Association of genetic risk factors for age-related macular degeneration with polypoidal choroidal vasculopathy and central serous chorioretinopathy

결절 맥락막 혈관병증 및 중심성 장액맥락망막병증 환자에서 연령관련 황반변성의 관련 유전 위험인자의 분석

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Association of genetic risk factors for age-related macular degeneration with polypoidal choroidal vasculopathy and central serous chorioretinopathy

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Abstract

Purpose: To investigate the association of known genetic risk factors for age-related macular degeneration (AMD) with polypoidal choroidal vasculopathy (PCV) and central serous chorioretinopathy (CSC).

Patients and Methods: Korean case-control group consisting of 112 PCV, 167 CSC patients and 395 control subjects were analyzed. Three single nucleotide polymorphisms (SNPs), ARMS2 rs10490924, HTRA1 rs11200638, and CFH rs1061170, were genotyped in PCV, CSC and control subjects. Differences in the observed genotypic distribution between the case and control groups were analyzed after adjusting for age and sex.

Results: ARMS2 rs10490924 and HTRA1 rs11200638 were significantly associated with PCV. There were no SNPs showing significant association with CSC. The genotype distribution of rs10490924 and rs11200638 also differed significantly between PCV and CSC. The risk allele frequency was higher in PCV patients compared to CSC.
Conclusions: Genetic risk factors for AMD showed significant association with PCV but not with CSC in Korean patients.

Keywords: Polypoidal choroidal vasculopathy; Central serous chorioretinopathy; Age-related macular degeneration; Genetic risk factor

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INTRODUCTION

Central serous chorioretinopathy (CSC) is a clinical entity characterized by serous detachment of the neurosensory retina at the posterior pole, in association with retinal pigment epithelium (RPE) dysfunction and choroidal vascular hyperpermeability. There are two phenotypes of CSC, acute or chronic, depending on the presence of persistent subretinal fluid (SRF). Acute CSC is known to resolve spontaneously with little sequelae, but chronic CSC, characterized by persistent SRF of at least 3 or 6 months, can cause extensive RPE damage, leading to photoreceptor damage, irreversible visual decline and even progression to age-related macular degeneration (AMD). Although the pathophysiology of CSC remains unclear to date, there is mounting evidence from new imaging techniques that the choroid and RPE play key roles.

Polypoidal choroidal vasculopathy (PCV), considered one of the subtypes of AMD, is a sight-threatening macular disease showing characteristic inner choroidal vascular networks ending in polypoidal lesions. Choroidal vascular hyperpermeability which was initially described as a characteristic finding in CSC has been demonstrated to be more frequently associated with PCV compared to AMD and recent studies using enhanced depth imaging optical coherence tomography (EDI OCT) have shown choroidal thickening...
in patients with PCV, in contrast to choroid thinning in those with exudative AMD. Also, a recent study found that PCV associated with choroidal vascular hyperpermeability had a thickened choroid and more frequent history of CSC. History of CSC itself has long been considered a risk factor for the development of PCV with the hypothesis that long-standing changes to the RPE in CSC, especially the chronic type, may predispose to the formation of polypoidal-like choroidal vascular changes.

Thus it may be hypothesized that CSC and PCV, both believed to originate from abnormalities of the inner choroidal vasculature, may in part, share a common pathogenic mechanism. The purpose of this study was to explore this theme at the genetic level, namely, to compare the genetic background of CSC and PCV by performing association analysis of known risk genetic variants for AMD with both PCV and CSC. To the best of our knowledge this is the first study to perform comparative analysis of genetic risk factors in PCV and CSC.
METHODS

Study design and patient subjects

PCV and CSC patients were recruited from the Retina clinic at Seoul National University Bundang Hospital (SNUBH). Study protocols were approved by the institutional review board of SNUBH and informed consent was obtained from all subjects before participation in the study.

A total of 279 cases were included, consisting of 112 PCV and 167 CSC patients. All patients underwent comprehensive ophthalmological evaluation, including measurement of best-corrected visual acuity, slit-lamp biomicroscopy, indirect fundus exam, fluorescein angiography (FA), indocyanine green angiography (ICGA, Heidelberg Retina Angiography, Heidelberg Engineering, Heidelberg, Germany), and optical coherence tomography (OCT, Spectralis, Heidelberg Engineering, Heidelberg, Germany). PCV was diagnosed by identifying hyperfluorescent polypoidal choroidal vasculature with branching vascular network on ICGA with concomitant exudation or hemorrhage. CSC was defined as macula-involving SRF with or without RPE detachment seen on OCT with multifocal RPE leaks evident on FA and choroidal vascular hyperpermeability on ICGA. A subgroup of CSC patients with persistent or progressive visual symptoms for more than 6 months was categorized as chronic CSC. Subjects with choroidal neovascularization (CNV) typical of
exudative AMD or CNV secondary to other causes such as myopic maculopathy were excluded.

For the control group, a total of 395 subjects were recruited, either from people visiting the SNUBH healthcare center for regular medical checkup or from participants of the Korean Longitudinal Study on Health and Aging (KLoSHA); randomly-sampled community-dwelling elderly Koreans aged 65 years or older. Normal control subjects underwent visual acuity examination, fundus photography and/or OCT to ensure that no intermediate sized drusen or RPE changes were present.

**Single nucleotide selection and genotype analysis**

Three SNPs previously reported to be associated with AMD, \textit{ARMS2} rs10490924, \textit{HTRA1} rs11200638, and \textit{CFH} rs1061170, were selected for analysis. DNA was extracted from leukocytes in the peripheral blood by DNA extraction kit (QIAamp® DNA Maxi kit, Qiagen Inc.). For PCV patients and control, SNPs were genotyped using multiplex PCR with single base extension primers (iPLEX Gold® kit and MassARRAY® software, Sequenom, San Diego, CA). CSC patients were genotyped on Illumina® Exome array (HumanExome Beadchip, Illumina Inc., San Diego, CA). The genotypes of the 3 SNPs were then used for statistical analysis.
**Statistical analysis**

Demographic data were compared using the *t*-test and $\chi^2$ test. Genotype allele frequencies were compared after adjusting for age and sex using the logistic regression method. SNP & Variation Suite (SVS) 7 (Golden Helix Inc., Bozeman, MA, USA) software program was used to perform all statistical analyses. Multiple testing correction for allelic associations was performed using the Bonferroni correction, taking the effective number of independent SNPs in a gene region into account. $P<0.025$ was considered to be statistically significant.
RESULTS

Patient demographic data are shown in Table 1. There were significant differences in the mean age and sex ratios between PCV and CSC patients. CSC patients were significantly younger and there were a higher percentage of males in the CSC group.

The risk allele frequencies, results of the association test and genotypic distribution in PCV, CSC and control subjects are shown in Table 2 and 3. In logistic analyses adjusted for age and sex, 2 SNPs, *ARMS2* rs10490924 and *HTRA1* rs11200638, showed significant association with PCV compared to control (P=1.84E-10 and 4.20E-9, respectively). However, there was no significant association between any of the 3 SNPs and CSC compared to control. The minor allele frequencies of the 3 SNPs were similar for chronic and total CSC. Significant differences were observed between PCV and CSC patients for all 3 SNPs.
Table 1. Baseline demographic data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCV</th>
<th>CSC</th>
<th>Control</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Chronic</td>
<td>Total</td>
<td>Chronic</td>
</tr>
<tr>
<td>Number</td>
<td>112</td>
<td>167</td>
<td>50</td>
<td>395</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>67.32 (7.31)</td>
<td>46.35 (7.16)</td>
<td>48.52 (7.24)</td>
<td>68.20 (10.13)</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>54.50%</td>
<td>71.30%</td>
<td>84.00%</td>
<td>50.10%</td>
</tr>
</tbody>
</table>

*PCV vs total CSC vs Control
Table 2. Allelic frequencies of the analyzed single nucleotide polymorphisms (SNPs) and results of the association test in subjects with polypoidal choroidal vasculopathy (PCV), central serous chorioretinopathy (CSC), and control.

<table>
<thead>
<tr>
<th>SNP / Gene</th>
<th>Minor allele frequency</th>
<th>Association test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARMS2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10490924</td>
<td>T</td>
<td>0.639</td>
</tr>
<tr>
<td>HTRA1</td>
<td>A</td>
<td>0.625</td>
</tr>
<tr>
<td>CFH</td>
<td>C</td>
<td>0.079</td>
</tr>
</tbody>
</table>

*Adjusted for age and sex
Table 3. Genotype distribution of SNPs in subjects with PCV, CSC and control.

<table>
<thead>
<tr>
<th>SNP / Gene</th>
<th>Genotype</th>
<th>PCV</th>
<th>CSC</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10490924 ARMS2</td>
<td>GG</td>
<td>15 (13.9)</td>
<td>80 (47.9)</td>
<td>25 (50.0)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>48 (44.4)</td>
<td>68 (40.7)</td>
<td>21 (42.0)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>45 (41.7)</td>
<td>19 (11.4)</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td>rs11200638 HTRA1</td>
<td>GG</td>
<td>18 (16.1)</td>
<td>75 (44.9)</td>
<td>25 (50.0)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>48 (42.9)</td>
<td>71 (42.5)</td>
<td>19 (38.0)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>46 (41.1)</td>
<td>21 (12.6)</td>
<td>6 (12.0)</td>
</tr>
<tr>
<td>rs1061170 CFH</td>
<td>TT</td>
<td>91 (84.3)</td>
<td>139 (83.2)</td>
<td>41 (82.0)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>17 (15.7)</td>
<td>25 (15.0)</td>
<td>7 (14.0)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0 (0)</td>
<td>3 (1.8)</td>
<td>2 (4.0)</td>
</tr>
</tbody>
</table>
DISCUSSION

This study is the first study to directly compare genetic risk factors for PCV and CSC. In the present study, we genotyped previously known genetic risk factors for AMD, ARMS2 rs10490924, HTRA1 rs11200638, and CFH rs1061170, in PCV and CSC patients and found significant association only with PCV.

Both PCV and CSC are clinically important macular diseases. PCV, regarded by some as a subtype of AMD and by others, a distinct disease entity, is known to be more prevalent in the Asian population with reports showing that 40-55% of Japanese exudative AMD, 25% of newly diagnosed Chinese AMD, and 31.7% of Korean exudative AMD patients to be PCV.23-27 CSC, the fourth most common retinopathy after AMD, diabetic retinopathy and retinal vein occlusions, is also believed to be more prevalent in Asians compared to Caucasians.28,29 Among the two phenotypes of CSC, acute and chronic, the latter form presents with more widespread RPE damage, leading to higher incidences of complication with CNV.30

PCV has been frequently reported to have clinical, FA and ICGA findings that are similar to CSC.10,14,31 Choroidal hyperpermeability, originally a characteristic finding in CSC, has been reported in as little as 9.3% or as high as 59.3% of patients with PCV.10,11,32 When Koizumi et al investigated associated clinical characteristics in PCV patients with
evidence of choroidal vascular hyperpermeability, they found higher incidence of bilateral involvement of the neovascular membrane and more frequent CSC history. Recent employment of enhanced depth imaging OCT (EDI-OCT) in PCV patients has also shown significantly thickened choroid when compared to those with typical exudative AMD, as is found in patients with CSC. These findings commonly imply a common pathogenic mechanism for CSC and PCV.

Due to clear differences in the associated clinical manifestations, patient demographics, the response to treatment and visual prognosis for PCV and exudative AMD, they are often regarded as distinct disease entities and there is yet no consensus on whether they are or not. Numerous studies have been conducted to investigate the association of well recognized AMD genetic risk factors with PCV, in order to gain insight on a genetic level. A recent meta-analysis found 5 SNPs in 4 genes, ARMS2 rs10490924, HTRA1 rs11200638, CFH rs1061170, CFH rs800292, and C2 rs547154, to be significantly associated with PCV, showing that PCV does share a common genetic etiology with AMD.

The pathophysiology of CSC remains unclear to date, although major theories implicate the choroid, RPE, and hormonal milieu as the major culprits. Also, although there are numerous reports of familial CSC in the literature, there have been no genetic studies investigating a genetic etiology for CSC to date.

In our study, 2 SNPs, ARMS2 rs10490924 and HTRA1 rs11200638, were
significantly associated with PCV patients, as has been confirmed in past studies. The lack of association between the CFH Y402H variant (rs1061170) and exudative AMD or PCV has already been reported in the Asian population. This is known to be due to ethnic differences in the frequency of the risk allele, 5~8% in Chinese, Japanese and Koreans, compared to 35% in Caucasians. The ARMS2 rs104904924 SNP has been extensively studied for association with PCV. It has been reported to be expressed in the mitochondria of human retina photoreceptors and may be related to RPE dysfunction. Interestingly, the ARMS2 rs10490924 was the only SNP that showed a statistical difference between PCV and AMD in a recent meta-analysis.

We investigated the allelic and genotypes frequencies of chronic CSC as a sub-analysis since it is more likely to share a common pathogenic mechanism with PCV, but found no differences when compared with the control group. Although the number of patient was relatively small, we believe some differences in the trend of risk allele frequencies would have been noted, if it had existed.

In this study, well recognized genetic risk factors for PCV were not associated with CSC. There is the possibility that other genetic factors are involved, environmental factors may play a greater role in the pathogeneses of the two diseases, and common anatomic changes of the choroid and RPE, observed in PCV and CSC, maybe secondary changes resulting from pathologies at a molecular level.
In conclusion, widely recognized genetic risk factors for AMD, ARMS2 rs10490924, HTRA1 rs11200638, CFH rs1061170, were associated with PCV but not CSC in Korean patients. Further research is warranted to elucidate the common mechanism of PCV and CSC.
REFERENCES


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국문초록

서론: 최근 들어 결절 맥락막 혈관변증 및 중심성 장액맥락망막병중의 병리기전에 맥락막혈관 이상이 관여하는 점이 조명되면서 두 질환의 연관성이 대두되고 있다. 본 연구는 결절 맥락막 혈관병증 및 중심성 장액맥락망막병증 환자를 대상으로 이미 알려진 연령관련 황반변성의 관련 유전 위험인자의 분석을 하고자 하였다.

방법: 한국인 환자군-대조군(case-control) 연구로 결절 맥락막혈관변증 환자 112명, 중심성 장액맥락망막병증 환자 167명, 그리고 395명의 대조군 환자를 대상으로 하였다. 3종류의 단일염기 다형성 (single nucleotide polymorphisms) ARMS2 rs10490924, HTRA1 rs11200638, and CFH rs1061170를 이들 환자군 및 대조군에서 유전자형 분석 하였다. 추출된 유전자형을 토대로 각 질환 및 대조군에서의 분포차이를 분석하였다.

결과: ARMS2 rs10490924 와 HTRA1 rs11200638 는 결절 맥락막혈관변증과 유의한 연관성이 있었다. 반면, 중심성 장액맥락
망막병증과 의미있는 상관관계를 지닌 유전자형은 존재하지 않았다. 유전자형 rs10490924와 rs11200638 분포에 있어서는 결절 맥락막 혈관변증과 중심성 장액맥락망막병증 사이에 확연한 차 이를 보였다. 중심성 장액맥락망막병증과 비교하여, 고위험 대립유전자 빈도는 (risk allele frequency)는 결절 맥락막 혈관변증 환자군에서 더 높게 나타났다.

결론: 연령관련 황반변성의 관련 유전 위험인자들은 한국인에서 결절 맥락막 혈관변증과는 의미있는 연관성을 보였으나, 중심성 장액맥락망막병증과는 연관성을 보이지 않았다.

주요어: 결절 맥락막 혈관병증, 장액맥락망막병증, 연령관련 황반변성, 유전 위험인자

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