Experimental Eye Research 108 (2013) 16–22

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Different impact of high-density lipoprotein-related genetic variants on polypoidal choroidal vasculopathy and neovascular age-related macular degeneration in a Chinese Han population

Xiongze Zhang [1], Meng Li [1], Feng Wen*, Chengguo Zuo, Hui Chen, Kunfang Wu, Renpan Zeng

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie South Road, Guangzhou 510060, China

ARTICLE INFO

Article history:
Received 10 July 2012
Accepted in revised form 12 December 2012
Available online 26 December 2012

Keywords:
high-density lipoprotein
age-related macular degeneration
polypoidal choroidal vasculopathy
genetic polymorphism

ABSTRACT

Neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV) are both major serosanguinous maculopathies among the Asian elderly. They are similar in phenotype. Genetic variants in high-density lipoprotein (HDL) pathway were discovered to be associated with AMD in two genome-wide association studies. In this study with a Chinese Han cohort, we investigated the impacts of these genetic variants on nAMD and PCV separately. The missense coding variants and previously identified variants at *LIPC*, *ABCA1*, *CETP*, *LPL* and *FADS1* loci were genotyped in 157 nAMD patients, 250 PCV patients and 204 controls without any macular abnormality. The known variants in *CFH*, *ARMS2* and near *HTRA1* were also genotyped. Fasting serum cholesterol levels were determined. The variants in *CFH*, *ARMS2* and near *HTRA1* were strongly associated with both PCV ($P < 10^{-6}$, 10^{-7} and 10^{-7} respectively) and nAMD ($P < 10^{-6}$, 10^{-16} and 10^{-17} respectively). None of the studied HDL-related variants were significantly associated with nAMD. A missense variant in *CETP*, rs5882, was significantly associated with PCV ($P = 2.73 \times 10^{-4}$). The rs5882 GG genotype had a 3.53-fold (95% CI: 1.93–6.45) increased risk for PCV, and conferred a significantly lower serum HDL-cholesterol level for PCV patients than the AA genotype ($P = 0.048$). These results suggest the need to separate PCV from nAMD in association studies especially with Asian cohorts, and that the HDL pathway may involve in the pathogenesis of PCV and nAMD differently.

1. Introduction

Polypoidal choroidal vasculopathy (PCV) and neovascular age-related macular degeneration (nAMD) are both the leading cause of irreversible vision impairment among Asian elderly (Byeon et al., 2008; Liu et al., 2007; Maruko et al., 2007; Wen et al., 2004). They are similar in clinical manifestation (Imamura et al., 2010; Laude et al., 2010). Indocyanine green angiography (ICGA) can be used to differentiate these two serosanguineous maculopathies morphologically. Although there is still some debate on the nature of PCV (Imamura et al., 2010), an increasing understanding of PCV and the widespread availability of ICGA has helped us to recognize PCV as a clinical entity, possibly distinct from nAMD. However, the differences between the pathogenesis of PCV and nAMD are mostly unknown. Genome-wide association studies (GWAS) for AMD have led to substantial discoveries, finding the risk loci such as the *CFH* and *HTRA1*/*ARMS2* (Peter and Seddon, 2010). However, in those cohorts, PCV is not identified using ICGA and investigated separately. Several genetic association studies (Laude et al., 2010) that were designed to compare these two entities aimed to discover if these two different phenotypes can be attributed to genetic differences that may reveal different underlying pathogenic mechanisms. The genes *complement factor 2* (*C2*) and *complement factor B* (*CFB*) (Lee et al., 2008) were found to be associated with nAMD, but not with PCV. Recently, we showed that a risk variant for intracranial aneurysm and coronary artery disease on chromosome 9p21 was associated with PCV, but not with nAMD (Zhang et al., 2011). In this study with an extended cohort, we investigated the serum cholesterol levels and the high-density lipoprotein (HDL)-related genetic variants, which were newly identified to be associated with AMD by two GWAS (Chen et al., 2010; Neale et al., 2010), and demonstrated the differential impacts of each on the risk of PCV and nAMD.

2. Patients and methods

2.1. Study population

This study was performed in accordance with the tenets of the Declaration of Helsinki. The study protocol was approved by the

* Corresponding author. Tel.: +86 20 87330292.
E-mail address: wenfeng208@yahoo.com.cn (F. Wen).
[1] These authors contributed equally to this work.

0014-4835/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2012.12.005

institutional review board at the Zhongshan Ophthalmic Center of Sun Yat-sen University. Written informed consent was obtained from all subjects for providing medical information and a blood sample.

All study subjects were unrelated Chinese Han individuals that were recruited from the Zhongshan Ophthalmic Center from June 2008 to July 2011. They were asked about their smoking status and alcohol consumption and were asked to provide a detailed medical history. All PCV and nAMD patients were newly diagnosed and treatment-naïve. They all underwent bilateral ophthalmic examinations including visual acuity measurements, slit-lamp biomicroscopy, ophthalmoscopy, color fundus photography, fluorescein angiography and ICGA. Diagnosis was based on the worst eye, but cases with comorbidity of any other retinal or choroidal disease in one or both eyes were excluded. The diagnosis of PCV was based on the identification of characteristic polypoidal choroidal vascular dilations with branching inner choroidal vascular network within the first 5 min after the injection of ICGA. Cases that were difficult to distinguish from nAMD and retinal angiomatous proliferation were excluded. The diagnosis of nAMD was based on the identification of typical choroidal neovascularization with both fluorescein angiography and ICGA. Patients with other neovascularized maculopathies, such as pathologic myopia, angioid streaks, multifocal choroiditis and punctate inner choroidopathy were excluded. All control subjects were aged $\geq$50 years and underwent ophthalmic examinations including visual acuity measurements, slit-lamp biomicroscopy, ophthalmoscopy and 50° color fundus photography. Those with macular degeneration of any cause, macular changes (such as drusen or pigment abnormalities), or media opacities preventing the clear visualization of the macula were excluded from the study.

2.2. Single nucleotide polymorphism (SNP) selection

Previously reported AMD-associated SNPs at HDL metabolism loci, as identified by GWAS (Chen et al., 2010; Neale et al., 2010), were selected. Meanwhile, to narrow down the candidate SNPs, only the missense coding SNPs, which would be more likely to influence the protein function, across the genes *hepatic lipase* (*LIPC*), *ATP-binding cassette sub-family A member 1* (*ABCA1*), *cholesteryl ester transfer protein* (*CETP*), *lipoprotein lipase* (*LPL*) and *fatty acid desaturase 1* (*FADS1*), with a minor allele frequency above 1% in HAPMAP-HCB (Han Chinese in Beijing, China, in the International HapMap project), were selected from the NCBI Entrez SNP database (http://www.ncbi.nlm.nih.gov/SNP/). The SNPs repeatedly confirmed to be associated with PCV and nAMD in *complement factor H* (*CFH*) (rs800292), *age-related maculopathy susceptibility 2* (*ARMS2*) (rs10490924) and near *HtrA serine peptidase 1* (*HTRA1*) (rs11200638) (Hayashi et al., 2010; Lee et al., 2008; Lima et al., 2010) were also included to confirm their association in the cohorts studied. Thus, a total of 17 candidate SNPs were selected and are listed in Supplementary Table 1.

2.3. Genotyping

The collection of a peripheral blood sample and the extraction of genomic DNA were performed as previously described (Li et al., 2010). The SNPs were genotyped using a Multiplex SNaPshot system with an ABI 3730XL genetic analyzer (Applied Biosystems, Foster City, CA). The genotypes of the SNPs were determined using Genemapper software v4.1 (Applied Biosystems, Foster City, CA). The sequences of the primers used for each SNP are provided in Supplementary Table 1. To confirm the accuracy of the Multiplex SNaPshot method, randomly selected subjects (10% of all samples) were analyzed by direct sequencing (Generay Biotech Co., Ltd., Shanghai, China). The primers that were used for the direct sequencing are available on request.

2.4. Serum analysis

Fasting serum samples were collected on the same day as the ophthalmic examinations and were analyzed for total cholesterol, HDL-cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), as measured on a Hitachi 7170A automatic analyzer (Hitachi Ltd., Tokyo, Japan) using the Cholesterol (CHO) Assay kit (Human GmbH, Wiesbaden, Germany), the Cholestest-N HDL kit and the Cholestest-LDL kit (Sekisui Medical Co., Ltd., Tokyo, Japan), respectively.

2.5. Covariates

Clinical information and family histories were collected from medical records and interviews. A smoker was defined as having smoked at least 1 cigarette per day for at least 6 months. An alcohol drinker was defined as having at least 1 drink of beer, wine, or liquor per week for at least 6 months. A person with hypertension was diagnosed by having a systolic blood pressure $\geq$140 mmHg, a diastolic blood pressure $\geq$90 mmHg, or because they were being treated with anti-hypertensive medication. Coronary artery disease was defined by a physician's diagnosis. Body weight and height were measured on the same day as the ophthalmic examinations. The body mass index was calculated as weight (kilograms) divided by height (meters) squared.

2.6. Statistical analysis

Differences in the demographic characteristics between cases and controls were assessed using unpaired Student's *t*-tests for means and chi-squared tests for proportions using SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL). Genetic association analyses were performed using the PLINK software package v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). Deviations from the Hardy–Weinberg equilibrium and allele frequencies between cases and controls were evaluated for each SNP using the exact test and the chi-square test in PLINK, respectively. The minor allele frequency was calculated based on all the case and control subjects. A Bonferroni correction was used to correct the *P* values obtained from the allele frequencies analysis. 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 chi-square test. Power calculation of single association was performed using PGA (Power for Genetic Association Analyses) software (Menashe et al., 2008), based on minor allele frequency and sample size, with relative risks at 1.8, disease prevalence of 0.56% for nAMD (Kawasaki et al., 2010) and 0.14% for PCV (Kawasaki et al., 2010; Liu et al., 2007), respectively, effective degrees of freedom of 17, and a false positive rate of 5%, under a co-dominant model (heterozygous vs. all homozygotes) with two degrees of freedom. Linkage disequilibrium patterns and haplotype association analyses were performed with the Haploview software package (Barrett et al., 2005) v4.2 (http://www.broadinstitute.org/haploview). Logistic regression analysis was used to estimate the adjusted odds ratio and 95% confidence intervals (CI) with the controlling factors known to be associated with the diseases. Model A was adjusted for gender, age, body mass index, smoking status, alcohol consumption and history of hypertension and coronary artery disease. Model B was adjusted for the same covariates as in model A plus the genotypes of rs800292 in *CFH* (CC, CT, TT), rs10490924 in *ARMS2* (TT, GT, GG) and rs11200638 near *HTRA1* (AA, AG, GG). The Cochran–Armitage trend test was used to estimate the trend of proportions. A value of $P < 0.05$ was considered to be statistically significant.