Association of Central Serous Chorioretinopathy with single-nucleotide polymorphisms in Complement Factor H Gene in a Chinese population

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Abstract

Background: To analyze the association between central serous chorioretinopathy (CSCR) and single-nucleotide polymorphisms in the complement factor H (CFH) gene in patients of Chinese descent. Methods: 437 CSCR patients and 510 controls were enrolled from the Department of Ophthalmology, People's Hospital of Peking University. We genotyped each patient for six single-nucleotide polymorphism (SNP) markers in CFH (rs800292, rs1061170, rs3753396, rs2284664, rs1329428, and rs1065489), and assessed each SNP's associations with CSCR. We also performed a meta-analysis of CFH SNPs associations with CSCR. Results: In our Chinese population sample, two CFH SNPs—rs800292 and rs1065489—were significantly associated with CSCR. We found that rs800292 had the strongest association, and rs1065489 had the second strongest association. From a meta-analysis of all existing case-control studies on CFH SNPs and CSCR, we also found significant associations between CFH SNPs rs1329428, rs1065489, rs2284664, and rs800292, and CSCR. Conclusions: Our results show a significant association between CSCR and two SNPs in the CFH gene, rs800292 and rs1065489, in a Chinese population. These findings suggest a role for CFH in CSCR pathogenesis. Further investigation into how CFH contributes to CSCR will improve our understanding of CSCR, and of CFH as a potential therapeutic target.

Background

Central serous chorioretinopathy (CSCR) is characterized by localized serous detachment of the neurosensory retina in conjunction with one or more focal lesions of the retinal pigmented epithelium. CSCR ranks among the most common vision-threatening retinopathies after age-related macular disease, diabetic retinopathy and branch retinal vein occlusion [1]. Acute CSCR often resolves spontaneously, but recurrent and chronic CSCR may lead to severe vision loss due to foveal attenuation, chronic macular edema, and damage to the foveal photoreceptor layer [2]. CSCR is more commonly seen in Asian, Caucasian, and Spanish populations [3]. In Asian populations, CSCR is frequent and severe, with bilateral and multifocal forms reported more frequently than in other ethnic groups [4]. CSCR itself is incompletely understood, with systemic associations, a multifactorial etiology, and a complex pathogenesis. Risk factors include genetic predisposition [5-12], corticosterioids [13-17], and psychopathology [18-22]. Choroidal circulation abnormalities are also thought to play a key role in CSCR pathogenesis, including choroidal hyper-permeability, choroidal vein dilation, and arterial filling delay.

CFH encodes the complement factor H protein, which binds to adrenomedullin, a calcitonin family peptide, eliciting choroid vasodilation and consequently increasing microvascular permeability [23,24]. These hemodynamic features are in accordance with the manifestation of indocyanine green angiography in CSCR patients. Several gene association studies support a potential role for *CFH* in CSCR pathogenesis: Miki *et al*. [12] reported five CSCR-associated SNPs (rs800292, rs3753394, rs2284664, rs1329428, and rs1065489) of *CFH* in a Japanese population; Moschos *et al*. [11] reported three CSCR-associated *CFH* SNPs (rs3753394, rs1329428, and rs1065489) in a Greek population; and de Jong *et al*. [10] reported three CSCR-associated *CFH* SNPs (rs800292, rs1329428, and rs1065489) in a Western

European population. Interestingly, although these three studies analyzed the same five SNPs of *CFH*, they did not all confirm exactly the same associations. In addition, the findings of Miki *et al*. [12] and de Jong *et al*. [10] suggest that rs1065489 is protective for CSCR, but Moschos *et al*. [11] showed the opposite result. In addition, the sample size of each study was relatively small: 140, 41, and 292, respectively. Furthermore, none of the three studies included patients of Chinese descent.

To the best of our knowledge, this is the first study to test for associations between *CFH* SNPs and CSCR in a Chinese population. Additionally, our enrollment of 437 CSCR patients makes this the largest sample size in such studies to date. With regard to the discrepant results in previous studies, which we attribute to small sample sizes, we also carried out a meta-analysis in order to amplify the overall sample size and more accurately evaluate the associations of individual *CFH* SNPs with CSCR. Furthermore, recent studies have confirmed associations between *CFH* SNPs and polypoidal choroidal vasculopathy (PCV) [25-27], another disease associated with choroidal thickening, raising the possibility that a similar genetic factor(s) may underpin both CSCR and PCV. We therefore compared our meta-analysis result with that of a meta-analysis of *CFH* SNP associations with PCV [25] to examine whether CFH acts similarly in both PCV and CSCR.

Methods

Patient Selection

The research was approved by the Clinic Institutional Review Board, and complied with the Declaration of Helsinki. Informed consent was obtained from each participating subject. We enrolled 437 CSCR patients and 510 control patients in the original population. All patients were recruited from the Eye Center of the People's Hospital of Peking University. Enrollees' demographic details are shown in Table 1.

All subjects underwent a comprehensive ophthalmic examination, including best-corrected visual acuity measurement, slit-lamp biomicroscopy, dilated fundoscopy, color fundus photographs, optical coherence tomography, flurorescein angiography, and indocyanine green angiography.

Our criteria for CSCR patient selection were as follows: serous subretinal fluid on optical coherence tomography, focal leakage spot (ink blot) or smokestack pattern or ≧1 area of multifocal diffuse leakage, or presence of irregular retinal pigment epithelium window defects on fluorescein angiography and corresponding hyperfluorescence on indocyanine green angiography[28,29]. Participants with uveitis, diabetic retinopathy, age-related macular degeneration, polypoidal choroidal vasculopathy, other retinal and choroidal vascular diseases, pathologic myopia were excluded.

Genotyping

Five milliliters of peripheral blood was collected from each participant and stored at -80℃ prior to DNA extraction. Genomic DNA was extracted from venous blood leukocytes, using a genomic extraction kit (Beijing eBios Biotechnology, Beijing, China). Genotyping was performed by matrix-assisted laser

desorption/ionization time-of-flight mass spectrometry, as previously described [11]. The primers used for *CFH* SNPs (rs800292, rs1061170, rs3753396, rs2284664, rs1329428, and rs1065489) were as follows:

SNP-1-F: CAGAAGGCACCCAGGCTATC

SNP-1-R: TGGCAATAGTGATATAATTCAGGCA

SNP-2-F: TCCTGAACTCCTCAATGGGAA

SNP-2-R: ACAGGTACTCTCCTCCACTATGT

SNP-3-F: TGTTTGCGTCATTAGATACCCT

SNP-3-R: TACAACCTTTACAGTTACCTCC

SNP-4-F: CTATTTGCTTTACGATCTATGG

SNP-4-R: CAGTGATACATCCAGGTACATTA

SNP-5-F: GTTGGTGACAGTCCGATAGACA

SNP-5-R: ATTTCCCACAGCAGTCCAGAATA

SNP-6-F: AGGGTTTCTTCTTGAAAATCACAGG

SNP-6-R: GAGTAGTGTACTTACTGACACGGA

Following amplification, the PCR products were used for locus-specific single-base extension reactions. The resulting products were desalted and transferred to a 384 SpectroCHIP array (Sequenom, San Diego, CA). Allele detection was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The mass spectrograms were analyzed using MassARRAY Typer software version 4.0 (Sequenom, San Diego, CA).

Statistical Analysis

The data were analyzed using SPSS (version 16.0; SPSS Science, Chicago, IL). All identified polymorphisms were assessed for Hardy-Weinberg equilibrium. Allelic association was evaluated for each SNP by Chi-square tests on 2 × 2 contingency tables. All odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated with respect to minor alleles in the controls. For the allelic association tests, we used a Bonferroni correction for multiple testing, in which nominal P values were multiplied by 6 (the number of SNPs). Values of $p < 0.05$ were considered statistically significant.

To begin the meta-analysis, we searched PubMed, CNKI, and Wanfang for studies on *CFH* and CSCR. We identified five articles, only three of which accorded with our inclusion criteria requiring case-control studies showing P values and 95% CIs. Both a fixed-effect model and a random-effect model were fitted

to the data in each study. Cochran's Q statistic and the companying I^2 index was used to access inter-cohort heterogeneity. For heterogeneity P values > 0.05, we assumed the effects were heterogeneous and used the fixed-effect model was selected; otherwise, we used the random-effect model. All statistical calculations were conducted using Stata version 13.0.

Results

There were 947 participants in our study, including 437 cases (mean age = 45.6 years, female-to-male ratio = 0.30) and 510 controls (mean age = 67.3 years, female-to-male ratio = 1.27). Table 1 shows the demographic characteristics of patients and controls.

Table 2 shows our SNP analysis results. The frequencies of all genotypes were in Hardy-Weinberg equilibrium in both the control and case patients (All the P value>0.05). Among the six SNPs examined (rs800292, rs1061170, rs3753396, rs2284664, rs1329428, and rs1065489), two were significantly associated with CSCR after Bonferroni correction: rs8000292 (allelic P = 0.0024, OR= 1.39, 95% CI= 1.15-1.66) and rs1065489 (allelic P = 0.0252, OR = 0.77, CI = 0.64-0.92). None of the other four SNPs we examined—rs1061170, rs3753396, rs2284664, and rs1329428 showed a significant association with CSCR.

We compared our results with those of three previous studies [10-12] and identified four SNPs (rs1329428, rs1065489, rs2284664, and rs800292) for which results differed between studies. With regard to these four SNPs, we performed a meta-analysis of our data plus the data in the three previous studies (910 cases, 3168 controls). The meta-analysis results revealed significant associations between rs1329428 (OR = 1.62, CI = 1.30-2.01, P < 0.001; I^2 = 70.3%, P = 0.009), rs1065489 (OR = 0.72, CI = 0.55-0.95, P = 0.020; I^2 = 76.9%, P = 0.002), rs2284664 (OR = 1.31, CI = 1.06-1.61, P = 0.012; I^2 = 65.8%, P = 0.020), and rs800292 (OR = 1.34, CI = 1.13-1.59, P = 0.001; I^2 = 50%, P = 0.091) and CSCR.

In addition, we compared our meta-analysis results with those of a previous meta-analysis of *CFH* SNP associations with PCV [27]. From that comparison, we found that rs1065489 was protective for CSCR, and rs1329428, rs2284664, and rs800292 conferred risk, which were precisely the opposite of their associations with PCV.

Discussion

To the best of our knowledge, this is the first study to focus on a Chinese population with 437 CSCR patients. We tested six SNPs (rs800292, rs1061170, rs3753396, rs2284664, rs1329428, and rs1065489), and found that two (rs1065489, rs800292) were significantly associated with CSCR, which was in accordance with the findings of Miki *et al*. (in a Japanese population), [12] de Jong *et al*. (in a Western

European population), [10] and our meta-analysis. Moschos *et al*. also confirmed an association for rs1065489 in their Greek population [11]. However, we found the minor allele rs1065489 T to be protective for CSCR, which was consistent with the results of Miki *et al*. [12] and de Jong *et al*. [10], but opposite to that of Moschos *et al*. [11], possibly due to that study's small sample size. Our meta-analysis results confirmed the protective role of the minor allele rs1065489 T in CSCR (OR = 0.72, CI = 0.55-0.95, P = 0.020). For rs800292, Moschos *et al*. [11] found no association with CSCR, in contrast to results of the other studies. Our meta-analysis confirmed the association for rs800292 (OR = 1.34, CI = 1.13-1.59, P = 0.001), and the minor allele rs800292 T showed a risk for CSCR. For rs1329428 and rs2284664, our initial analysis did not find associations with CSCR, but the meta-analysis amplified the sample size and showed an association (rs1329428: OR = 1.62, CI = 1.30-2.01, P<0.001; rs2284664: OR = 1.31, CI = 1.06-1.61, P=0.012). This difference may indicate that our control sample size was not large enough. For rs1061170, our study showed no significant association with CSCR, which was consistent with the study of de Jong *et al*. [10]. We are also the first to examine rs3753396 for possible association with CSCR.

Owing to interest in the pachychoroid spectrum disease, we asked whether similar genetic factors underlie both CSCR and PCV. We therefore compared our meta-analysis results for *CFH* SNPs and CSCR with Li Ma *et al*.'s meta-analysis results [27] for *CFH* SNPs and PCV. We found that four SNPs were associated with both CSCR and PCV—rs1329428 T, rs1065489 T, rs2284664 T, and rs800292 A— but the correlation with PCV was much stronger than that with CSCR. Moreover, the associative results were exactly the opposite for rs1329428, rs1065489, rs2284664, and rs800292; those that conferred CSCR risk were protective against PCV, and vice versa. The different effects of these four SNPs on CSCR and PCV suggest that these two diseases have different pathogenesis mechanisms and/or different genetic factors.

Although many studies on CSCR have been conducted, its exact pathogenesis remains unknown. Our findings support a potential role for CFH in CSCR. Complement factor H, the *CFH* gene product, helps regulate the complement system. Specifically, CFH negatively regulates the complement system, protecting healthy cells by preventing the complement system from being activated when it is not needed. Whether the four SNPs (rs1329428, rs1065489, rs2284664, rs800292) influence the quality and quantity of CFH protein is still unknown. Perhaps they weaken the regulatory role of CFH protein in the complement system, or affect its binding with adrenomedullin.

One potential limitation of our study is the lack of participants from all Chinese provinces, so larger-scale studies are needed to confirm our results. Moreover, both acute and chronic phenotype CSCR patients were enrolled in our study, but our methodology did not distinguish between the two, so we could not analyze the association between *CFH* SNPs and different CSCR phenotypes.

Conclusions

In summary, our study demonstrates a significant association between multiple SNPs in the *CFH* gene (rs1329428, rs1065489, rs2284664, and rs800292) and CSCR. These findings support CFH's potential

role in CSCR pathogenesis. Additional studies are needed to confirm our results in other populations and clarify the mechanisms and pathways through which CFH contributes to CSCR pathogenesis. Functional studies of each independent SNP will be necessary to provide further information regarding altered CFH activity and its role in CSCR. In addition, our results comparing *CFH* SNP associations with CSCR and PCV provide intriguing insights into the underlying genetic characteristics and pathophysiology of the development of these two diseases.

Abbreviations

CSCR: Central serous chorioretinopathy; CFH: complement factor H; SNP: single-nucleotide polymorphism; PCV: polypoidal choroidal vasculopathy; OR: odds ratio; CI: confidence interval; SD: standard deviation; MAF: Minor Allele Frequency

Declarations

Ethics approval and consent to participate

All authors state that subjects have given their written informed consent and that the study protocol was approved by the Clinic Institutional Review Board of Peking University People's Hospital.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Dandan Linghu and Hui Xu wrote the manuscript and contributed equally to this work. Yu Cao is responsible for statistic analysis. Enzhong Jin, Tingting Gao and Yuou Yao conducted the study and enrolled participants. Jinfeng Qu, Lvzhen Huang and Mingwei Zhao are responsible for conceptual idea. All authors read and approved the final manuscript.

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Tables

Table 1 Characteristics of the study population

parameters	CSCR patients	controls
Females,n(%)	100(22.9%)	285(55.9%)
Males,n(%)	337(77.1%)	225(44.1%)
Age* ranges(Years)	25-77	45-96
Mean age±SD**(Years)	45.6±7.7	67.3±9.6

Age*, age of presentation. ** SD, standard deviation

Table 2 The genotype Distribution of CFH SNPs and association analysis in CSCR patients and controls

Genotype	Case (n=437)	Control (n=510)	&MAF (Cases)	MAF (Controls)	Nominal Allelic P Value	Allelic OR (95% CI)	*Bonferroni-corrected P Value
CC	0	1	0.089	0.064	0.0446	1.42(1.01-2.00)	0.2676
CT	78	63					
TT	359	446					
GG	157	134	0.411	0.478	0.0042	0.77(0.64-0.92)	0.0252
GT	200	264					
TT	80	112					
AA	87	81	0.436	0.395	0.0655	1.19(0.99-1.43)	0.3930
GA	207	241					
GG	143	188					
AA	149	141	0.423	0.470	0.0386	0.83(0.69-0.99)	0.2316
GA	206	259					
GG	82	110					
CC	112	177	0.487	0.406	0.0004	1.39(1.15-1.66)	0.0024
CT	224	252					
TT	101	81					
AA	103	109	0.485	0.531	0.0447	1.20(1.00-1.44)	0.2682
AG	244	260					
GG	90	141					

single-nucleotide polymorphisms

& Minor Allele Frequency

*Bonferroni-corrected P value was calculated by multiplying the nominal P by 6 (Because there were 6 SNPs in our study).

Figures

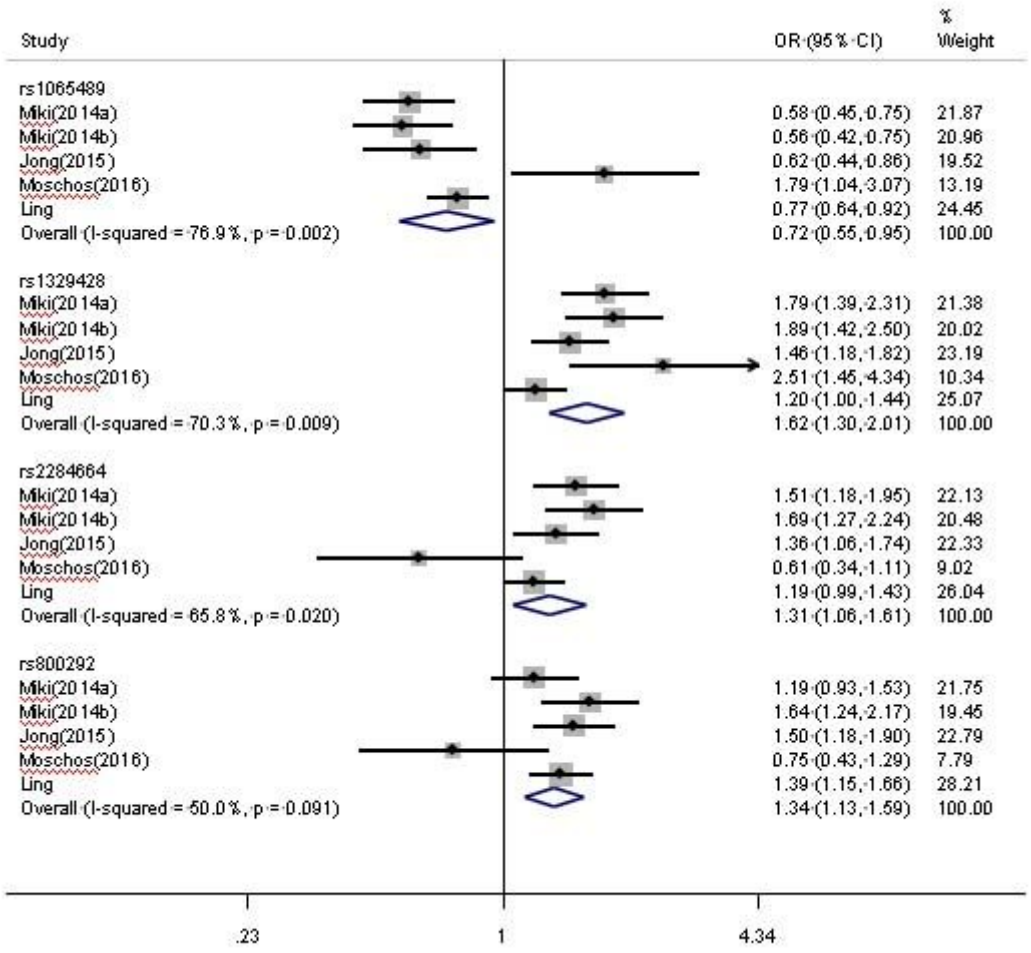

Figure 1

Meta analysis of rs1065489, rs1329428, rs2284664, rs800292 and central serous chorioretinopathy (CSCR) Forest-plot (910 cases, 3168 controls): Meta-analysis results revealed significant associations between rs1329428 (OR = 1.62, CI = 1.30-2.01, P < 0.001; I2 = 70.3%, P = 0.009), rs1065489 (OR = 0.72, CI = 0.55-0.95, P = 0.020; I2 = 76.9%, P = 0.002), rs2284664 (OR = 1.31, CI = 1.06-1.61, P = 0.012; I2 = 65.8%, P = 0.020), and rs800292 (OR = 1.34, CI = 1.13-1.59, P = 0.001; I2 = 50%, P = 0.091) and CSCR.