fluorescein angiography (FFA), and ICGA. All study patients had well-delineated lesions of CNV or PCV, as defined by angiography. The diagnosis of PCV was based on identification of polypoidal choroidal vascular dilations, with or without branching inner choroidal vessels on ICGA, that met the criteria proposed by the Japanese Study Group of PCV. Patients with secondary CNV diseases—angiod streaks, idiopathic CNV, degenerative high myopia, retinal angiomatous proliferation, presumed ocular histoplasmosis, or ocular trauma—were excluded from the study.

The control group comprised individuals with no macular degeneration, no retinal changes such as drusen or pigment abnormalities, and no family history of AMD. Each control subject underwent a detailed eye examination that included a fundus examination. All control subjects were >50 years of age and were unrelated to study patients.

**SNP Genotyping**

Peripheral blood samples from the antecubital vein of all study participants were collected and placed in tubes containing ethylene diamine tetraacetic acid. Genomic DNA was isolated from whole blood using the Nucleospin Blood XL kit (Macherey-Nagel GmbH & Co., KG Düren, Germany) and stored at −20°C. Met72Thr (rs1136287) was genotyped using the Multiplex SNaPshot System with an ABI 3730XL genetic analyzer (Applied Biosystems, Foster City, CA, USA). The primer sequence used for the SNP was forward 5′- TCTTCTGACCTGAGACACTAC-3′ and reverse 5′-ACTTCGGCTATGACCTGTAC-3′. The extension primer was TTTTTTTTTTTTT TTTTTTTCACGTGTCGTCGTGGGGCTC. Randomly selected subjects (10% of all groups) were analyzed by direct sequencing (Shanghai Generay Biotech).
The observed genotype distributions in each group were in accordance with those predicted by the Hardy–Weinberg equilibrium (p > 0.1000) (Table 2). Allele and genotype frequencies of Met72Thr were not significantly different for PCV or nAMD; frequencies of the C allele were 48.55 and 45.45%, respectively (Table 2). The OR for the risk allele C of rs1136287 was 1.14 (95% CI, 0.8501–1.528; p = 0.3822) for PCV and 1.004 (95% CI, 0.7296–1.381; p = 0.9822) for nAMD (Table 2). None of the p-values for the additive, dominant, or recessive model was statistically significant for PCV or nAMD (Table 3).

For power calculations, the present sample size revealed >80% power to detect significant associations (α < 0.017), with an effect size index of 0.2 (corresponding to a weak-to-moderate gene effect). Allele powers for the PCV and nAMD groups, compared separately to controls, were 91.97 and 87.53%, respectively; genotype powers, compared separately to controls, were 86.19 and 80.05%, respectively.

**DISCUSSION**

There is evidence to suggest that an imbalance between PEDF and VEGF induces growth of CNV. PEDF could down-regulate VEGF expression and inhibit CNV. Bhutto et al. reported that a decrease in PEDF disrupted the normal balance between PEDF and VEGF, and permitted occurrence of CNV in nAMD. On the other hand, some recent studies have demonstrated that intravitreal or pericocular injection of the PEDF gene inhibits CNV. Taken together, these facts suggest that PEDF not only plays an important role in the occurrence, development, and subsequent therapy of CNV, but also is an important candidate gene for CNV.

**RESULTS**

Clinical baseline characteristics (including gender, age) of study participants are presented in Table 1. There was no statistical difference between the control group and either the PCV or nAMD group with regard to gender. The mean age (65 ± 8.45 years) of patients in the PCV group was lower than that (68 ± 9.17 years) of the control group (p < 0.0010).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Status</th>
<th>Minor allele</th>
<th>HWE</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met72Thr</td>
<td>1620026</td>
<td>Control</td>
<td>C</td>
<td>0.1003</td>
<td>0.4530</td>
<td>1.140 (0.8501–1.5280)</td>
<td>0.3822</td>
</tr>
<tr>
<td>(rs1136278)</td>
<td></td>
<td>PCV</td>
<td></td>
<td>0.3666</td>
<td>0.4855</td>
<td>1.004 (0.7296–1.3810)</td>
<td>0.9822</td>
</tr>
<tr>
<td>nAMD</td>
<td></td>
<td></td>
<td></td>
<td>0.1616</td>
<td>0.4545</td>
<td>1.004 (0.7296–1.3810)</td>
<td>0.9822</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 frequencies were calculated based on all study participants.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Group</th>
<th>Genotype</th>
<th>Genotype distribution n (%)</th>
<th>OR (95% CI)</th>
<th>p-values</th>
<th>Genotype</th>
<th>Genotype distribution n (%)</th>
<th>OR (95% CI)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met72Thr</td>
<td>PCV</td>
<td>TT</td>
<td>50 (28.3) 60 (33.0)</td>
<td></td>
<td></td>
<td>Additive</td>
<td>1.126 (0.8513–0.4046)</td>
<td>0.4046</td>
<td></td>
</tr>
<tr>
<td>(rs1136278)</td>
<td></td>
<td>CT</td>
<td>82 (46.3) 79 (43.4)</td>
<td>1.2456 (0.7660–2.0253)</td>
<td>0.3757</td>
<td></td>
<td>1.242 (0.7964–1.9593)</td>
<td>0.3323</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>45 (25.4) 43 (23.6)</td>
<td>1.2558 (0.7161–2.0204)</td>
<td>0.4265</td>
<td></td>
<td>1.102 (0.6812–1.7829)</td>
<td>0.6923</td>
<td></td>
</tr>
<tr>
<td></td>
<td>nAMD</td>
<td>TT</td>
<td>43 (32.8) 60 (33.0)</td>
<td></td>
<td></td>
<td>Additive</td>
<td>1.003 (0.7425–1.355)</td>
<td>0.9832</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>57 (43.5) 79 (43.4)</td>
<td>1.0068 (0.5991–1.6918)</td>
<td>0.9797</td>
<td></td>
<td>1.0065 (0.624–1.6233)</td>
<td>0.9789</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>31 (23.7) 43 (23.6)</td>
<td>1.0059 (0.5490–1.8431)</td>
<td>0.9837</td>
<td></td>
<td>1.0021 (0.5907–1.6999)</td>
<td>0.9938</td>
<td></td>
</tr>
</tbody>
</table>

SNP = single nucleotide polymorphism; PCV = polypoidal choroidal vasculopathy; nAMD = neovascular age-related macular degeneration; OR = odds ratio; 95% CI = 95% confidence interval.
disorders, e.g., PCV and nAMD. Results of the present study demonstrate that the PEDF Met72Thr variant (rs1136287) SNP is not significantly associated with either PCV or nAMD.

In recent years, there have been many studies demonstrating that SNPs associated with inflammation, oxidative stress, angiogenesis, and other pathological processes are linked to PCV and nAMD.\(^\text{[5]}\)\(^\text{[6]}\)\(^\text{[7]}\)\(^\text{[8]}\) The PEDF Met72Thr (rs1136287) variant, a methionine-to-threonine polymorphism at codon 72 in exon 3, results in the formation of a BstSI restriction site.\(^\text{[9]}\) As mentioned, several studies attempted to determine if variants of the PEDF gene are associated with exudative AMD or PCV. Only Lin et al.\(^\text{[10]}\) reported the Met72Thr variant to be a novel risk factor for exudative AMD. Three other studies—exudative AMD in a White European population, exudative AMD in a Chinese population, and PCV and nAMD in a Japanese population—failed to replicate Lin’s findings (Table 4). Similarly, our study of a Han Chinese population revealed no significant association of the PEDF Met72Thr variant (rs1136287) with either PCV or nAMD. Conflicting results have been reported from studies of VEGF gene polymorphisms. Lin et al.\(^\text{[11]}\) reported that the VEGF +936 C/T polymorphism was associated with wet AMD in a Taiwanese Chinese population \(p=1.45 \times 10^{-5}\). However, Qu Y et al.\(^\text{[12]}\) reported that none of the tag SNPs (including the +936C/T polymorphism) had any significant association with nAMD in a Chinese cohort. There are several reasons for these discrepancies: (i) differences in case-selection criteria, (ii) insufficient numbers of cases and controls, and (iii) differences in genotyping methods. There is also a lack of data detailing how ethnic differences might contribute to the degree of associative significance. It is, thus, important to conduct repeated trials on different ethnic lines. To date, the degree to which gene polymorphisms and genetic–environmental interactions affect expression and activities of PEDF in the retina and choroid remains unknown.

Several potential limitations to our study should be mentioned. First, while we had sufficient post-hoc power analysis data, the sample size was still relatively small. Thus, we could not completely exclude the existence of weak associations between PCV, nAMD, and the PEDF Met72Thr variant. Further studies of larger sample sizes are needed. Second, no mechanistic or functional evaluation was performed. Finally, a complete survey of all tag SNPs on the PEDF gene was not carried out.

In conclusion, we found no evidence to support a role for the PEDF Met72Thr (rs1136287) variant in susceptibility to PCV or nAMD in a Han Chinese population. This supposition implies that the PEDF Met72Thr (rs1136287) variant is not a major functional candidate gene for conferring risk for PCV or nAMD. Moreover, our research highlights the importance of and difficulty in replicating results from genetic association studies of complex human diseases. The fundamental pathogenic mechanism(s) underlying disturbances in the equilibrium between PEDF and VEGF needs further elucidation.

**ACKNOWLEDGMENTS**

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

**REFERENCES**


[8] Byeon SH, Lee SC, Oh HS, Kim SS, Kohn HJ, Kwon OW. Incidence and clinical patterns of polypoidal...


