

Original Article

COL8A1 rs13095226 polymorphism shows no association with neovascular age-related macular degeneration or polypoidal choroidal vasculopathy in Chinese subjects

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Abstract: Purpose: Age-related macular degeneration (AMD) is the main cause of visual impairment and legal blindness in older individuals. COL8A1 rs13095226 variants have recently been implicated associated with neovascular age-related macular degeneration (nAMD) and Polypoidal Choroidal Vasculopathy (PCV) in American studies. The aim of this study was to investigate the association between the COL8A1 rs13095226 Polymorphism and neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV) in Chinese people. Methods: 900 Chinese subjects-300 cases with nAMD, 300 cases with PCV and 300 controls, were enrolled in a cross-sectional observational study. The diagnoses of nAMD and PCV were confirmed by Fundus photography, Fluorescence Fundus Angiography (FFA) and Indocyanine Green Angiography (ICGA). Genomic DNA was extracted from venous blood leukocytes and genotypes of rs13095226 were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Differences in allele distribution between cases and controls were tested by chi-square tests, with age and gender adjusted by logistic regression analysis. Result: The COL8A1 rs13095226 polymorphism was not statistically significantly different from the nAMD or PCV to the normal controls ($P>0.05$) in Chinese Population. The association remained insignificant after adjustment for age and gender differences ($P>0.05$). Conclusions: This case-control study indicated that the COL8A1 rs13095226 polymorphism is not associated with nAMD or PCV, which suggesting this gene maybe not a susceptibility gene locus for nAMD or PCV in Chinese subjects.

Keywords: Neovascular age-related macular degeneration, polypoidal choroidal vasculopathy, COL8A1, genetics

Introduction

Age-related macular degeneration (AMD), which causes substantial and progressive vision impairment, is a leading cause of blindness in the elderly worldwide [1]. AMD is a multifactorial disease including a complex interaction of genetic and environmental, and it is characterized as chronic and progressive degeneration of photoreceptors, the underlying retinal pigment epithelium, Bruch's membrane and potentially the chorio-capillaris in the macula [2]. Advanced AMD has been classified into two subtypes, neovascular AMD (or wet, exudative, nAMD) and non neovascular AMD (or dry, non

exudative). And the neovascular AMD causes much more cases of severe visual loss and legal blindness among AMD [3]. Polypoidal choroidal vasculopathy (PCV) is associated with a decrease in vision in the elderly Asian population, and is characterized by a network of vessels with two distinct components: a complex of branching vessels and multiple, terminal, reddish-orange polypoidal lesions [4-6]. PCV has been described as a distinct clinical entity from AMD and the other diseases associated with subretinal neovascularization [7]. Nevertheless, whether PCV represents a subtype of neovascular AMD remains controversial [8]. Evidence suggests that AMD and PCV, despite their dif-

Table 1. Demographic distribution of the study subjects

| | nAMD (n=300) | PCV (n=300) | Control (n=300) |
|---------------------------|----------------|----------------|-----------------|
| Females, n (%) | 111 (37.0%) | 112 (37.3%) | 158 (52.7%) |
| Males, n (%) | 189 (63.0%) | 188 (62.7%) | 142 (47.3%) |
| Age range (Years)* | 50-90 | 42-85 | 45-95 |
| Mean age \pm SD (Years) | 69.4 \pm 8.9 | 66.8 \pm 9.7 | 65.1 \pm 9.5 |

*Age of presentation.

ferent phenotypic manifestations, may share common genetic risk factors [9-13].

The extensive family of COL gene products (collagens) is composed of several chain types, including fibril-forming interstitial collagens (types I, II, III and V) and basement membrane collagens (type IV), each type containing multiple isoforms, several collagens also play a role in cell adhesion, important for maintaining normal tissue architecture and function [14, 15]. Recent studies based on European and American indicated the COL8A1 gene was associated with AMD [16-19]. However, no related AMD and PCV studies of COL8A1 in Chinese population have been carried on thus far. As it is well documented that genotype-phenotype associations may vary in different populations, it is imperative to investigate further in the Chinese population. Thus far, we analyzed the COL8A1 rs13095226 polymorphism to identified the potential association with nAMD and PCV in the Chinese population.

Subjects and methods

Subjects

A total of 900 unrelated Chinese subjects were studied in this case-control cohort. Three hundred patients had nAMD and three hundred patients had polypoidal choroidal vasculopathy (PCV); Three hundred individuals without age-related maculopathy (ARM) were studied as controls. The genders and ages of the controls and cases are given in **Table 1**. The study participants were recruited at the Department of Ophthalmology in the Peking University People's Hospital, and the study was approved by the Ethical Committee of Peking University People's Hospital. An informed consent process was established following the guidelines of the Helsinki Declaration, and written informed consent was obtained from all subjects. All subjects received a comprehensive ophthalmic

examination, including visual acuity measurements, slit-lamp biomicroscopy and dilated fundus examination performed by a retinal specialist. All cases with nAMD and PCV underwent fluorescein angiography, optic coherence tomography (OCT), and indocyanine green angiograms with HRA2 (Heidelberg Engin-

eering, Heidelberg, Germany). The diagnosis of nAMD or ARM was defined by the International Classification System for ARM [20]. The diagnosis of PCV was based on indocyanine green angiography (ICGA) results that showed a branching vascular network terminating in aneurysmal enlargements, which typify polypoidal lesions. Exclusion criteria included any eye with any other macular abnormalities, such as pathologic myopia, idiopathic choroidal neovascularization (CNV), presumed ocular histoplasmosis, angioid streaks, and any other secondary CNV. Normal controls were defined as having no clinical evidence of AMD or PCV in either eye or any other eye diseases, excluding mild age-related cataracts. Subjects with severe cataracts were excluded from the study.

Genetic analysis

Blood samples were collected from all participants and stored at -80°C before DNA was extracted. Genomic DNA was extracted from venous blood leukocytes using a genomic extraction kit (Beijing eBios Biotechnology, Beijing, China), and genotyping was performed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as previously described [21]. Briefly, approximately 30 ng of genomic DNA was used to genotype each sample. The primer sequences were 5'-ACGTTGGATGCCAAGGCTTCTGTGGAAAGT-3' and 5'-ACGTTGGATGCACCAGTCATTACCTAAC-3'. The DNA samples were amplified, and the PCR products were used for locus-specific single-base extension reactions. The resulting products were desalted and transferred to a 384 Spectro CHIP array (Sequenom, San Diego, CA, USA). Allele detection was performed using matrix-assisted laser desorption/ionization time-off light mass spectrometry. The mass spectrograms were analyzed using Mass ARRAY Typer software version 4.0 (Sequenom, San Diego, CA, USA).

Table 2. The COL8A1 rs13095226 genotype and allele frequency distribution and the results of association tests

| Frequency distribution | | nAMD vs. control | | | | PCV vs. control | | | | |
|------------------------|-------|------------------|----------------|--------------------|-------|----------------------|-------|-------|----------------------|-------|
| | | nAMD (n=300) | PCV (n=298) | Control (n=300) | P | OR (95% CI) | Power | p | OR (95% CI) | Power |
| Genotype | TT | 268 (89.33) | 274 (91.95) | 265 (88.33) | 0.925 | - | - | 0.218 | - | - |
| | CT | 31 (10.33) | 22 (7.38) | 34 (11.33) | | | | | | |
| | CC | 1 (0.33) | 2 (0.67) | 1 (0.33) | | | | | | |
| Allele | T | 567 (94.50) | 570 (95.64) | 564 (94.00) | 0.710 | 0.912 (0.561-1.483) | 0.066 | 0.202 | 0.715 (0.426-1.199) | 0.204 |
| | C | 33 (5.50) | 26 (4.36) | 36 (6.00) | | | | | | |
| Dominant | TT | 268 (89.33) | 274 (91.95) | 265 (88.33) | 0.697 | 0.904 (0.544-1.503) | 0.067 | 0.139 | 0.663 (0.384-1.145) | 0.317 |
| | CT+CC | 32 (10.67) | 24 (8.05) | 35 (11.67) | | | | | | |
| Recessive | TT+CT | 299 (99.67) | 296 (99.33) | 299 (99.67) | 1.000 | 1.000 (0.062-16.062) | 0.050 | 0.559 | 2.020 (0.182-22.400) | 0.091 |
| | CC | 1 (0.33) | 2 (0.67) | 1 (0.33) | | | | | | |

The dominant model compared a combination of heterozygotes and rare homozygotes to the common homozygotes. The recessive model compared the rare homozygotes to a combination of common homozygotes and heterozygotes. $P < 0.05$ was considered significant.

Table 3. The adjusted p values and odds ratios for age, gender, different genotypes and other various genetic models in each disease

| | | nAMD versus Control | | PCV versus Control | |
|---------------|-----------|---------------------|----------------------|--------------------|----------------------|
| | | P | OR (95% CI) | P | OR (95% CI) |
| Genotype | TT | - | 1.00 | - | 1.00 |
| | CT | 0.716 | 1.104 (0.648-1.883) | 0.106 | 0.625 (0.353-1.106) |
| | CC | 0.775 | 1.504 (0.092-24.610) | 0.845 | 1.272 (0.113-14.274) |
| Genetic model | Dominant | 0.686 | 1.115 (0.659-1.887) | 0.126 | 0.649 (0.372-1.129) |
| | Recessive | 0.781 | 1.488 (0.091-24.318) | 0.818 | 1.327 (0.118-14.888) |

The dominant model compared a combination of heterozygotes and rare homozygotes to the common homozygotes. The recessive model compared the rare homozygotes to a combination of common homozygotes and heterozygotes. $P < 0.05$ was considered significant.

Statistical analysis

The data were analyzed using SPSS (version 16.0; SPSS Science, Chicago, IL, USA). All of the identified polymorphisms were evaluated for Hardy-Weinberg equilibrium using chi-square tests. Single-marker association analyses were performed using chi-square tests or Fisher's exact tests under various genetic models. Logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval (CI) of nAMD or PCV, comparing the case groups to the control group. Age- and gender-adjusted p values and ORs were also calculated. The statistical power was also calculated. Values of $P < 0.05$ were considered statistically significant.

Results

A total of 900 subjects were enrolled in this study, including 300 control subjects (mean age \pm SD, 65.1 \pm 9.5 years; ratio of women to

men, 52.7:47.3), 300 cases with nAMD (mean age \pm SD, 69.4 \pm 8.9 years; ratio of women to men, 37.0:63.0) in one or both eyes, and 300 cases with PCV (mean age \pm SD, 66.8 \pm 9.7 years; ratio of women to men, 37.3:62.7) in at least one eye. The general characteristics of the study subjects are summarized in **Table 1**.

The rs13095226 genotype call rates were 99.78% (898/900). The allele frequencies and genotype frequencies for patients and controls and single-SNP association analysis results are shown in **Table 2**. The genotype and allele distribution of the investigated SNP showed no significant association from the Hardy-Weinberg equilibrium in both the controls and the nAMD and PCV cases ($P > 0.05$; **Table 2**), even after correction for age, gender, different genotypes, and various genetic models based on a logistic regression model, the associations remained insignificant (all $P > 0.05$; **Table 3**). The statistical powers to detect the association between the SNP and nAMD or PCV ranged from 0.05 to

0.32, assuming conventional levels of statistical significance (Table 2).

Discussion

AMD is a complex disease caused by multiple environmental and genetic risk factors. Several genes have been proved to be associated with nAMD and PCV [22, 23]. The SNP rs13095226 is on chromosome 3 in the COL8A1 gene, which encodes one of the two alpha chains of type VIII collagen, a major component of the multiple basement membranes in the eye, including Bruch's membrane and the choroidal stroma [24]. Bruch's membrane is located directly below the retinal pigment epithelium and plays a central role in the pathogenesis of AMD [25].

Several studies [16-19, 26] have been performed to explore the interactions between SNP rs13095226 as risk factors in nAMD. Moreover, few studies explore the interactions of rs13095226 in the development of nAMD and PCV in Chinese population. To our knowledge, this is the first investigation of an association between COL8A1 rs13095226 polymorphism and nAMD or PCV risk in a large Chinese subjects. In this study, we evaluated SNP rs13095226 in the COL8A1 gene in nAMD and PCV subjects from a Chinese population and found that rs13095226 variants in the COL8A1 gene were not associated with either nAMD or PCV in Chinese population.

COL8A1 rs13095226 variants has previously been observed associated with nAMD and PCV in an American study. A Genome-wide association study (GWAS) of advanced age-related macular degeneration, which included 979 advanced AMD cases and 1,709 controls, indicated genome-wide association at rs13095226 ($P=2.5e-06$) in European AMD populations [26]. And a GWAS meta-analysis of advanced AMD, which included 2,594 advanced AMD cases and 4,134 controls also supported the previously suggestive association to advanced AMD ($P=9.7e-07$) [16]. In contrast, we were unable to observe any trend towards an association for rs13095226 and AMD in our subjects.

The allele frequency at rs13095226 differed between American and Chinese subjects. The minor-allele (C) frequency (MAF) was shown by Benjamin M. Neale et al. to be as high as 11.6% in the discovery sample [26]. In our current

study, we note that the frequency of the allele (C) for rs13095226 was only 5.5% for nAMD, 4.3% for PCV and 6.0% for controls, a finding entirely consistent with that seen in the Americans despite the discrepant minor allele frequencies between Americans and Asians. The different allele frequencies for rs13095226 may influence the final effects between American and Asians. Anyhow, since the low statistical power, a large sample size is required to get more conclusions. Alternatively, the fact that genetic variants associated with a particular disease in this population may not necessarily be associated in other populations must be considered. Moreover, the gene-disease association of rs13095226 in populations from East Asia could be weak or absent compared with American populations. Anyhow, since the low statistical power, a large sample size is required to get more conclusions.

In conclusion, we found no evidence to support a significant association of the COL8A1 rs13095226 polymorphism with nAMD or PCV in this study, suggesting that this polymorphism is unlikely to be a major susceptibility gene locus for these diseases in the Chinese population. Whether there is a weak association between the COL8A1 rs13095226 polymorphism and nAMD or PCV in Chinese must be confirmed with future studies with much larger sample sizes.

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Disclosure of conflict of interest

None.

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